e-ISSN 2544-1361

European Journal of Clinical and Experimental Medicine

Formerly: Medical Review

Quarterly

Vol. 21, No. 3

Publication date: September 2023

Rzeszów, Poland 2023

Technical development, layout and interior design: Wojciech Pączek
Cover design: Wiesław Grzegorczyk

ICV 2021: 100.00
MEiN: 40.00

Indexing:
ARIANTA – Science and branch Polish electronic journals
Bielefeld Academic Search Engine
The Central European Journal of Social Sciences and Humanities (CEJSH)
Central Medical Library (Poland), The Directory of Open Access Journals (DOAJ)
EBSCO, Free Medical Journals
Harvard Libraries – University of California Libraries
Index Copernicus, J-Gate
Main Medical Library in Warszawa
Ministry of Education and Science
MOST WIEDZY, POL-Index
Scopus

e-ISSN 2544-1361

EDITORIAL CORRESPONDENCE
European Journal of Clinical and Experimental Medicine Editorial Office
35-959 Rzeszów, ul. Kopisto 2A,
tel. 17 851 68 38, fax 17 872 19 30
http://www.ejcem.ur.edu.pl
e-mail: ejcemur@gmail.com
https://mc04.manuscriptcentral.com/pmur

PUBLISHER: PUBLISHING OFFICE OF THE UNIVERSITY OF RZESZÓW
35-959 Rzeszów, ul. prof. S. Pigonia 6,
tel./fax 17 872 14 26, e-mail: wydaw@ur.edu.pl

Wydawnictwo UR 2023
ISSN 2544-1361 (online)

http://www.ejcem.ur.edu.pl
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2023; 21 (3): 440

Contents

Wydawnictwo UR 2023
ISSN 2544-1361 (online)
doi: 10.15584/ejcem.2023.3.1

http://www.ejcem.ur.edu.pl
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2023; 21 (3): 442–449

ORIGINAL PAPER

Synthesis and characterization of Fulvestrant and Paclitaxel conjugates with polyamidoamine dendrimer fourth generation

Konrad Wróbel, Stanisław Wołowiec

Institute of Medical Sciences, Medical College, Rzeszów University, Rzeszów, Poland

ABSTRACT

Introduction and aim. Poorly soluble anticancer drugs can be attached covalently into biologically inert macromolecule in order to administrate a drug as water soluble form. It was proven that covalent linkers, for instance amide or carbamate bonds are susceptible to hydrolysis. Thus the attached drug can be released from the conjugates in tissue, specifically within the targeted cell. We aimed at construction of water soluble conjugates of Fulvestrant and Paclitaxel with PAMAM G4 dendrimer. In order to obtain water soluble conjugates the amine groups were substituted with *R*-glycidol.

Material and methods. Polyamidoamine dendrimer of fourth generation was synthesized and examined by detailed NMR analysis in water and in DMSO. The conjugates were covalently linked to amine groups of PAMAM after activation of Fulvestrant 17-OH group with 4-nitrophenylchloroformate and activation of end-carboxyl group of Paclitaxel succinate.

Results. The method of binary conjugate PAMAMG4-Fulvestrant-Paclitaxel synthesis was elaborated and the product was characterized by physicochemical methods.

Conclusion. The glycidylated PAMAMG4-Fulvestrant-Paclitaxel conjugate is better soluble in water than unconverted drugs.

Keywords. PAMAM dendrimer, double-conjugation, Fulvestrant, Paclitaxel, anticancer drug delivery system

Introduction

It is estimated that nearly 2 million of US inhabitants will be diagnosed with cancer in 2022 and more than 600 000 deaths (due to cancer) will be reported. In comparison WHO data from 2020 shows that cancers mortality reached 10 million (nearly one in six of all reported deaths globally was due to cancer). After heart disease cancer is second leading cause of death in US. Since early 1960s the 5-year relative survival rate (all cancers combined) has increased from 39% to 68% among White population and 27% to 63% among Black population of Americans as a result of improvements in treatment and diagnostic approaches. Main conventional methods of treating cancer could be distinguish as chemotherapy, radiotherapy and surgical treatment. In recent years some novel strategies such as targeted drug therapy, stem cells therapy, gene therapy or therapy based on systems for drug delivery have been developed for further improvement of survival rate and to overcome some limitations of using „traditional" methods.[1-4]

PAMAM dendrimers are one of extensively tested drug delivery systems for improving outcomes of chemotherapy by increasing water solubility, absorption and cellular uptake of drugs applied in cancer systemic treatment. These spherical, highly branched molecules build up with repeatedly added ethylenediamine and methyl acrylate to diamine core are suitable for functionalization with anticancer drugs because of having superficial functions such as amine or hydroxyl groups which may be utilized to covalently bond dendrimer with other molecules (via direct bonding or cleavable linkers). It was shown that PAMAM G4 functionalization with Paclitaxel (P)

Corresponding author: Stanisław Wołowiec, e-mail: swolowiec@ur.edu.pl

Received: 12.04.2023 / Revised: 17.05.2023 / Accepted: 18.05.2023 / Published: 30.09.2023

Wróbel K, Wołowiec S. *Synthesis and characterization of Fulvestrant and Paclitaxel conjugates with polyamidoamine dendrimer fourth generation.* Eur J Clin Exp Med. 2023;21(3):442–449. doi: 10.15584/ejcem.2023.3.1.

and omega-3 fatty acids enhance anticancer activity of P against human esophageal cancer in vitro model. Moreover it was recently proved that Fulvestrant and Lapatinib conjugated with partially glucoheptoamidated PAMAM G3 gain ability to promote apoptosis in senescent breast cancer cell lines of different receptor status.[4-8]

Paclitaxel, as one of the taxanes, is commonly used in treatment of cancer. P is approved by FDA to treat breast, ovarian, lung cancers and also Kaposi's sarcoma. Moreover, it could be used in systemic therapy (as off-label treatment) of gastroesophageal, endometrial, cervical, prostate, head and neck cancers, as well as sarcoma, lymphoma, and leukemia. It was commonly believed that predominant mechanism of P anticancer effect emerges from its ability to disruption in microtubule stabilization/destabilization process (inducing polymerization and stabilization of microtubules) resulting in mitotic arrest. However, such conception changes in recent years due to extensive investigations of P on biological models. It was shown that P concentrations in primary breast tumors are below level required to elicit sustained mitotic arrest. Alternative hypothesis suggests that P efficacy may be explained by its ability to cause cell death in interphase instead of affecting mitosis (due to interference with cell signaling, trafficking, and microtubule-mediated transport). It was proved (in primary human breast cancers models) that P efficacy could be effectively explained by its ability to cause chromosome missegregation on abnormal mitotic spindles. In addition it is suggested that P cytotoxicity could be result of some other factors for example its ability to hyperphosphorylation of Bcl-2, induction of calcium ions release by mitochondria or modulatory effect on miRNA expression profiles. P bioavailability is significantly limited by poor water solubility of this drug and first-pass metabolism. To improve P pharmacokinetic Nab-paclitaxel, a P complex with albumin was approved by FDA to treat metastatic breast cancer in 2005.[9-14]

One of drugs commonly used in hormonal treatment of hormone sensitive breast cancers is Fulvestrant. Due to high affinity to estrogen receptor (ER) and lack of intrinsic activity Fulvestrant acts as competitive antagonist. In addition Fulvestrant accelerate ER protein degradation and therefore it is defined as selective estrogen receptor degrader (SERD). Instead of proven high efficacy in systemic therapy of patients with hormone receptor-positive locally advanced or metastatic breast cancer (who have not received hormonal treatment before) application of Fulvestrant has some significant limitations. The main limitation is poor water solubility of Fulvestrant which leads to necessity of intramuscular application and such route of administration limits volume and dose of drug.[15-18]

Combination therapy is intensively tested approach for increase efficacy of anticancer treatment by synergistic or additive effect of drug combination. Application of two or more drugs (combined) of different mechanisms of action and toxicity profiles may lead to overcome drug resistance of cancer cells, enhancement of anticancer effect and also decrease systemic toxicity of such therapy (for example by reduced doses of each applied drug). Combination of Lapatinib and Capecitabine was proved to be significantly more effective than Capecitabine alone in women with advanced HER2- positive breast cancer (treated before with anthracycline, taxane and even Trastuzumab). In mentioned study elongation of median time to progression from 4.4 months for Capecitabine alone to 8.4 months for combination with Lapatinib was reported.[19,20]

The aim of our study was to conjugate polyamidoamine dendrimer of fourth generation (PAMAM G4) with P by succinate linker and Fulvestrant through carbonyl linker and to characterize conjugates using 1-D and 2-D NMR spectroscopy (^{1}H,^{13}C, COSY, HSQC, HMBC). Generally the PAMAM and other type dendrimers are currently extensively studied as macromolecular carriers of drugs due to their radial geometry, strictly defined molecular weight and molecular size in contrary to other polymers, which are polydispersed macromolecules. We have elaborated an effective protocol to obtain the conjugate, which are very well water-soluble due to substitution of PAMAM amine groups with 2,3-dihydroxyproyl substituents by reaction with *R*-glycidol. The degree of PAMAM G4 substitution with drugs was low enough to maintain the solubility of the conjugates from one side and to obtain high drug load of macromolecular carrier. Additionally we have applied PAMAM G4 as the carrier instead of previously used PAMAM G3 as core for conjugates of Lapatinib, Fulvestrant, Simvastatin, Celecoxib, or Nimesulide.[8,21,22]

Aim

Paclitaxel and Fulvestrant are commonly used anticancer drugs. They are the compounds which are highly hydrophobic and therefore sparingly soluble in water. We aimed at synthesis of potential drug delivery system by conjugating both drugs with high molecular weight polyamidoamine dendrimer of fourth generation (G4) in order to increase the solubility of the drugs. Especially we aimed at the conjugate with both drugs attached to one macromolecule as combination therapy approach (binary conjugate). In order to reach the goal we have applied known methods of activation and linking the drugs into the G4 carrier, and characterized the final product and all compounds used to construct binary conjugate by nuclear magnetic resonance spectroscopy and determined the size of conjugates by dynamic light scattering method.

Material and methods

Materials

PAMAM G4 dendrimer was synthesized according to the general procedure described by Tomalia et al.[23] The

chemicals used for synthesis: Paclitaxel, Fulvestrant, dimethyaminepyridine (DMAP), 1-methyl-2-chloropyridine iodide (Mukaiyama reagent), succinic anhydride, *p*-nitrophenyl-chloroformate (NPCF), bulk solvents and triethylamine were purchased from Merck (KGaA, Darmstadt, Germany).

Syntheses

Paclitaxel succinate (P-suc)

Paclitaxel was converted into 2-O-succinyl derivative according to the published method.[24] In specific 75 mg P (87.7 μmoles) was dissolved in 5 ml $CDCl_3$ and 250 μl pyridine. Succinic anhydride (10.0 mg; 100 μmoles) was added and the mixture refluxed for 8 hours. Then solvents were removed under vacuum and the solid residue dissolved in 2 ml DMSO.

P-suc conjugation to PAMAM G4 and glycidylation

244 mg G4 (17.5 μmol) was dissolved in 3 ml DMSO. Meantime P-suc (87.7 μmoles) was activated by addition of 42 mg DMAP (337 μmoles) and 43 mg of N-methyl-2-chloropyridinium iodide (168 μmoles) for 1 hr at room temperature. Activated P-suc was added dropwise into G4 solution in methanol and stored at 50 °C for 6 hours. Afterwards, the mixture was transferred to dialytic bag (cellulose MW_{cutoff} = 3.5 kDa) and dialyzed for three days against water. The isolated yield was 217.1 mg. The stoichiometry of the conjugate was estimated based on the ^{1}H NMR spectroscopy. It was found that 5 P-suc was attached per one equivalent of G4. According to the molecular formula of this conjugate: $G4^{5P}$, 18.9 kDa, 11.5 μmoles of the conjugate was obtained; 65.7 % per dendrimer. Hydrodynamic diameter of nanoparticle by DLS: numbered-averaged d(N) = 99.20 (± 7.28) nm. Polydispersity index (PDI) = 0.134 (0.016). Potential zeta (ξ) = 13.57 (± 0.43) mV. In repeated synthesis (starting with 60 μmoles of P and 15 μmoles (209 mg) G4) aimed at lower substitution the $G4^{4P}$ was obtained and further remaining amine groups were converted by addition of *R*-glycidol (200 μL; 223 mg; 3 mmoles). The mixture was stored at ambient temperature for 12 hours and the solid product was identified as $G4^{4P110gl}$ by NMR spectroscopy. Theoretical molecular weight – 30.0 kDa. The isolated yield was 279.2 mg (9.32 μmoles); 81 %.

Fulvestrant conjugation to PAMAM G4

Fulvestrant (F) was substituted with *p*-nitrophenyl-chloroformate (NPCF) as described before.[8] In specific, 121.4 mg F (200 μmoles) was dissolved in 2 ml $CHCl_3$ and 50 μL pyridine. NPCF (220 μmole, 44.3 mg) was added stepwise and the mixture was refluxed for 2 hours. The solvents were removed *in vacuo* and the solid residue was dissolved in 1 ml DMSO. This solution was added into 185 mg G4 (13 μmoles) in 2 ml DMSO. The mixture was stored at 45°C for 12 hours followed by isolation of the product by dialyzis. The product subjected to glycidylation by addition of 100 μL *R*-glycidol and reacted 12 hours in methanol at ambient temperature. The product was worked up by dialysis, dried and identified by the ^{1}H NMR as PAMAM G4 bearing average 15 equivalents of succinate linked F and 66 equivalents of glycidol (gl); $G4^{15F66gl}$. Theoretical molecular weight - 24.5 kDa. Yield: 279.2 mg (11.4 μmoles); 87.6 %. Hydrodynamic diameter of nanoparticle by DLS d(N) = 138.23 (± 6.24) nm. (PDI) = 0.155 (0.022). Potential ξ = 38.02 (± 0.54) mV.

Double conjugate G4- Paclitaxel – Fulvestrant

Fulvestrant (17.6 mg, 29 μmoles) was activated with 7.1 mg NPCF (35 μmoles) as before and added into 35.9 mg of $G4^{5P}$ (1.9 μmol) obtained before. The mixture was stored at 45 °C for 12 hours followed by isolation of the product by dialysis. The product was further converted by glycidylation with *R*-glycidol (100 μL) for 12 hours in methanol at ambient temperature. Solvents were removed and the solid residue was worked-up by dialysis. The solid conjugate was identified by the ^{1}H NMR spectroscopy as the binary conjugate containing 5 equivalents of succinate linked Paclitaxel, 10 equivalents of carbamide linked Fulvestrant and 98 equivalents of 2,3-dihyroxypropyl residues attached to terminal nitrogen of the remaining PAMAM G4 primary nitrogen, $G4^{5P10F98gl}$. Theoretical molecular weight –35.0 kDa. Yield: 42.8 mg (1.22 μmole); 64 % per dendrimer. Hydrodynamic diameter of nanoparticle by DLS d(N) = 113.41 (± 5.48) nm. (PDI) = 0.122 (0.015). Potential ξ = 29.29 (± 0.72) mV.

Methods

NMR Spectroscopy

The 1-D ^{1}H and ^{13}C NMR spectra as well as 2-D ^{1}H-^{1}H correlations spectroscopy (COSY), ^{1}H-^{13}C heteronuclear single quantum correlation (HSQC), and heteronuclear multiple bond correlation spectra (HMBC) were recorded in deuterated water using Bruker 300 MHz (Rheinstetten, Germany) and worked up with TopSpin 3,5 software at College of Natural Sciences, University of Rzeszów.

Dynamic light scattering

Hydrodynamic diameter and zeta potential were measured in aqueous solution using a Zetasizer nano ZS instrument for 1 mM conjugate concentration.

Results

The ^{1}H and ^{13}C NMR spectra of PAMAM G4 dendrimer

The PAMAM G4 dendrimer has an effective radial symmetry. The ^{1}H NMR spectrum consists only of 6 methylene group resonances (broad triplets), the overlapped $a_0a_1a_2a_3$ a_4, overlapped $b_0b_1b_2b_3$,b_4 (both of 248 [H] in-

tegral intensity) overlapped $c_0c_1c_2c_3$, and overlapped $d_0d_1d_2d_3$, (120 [H] intensity each), all from inner sphere shells and the separated triplets of c_4 and d_4 (both of 128 [H] integral intensity) from outer sphere fragment of terminal ethylenediamine group (for atom numbering see Fig. 1). G4 is well soluble in water, methanol and dimethylsulfoxide. We have taken the ^{1}H NMR spectra of G4 in D_2O and in DMSO-d_6 in order to characterize the G4 as later we will use it as macromolecular carrier to bind various substituents into terminal primary amine groups. The detailed peak assignment of ^{1}H and ^{13}C resonances was performed based upon 2-D ^{1}H-^{1}H COSY spectra as well as heteronuclear HSQC and HMBC measurements. The combined HSQC/HMBC for G4 in D_2O and in DMSO-d_6 are presented at Fig. 2 and Fig. 3, respectively.

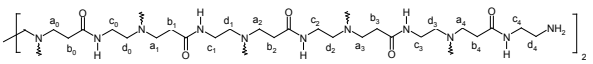

Fig. 1. Schematic representation of one arm of G4 PAMAM dendrimer with atom numbering. The winding lines represent branching at nitrogen atoms. involved in branching

The characteristic feature of the ^{1}H spectra presented as upper trace in 2-D maps at Fig.2 and 3 is the sharp triplet structure of outer sphere resonances of methylene d_4 (depicted as d*) and c_4 (c*) protons. These resonances are of special importance due to their proximity to terminal amine groups. The ^{1}H NMR spectrum taken in D_2O shows especially sharp triplets of d* and c* (both 128 [H[intensity) if compare with those at spectrum taken in DMSO (Fig.3). Thus further conversion of synthesized G4 by covalent attachment of substituents into terminal primary amine groups can be easily detected by changes of chemical shifts for c_4 and d_4 (c* and d* at Fig.2 and 3) promised the convenient experimental evidence to follow the structural changes of PAMAM carrier upon chemical changes. The full description of the ^{1}H and ^{13}C NMR is given in Table 1.

Table 1. The ^{1}H and ^{13}C NMR chemical shifts of PAMAM G4 dendrimer in D_2O and DMSO-d_6

Solvent: D_2O								
locant ®	a		b		c		d	
shell ¯	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H
0 - 3	49.1	2.75	32.9	2.36	36.9	3.23	51.4	2.56
4	49.1	2.75	32.9	2.36	41.8	3.17	39.9	2.65
Solvent: DMSO-d_6								
0 - 3	49.7	2.64	33.3	2.19	36.9	3.08	52.2	2.42
4	49.7	2.64	33.3	2.19	42.1	3.03	41.1	2.54

G4 conjugates with Paclitaxel and Fulvestrant

In our previous study we have applied the NMR spectroscopy to characterize the low molecular weight

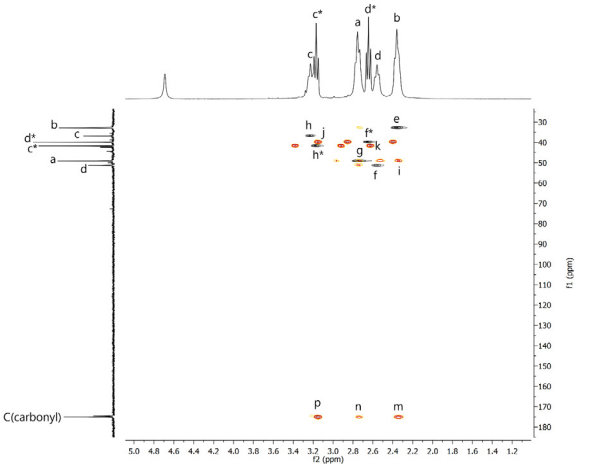

Fig. 2. Combined HSQC/HMBC map of PAMAM G4 in D_2O. The ^{1}H and ^{13}C resonances of c_4 and d_4 are described respectively as c* and d*, whereas c represents overlapped resonances of $c_0c_1c_2c_3$, analogously overlapped resonances of $d_0d_1d_2d_3$ are presented as d. There are no significant differences in chemical shifts for ^{1}H and ^{13}C resonances of a_0, a_1, a_2, a_3, and a_4 and b_0, b_1, b_2, b_3, and b_4 (both protons and carbons) therefore the resonances are labeled simply as a and b. The relevant HSQC coupling peaks (through one bond) are shown in grey scale as follows: e – H^b-C^b; f – H^d-C^d; f* - H^{d*}-C^{d*}; g – H^a-C^a; h* - H^{c*}-C^{c*}; h – H^c-C^c. The significant HMBC coupling peaks (long-range coupling) are represented in red/yellow scale as follows: i – H^b-C^a; k – H^{d*}-C^{c*}; j – H^{c*}-C^{d*}; m – H^b-C(O); n – H^a-C(O); p – H^{c*}-C(O)

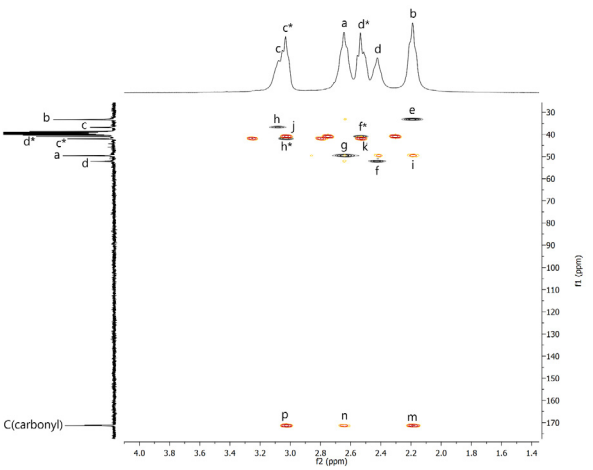

Fig. 3. Combined HSQC/HMBC map of PAMAM G4 in DMSO. The ^{1}H and ^{13}C resonances of c_4 and d_4 are described respectively as c* and d*, whereas c represents overlapped resonances of c_0,c_1,c_2 and c_3, analogously overlapped resonances of d_0,d_1,d_2 and d_3 are represented as d. There are no significant differences in chemical shifts for ^{1}H and ^{13}C resonances of a_0,a_1,a_2,a_3,a_4 and b_0,b_1,b_2,b_3,b_4 (both protons and carbons) therefore the resonances are labeled simply as a and b. The relevant HSQC coupling peaks are shown in grey scale as follows; e – H^b-C^b; f – H^d-C^d; f* - H^{d*}-C^{d*}; g – H^a-C^a; h* - H^{c*}-C^{c*}; h – H^c-C^c. The significant HMBC coupling peaks are represented in red/yellow scale as follows: i – H^b-C^a; k – H^{d*}-C^{c*}; j – H^{c*}-C^{d*}; m – H^b-C(O); n – H^a-C(O); p – H^{c*}-C(O)

PAMAM G3 conjugates with covalently attached biotin.[25] Later we used PAMAM G3 macromolecular carrier to construct the drug delivery system (DDS) for anticancer drugs: Lapatinib (L) and Fulvestrant (F) and tested those conjugates against three breast cancer cell lines in vitro.[7] We have found that G3-L-F binary conjugate promoted apoptosis in chemotherapy-induced senescent breast cancer cells with different receptor status.[8] Here we have stepped further to increase the payload of PAMAM carrier by applying generation 4 dendrimer (G4). In previous G3-L-F conjugate the amine groups of the carrier were blocked by substitution with glucoheptonoamidate residues in order to maximize the conjugate solubility in water. According to our further discovery, that PAMAM dendrimer glycidylated with *R*-glycidol, in contrary to *S*-isomer, enantioselectively penetrated cell membranes of both normal and cancer lines with high efficiency,[26] we applied this DDS to construct the binary conjugate with celecoxib and simvastatine and observed their increased additive cytotoxicity for cancer cell lines.[21] Considering mentioned experience we applied G4 dendrimer as carrier for Paclitaxel, and for Paclitaxel and Fulvestrant anticancer drugs and converted them into *R*-glycidylated derivatives. We elaborated synthetic path for both and characterized the derivatives by NMR spectroscopy and estimated the size and potential zeta of nano-DDS by DLS method to fully characterize the obtained conjugates. The general formula of studied conjugates are presented at Fig.4.

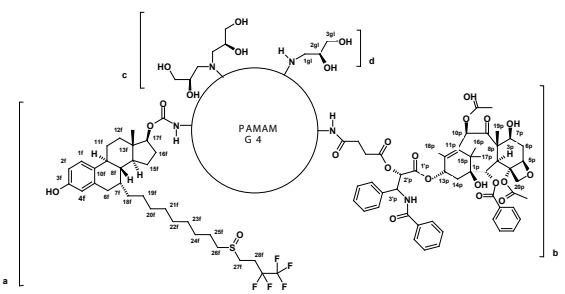

Fig. 4. The general formula of obtained conjugates with atom numbering: G4^{5P} (a = 0, b = 5, c, d = 0); G4^{4P110gl} a = 0; b = 4; c = 50; d = 10; G4^{15F66gl} a = 15; b = 0; c = 17; d = 32; G4^{5P10F98gl} (a = 10; b = 5; c = 49; d = 0)

Paclitaxel – G4 conjugate

In order to attach covalently Paclitaxel (P) to amine groups of G4 an additional functionalization was needed. It was already demonstrated that succinic and glutaric anhydrides attached to 2' oxygen of P are convenient linkers.[27,28] We have obtained 2'-o-succinated derivatives and used that to attach obtained P-suc using Mukaiyama reagent to activate pending carboxyl group of succinate linker. We have obtained the conjugate containing 4 molecules of amide attached P-suc, G4^{5P}. The compound was well soluble in DMSO and slightly less soluble in water (ca 0.1 mM aqueous solution could be obtained). The conjugate was characterized by 1-D and 2-D ^{1}H and ^{13}C NMR spectroscopy. COSY spectrum of G4^{5P} is presented at Fig. 5. Observed scalar coupling cross-peaks a, b, and c between methylene protons of PAMAM G4 core were analogous to those found for G4 alone. The cross-peaks d – j enabled to assign the ^{1}H resonances of P residue, even though some of them are hidden under the broad residual peak of HDO. In order to further characterize the conjugate the heteronuclear 2-D ^{1}H-^{13}C HSQC and HMBC spectra were recorded and presented as combined map at Fig.6. One-bond coupling cross-peaks are in grey; all the G4 core were well visible and additionally some peaks of P were found. Although full ^{13}C resonances were not assigned to P residues, the long-range couplings (in yellow-red color) enabled to confirm the assignments of all ^{1}H resonances of P in G4^{5P} conjugate. The conjugate G4^{5P} was a semi-product to further attachment of another drug, namely Fulvestrant.

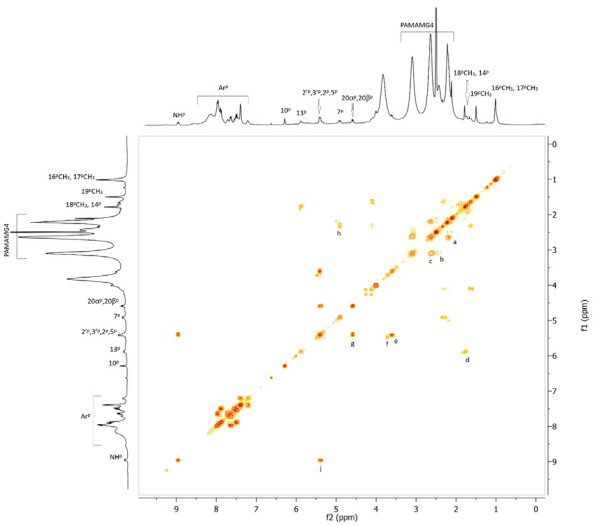

Fig. 5. The ^{1}H-^{1}H- COSY spectrum of G4^{5P} conjugate in DMSO-d$_6$. The relevant cross-peaks are: a – H^{b}(G4) / H^{a}(G4); b – H^{d}(G4) / H^{c}(G4); c –H^{d*}(G4) / H^{c}(G4); d – 14H^{P} / 13H^{P}; e – 3H^{P} / 5H^{P} ; f – 3H^{P} / 2H^{P} ; g - 20αH^{P} and 20βH^{P}/5H^{P} ; h – 7H^{P} / 6H^{P} ; j – 3'H^{P} / NH^{P}. The resonances from P are labeled with upper ^{P}, the resonances from PAMAM G4 core are designated in bracket as (G4). For atom numbering see Fig. 4

Glycidylated Fulvestrant – G4 conjugate

We aimed at construction of double-drug conjugate, with P and F (Fulvestrant). Previously we have elaborated the method of covalent attachment of F to G3.[8] We applied the same pathway, namely substitution of F with NPCF followed by reaction of functionalized F with G4. The highly substituted G4 with 15 equivalents of F was obtained and converted by reaction with *R*-glycidol to obtain finally G4^{15F66gl}. The ^{1}H NMR spectrum of G4^{15F66gl} is presented at Fig.7A. The number of F residues and 2,3-dihydroxopropyl residues (gl, from *R*-glycidol)

was defined by integration of the aromatic resonances of F (for the formula and atom numbering see Fig.4) and 3'- and 2'-gl resonances related to internal PAMAM G4 b resonance at 2.3 ppm, corresponding to [248H].

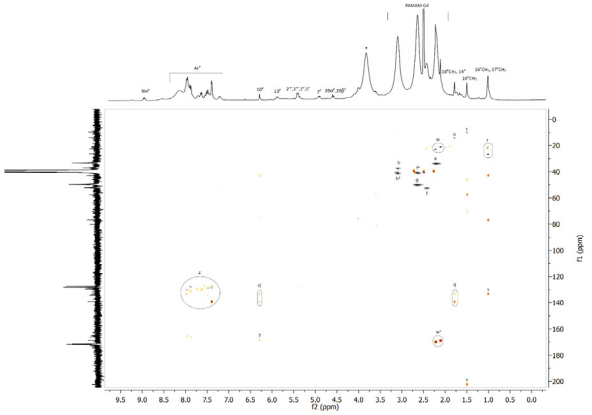

Fig. 6. Combined HSQC/HMBC map of PAMAM $G4^{5P}$ in DMSO-d_6. The relevant one bond coupling peaks are shown in grey scale as follows: e –H^b(G4) / C^b(G4); f – H^d(G4) / C^d(G4); f*- H^{d*}(G4) / C^{d*}(G4); g –H^a(G4) / C^a(G4); h –H^c(G4) / C^c(G4); h* - H^{c*}(G4) / C^{c*}(G4); r – $16CH_3{}^P$ / 16PC and 17^PCH_3 / 17CP; s – $19CH_3{}^P$ / 19CP; u – 18HP/ 18CP; w – 10HP-OAcH / 10P-OAcC and 4HP-OAcH / 4CP-OAcC. The significant HMBC coupling peaks are represented in red/yellow scale as follows: s – $16CH_3{}^P$ or $17CH_3{}^P$ / 11CP; q – $18CH_3{}^P$ / 11CP and 12CP, q' – 10HP / 11CP and 12PC, w* - 10HP (OAcH) / C(O)P and 4HP(OAcH) / C(O)P ; x – $19CH_3{}^P$ / 9PC; y – 10HP / C(O)P; z – a group of HMBC/HSQC aromatic cross-peaks of P residues are represented. Residual HDO is labeled with asterisk in upper trace of the map

Glycidylated Paclitaxel – G4 conjugate

The $G4^{4P}$ was obtained in separate synthesis as previously described $G4^{5P}$ and further derivatized with *R*-glycidol. The ^{1}H NMR spectrum of the product is presented at Fig.7C. The integration of resonances from P and gl protons related to [248H] G4 resonance enabled to identify the stoichiometry of the conjugate as $G4^{4P110gl}$.

Binary glycidylated Paclitaxel – Fulvestrant – G4 conjugate

The $G4^{5P}$ was used to attach covalently Fulvestrant activated with NPCF. After the condensation step, the *R*-glycidylation was performed and afterwards the product was purified by extensive dialysis against water. The product was analyzed by ^{1}H NMR spectroscopy in order to determine the number of P, F, and gl residues. The ^{1}H NMR spectrum of binary conjugate is presented at Fig.7B. The integration of aromatic resonance from P, and well separated aromatic resonances of F enabled to determine the average number of P and F residues and gl substituents to obtain final formula $G4^{5P10F98gl}$. The compound was also fully characterized by 2-D spectra. The COSY spectrum is presented at Fig.8. Two crucial cross-peaks (a and b) were observed gl residues and additional cross-peak c allowed to identify the F resonances. Some additional information gained from heteronuclear ^{1}H-^{13}C HSQC and HMBC experiments (Fig.9) completed the assignments of ^{1}H resonances of PAMAM, gl, F, and P.

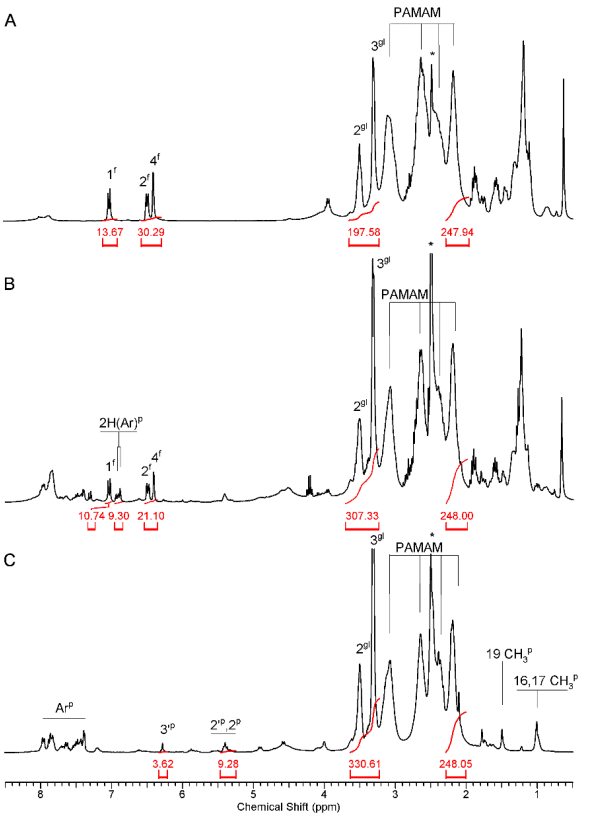

Fig. 7. The ^{1}H NMR spectra of: $G4^{15F66gl}$ (A) , $G4^{5P10F98gl}$ (B), and $G4^{4P110gl}$ (C) in DMSO-d_6. For atom numbering see Fig. 4. The $HD_2S(O)CD_3$ resonance is labeled with asterisk. The resonances from Paclitaxel, Fulvestrant and glycidol are upper indexed with p, f, and gl

Obtained conjugates: $G4^{15F66gl}$, $G4^{4P110gl}$, and $G4^{5P10F98gl}$ were designed as DDS. All conjugates are water soluble at ca 100 μM concentration. The stoichiometry of conjugates was determined by NMR spectroscopy in DMSO-d_6. They can be stored in frozen solvent for months and are stable. The stock solutions in DMSO (5 mM concentration) can be diluted with water 200 times without precipitation. The glycidylated conjugates have some tendency to associate in water. The measurements of conjugate hydrodynamic diameters by Dynamic Light Scattering method evidenced that size of nanoparticles was around 100 nm, while the size of G4 dendrimer is 5 nm in monomolecular disperse. Nevertheless, the conjugates can be active as DDS against breast cancer cells, as we observed before in case of glucoheptoamidated G3 conjugated with Fulvestrant and Lapatinib.[8] Gener-

ally, the solubility of conjugates is one order of magnitude higher than free drugs. High load of obtained DDSs with F and P is promising factor in anticancer activity, which will be tested and reported separately.[29]

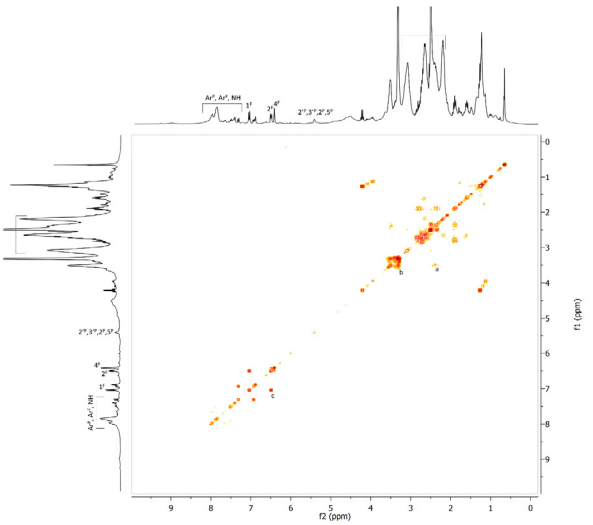

Fig. 8. ^{1}H-^{1}H COSY spectrum of G4^{5P10F98gl} in DMSO-d$_6$.The significant cross-peaks are as follows:
a – H^{1gl} / H^{2gl}; b – H^{2gl} / H^{3gl}; c – 2H^{F}/1H^{F}

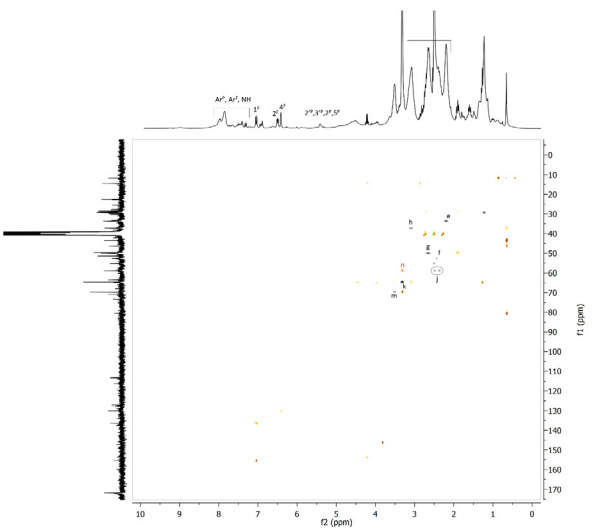

Fig. 9. Combined HSQC/HMBC map of G4^{5P10F98gl} in DMSO-d$_6$. The relevant one bond coupling peaks are shown in grey scale as follows: e – H^{b}(G4) / C^{b}(G4); f –H^{d}(G4) / C^{d}(G4); g – H^{a}(G4) / C^{a}(G4); h –H^{c}(G4) / C^{c}(G4); j – H^{1gl} / C^{1gl}; k – H^{3gl} / C^{3gl}; m – H^{2gl} / C^{2gl}. The significant HMBC coupling peak is n – H^{3gl} / C^{2gl}

Conclusion

The anticancer DDS was constructed based on PAMAM dendrimer of fourth generation. Well water soluble forms of Fulvestrant and Paclitaxel were obtained by covalent attachment of the drugs into macromolecular carrier. High water solubility of obtained macromolecular DDS was achieved by addition of *R*-glycidol into primary amine groups of PAMAM dendrimer, which otherwise are responsible for general toxicity of such DDSs. The binary conjugate, containing both Paclitaxel and Fulvestrant was deliberately designed as DDS against breast cancer cell models expressing estrogen receptor α such as MCF7 and BT474.

Declarations

Funding

The research was funded by University of Rzeszów from General Status Activity Fund.

Author contributions

Conceptualization, K.W.; Methodology, S.W.; Software, K.W.; Validation, S.W.; Formal Analysis, S.W.; Investigation, K.W.; Resources, S.W.; Data Curation, K.W.; Writing – Original Draft Preparation, K.W.; Writing – Review & Editing, S.W.; Visualization, K.W.; Supervision, S.W.; Project Administration, S.W.; Funding Acquisition, S.W.

Conflicts of interest

Authors declare no conflict of interest.

Data availability

The data presented (1-D and 2-D NMR spectra are available) from Authors upon request.

Ethics approval

Non applicable.

References

1. www.cancer.org/research/cancer-facts-statistics/. Accessed February 14, 2023.
2. World Health Organization. https://www.who.int/news-room/fact-sheets/detail/cancer. Accessed February 14, 2023.
3. Debela DT, Muzazu SG, Heraro KD, et al. New approaches and procedures for cancer treatment: Current perspectives. *SAGE Open Med.* 2021; 9:20503121211034366. doi: 10.1177/20503121211034366
4. Patra JK, Das G, Fraceto LF, et al. Nano based drug delivery systems: recent developments and future prospects. *J Nanobiotechnol.* 2018;16:71. doi: 10.1186/s12951-018-0392-8
5. Oddone N, Lecot N, Fernández M, et al. In vitro and in vivo uptake studies of PAMAM G4.5 dendrimers in breast cancer. *J Nanobiotechnol.* 2016;14:45. doi: 10.1186/s12951-016-0197-6
6. Abedi-Gaballu F, Dehghan G, Ghaffari M, et al. PAMAM dendrimers as efficient drug and gene delivery nanosystems for cancer therapy. *Appl Mater Today.* 2018;12:177-190. doi: 10.1016/j.apmt.2018.05.002
7. Dichwalkar T, Patel S, Bapat S, et al. Omega-3 Fatty Acid Grafted PAMAM-Paclitaxel Conjugate Exhibits Enhanced

Anticancer Activity in Upper Gastrointestinal Cancer Cells. *Macromol Biosci.* 2017;17:1600457. doi: 10.1002/mabi.201600457

8. Lewińska A, Wróbel K, Błoniarz D, et al. Lapatinib- and fulvestrant-PAMAM dendrimer conjugates promote apoptosis in chemotherapy-induced senescent breast cancer cells with different receptor status. *Biomater Adv*. 2022;140:213047. doi: 10.1016/j.bioadv.2022.213047
9. Weaver BA. How Taxol/paclitaxel kills cancer cells. *Mol Biol Cell*. 2014;25:2677-2681. doi: 10.1091/mbc.E14-04-0916
10. Zasadil LM, Andersen KA, Yeum D, et al. Cytotoxicity of paclitaxel in breast cancer is due to chromosome missegregation on multipolar spindles. *Sci Transl Med*. 2014;6(229):229ra43. doi: 10.1126/scitranslmed.3007965
11. Abu Samaan TM, Samec M, Liskova A, Kubatka P, Büsselberg D. Paclitaxel's Mechanistic and Clinical Effects on Breast Cancer. *Biomolecules.* 2019;9(12):789. doi: 10.3390/biom9120789
12. Haldar S, Chintapalli J, Croce CM. Taxol Induces bcl-2 Phosphorylation and Death of Prostate Cancer Cells. *Cancer Res.* 1996;56:1253-1255.
13. Torne SJ, Ansari KA, Vavia PR, Trotta F, Cavalli, R. Enhanced oral paclitaxel bioavailability after administration of paclitaxel-loaded nanosponges. *Drug Delivery*. 2010;17(6):419-425. doi: 10.3109/10717541003777233
14. https://www.accessdata.fda.gov/drugsatfda_docs/label/2020/021660s047lbl.pdf. Accessed February 27, 2023.
15. Nathan MR, Schmid P. A Review of Fulvestrant in Breast Cancer. *Oncol Ther*. 2017;5(1):17-29. doi: 10.1007/s40487-017-0046-2
16. Johnston SJ, Cheung KL. Fulvestrant - a novel endocrine therapy for breast cancer. *Curr Med Chem*. 2010;17(10): 902-914. doi: 10.2174/092986710790820633
17. Robertson JFR, Bondarenko IM, Trishkina E, et al. Fulvestrant 500 mg versus anastrozole 1 mg for hormone receptor-positive advanced breast cancer (FALCON): an international, randomised, double-blind, phase 3 trial. *Lancet*. 2016;388(10063):2997-3005. doi:10.1016/s0140-6736(16)32389-3
18. Rocca A, Maltoni R, Bravaccini S, Donati C, Andreis D. Clinical utility of fulvestrant in the treatment of breast cancer: a report on the emerging clinical evidence. *Cancer Manag Res*. 2018;10:3083-3099. doi: 10.2147/CMAR.S137772
19. Bayat Mokhtari R, Homayouni TS, Baluch N, et al. Combination therapy in combating cancer. *Oncotarget*. 2017;8(23):38022-38043. doi: 10.18632/oncotarget.16723
20. Geyer CE, Forster J, Lindquist D, et al. Lapatinib plus capecitabine for HER2-positive advanced breast cancer. *N Engl J Med*. 2006;355(26):2733-2743. doi: 10.1056/NEJMoa064320
21. Wróbel K, Wołowiec S, Markowicz J, Wałajtys-Rode E, Uram Ł. Synthesis of biotinylated PAMAM G3 dendrimers substituted 2 with R-glycidol and celecoxib/simvastatin as repurposed drugs and demonstration of their increased additive cytotoxicity for cancer cell lines. *Cancers.* 2022;14 (3):714. doi: 10.3390/cancers14030714
22. Uram Ł, Filipowicz-Rachwał A, Misiorek M, Winiarz A, Wałajtys-Rode E, Wołowiec S. Synthesis and Different Effects of Biotinylated PAMAM G3 Dendrimers Substituted with Nimesulide in Human Normal Fibroblasts and Squamous Carcinoma Cells. *Biomolecules.* 2019;9:437. doi: 10.3390/biom9090437
23. Tomalia D, Baker H, Dewald J, et al. A new Class of Polymers: Starburst-Dendritic Macromolecules. *Polym J*. 1985;17:117-132.
24. Satsangi A, Roy SS, Satsangi RK, Vadlamudi, RK, Ong JL. Design of a Paclitaxel Prodrug Conjugate for Active Targeting of an Enzyme Upregulated in Breast Cancer Cells. *Mol Pharmaceutics.* 2014;11:1906-1918. doi: 10.1021/mp500128k
25. Wróbel K, Wołowiec S. Low generation polyamidoamiine dendrimers (PAMAM) and biotin PAMAM conjugate – the detailed structural studies by ^{1}H and ^{13}C nuclear magnetic resonance spectroscopy. *Eur J Clin Exp Med.* 2020;18:281-285. doi: 10.15584/ejcem.2020.4.4
26. Malinga-Drozd M, Uram Ł, Wróbel K, Wołowiec S. Chiral recognition of homochiral poly(amidoamine) dendrimers substituted with R- and S-glycidol by keratinocyte (HaCaT) and squamous carcinoma (SCC-15) cells in vitro. *Polymers.* 2021;13:1049. doi: 10.3390/polym13071049
27. Cline EN, Li M-H, Choi SK, et al. Paclitaxel-Conjugated PAMAM Dendrimers Adversely Affect Microtubule Structure through Two Independent Modes of Action. *Biomacromolecules.* 2013;14:654-664. doi: 10.1021/bm301719b
28. Teow HM, Zhou ZY, Najlah M, Yusof SR, Abbott NJ, D'Emanuele A. Delivery of paclitaxel across cellular barriers using a dendrimer-based nanocarrier. *Int J Pharm.* 2012;441:701-711. doi: 10.1016/j.ijpharm.2012.10.024
29. Dai X, Cheng H, Bai Z, Li J. Breast Cancer Cell Line Classification and Its Relevance with Breast Tumor Subtyping. *J Cancer*. 2017;8(16):3131-3141. doi: 10.7150/jca.18457

Wydawnictwo UR 2023
ISSN 2544-1361 (online)
doi: 10.15584/ejcem.2023.3.2

http://www.ejcem.ur.edu.pl
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2023; 21 (3): 450–457

ORIGINAL PAPER

Biochemical and cellular (liver and kidney) restorative properties of garlic (*Allium sativum*) aqueous extract in cow brain-induced hypercholesterolemic model Swiss albino mice

Swarup Kumar Kundu [1,2], Md. Abu Hadi Noor Ali Khan [3], Shonkor Kumar Das [2]

[1] Department of Anatomy and Histology, Faculty of Veterinary, Animal and Biomedical Sciences, Khulna Agricultural University, Khulna, Bangladesh
[2] Department of Anatomy and Histology, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh, Bangladesh
[3] Department of Pathology, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh, Bangladesh

ABSTRACT

Introduction and aim. Garlic is one of the most popular traditional medicinal herbs which has a number of desirable health benefits. The study was designed to depict the improvement of serum biochemical parameters as well as the histomorphological recovery potential of garlic aqueous extract in hypercholesterolemic mice.

Material and methods. A total of thirty Swiss albino mice weighing 24±5g and aged 5 weeks were randomly divided into three groups. Group A: supplied standard mice pellet and water; Group B: standard mice pellet + hypercholesterolemic diet (cow brain: 2 g/kg b.w.t.); and Group C: standard mice pellet + hypercholesterolemic diet (cow brain: 2 g/kg b.w.t.) + garlic extract (25 ml/kg b.w.t.). After four weeks of experimental tenure, samples (blood, liver, and kidney) were collected from each group of mice for serum biochemical analysis and histomorphological study.

Results. Compared with hypercholesterolemic mice, total cholesterol (TC), triglyceride (TG) concentration, and low-density lipoprotein (LDL) levels significantly decreased respectively by 7%, 20% and 48% along with high-density lipoprotein (HDL) levels significantly increased by 47% in garlic extract supplemented group. Based on the histological evaluation in the liver sample of group C, both portal and central veins were normal, and fat droplets were not found in the hepatocytes which were found in the liver of group B. On the other hand, unchanged renal cortex, glomerulus, Bowman's space, and kidney tubules were seen in group C.

Conclusion. Therefore, the above findings of the present research would assist to provide affirmation about the cholesterol-decreasing and cellular restoration potentiality of garlic aqueous extract.

Keywords. cellular restoration, garlic extract, health beneficial, lipid profile, Swiss albino mice

The list of abbreviations:

CHD – coronary heart disease, **PART** – peri–renal adipose tissue, **TC** – total cholesterol, **TG** – triglyceride, **HDL** – high–density lipoprotein, **LDL** – low–density lipoprotein, **WHO** – World Health Organization

Introduction

Liver and kidney are the most essential organs in the body for performing multiple functions like excretion of waste products, hormonal regulation, digestion, and detoxification of harmful drugs.[1] According to the latest World Health Organization (WHO) records released in

Corresponding author: Swarup Kumar Kundu, e-mail: swarupkundu95@gmail.com

Received: 21.04.2023 / Revised: 12.05.2023 / Accepted: 16.05.2023 / Published: 30.09.2023

Kundu SK, Khan MAHNA, Das SK. *Biochemical and cellular (liver and kidney) restorative properties of garlic (Allium sativum) aqueous extract in cow brain-induced hypercholesterolemic model Swiss albino mice*. Eur J Clin Exp Med. 2023;21(3):450–457. doi: 10.15584/ejcem.2023.3.2.

2020, about 1.51% of total deaths were found due to kidney disease as well as 2.94% of total deaths due to liver disease in Bangladesh.[2] Diets rich in highly saturated fat have been identified as one of the major risk factors for developing visceral adiposity like cardiovascular problems, fatty liver syndrome, obesity-related glomerulopathy, etc. Obesity is a burden issue nowadays and the liver and kidneys frequently develop obesity-related different disorders.[3] Cow brain is a high-cholesterol meal that is rich in saturated fat.[4-7] People in Bangladesh, in particular, can purchase this commodity from local marketplaces as well as various online retailers. It has been recorded that consumption of the cow brain is a potential means of transmission of prion disease to individuals in different countries who consume cow brain products in various food preparations.[5] Apart from this, people of Bangladesh are involved in various types of fast food culture like beef pizza, chicken patties, beef brain patties, all sweets, chicken or beef burgers, etc. which are considered a source of excess cholesterol, and saturated fat. People are suffering from various coronary artery diseases, obesity problems, fatty liver syndrome, kidney complications, etc. by consuming these food items.[6] Cholesterol-rich food is the key factor in changing liver pathology. Because large amounts of triglyceride accumulate in the hepatocytes for a high-fat diet.[8] In the present research, the potential author focused more on pathological alteration rather than hepatosteatosis. Accumulation of adipocytes and mononuclear cell infiltration in the renal cortices have been seen previously in the high-fat diet rat, but the present author also revealed a few more pathological alterations in the current study.[9] Different studies revealed that garlic bulbs contain a substance called alliin (S-allyl cysteine sulfoxide) which is converted into sulfur-based antioxidant compound allicin when in contact with air. This allicin is the key product of recovering cholesterol levels. Garlic is also commonly used for diabetes mellitus, hypercholesterolemia, and cancer treatment.[10] Some medicinal plants are rich in antioxidants and garlic belongs to this group.[11,12] Garlic extract has the capacity of restoring mesangial cell proliferation in the glomerulus and basement membrane thickening.[11] In addition, garlic extract has a protective effect against cardiac ischemia, diet-induced oxidative damage in hypercholesterolemia, dilated sinusoids, hepatic steatosis, cellular infiltration, etc.[13]

Aim

Therefore, on the basis of the above description main objectives of the present research were to determine:

I. The negative effects of high-cholesterol food (cow brain) on liver and kidney cellular levels and serum biochemical markers.

II. The restorative properties of garlic extract on biochemical profiles as well as the pathological changes of the liver and kidney in diet-induced hypercholesterolemic mice.

Material and methods

Participants and ethical approval

The current research activity was conducted in the Department of Anatomy and Histology, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh-2202. The Ethical Committee of Bangladesh Agricultural University approved the study protocol (AWEEC/BAU/2019-53). Experimental Swiss albino mice at the age of 5 weeks old were collected from International Center for Diarrheal Disease Research (ICDDR'B), Mohakhali, Dhaka, Bangladesh. The initial weight of the collected mice was recorded at about 24±5 g.

Preparation of garlic aqueous extract

Allium sativum, a plant, is the scientific name for garlic. Its aqueous extract is the source of phenolic compounds which are antioxidant and biologically active.[14] A total of 1 kg of fresh garlic (Variety: BARI Roshun 3) was collected from the Bangladesh Agricultural University horticulture center. Then all the papery skin from the outside of the head of garlic was removed. All the cloves of garlic were separated from each other. Then 250 g of garlic cloves were weighed and crushed in mortar and pestle for 1 min together with 500 ml of distilled water and allowed them standing for 1 h at room temperature. Filtration was done through Whitman no. 4 filter paper to remove the impurities and get up to 100 ml of aqueous garlic extract. Finally, the prepared aqueous garlic extract was taken in a glass container, sealed at its mouth, and stored in the refrigerator for future use.

Study design

Before the commencement of the research, a total of healthy 30 collected Swiss albino mice of either sex were reared in 20x30x10 cm plastic box cages at a standard room temperature (23±2°C), 52±5% relative humidity on a 12 h light and 12 h dark cycle were maintained for 7 days. The rearing boxes were filled with sawdust and changed regularly to maintain hygiene and comfort. At that time mice pellet and fresh drinking water ad libitum were supplied to each mouse. After 7 days of acclimatization, collected Swiss albino mice were randomly categorized into three groups and each group consisted of ten (10) mice.

Group A: Considered as the control group, and given normal mice pellets, and fresh water.

Group B: Cow brain at a dose of 2 g/kg b.w.t as hypercholesterolemic food+ mice pellets+ fresh water.

Group C: Hypercholesterolemic food (cow brain at a dose of 2g/kg b.w.t) +garlic extract at a dose of 25ml/kg b.w.t orally + mice pellets +fresh water (Fig. 1)

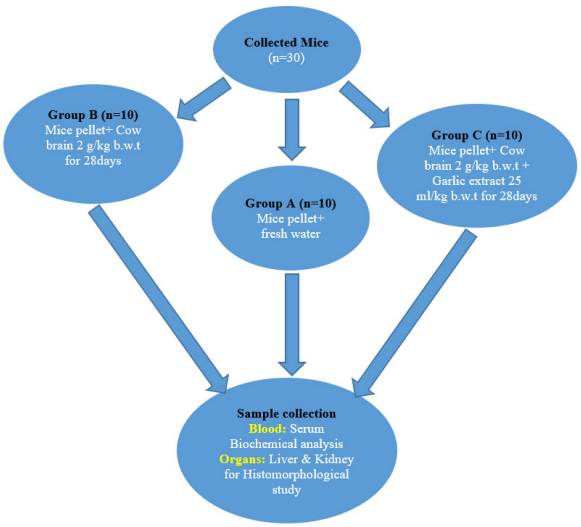

Fig. 1. Graphical distribution of collected Swiss albino mice into different groups

For the 28 days of research tenure, mice of the control group were supplied standard mice pellets (collected from ICDDR'B) and fresh water. In contrast, cow brain at a dose of 2 g/kg b.w.t was given to both groups B and C of mice as hypercholesterolemic food. Cow brain is a definite source of cholesterol, approximately 100g of cow brain contains 3100mg of cholesterol.[4] Eating cow brains in the daily diet is a natural habit of Asian people, especially Bangladeshi that causes pathological changes in the liver and kidney and enhance total cholesterol (TC) and triglyceride (TG) level.[4,8] In addition to hypercholesterolemic feed, mice of group C were also given garlic extract at a dose of 25 ml/kg. Garlic has the potential beneficial effects against hypercholesterolemia. About 8-10% of cholesterol can be reduced by the consumption of 1-2 cloves of garlic in a day.[15] Weight of each group of mice was measured on the 1st, 7th, 14th, 21st, and 28th days of the consecutive days of the study period.

Blood collection, sample processing, and staining

After the experimental tenure (28 days), the mice of each group were sacrificed ethically by anesthesia with isoflurane (2%) and placed on the autopsy plate. The blood sample was taken from the ventricle of the heart and kept in a common blood collection tube with EDTA for preventing coagulation. Then the Liver and Kidney samples were collected from each group of mice and preserved in 10% formalin. Color and weight of the samples were carefully taken into consideration for the gross study. After washing with 0.9% saline the samples were dehydrated in ascending grades of ethanol (70%, 80%, 95%, 100%, 100%, and 100%). The incubation period for ethanol was 2hr. Xylene was used for clearing the tissues. 5-µm thick slices of tissue were taken using an American Optical Spencer model 820 microtome. Finally, hematoxylin and eosin (H&E) staining was done for histopathological analysis of the collected organs. Necessary photographs were randomly taken at 10X and 40X focuses to get better illustration and pictures were captured by photomicroscope (Model: SKU: B120C-E520200610, AMSCOPE LOS ANGLES USA).

Biochemical analysis

Determination of total cholesterol

The TC was determined after enzymatic hydrolysis and oxidation (CHOD-POD method). The indicator quinoneimine was formed from hydrogen peroxide and 4-Aminophenazone in the presence of phenol and peroxidase. Both reagent and sample were kept at room temperature and mixed 1 ml reagent with 10 µl samples in the test tube. Waited for 10 minutes and placed the mixture in the cuvette. The cuvette was placed in a spectrophotometer at 550 nm and recorded the reading. The reading was calculated by comparing it with the standard value and multiplying it by 200 mg/dL. So the result was expressed as mg/dL (catalogue no. of the used reagent: MO-165218).

Determination of triglyceride

The TG was determined after enzymatic hydrolysis with lipase (GPO-POD method). The indicator is a quinoneimine formed from hydrogen peroxide, 4-Aminophenazone, and 4-chlorophenol under the catalytic influence of peroxide. The fatty acid can be hydrolyzed by lipoprotein lipase of an experimental reagent and ultimately produced quinoneimine proportional to triglyceride. This quinoneimine absorbs light of 500 nm. 10 µl samples were mixed with 1ml reagent and kept in a standing position for 15 minutes at room temperature. The mixture was placed in a cuvette of the spectrophotometer and the reading was recorded. This reading was divided by the standard reading and multiplied by 200 mg/dL (catalogue no. of the used reagent: MO-165221).

Determination of LDL-cholesterol

This was assayed in each sample by using RANDOX kit (UK). The reagent and sample were kept at room temperature and mixed 0.4 ml precipitating reagent with 0.2 ml sample. After 10 minutes, this was centrifuged at 12000 rpm for 2 minutes. Then the supernatant was separated. This supernatant was used for LDL determination. 50 **µl** supernatant was mixed with 1 ml cholesterol reagent and stand for 10 minutes at room temperature. This mixture was set in a spectrophotometer by using a cuvette at 500 nm wavelength. The reading was recorded and calculated by comparing standard readings and the fraction was multiplied by 50 mg/dL.

Determination of HDL-cholesterol

The HDL cholesterol was assayed in each sample by using RANDOX kit (UK). Low-density lipoproteins (LDL

and VLDL) and chylomicron fractions were precipitated quantitatively by the addition of phosphotungstic acid in the presence of magnesium ions. After centrifugation, the cholesterol in the HDL fraction, which remains in the supernatant, was determined. The HDL-cholesterol was determined on the principle of estimation of TC (catalogue no. of the used reagent: 1133010).

Statistical analysis

All the collected data were analyzed by using Statistical Package for the Social Sciences (SPSS: version 25, IBM, Armonk, NY, USA) software and revealing the results in tabular form. Statistical analysis was performed using one-way analysis of variance (ANOVA). Results were expressed as mean±SE. Differences between groups were considered significant at $^{**}p<0.001$ and $^{*}p<0.05$ levels.

Results

Body weight

The mean body weight of the hypercholesterolemic group (39.9 g) of mice was significantly higher than the control group (31.4 g) of mice. Garlic extract supplementation successfully maintained the average normal body weight in group C (33.1 g) of mice compared to group B.

Gross morphometric study

Liver assessment

In the current study, no irregular surface, red-brownish color, no detectable pathological lesions, and normal shape of lobes were found in the control group (Group A) of the liver (Fig. 2). Liver become slightly enlarged, and pale yellowish discoloration of the surface was found in the hypercholesterolemic (Group B) group of mice (Fig. 2). In contrast, garlic extract supplementation fully restored the morphological changes in the liver. No detectable abnormalities were seen on the liver surface of group C of mice (Fig. 2). On the other hand, liver weight of the garlic extract-supplemented group was seen as normal compared to the control group of mice but the liver weight of group B of mice was found considerably high (Fig. 3).

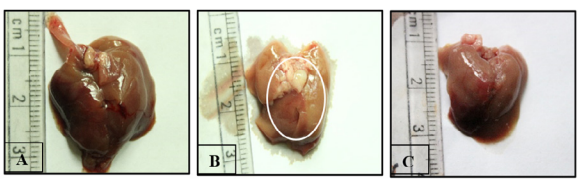

Fig. 2. Gross view of liver of A: control (group A) B: hypercholesterolemic (group B) and C: garlic (group C) extract-supplemented mice. No considerable lesions were observed in the photograph of A and C of the liver. B: Pale and slight hepatomegaly (white oval) seen in the hypercholesterolemic liver

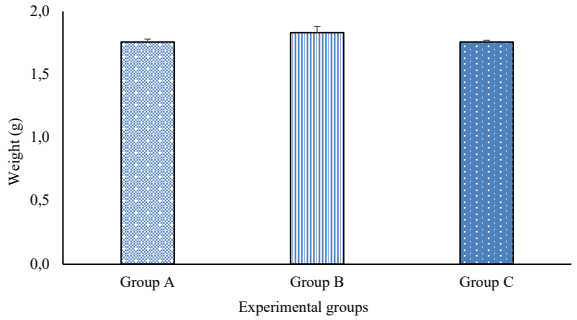

Fig. 3. Diagram represents the liver weight of control (group A), hypercholesterolemic (group B), and garlic extract-supplemented (group C) mice. Liver weight of the garlic extract-supplemented group was shown normal as the control group of mice (mean ± standard error)

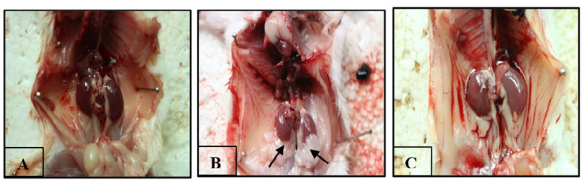

Fig. 4. Gross view of kidney of A: control (group A) B: hypercholesterolemic (group B) and C: garlic (group C) extract-supplemented mice. Surface architecture was observed normal in the photograph of A and C of the kidney. B: Profuse perirenal adipose tissue (black arrow) seen in the hypercholesterolemic kidney

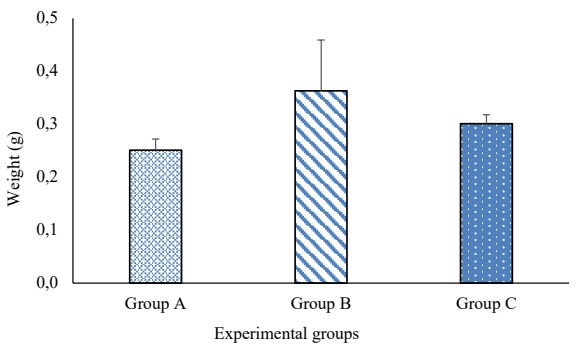

Fig. 5. Diagram represents the kidney weight of control (group A), hypercholesterolemic (group B), and garlic extract-supplemented (group C) mice. Kidney weight of the garlic extract-supplemented group was shown normal as the control group of mice (mean ± standard error)

Kidney assessment

Kidney of the control and garlic extract-treated group of mice showed a normal appearance, brownish-red color, and normal position and shape. No detectable pathological lesions were seen (Fig. 4). Excess peri-renal adipose tissue (PART) accumulated around the lower end of the kidney and the size of the kidney slightly increased compared to other groups of mice (Fig. 4). The weight of the kidney in group C (garlic extract supplemented) of mice

showed normal but hypercholesterolemic feed increased the kidney weight in group B compared to group A of mice (Fig. 5).

Histopathological study

Histological analysis was performed by H&E staining for the confirmation of hepatoprotective and renoprotective effects of garlic aqueous extract.

Hepatoprotective properties of garlic extract

Histological study of the section of the liver of hypercholesterolemic (Group B) mice exhibited narrowing of the portal vein, hypertrophied hepatocytes, radiated hepatocytes from the central vein and formed coherent groups, merging of the central and portal vein which made it difficult to separate them, Kupffer cells in the hepatic sinusoids, mild to moderate congestion in both central and portal vein, dilated central vein, fat droplets in the hepatocytes (Fig. 6). Surprisingly, following the supplementation of garlic extract to group C of mice liver section showed normal central and portal veins, normal hepatocytes (no fat droplets) as well as congestion was not found in the central and portal veins (Fig. 6). Bi-nucleated hepatocytes were found in every section of the liver.

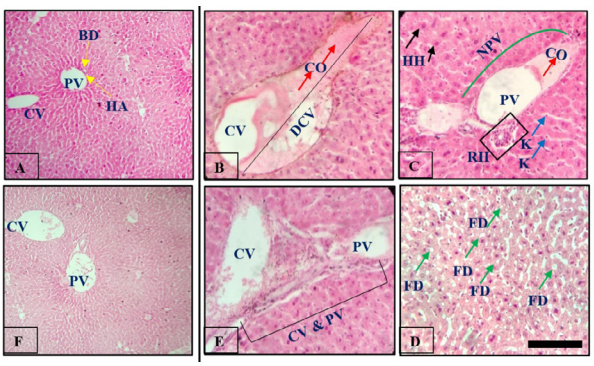

Fig. 6. Transverse section of representative photomicrographs of liver of A: control (group A), B-E: hypercholesterolemic (group B), and F: garlic extract supplemented (group C) mice (H&E stained) at 40X magnification. CV – central vein; PV – portal vein; BD – bile duct; HA – hepatic artery; (B-E): NPV – narrowing portal vein (green arch); HH – hypertrophied hepatocytes (black arrow); RH – radiated hepatocytes (rectangle); CV&PV – merges of the central and portal vein (bracket); K – Kupffer cells (blue arrow); CO – congestion (red arrow); DCV – dilated central vein (line); FD – fat droplets (green arrow); (A, F): Normal CV and PV in the liver of control and garlic extract-supplemented mice. The scale bar stands for 100 µm

Reno-protective properties of garlic extract

Normal architecture of the cortex and medulla was found in the control group of mice. Due to the garlic extract supplementation, glomerular and tubular fatty infiltration, degenerated renal tubules, and hypertensive glomerulosclerosis was successfully restored in group C of mice (Fig. 7). Glomerulus, Bowman's space was also seen as normal like the control group of mice (Fig. 7).

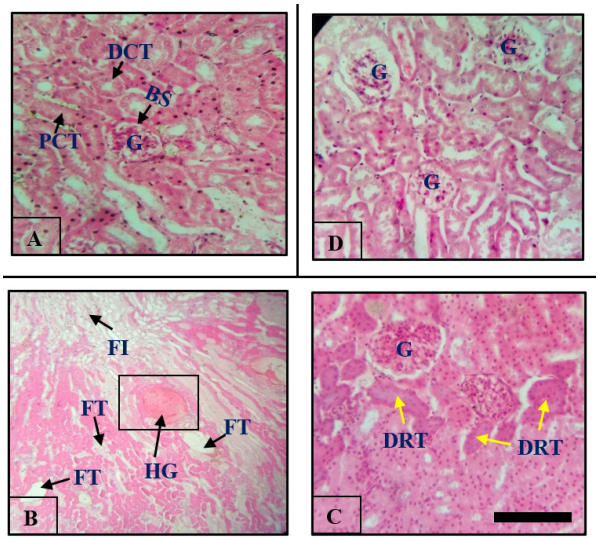

Fig. 7. Transverse section of representative photomicrographs of kidney of A: control (group A), B, C: hypercholesterolemic (group B), and D: garlic extract supplemented (group C) mice (H&E stained) at 40X magnification. DCT – distal convoluted tubule; PCT – proximal convoluted tubule; G – glomerulus; BS – Bowman's space; (B&C): FT – fatty infiltration (black arrow); HG – hypertensive glomerulosclerosis (rectangle); DRT – degenerated renal tubules (yellow arrow); (A, D): Glomerulus, renal tubules, and Bowman's space were found normal. Fatty infiltration and degenerated tubules were also restored by garlic extract supplementation in the C group of mice. The scale bar stands for 100 µm

Study of lipid profile

Improvement of serum lipid profile by garlic extract supplementation

In the present study, the mean value of TC concentration increased by 27% in group (B) (179.044±0.412 mg/dL) (Table 1) compared to group (A) (141.296±0.318 mg/dL) (Table 1) of mice. There was a reduction of TC concentration by 7% in group (C) (166.396±0.249 mg/dL) (Table 1) compared to group (B) of mice. TG concentration increased by 72% in group (B) (231.294±0.258 mg/dL) (Table 1) compared to group (A) (115.108±1.067 mg/dL) (Table 1) of mice. However, group (C) (162.312±1.596 mg/dL) (Table 1) showed a decreased value of TG concentration by 20% compared to group (B) of mice. HDL level (78.906±0.417 mg/dL) (Table 1) decreased by 31% and LDL level (46.544±0.203 mg/dL) (Table 1) increased by 197% in group (B) mice compared to group (A) (94.481±0.395 mg/dL & 24.892±0.273 mg/dL) (Table 1) of mice. Surprisingly, group (C) showed a higher

level of HDL (96.06±0.221 mg/dL) (Table 1) at 47% and a decreased level of LDL (33.02±0.465 mg/dL) (Table 1) at 48% compared to group (B) of mice. Statistically, TC, TG, and LDL levels showed a significant **p<0.001 increase in group B (Fig. 8) compare to control group A, as well as a significant **p<0.001 reductions seen in HDL level of group (B) of mice (Fig. 8). In contrast, garlic extract supplementation (group C) significantly **p<0.001 reduced TC, TG, and LDL concentration and significantly **p<0.001 increased HDL concentration compare to group B (Fig. 8). In addition, the concentration difference of TC, TG, and LDL between the control (group A) and group C was significant at the level of *p<0.05 and LDL concentration was almost similar in both the A and C groups of mice (Fig. 8).

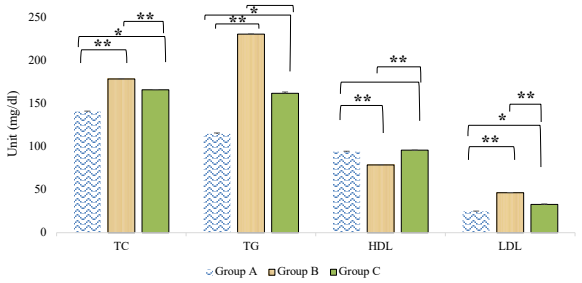

Fig. 8. Diagram represents the analysis of the protective effects of garlic extract in lowering the serum lipid profile. TC – total cholesterol; TG – triglyceride; HDL – high-density lipoprotein; LDL – low-density lipoprotein. Results were expressed as mean ± standard error. (**) indicates a statistically significant difference at the level of **p<0.001; (*) indicates a statistically significant difference at the level of *p<0.05 from the control (group A) of mice

Table 1. TC, TG, HDL, and LDL values in control, diet-induced hypercholesterolemic, and garlic extract-supplemented groups[a]

Parameters (mg/dl)	Group A	Group B	Group C
TC, mg/dL	141.296±0.318	179.044±0.412**	166.396±0.249*
TG, mg/dL	115.108±1.067	231.294±0.258**	162.312±1.596*
HDL, mg/dL	94.481±0.395	78.906±0.417**	96.06±0.221
LDL, mg/dL	24.892± 0.273	46.544±0.203**	33.02±0.465*

[a] (**) and (*) denotes a statistically significant difference at the level of **p<0.001 and *p<0.05 respectively

Discussion

The present study showed that consumption of a cholesterol-rich diet (cow brain) alters the gross and histoarchitecture of the liver, which is in complete agreement with the results of Korish and Arafah. It was reported that regular high-cholesterol feed consumption causes excess energy intake, growing obesity, and a tendency to inactivity. Liver weight was significantly increased compared to the control group. Liver become pale and showed slight hepatomegaly, degenerated hepatocytes, and fatty droplets in the hepatocytes.[16] Kundu et al. reported similar results in their study where cow brain was used as a cholesterol-rich diet.[4,7] Our study exhibited that garlic extract feeding surprisingly restored gross and cellular changes in the hypercholesterolemic liver. Garlic extract supplementation eliminates fat droplet accumulation in hepatocytes and reduces organ weight. The study conducted by Zhang et al. also agrees with our statement. They found a significant reduction in N-nitrosodiethylamine-induced liver weight, surface nodule, and liver enlargement, and improvement in the hepatocellular architecture from hyperplastic nodules and destroyed hepatocytes after garlic oil application during the study period.[17] Tran et al. also speculated in their research, the aqueous extract of garlic reduced the weight and restore the texture of the CCl4-intoxicated liver, and reduced the proliferation of mononuclear cells around the hepatic veins.[18] In addition to this, our current research showed an accumulation of PART, a slight increase in kidney weight, and several fatty changes in the cow brain-induced hypercholesterolemic kidney tubules and glomerulus. In the previous study conducted by Salim et al. stated that a high-fat diet leads to hypercholesterolemia and disturbance of lipid profile, impaired kidney function, and accumulation of fatty droplets in the glomerulus and kidney tubules. There is a close relationship between TG concentration and fat deposition in organs. A higher level of triglyceride is the key factor for the deposition of fat in organs.[19] Increased hypercholesterolemia alters the cellular structure of the tubules and deposits fatty droplets in the tubules that ultimately inhibit cell metabolism and impede filtration.[20] Alansari et al. were also in agreement with our current finding. Tubular deformities, atrophied glomerular capillaries, and vacuolar degeneration in tubules frequently appear due to a high-fat diet.[21] But garlic extract supplementation mitigated the cellular changes induced by a high-fat diet and completely restored fatty infiltration in both tubules and glomerulus and ultimately improved filtration capacity in the current findings. El-Shenawy and Hassan also revealed a statement in a study that garlic extract improved the cellular alteration of kidneys induced by a hypercholesterolemic diet as well as recovered the damages from fatty infiltration in renal tubules.[22]

From the biochemical analysis of the present study, it has been stated that TC and triglyceride TG concentration was significantly reduced by garlic extract consumption. However, this statement was in agreement with Ali and Thomson who mentioned that administration of garlic to hypercholesterolemic rats, humans, and cell cultures is effective in decreasing TC and TG concentration.[23] Sher et al. also observed similar findings. They described in the study, levels of TC (32.8±0.7 mg/dL) and TG (44.0±0.9 mg/dL) concentration significant-

ly ($p<0.001$) decreased after 4 hours of garlic extract administration in rabbits.[24] Adler and Holub also reported in support of the reduction of TC-level and LDL-cholesterol levels respectively by 11.5% and 14.2% by using garlic aqueous extract.[25] Serum TC, LDL, and TG levels were found to be significantly lowered but HDL cholesterol levels increased after regular feeding of garlic extract in the study conducted by Sun et al. and Durak et al.[15,26] Rahman and Lowe also showed that garlic administration suppressed LDL oxidation and increased HDL which may be one of the beneficial effects of garlic in cardiovascular diseases.[27] In our present study, garlic extract supplementation significantly reduced LDL levels which were also revealed in the study of Shela et al., Merat and Fallahzadeh, Aslani et al, and Yeh et al. They observed that garlic administration in rats suffering from hypercholesterolemia induced by a high-cholesterol diet significantly reduced LDL levels.[28-31] In the current study, feeding garlic extract to an induced hypercholesterolemic rat caused a significant rise in HDL levels. This was in agreement with the statement of Aouadi et al.[32] The protective mechanisms of the beneficial effects of garlic in CVDs may be achieved by suppressing LDL oxidation, increasing HDL as well as decreasing TC and TG reported by Katsuki et al. and Gardner et al.[33,34] Therefore, according to the above description, it can be suggested that garlic extract has a protective function to restore serum biochemical parameters and to restore the ability of gross and histomorphological changes of organs (liver and kidney) in hypercholesterolemia induced by a cholesterol-rich diet.

Conclusion

Cholesterol-rich food intake has several adverse health effects including changes in lipid profile, changes in organ weight, fat accumulation in various organs, especially the liver and kidney, various pathological lesions in gross and microscopic aspects, etc. In our society, people are not aware of the tremendous beneficial effects of medicinal herbs like garlic. In the present study, garlic extract supplementation restored the lipid profile, excess organ weight, and histopathological lesions of the liver and kidney, specifically removing fat deposits in the cells. However, further studies on a molecular basis are needed to investigate the pathophysiological mechanism of garlic extract in restoring pathological changes as well as elevated lipid profiles in hypercholesterolemic mice.

Acknowledgments

The authors are highly grateful to the Department of Anatomy and Histology, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh-2202 as well as the university central laboratory for the technical support. Special thanks to MoST (Ministry of Science and Technology), Bangladesh for providing the NST (National Science and Technology fellowship as financial support to conduct the research efficiently.

Declarations

Funding

MoST (Ministry of Science and Technology) Bangladesh; NST (National Science and Technology, 2018-2019) (NST SL No. 303 and ID No. 4110).

Author contributions

Conceptualization, S.K.K. and S.K.D.; Methodology, S.K.K.; Software, S.K.K.; Validation, S.K.K., S.K.D. and M.A.H.N.A.K.; Formal Analysis, S.K.K.; Investigation, S.K.K., S.K.D. and M.A.H.N.A.K.; Resources, S.K.K. and S.K.D.; Data Curation, S.K.K.; Writing – Original Draft Preparation, S.K.K.; Writing – Review & Editing, S.K.K., S.K.D. and M.A.H.N.A.K.; Visualization, S.K.K.; Supervision, S.K.K., S.K.D. and M.A.H.N.A.K.; Project Administration, S.K.K. and S.K.D.; Funding Acquisition, S.K.K.

Conflicts of interest

Nothing to disclose.

Data availability

Datasets analyzed during the present study and/or are available from the corresponding author upon reasonable request.

Ethics approval

The Ethical Committee of Bangladesh Agricultural University approved the study protocol (AWEEC/BAU/2019-53).

References

1. Lonardo A, Mantovani A, Targher G, Baffy G. Nonalcoholic Fatty Liver Disease and Chronic Kidney Disease: Epidemiology, Pathogenesis, and Clinical and Research Implications. *Int J Mol Sci.* 2022;23(21):13320. doi: 10.3390/ijms232113320
2. World Health Rankings site. https://www.worldlifeexpectancy.com/country-health-profile/bangladesh. Accessed February 20, 2023.
3. Ix JH, Sharma K. Mechanisms linking obesity, chronic kidney disease, and fatty liver disease: the roles of fetuin-A, adiponectin, and AMPK. *J Am Soc Nephrol.* 2010;21(3):406-412. doi: 10.1681/ASN.2009080820
4. Kundu SK, Khan MA, Das SK. Cow brain consumption causes hypercholesterolemia: An in vivo study. *Asian Journal Of Dairy And Food Research*. 2021;40(2):177-180. doi: 10.18805/ajdfr.DR-216
5. Bengal Meat Beef Brain: https://jarlimited.com/product/bengal-meat-beef-brain-500-gm/. Accessed March 29, 2023.
6. Nessa MU, Rahim AT, Kabir Y. Fatty acid profile of fast foods, traditional snacks and sweetmeats of Bangladesh. *KU Studies*. 2008;29:85-90. doi 10.53808/KUS.2008.9.1.0820-L

7. Kundu SK, Khan MA, Das SK. Beneficial Role of Mushroom in Recovering Complications of Hypercholesterolemia. *IDJPCR*. 2021;4(2):1-4. doi: 10.32734/idjpcr.v4i2.6467
8. Serviddio G, Bellanti F, Villani R, et al. Effects of dietary fatty acids and cholesterol excess on liver injury: A lipidomic approach. *Redox Biol.* 2016;9:296-305. doi: 10.1016/j.redox.2016.09.002
9. Altunkaynak ME, Özbek E, Altunkaynak BZ, Can İ, Unal D, Unal B. The effects of high-fat diet on the renal structure and morphometric parametric of kidneys in rats. *J Anat*. 2008;212(6):845-852. doi: 10.1111/j.1469--7580.2008.00902.x
10. Ried K, Frank OR, Stocks NP. Aged garlic extract reduces blood pressure in hypertensives: a dose-response trial. *Eur J Clin Nutr*. 2013;67(1):64-70. doi: 10.1038/ejcn.2012.178
11. Shiju TM, Rajesh NG, Viswanathan P. Renoprotective effect of aged garlic extract in streptozotocin-induced diabetic rats. *Indian J Pharmacol.* 2013;45(1):18. doi: 10.4103/0253-7613.106429
12. Nani D, Proverawati A, Taufik A. Black solo garlic protects hepatic and renal cell function in streptozotocin-induced rats. *Front Nutr*. 2022;9:962993. doi.org/10.3389/fnut.2022.962993
13. Naji KM, Al-Shaibani ES, Alhadi FA, Al-Soudi SA, D'souza MR. Hepatoprotective and antioxidant effects of single clove garlic against CCl4-induced hepatic damage in rabbits. *BMC Complement Altern Med*. 2017;17(1):1-2. doi: 10.1186/s12906-017-1916-8
14. Shang A, Cao SY, Xu XY, et al. Bioactive compounds and biological functions of garlic (Allium sativum L.). *Foods*. 2019;8(7):246. doi: 10.3390/foods8070246
15. Sun YE, Wang W, Qin J. Anti-hyperlipidemia of garlic by reducing the level of total cholesterol and low-density lipoprotein: A meta-analysis. *Medicine*. 2018;97(18):e0255. doi: 10.1097/MD.0000000000010255
16. Korish AA, Arafah MM. Camel milk ameliorates steatohepatitis, insulin resistance and lipid peroxidation in experimental non-alcoholic fatty liver disease. *BMC Complement Altern Med*. 2013;13(1):1-2. doi: 10.1186/1472-6882-13-264
17. Zhang CL, Zeng T, Zhao XL, Yu LH, Zhu ZP, Xie KQ. Protective effects of garlic oil on hepatocarcinoma induced by N-nitrosodiethylamine in rats. *Int J Biol Sci.* 2012;8(3):363. doi: 10.7150/ijbs.3796
18. Tran GB, Dam SM, Le NT. Amelioration of single clove black garlic aqueous extract on dyslipidemia and hepatitis in chronic carbon tetrachloride intoxicated Swiss Albino mice. *Int J Hepatol.* 2018;2018:9383950. doi: 10.1155/2018/9383950
19. Salim HM, Kurnia LF, Bintarti TW. The effects of high--fat diet on histological changes of kidneys in rats. *BHSJ*. 2018;1(2):109-112. doi: 10.20473/bhsj.v1i2.9675
20. Curthoys NP, Moe OW. Proximal tubule function and response to acidosis. *CJASN*. 2014;9(9):1627-1638. doi: 10.2215/CJN.10391012
21. Alansari AK, ALmohaimeed HM, Baradea SM, Ali SS. Reno-protective effects of fresh garlic against high fat diet induced damage. *J Health Sci.* 2020;2(2):85-67. doi: 10.4103/1658-8312.347586
22. El-Shenawy SM, Hassan NS. Comparative evaluation of the protective effect of selenium and garlic against liver and kidney damage induced by mercury chloride in the rats. *Pharmacol Rep.* 2008;60(2):199.
23. Ali M, Thomson M. Consumption of a garlic clove a day could be beneficial in preventing thrombosis. *Prostaglandins Leukot Essent Fatty Acids*. 1995;53(3):211-212. doi: 10.1016/0952-3278(95)90118-3
24. Sher A, Fakhar-ul-Mahmood M, Shah SN, Bukhsh S, Murtaza G. Effect of garlic extract on blood glucose level and lipid profile in normal and alloxan diabetic rabbits. *Adv Clin Exp Med.* 2012;21(6):705-711.
25. Adler AJ, Holub BJ. Effect of garlic and fish-oil supplementation on serum lipid and lipoprotein concentrations in hypercholesterolemic men. *AJCN*. 1997;65(2):445-450. doi: 10.1093/ajcn/65.2.445
26. Durak I, Kavutcu M, Aytaç B, et al. Effects of garlic extract consumption on blood lipid and oxidant/antioxidant parameters in humans with high blood cholesterol. *J. Nutr Biochem*. 2004;15(6):373-377. doi: 10.1016/j.jnutbio.2004.01.005
27. Rahman K, Lowe GM. Significance of garlic and its constituents in cancer and cardiovascular disease. *J Nutr*. 2006;136(3):736-740.
28. Gorinstein S, Leontowicz H, Leontowicz M, et al. Raw and boiled garlic enhances plasma antioxidant activity and improves plasma lipid metabolism in cholesterol-fed rats. *Life Sci.* 2006;78(6):655-663. doi: 10.1016/j.lfs.2005.05.069
29. Merat A, Fallahzadeh M. Effect of garlic on some blood lipids and HMG-CoA reductase activity. *Iran J Med Sci*. 1996;21:141-146.
30. Aslani N, Entezari MH, Askari G, Maghsoudi Z, Maracy MR. Effect of Garlic and Lemon Juice Mixture on Lipid Profile and Some Cardiovascular Risk Factors in People 30-60 Years Old with Moderate Hyperlipidaemia: A Randomized Clinical Trial. *Int J Prev Med.* 2016;7:95. doi: 10.4103/2008-7802.187248
31. Yeh YY, Yeh SM. Garlic reduces plasma lipids by inhibiting hepatic cholesterol and triacylglycerol synthesis. *Lipids.* 1994;29(3):189-193. doi:10.1007/BF02536728
32. Aouadi R, Aouidet A, Elkadhi A, et al. Effect of fresh garlic (Allium sativum) on lipid metabolism in male rats. *Nutr Res.* 2000;20(2):273-280. doi.org/10.1016/S0271-5317(99)00159-1
33. Amagase H. Significance of garlic and its constituents in cancer and cardiovascular disease. *J Nutr*. 2006;136(3):716-725. doi: 10.1093/jn/136.3.v
34. Gardner CD, Chatterjee LM, Carlson JJ. The effect of a garlic preparation on plasma lipid levels in moderately hypercholesterolemic adults. *Atherosclerosis*. 2001;154(1):213-220. doi: 10.1016/s0021-9150(00)00466-4

Wydawnictwo UR 2023
ISSN 2544-1361 (online)
doi: 10.15584/ejcem.2023.3.3

http://www.ejcem.ur.edu.pl
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2023; 21 (3): 458–464

ORIGINAL PAPER

Evaluation of nonpharmacological nursing practices related to thirst and the thirst of patients in the intensive care unit

Sevim Çelik [1], Münevver Şengül [2], Elif Karahan [1]

[1] Department of Nursing, Faculty of Health Sciences, Bartin University, Bartin, Turkey
[2] Department of Nursing, Graduate Education Institute, Bartin University, Bartin, Turkey

ABSTRACT

Introduction and aim. Thirst is a significant symptom and stressor among patients in the intensive care unit. The aim of this study is to assess the severity of thirst and associated symptoms experienced by patients in the intensive care unit, and to evaluate the nursing practices related to addressing thirst.

Material and methods. This descriptive and correlational study involved a total of 66 patients in the intensive care unit. Severity of thirst experienced by these patients was assessed using a numeric rating scale. The nursing practices related to assessing thirst severity on admission to the unit, on the third day, and on the seventh day were also recorded.

Results. Our findings showed a statistically significant increase in the levels of thirst, dry mouth, bad taste and odor in the mouth, as well as sensitivity and dryness in the throat among patients on the seventh day of hospitalization ($p<0.05$).Among nursing interventions, communication with the patient was the most frequently applied intervention during the seven-day period, with a range of 98.5% to 100%. biochemical control (100%), ventilation of the unit (100%), and temperature regulation (100%) were also frequently applied.

Conclusion. The patients had all the symptoms of thirst.The frequency of nursing interventions increased in parallel with the severity of thirst.

Keywords. intensive care patient, nurse, thirst, nursing practices

Introduction

Thirst is a significant symptom and stressor among patients in the intensive care unit.[1] Various factors contribute to the development of thirst in this population, including life-threatening illnesses, endotracheal tubes, pain, immobility, sleep disturbances, frequent examinations or touching, communication difficulties, fear, and anxiety.[2,3] In addition, changes in consciousness, bleeding, preoperative fasting, anesthetic drugs, oxygen therapy, anticholinergics, diuretics, opioids, antihypertensives, hypovolemia, and inadequate oral hydration can also contribute to the development of thirst.[4-8] Studies have reported that a significant proportion of patients in the intensive care unit experience intense or moderate thirst, with prevalence ranging from 66% to 70%.[6,9]

Patients who experience thirst in the intensive care unit may present with a range of complaints, including dryness of the mouth, difficulty in swallowing, tenderness in the throat, changes in kidney function and electrolyte levels, and bad taste and odor in the mouth.[4,10,11] Several studies have investigated the prevalence and severity of thirst in this population. For instance, Vonstein et al. found that the average level of thirst, as measured on a 0-10 rating scale, was 5.5 among non-mechanically ventilated patients and 6.1 among patients who were not

Corresponding author: Sevim Çelik, e-mail: sevimakcel@gmail.com

Received: 12.04.2023 / Revised: 30.04.2023 / Accepted: 12.05.2023 / Published: 30.09.2023

Çelik S, Şengül M, Karahan E. *Evaluation of nonpharmacological nursing practices related to thirst and the thirst of patients in the intensive care unit.* Eur J Clin Exp Med. 2023;21(3):458–464. doi: 10.15584/ejcem.2023.3.3.

fed by mouth, with 66% of patients reporting dryness of the mouth.[12] Similarly, Negro et al. reported that 76.1% of intensive care patients experienced thirst, with a mean thirst level of 5.37 out of a maximum of 10 points.[5]

Prolonged thirst lasting more than 24 hours can have significant negative impacts on the comfort of patients during their healing process, potentially causing fluid and electrolyte imbalances, delirium, and even post-traumatic stress disorder.[5,9,11,13,14] As such, regular assessment of thirst is crucial in order to prevent or mitigate these symptoms.[4] However, the assessment and management of thirst in intensive care patients is often neglected. In addition, nurses may prioritize vital signs over the recognition of thirst.[1,15] When thirst is identified, effective management strategies may be limited by factors such as fluid restriction, concerns about aspiration or vomiting, and the presence of endotracheal tubes.[1]

Intensive care specialists strongly recommend the management of thirst as an important factor in patient prognosis. Various nursing interventions have been found to be effective in managing thirst, such as cooling the oral mucosa, using menthol moisturizer, providing effective oral care, regulating ambient temperature and humidity, gargling with salt water, and moistening the mouth and lips. These interventions have been supported by several studies.[2,7,12,15,18]

Aim

The aim of this study was to assess the severity of thirst and associated symptoms experienced by intensive care patients, as well as to evaluate the nursing interventions used to manage thirst.

Material and methods

Ethical approval

The study received ethical approval from the Social and Human Sciences Ethics Committee of the University (date:07.01.2022, approval number:21). Written consent was obtained from the state hospital affiliated with the provincial health directorate. Informed consent was obtained from the nurses, physicians, and patients for their participation in the study.

Design and sample

A descriptive and correlational design was used. Based on the literature and using the G-Power 3.1.9.7 program, a sample size of 66 patients was calculated to achieve a power of 95% with an alpha level of 0.05 and an effect size of 0.636.[19]

The inclusion criteria for the study were as follows: (i) Patients who were 18 years of age or older, (ii) Patients who were hospitalized in the intensive care unit, (iii) Patients who were treated in the intensive care unit for at least 7 days, (iv) Patients who were able to communicate, (v) Patients who were conscious, (vi) Patients who had no diagnosed mental or mental illness, (vii) Patients who volunteered to participate in the study.

Excluding unconscious patients (n=5) and patients (n=4) hospitalized for less than 7 days ensures that the patients included in the study are able to communicate effectively and have experienced thirst symptoms for a sufficient duration.

Instruments

Survey form

The survey form used in the study had five parts: The first part evaluated the reason for the patient's admission to the intensive care unit and their habits and characteristics before admission. The second part evaluated the characteristics of patients who underwent surgical intervention. The third part evaluated the factors that may cause the patient's thirst and the hemodynamic parameters that may occur as a result of thirst. The fourth part recorded thirst markers using the numeric rating scales (NRS). The last part recorded the practices of nurses for managing thirst in the patients.[19]

Numeric rating scales

The NRS were used to record the intensity of various thirst symptoms on the 0th, 3rd, and 7th days of the patient's stay in the intensive care unit. The symptoms evaluated using the NRS included thirst expression, dryness of mouth, dryness of throat, sensitivity in the throat, difficulty in swallowing, difficulty in speaking, feeling of bad taste and bad smell in the mouth, deterioration in the oral mucosa, and moistness of the lips. The NRS is a reliable and safe tool for evaluating thirst symptoms, and the mean scores were interpreted as mild (0-3 points), moderate (4-6 points), and severe (7-10 points) thirst.[19-21]

Procedure

The data were collected between March and October 2022 in the intensive care units at a state hospital in Turkey. The demographic and clinical characteristics of the patients on the 0th day of hospitalization in the intensive care unit were recorded using self-reports and medical reports of the patients. Thirst symptoms, except for mouth odor, deterioration of mucosa, and moistness of lips, were recorded using the NRS according to the patients' self-reports on the 0th, 3rd, and 7th days of hospitalization in the intensive care unit. On the other hand, mouth odor, deterioration of mucosa, and moistness of lips were recorded using the NRS by the researcher. The clinical status of the patients on the 3rd and 7th days was documented based on their medical reports. Additionally, the nursing practices related to the severity of thirst, as reported by the nurses themselves, were recorded on the 3rd and 7th days.

Table 1. The demographic characteristics of the patients (n=66)

Characteristic		Mean (SD)
Age		73.67 (11.46)
		n (%)
Gender	Woman	30 (45.5)
	Male	36 (54.5)
Chronic disease (n=59; 89.4%)	Neurological	18 (27.3)
	Cardiovascular	47 (71.2)
	Respiratory	25 (37.9)
	Endocrine	26 (39.4)
	Urogenital	6 (9.1)
	Other (musculoskeletal, psychiatric)	4 (6)
Drug used continuously (n=57; 86.4%)	Antiepileptic	4 (6.1)
	Parasympathomimetic	6 (9.1)
	Proton pump inhibitor	30 (45.5)
	Inhaler	20 (30.3)
	Antihypertensive	37 (56.1)
	Antidiabetic	23 (34.8)
	Diuretic	3 (4.5)
	Anticoagulant	18 (27.3)
	Betablocker	19 (28.8)
	Antipsychotic	1 (1.5)
	Other (Lipid lowering, urological)	7 (10.6)
Alcohol use	Yes	5 (7.6)
	No	61 (92.4)
Smoking	Yes	13 (19.7)
	No	53 (80.3)
Intensive care unit	COVID	35 (53)
	1st level	19 (28.8)
	General	7 (10.6)
	Emergency	5 (7.6)
Diagnosis*	COVID-19 infection	35 (53)
	Acute respiratory failure	17 (25.8)
	Dyspnea	28 (42.4)
	Pneumonia	26 (39.4)
	Pulmonary embolism	4 (6.1)
	Pulmonary edema	5 (7.6)
	Aspiration pneumonia	3 (4.5)
	Acute kidney failure	6 (9.1)
	Atrial fibrillation	6 (9.1)
	Gastrointestinal bleeding	3 (4.5)
	General condition disorder	15 (22.7)
Feeding	Oral	39 (59.1)
	Nasogastric tube	13 (19.7)
	Percutaneous endoscopic	1 (1.5)
	gastostomy	3 (4.5)
	Total paranteral nutrition	10 (15.2)
	Peripheral nutrition	

*more than one answer was given, the percentages were taken according to the number n (66)

Data analysis

The data were analyzed using the SPSS 22.0 package program (IBM, Armonk, NY, USA). The normal distribution was assessed using the Kolmogorov-Smirnov test. Descriptive statistical analysis methods and the chi-square test were used to analyze the data. For data that did not follow a normal distribution, the Friedman test for repeated measures and Spearman correlation analysis were conducted. Pairwise comparisons were examined using the Wilcoxon sign-rank test. The level of significance was set at $p<0.05$.

Results

The study included patients with a mean age of 73.67, where 54.5% were male. A high percentage of patients (89.4%) had a chronic disease and 86.4% were taking medications regularly. The most common chronic disease among the patients was related to the cardiovascular system (71.2%), and more than half of the patients (56.1%) were using antihypertensive drugs. Majority of the patients did not consume alcohol (92.4%) or smoke (80.3%). The majority of patients (53%) were admitted to the COVID intensive care unit (ICU) for the treatment and care of COVID-19, followed by diagnoses of dyspnea and pneumonia (Table 1).

The study found that patients diagnosed with acute respiratory failure, male patients, and patients using continuous medication experienced statistically significantly higher levels of thirst at the time of hospitalization compared to other patients ($Z=-2.292$, $p=0.022$; $Z=-2.412$, $p=0.016$; $Z=-2.137$, $p=0.033$, respectively). There was also a weak negative correlation found between the mean age of the patients and the severity of thirst on the 0th day of hospitalization ($rho=-0.250$, $p=0.043$). However, there was no statistically significant difference found between the severity of thirst at hospitalization and other descriptive characteristics of the patients ($p>0.05$).

Table 2 displays the results of the clinical characteristics of the patients during the seven-day follow-up period after their admission to the intensive care unit. No statistically significant difference was found in the vital signs and biochemical analyses of the patients ($p>0.05$). However, there was a statistically significant increase in the mean scores of oxygen saturation and Glasgow Coma Scale ($p<0.001$).

Table 2. The clinical characteristics of the patients for 7 days (n=66) *

Clinical characteristics	0th day Mean (SD)	3rd day Mean (SD)	7th day Mean (SD)	c^2 p
Body temperature (°C)	36.14 (0.28)	36.44 (0.31)	36.45 (0.30)	3.759; 0.153
Heart rate (beats/min)	90.09 (15.25)	87 (13.18)	86.94 (12.65)	3.512; 0.173
Systolic blood pressure (mmHg)	122.97 (18.22)	120.71 (18.11)	119.39 (18.33)	3.567; 0.168
Diastolic blood pressure (mmHg)	53.52 (7.32)	52.85 (9.21)	51.77 (9.49)	5.567; 0.056
SpO_2 (%)	94.12 (2.08)	94.94 (2.07)	95.53 (2.17)	22.183; <0.001
GCS score	13.88 (1.07)	14.14 (1.02)	14.17 (1.07)	23.439; <0.001
Na^+ (mEq/L)	137.64 (4.99)	137.02 (4.03)	136.68 (3.77)	5.653; 0.059
Cl^- (mEq/L)	102.91 (6.42)	102.05 (5.61)	101.68 (4.75)	3.119; 0.21
K^+ (mEq/L)	4.05 (0.68)	4.72 (4.94)	4.13 (0.64)	0.593; 0.743
Hct (%)	33.60 (6.07)	33.26 (5.56)	32.98 (5.24)	3.848; 0.146
BUN (mg/dl)	70.50 (33.59)	76.36 (32.69)	75.62 (43.39)	7.870; 0.02

* c^2 – Friedman test; GCS – Glasgow Coma Scale

Table 3 presents the results of the thirst indicators that were monitored for the 7-day hospitalization period in the intensive care units. According to the NRS, the severity of thirst and thirst-related symptoms experienced by the intensive care patients decreased significantly, except for difficulties in swallowing and speaking on the 0th day of hospitalization ($p<0.05$). However, it was not clinically significant.

The results of the Wilcoxon signed-rank test showed that the pairwise comparisons of thirst levels were statistically significant between the third day versus admission day (Z=-3.120; p=0.002) and on the seventh day versus admission day (Z=-2.380; p=0.017). Similarly, dryness of mouth levels were statistically significant between the third day versus admission day (Z=-3.440; p=0.001) and on the seventh day versus third day (Z=-2.041; p=0.041). Throat tenderness levels were statistically significant between the third day versus admission day (Z=-3.801; p<0.001) and on the seventh day versus admission day (Z=-3.229; p=0.001). Bad taste in the mouth levels were statistically significant between the third day versus admission day (Z=-3.840; p<0.001) and on the seventh day versus admission day (Z=-2.480; p=0.013). Bad smell in the mouth levels were statistically significant between the third day versus admission day (Z=-3.397; p=0.001) and on the seventh day versus admission day (Z=-1.199; p=0.046). The deterioration of the oral mucosa levels were statistically significant between the third day versus admission day (Z=-5.945; p<0.001) and on the seventh day versus admission day (Z=-5.059; p<0.001). However, there was no statistically significant difference in the comparison between thirst, dryness of mouth, throat tenderness, bad taste in the mouth, bad smell in the mouth, and deterioration of the oral mucosa levels on the seventh and third days (p>0.05).

Table 3. Thirst indicators for 7 days in patients (n=66) *

Indicators	0th day Mean (SD)	3rd day Mean (SD)	7th day Mean (SD)	c^2 p
Expression of thirst	3.45 (1.25)	4.02 (1.70)	3.95 (1.58)	7.011; 0.03
Dryness of mouth	3.73 (1.37)	4.48 (1.72)	4.18 (1.65)	14.468; 0.001
Dryness of throat	2.89 (1.20)	3.47 (1.64)	3.26 (1.47)	15.362; <0.001
Throat tenderness	2.67 (1.50)	3.48 (1.93)	3.35 (1.82)	16.038; <0.001
Difficulty swallowing	2.79 (2.28)	3.03 (2.3)	3.00 (2.41)	2.430; 0.297
Speech difficulties	1.94 (2.38)	1.82 (2.41)	1.92 (2.66)	3.885; 0.143
Bad taste in the mouth	2.29 (1.29)	2.94 (1.39)	2.74 (1.26)	16.247; <0.001
Bad smell in the mouth	2.02 (1.33)	2.56 (1.62)	2.38 (1.6)	11.071; 0.004
Deterioration of the oral mucosa	2.74 (1.2)	4.20 (1.56)	4.00 (1.66)	56.000; <0.001
Moisture of lips	2.88 (1.07)	3.61 (1.42)	3.38 (1.36)	23.545; <0.001

* c^2 – Friedman test

The dryness of throat levels on the third day versus admission day (Z=-3.170; p=0.002) and on the seventh day versus admission day (Z=-2.358; p=0.018) were statistically significant. However, the comparison between dry throat levels on the seventh and third days was not statistically significant (p>0.05). The moisture of lips levels on the third day versus admission day (Z=-4.114; p<0.001), on the seventh day versus admission day (Z=-3.156; p=0.002), and on the seventh day versus third day (Z=-2.501; p=0.012), were statistically significant (Table 3).

It seems that there is a weak positive correlation between blood urea nitrogen levels and the severity of thirst experienced by the patients on admission (rho=0.305; p=0.013), the third day (rho=0.258; p=0.036), and the seventh day (rho=0.351; p=0.004) of hospitalization. There is also a weak positive correlation between sodium values on the third day and thirst severity (rho=0.306; p=0.012). However, there is a weak negative correlation between SpO_2 values and thirst levels on the seventh day (rho=-0.298; p=0.015). No significant correlation was found between the follow-up results of other clinical characteristics and thirst levels (p>0.05) (Table 4).

Table 4. The relationship between the results of the 7 day follow-up of the clinical characteristics of the patients and the severity of thirst (n=66) *

Clinical characteristics	0th day Thirst level rho p	3rd day Thirst level rho p	7th day Thirst level rho p
Body temperature (C)	-0.082 0.515	0.039 0.755	-0.152 0.222
Heart rate (beats/min)	-0.007 0.957	0.098 0.434	0.105 0.402
Systolic blood pressure (mmHg)	0.076 0.546	0.200 0.107	0.139 0.265
Diastolic blood pressure (mmHg)	0.022 0.863	0.235 0.058	0.035 0.782
SpO_2 (%)	-0.184 0.14	-0.110 0.380	-0.298 0.015
GCS score	-0.028 0.822	-0.040 0.75	-0.208 0.094
Na^+ (mEq/L)	-0.057 0.649	0.306 0.012	0.086 0.491
Cl^- (mEq/L)	0.010 0.936	0.015 0.902	-0.097 0.438
K^+ (mEq/L)	0.114 0.362	0.061 0.626	0.167 0.181
Hct (%)	-0.041 0.746	-0.105 0.402	0.105 0.402
BUN (mg/dl)	0.305 0.013	0.258 0.036	0.351 0.004

* rho – Spearman correlation analysis; GCS – Glasgow Coma Scale

It appears that the most frequently applied nursing interventions by nurses for 7 days were communication with the patient (range 98.5-100%), biochemistry control (100%), ventilation of the unit (100%) and lowering the temperature (100%). In addition, giving oral care was followed between 48.5% and 84.8% of the time, and increased oral intake was followed between 77.3% and 75.8% of the time (Table 5).

It seems that there was no significant difference between the severity of thirst at hospitalization and the nursing interventions applied on the day of hospitalization (p>0.05). However, there was a significant difference between the severity of thirst on the third day and the frequency of oral care on the third day (Z=-2.162; p=0.031), as well as between the severity of thirst on the seventh day and the frequency of increasing oral intake

on the seventh day (Z=-2.193; p=0.028). There was no significant difference between the severity of thirst and the frequency of other nursing interventions performed on hospitalization, 3rd and 7th days (p>0.05).

Table 5. Nursing practices for thirst for 7 days in patients

Nursing practices	0th day n (%)	3rd day n (%)	7th day n (%)
Effective communication with the patient	65 (98.5)	65 (98.5)	66 (100)
Questioning the presence of thirst	36 (54.5)	45 (68.2)	44 (66.7)
Giving oral care	32 (48.5)	52 (78.8)	56 (84.8)
Using a humidifier	20 (30.3)	34 (51.5)	47 (71.2)
Wetting the lips	21 (31.8)	28 (42.4)	34 (51.5)
Evaluate your agitation	36 (54.5)	38 (57.6)	39 (59.1)
Biochemistry control	66 (100)	66 (100)	66 (100)
Increasing oral intake	51 (77.3)	51 (77.3)	50 (75.8)
Lowering the unit temperature	66 (100)	66 (100)	66 (100)
Venting the unit	66 (100)	66 (100)	66 (100)

Discussion

Thirst is a common stressor in the intensive care unit and can negatively impact the healing process and patient comfort. Nurses play a crucial role in addressing this issue by implementing interventions to manage thirst and its associated symptoms such as dry mouth, bad taste, and throat tenderness.[8,22,23] Effective communication with patients and monitoring of biochemical parameters such as blood urea nitrogen and sodium levels are important in managing thirst. Additionally, interventions such as oral care and increasing oral intake can help alleviate symptoms associated with thirst.

This study evaluated the severity of thirst in intensive care patients and the nursing interventions for preventing thirst. The findings revealed that patients experienced mild to moderate thirst on the day of hospitalization and on the 3rd and 7th days after hospitalization. However, the severity of thirst increased significantly on the 3rd and 7th days compared to the hospitalization day (p<0.05). Previous studies by Leemhuis et al. reported moderate thirst in intensive care patients with 3.57 points, whereas VonStein et al. and Negro et al. reported severe thirst.[5,12,17] These results demonstrate that thirst is a significant problem for intensive care patients that cannot be overlooked.

The study found no significant changes in the vital signs and biochemistry results of patients, except for SpO_2 and GCS scores during the three follow-up periods. The mean SpO_2 and GCS scores of patients showed positive clinical improvements. The blood urea nitrogen value increased as the severity of thirst increased (p<0.05). Additionally, there was a positive correlation between sodium values on the third day and severity of thirst. However, the relationship between the severity of thirst on the seventh day and SpO_2 values was negative, with SpO_2 levels decreasing as the severity of thirst increased. It is important to note that the feeling of thirst occurs with a 1% increase in osmolality in the body. The increase in osmolality in the body affects the endocrine, renal, and nervous system functions and disrupts the fluid-electrolyte balance.[24] Hypernatremia is a symptom commonly observed in intensive care patients experiencing thirst due to physiological processes.[25] Previous studies have reported increased sodium and blood urea nitrogen levels as the severity of thirst increases.[5,16] Eren also reported that a decrease in the severity of thirst was accompanied by an increase in SpO_2 values among patients.[19]

The study found that on the day of hospitalization, and on the third and seventh days after hospitalization, thirst was accompanied by dryness of mouth, throat sensitivity, bad taste in the mouth, and deterioration of the oral mucosa at the highest rate (p<0.05). Dryness of mouth was found to be the most important symptom accompanying thirst in intensive care patients. The risk of dry mouth in intensive care patients increases due to factors such as old age, chronic diseases, oxygen therapy methods, dependence on mechanical ventilators, inability to express their wishes, and communication difficulties. Patients with dry mouth may experience irritation of the oral mucosa, tenderness in the throat, and bad taste and odor in the mouth, as reported in previous studies.[5,7,11] VonStein et al. reported severe dry mouth with a score of 6.68 in 103 intensive care patients.[12] Kjeldsen et al. used the phrase "I feel like I have a carpet in my mouth" when patients expressed dryness of mouth in their study.[11] In another study, Negro et al. emphasized that lack of oral hydration, use of high-dose diuretics, and failure to prevent dryness of mouth were the causes of thirst intensity in patients.[5] These findings suggest that thirst is often overlooked in intensive care patients, and there is a deficiency in the practices implemented to manage it. Zhang et al. concluded that better management of thirst perception, increased recognition of thirst symptoms, and interventions should be implemented without increasing the nursing workload.[7] These findings suggest that the issue of thirst in ICU patients should be addressed with greater attention and improved measures are required to manage patients' thirst effectively.

Thirst is a crucial issue that patients in intensive care units often face, yet it may be overlooked due to the focus on other vital risks and intensive care procedures. However, thirst can have a significant impact on the healing process. Nurses are among the healthcare professionals who are directly responsible for addressing patients' thirst.[23,26] Li et al. noted that nurses' perceptions can be a barrier to identifying and addressing patients' thirst, as they may prioritize other more pressing issues and feel overwhelmed by workload and staffing shortages. As a result, they may fail to recognize thirst as a significant concern without patients explicitly expressing their discomfort.[15] We found that the most

common practice among nurses to prevent thirst was to communicate with each patient. Since biochemistry control, ambient ventilation, and temperature regulation are already routine operations in clinics, communication is the most common practice after these.

The results suggest that oral care may be an effective intervention in relieving thirst in ICU patients. Studies have shown that oral care with menthol solution or sticks can alleviate dryness of mouth and improve the oral mucosa, which may in turn alleviate thirst symptoms.[16,18,19] The fact that nurses provided more oral care on the third and seventh days, and that this practice increased with the severity of thirst, suggests that nurses may recognize the importance of oral care in managing thirst in ICU patients. However, further research is needed to evaluate the effectiveness of different oral care interventions in relieving thirst and improving patient outcomes. The finding from this study suggests that nurses provide oral care to relieve thirst rather than prevent it. Previous studies have also investigated the effectiveness of oral care in reducing thirst, such as the study by Zhang et al. which found that the severity of thirst decreased on average from 6.59 to 5.42 with the use of oral care and lip moisturizing methods such as vitamin C spray and boiled chilled water.[7]

This study found that there was an effort to prevent thirst by increasing oral intake, which was increased on the seventh day compared to the day of admission. A significant correlation was found between the severity of thirst and the attempt to increase oral intake on the seventh day ($p<0.05$). The attempt of nurses to increase oral fluid intake to prevent thirst is an encouraging finding. Previous research has shown that the severity of thirst is lower in patients who receive oral hydration.[5]

Study limitations

There are several limitations to the present study. Firstly, the study was restricted to conscious patients, which may limit the generalizability of the findings to unconscious patients. Secondly, almost half of the sample consisted of COVID-19 patients, which may limit the generalizability of the findings to other types of patients. Moreover, the mean age of the patients may have limited the generalizability of the findings as elderly patients may have different thirst levels and nursing practices compared to younger patients. Furthermore, the study was conducted in a single center, which may limit the generalizability of the findings to other settings. The study relied on self-reporting by patients, which may have introduced some bias. The study also did not investigate the impact of thirst management practices on patient outcomes, such as length of hospital stay or mortality. Finally, the study did not examine the impact of other factors, such as medications or comorbidities, on thirst and thirst management practices.

Conclusion

The study concluded that patients in the intensive care unit experienced significant symptoms of thirst that worsened during hospitalization. Furthermore, nursing practices were more frequent as patients' reported thirst levels increased.

In future research, a wider range of age groups could be included in the sample, and subgroup analyses conducted to control for the effects of age on thirst and nursing practices. Moreover, results could be compared with previous studies that included patients of different ages. Additionally, to increase generalizability, future studies could be conducted in multiple centers. Objective measures, such as biomarkers or physiological tests, could be utilized to assess thirst and management practices. The impact of thirst management practices on patient outcomes, such as length of hospital stay and mortality, could also be explored. Finally, the influence of other factors, such as medications and comorbidities, on thirst and thirst management practices could be investigated in future studies.

Declarations

Funding

This study received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Author Contributions

Conceptualization, S.Ç.; Methodology, S.Ç.; Software, S.Ç. and E.K..; Validation, S.Ç; Formal Analysis, S.Ç. and E.K.; Investigation, M.Ş.; Resources, S.Ç. and M.Ş.; Writing – Original Draft Preparation, S.Ç., M.Ş. and E.K.; Visualization, S.Ç. and M.Ş.; Supervision, S.Ç.; Project Administration, S.Ç. and M.Ş.; Funding Acquisition, M.Ş.

Conflicts of interest

The author declares no conflicts of interest.

Data availability

Data will be made available on request.

Ethics approval

The study received ethical approval from the Social and Human Sciences Ethics Committee of the University on 07.01.2022/21.

References

1. Clark J, Archer SK. Thirst Interventions in Adult Acute Care—What Are the Recommended Management Options and How Effective Are They? A Systematic Review. *Dimens Crit Care Nurs.* 2022;41(2):91-102. doi. 10.1097/DCC.0000000000000511
2. Gültekin Y, Özçelik Z, Akinci SB, Yorganci HK. Evaluation of stressors in intensive care units. *Turk J Surg.* 2018;34(1):5-8. doi: 10.5152/turkjsurg.2017.3736

3. Zengin N, Ören B, Üstündag H. The relationship between stressors and intensive care unit experiences. *Nurs Crit Care*. 2020;25(2):109-116. doi: 10.1111/nicc.12465.
4. Lin R, Li H, Chen L, He J. Prevalence of and risk factors for thirst in the intensive care unit: An observational study. *J Clin Nurs*. 2022;23. doi: 10.1111/jocn.16257
5. Negro A, Villa G, Greco M, et al. Thirst in patients admitted to intensive care units: an observational study. *Ir J Med Sci*. 2022;191(5):2283-2289. doi: 10.1007/s11845-021-02817-7
6. Sharma K, Kumar A. Effectiveness of thirst bundle on thirst and dry mouth among patients admitted in intensive care units. *Int J Nurs Care*. 2020;8(1):33-39. doi: 10.37506/ijonc.v8i1.8606
7. Zhang W, Gu Q, Gu Y, Zhao Y, Zhu L. Symptom management to alleviate thirst and dry mouth in critically ill patients: A randomised controlled trial. *Aust Crit Care*. 2022;35(2):123-129. doi: 10.1016/j.aucc.2021.04.002
8. Baumstarck K, Boucekine M, Estagnasie P, et al. Assessment of patients' self-perceived intensive care unit discomforts: Validation of the 18-item version of the IPREA. *Health Qual Life Outcomes*. 2019;7:17(29):1-9. doi: 10.1186/s12955-019-1101-5
9. Sato K, Okajima M, Taniguchi T. Association of persistent intense thirst with delirium among critically ill patients: a cross-sectional study. *J Pain Symptom Manage*. 2019;57(6):1114-1120. doi: 10.1016/j.jpainsymman.2019.02.022
10. Goodman SPJ, Marino FE. Thirst perception exacerbates objective mental fatigue. *Neuropsychologia*. 2021;150:107686. doi: 10.1016/j.neuropsychologia.2020. 107686
11. Kjeldsen CL, Hansen MS, Jensen K, Holm A, Haahr A, Dreyer P. Patients' experience of thirst while being conscious and mechanically ventilated in the intensive care unit. *Nurs Crit Care*. 2018;23(2):75-81. doi: 10.1111/nicc.12277
12. VonStein M, Buchko BL, Millen C, Lampo D, Bell T, Woods AB. Effect of a scheduled nurse intervention on thirst and dry mouth in intensive care patients. *Am J Crit Care*. 2019;28(1):41-46. doi: 10.4037/ajcc2019400
13. Adams JD, Myatich AI, McCullough AS. Thirst as an ingestive behavior: A brief review on physiology and assessment. *Nutr Health*. 2020;26(3):271-274. doi: 10.1177/0260106020916972
14. Hughes F, Mythen M, Montgomery H. The sensitivity of the human thirst response to changes in plasma osmolality: a systematic review. *Perioper Med*. 2018;17(1):1-11. doi: 10.1186/s13741-017-0081-4
15. Li S, Mi J, Tang Y. A qualitative study of nurses' perception on patients' thirst in intensive care units. *Intensive Crit Care Nurs*. 2022;69:103184. doi: 10.1016/j.iccn.2021.103184
16. Doi S, Nakanishi N, Kawahara Y, Nakayama S. Impact of oral care on thirst perception and dry mouth assessments in intensive care patients: An observational study. *Intensive Crit Care Nurs*. 2021;66:103073. doi: 10.1016/j.iccn.2021.103073
17. Leemhuis A, Shichishima Y, Puntillo K. Palliation of thirst in intensive care unit patients: translating research into practice. *Crit Care Nurse*. 2019;39(5):21-28. doi: 10.4037/ccn2019544
18. Serato VM, Fonseca LF, Birolim MM, Rossetto EG, Mai LD, Garcia AKA. Package of menthol measures for thirst relief: a randomized clinical study. *Rev Bras Enferm*. 2019;72(3):600-608. doi: 10.1590/0034-7167-2018-0057
19. Eren E. The effect of oral water and ice application in the management of early postoperative thirst. Istanbul University Institute of Health Sciences, Surgical Diseases Nursing Program. Ph.D. Thesis. 2018. Istanbul.
20. Arai SR, Butzlaff A, Stotts NA, Puntillo KA. Quench the thirst: lessons from clinical thirst trials. *Biol Res Nurs*. 2014;16(4):456-66. doi: 10.1177/1099800413505900
21. Oh KE, Song AR, Sok SR. Effects of aroma gargling, cold water gargling, and wet gauze application on thirst, halitosis, and sore throat of patients after spine surgery. *Holist Nurs Pract*. 2017;31(4):253-259. doi: 10.1097/HNP.0000000000000219
22. Gulia S, Kumari V, Khatri N. Effectiveness of an intervention bundle on thirst intensity and dry mouth among patients admitted in ICU. *Int J Health Sci Res*. 2019;9(5):397-408.
23. Flim M, Hofhuis J, Spronk P, Jaarsma T. Measuring thirst distress of patients in the intensive care unit. *Nurs Crit Care*. 2022;27(4):576-582. doi: 10.1111/nicc.12719
24. Bernal A, Zafra MA, Si-mon MJ, Mahía, J. Sodium homeo-stasis, a balance necessary for life. *Nutrients*. 2023;15(2):395. doi: 10.3390/nu15020395
25. Arora SK. Hypernatremic disorders in the intensive care unit. *J Intensive Care Med*. 2013;28(1):37-45. doi: 10.1177/0885066611403994
26. Shikha G, Vinay K, Neetu K. Effectiveness of an intervention bundle on thirst intensity and dry mouth among patients admitted in intensive care units. *Indian J Public Health Res Dev*. 2020;11(4):101-107.

Wydawnictwo UR 2023
ISSN 2544-1361 (online)
doi: 10.15584/ejcem.2023.3.4

http://www.ejcem.ur.edu.pl
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2023; 21 (3) : 465–475

ORIGINAL PAPER

Hygienic assessment of the occurrence and development of emotional burnout syndrome among medical students and its prevention

Valentyna Chorna [1], Hanna Syrota [1], Mariia Syrota [1], Svitlana Khliestova [1], Vlada Poliarush [1], Lyudmyla Hudzevych [2]

[1] Medicine National Pirogov Memorial Medical University, Vinnytsia, Ukraine
[2] Mykhailo Kotsyubynsky State Pedagogical University of Vinnytsia, Vinnytsia, Ukraine

ABSTRACT

Introduction and aim. Emotional burnout syndrome has become increasingly common in recent decades, regardless of the field of professional activity. The purpose of our study was to compare the prevalence of emotional burnout in medical students of Vinnytsia National Medical University before the beginning of the academic semester and during the passing of exams.
Material and methods. An anonymous, voluntary survey before the start of study was taken by 300 students, among them 82.3% were women, 17.7% were men.
Results. During the exams, 362 students took part, including 76.2% women and 24.1% men. According to the results of the study, the proportion of the "average" degree of exhaustion increased in second-year students by 15.6%; third-year students by 44.4%. The "high" degree of emotional exhaustion according to the results of the study before the start of training was found in male students of the third year – 44.4% and 36.4% of male students of the sixth year. During the exams, the rate of "high" emotional exhaustion was observed in first-year students, increasing from 12.5% to 18% and in sixth-year students from 36.4% to 50%.
Conclusion. Thus, it was found that female students are the most adapted to the educational load, to passing exams, which affects the psychosomatic state of future doctors.
Keywords. COVID-19 pandemic, doctors, emotional burnout syndrome, exhaustion, medical students, stress

Introduction

Emotional burnout syndrome (EBS) has been increasingly common in recent decades, regardless of the field of professional activity. Most often, we observe EBS among the following professions: teachers, doctors, social workers, psychologists, etc., which are based on person-to-person communication and involve constant work with people and direct interaction with them in the course of performing professional duties.[1] Due to the high demands placed on such workers, these professions are the most potentially exhausting in terms of psychological health and progressively increase the likelihood of emotional burnout, exhaustion, and stress. There is a large number of scientific studies around the world on this phenomenon.[2]

An advantage in the study of EBS is given to the study of its prevalence in the healthcare sector. Reviews of domestic and foreign literature emphasize the problematic and multifactorial nature of burnout for various professions in the medical field: doctors, residents, nurses, students of higher education institutions (HEIs), and mental health workers.[3,4]

Corresponding author: Valentyna Chorna, e-mail: valentina.chorna65@gmail.com

Received: 29.03.2023 / Revised: 29.04.2023 / Accepted: 6.05.2023 / Published: 30.09.2023

Chorna V, Syrota H, Syrota M, Khliestova S, Poliarush V, Hudzevych L. *Hygienic assessment of the occurrence and development of emotional burnout syndrome among medical students and its prevention*. Eur J Clin Exp Med. 2023;21(3):465–475. doi: 10.15584/ejcem.2023.3.4.

With the full-scale war in Ukraine, a number of major changes have recently taken place, especially the increase in people in need of medical care for mental health problems. As a result, domestic doctors, combat medics, students, interns, and health care workers involved in providing medical care to soldiers and internally displaced persons (IDPs) suffer from stressful situations and conditions.[5] This is manifested by excessive workload, high occupational stress, work-life imbalance, lack of time to provide care, potential health risks, etc. Extreme fatigue and exhaustion of doctors can easily provoke negative consequences in the future, including deterioration of doctor-patient and colleague relations and escalation of conflicts in the healthcare system. An endless series of problems and a range of negative emotions caused by professional burnout are likely to reduce the job satisfaction of healthcare workers, which ultimately leads to a decrease in motivation to continue working and building a career in medicine. Professional burnout in the field of medicine attracts constant attention of scientists around the world to develop preventive measures.[6]

The study of the peculiarities of the manifestation of EBS, symptoms and factors that determine its formation and progression in both experienced doctors and medical students, with the aim of implementing scientifically proven health programs aimed at preventing occupational stress and diseases, as well as restoring the psychoenergetic potential of the individual and society.[7]

Aim

We have conducted a study of the socio-psychological determinants of the phenomenon of emotional burnout in medical students before the beginning of the academic semester (September) and during the passing of tests, exams, and modules (December). Medical students of the 1st-6th years of study at Pirogov National Medical University took part in the voluntary, anonymous survey – 661 applicants, including 78.9% of women (n=522) and 21,1% of men (n=140).

Material and methods

An anonymous, voluntary survey before the start of study (September 2022 - the first survey) was taken by 300 students of Vinnytsia National Medical University named after Pirogov, among them 82.3% were women, 17.7% were men. During the exams/modules (December 2022 – second survey), 362 students took part, including 76.2% women and 24.1% men. The degree of emotional burnout of university students was determined by the Gibson's Assessment of Personal Burnout Potential. This methodology contains 18 questions that define three components: emotional exhaustion, depersonalization, and personal satisfaction. Medical students are asked to choose one of 6 answers to each question. For each component, the average score is calculated and the degree of "low", "medium", and "high" is determined. A search was conducted on the basis of PubMed and Google Scholar databases for the period 2015-2022 on SEB in healthcare professionals and medical students.

Results

An anonymous, voluntary survey before the start of study (September 2022 - the first survey) was taken by 300 students of Vinnytsia National Medical University named after Pirogov, among them 82.3% were women, 17.7% were men. During the exams/modules (December 2022 – second survey), 362 students took part, including 76.2% women and 24.1% men.

Emotional exhaustion was identified as the first component of the SES and is the main component in medical students. The manifestations of emotional exhaustion are indifference to duties, environment, studies, deterioration of health, mood, motivation and overwhelm in the emotional background. The research data showed the following (Fig. 1 and 2).

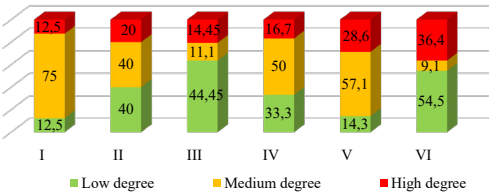

Fig. 1. The proportion of emotional exhaustion before the start of medical students' training (male), %

According to the results of the study, the proportion of the "average" degree of exhaustion increased in second-year students by 15.6%; third-year students by 44.4%.

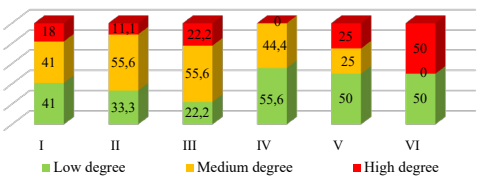

Fig. 2. The proportion of emotional exhaustion during exams/modules of medical students (male), %

The "high" degree of emotional exhaustion according to the results of the study before the start of training was found in male students of the third year – 44.4% and 36.4% of male students of the sixth year. However, during the exams/modules, the rate of "high" emotional exhaustion was observed in first-year students, increasing from 12.5% to 18% and in sixth-year students from 36.4% to 50%.

The increase in both "medium" and "high" degree of emotional burnout during the study may be a risk of de-

terioration of mental health of medical students, which will negatively affect academic performance.

According to the questionnaire data from female students, we found changes in emotional exhaustion. The "average" degree of emotional exhaustion in female students was observed in all courses before the start of training compared to male students (Fig. 3).

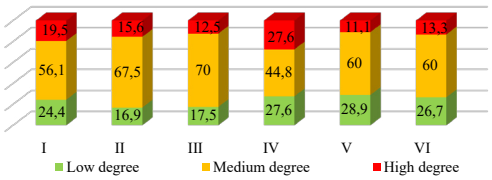

Fig. 3. The proportion of emotional exhaustion before the start of medical students' studies (female), %

According to the results of our study, during the exams/modules, female students' rates of "high" emotional exhaustion increased, namely, in the third year students by 25.7%; in the fifth year – 16.2%; in the second year – 11.6% (Fig. 4).

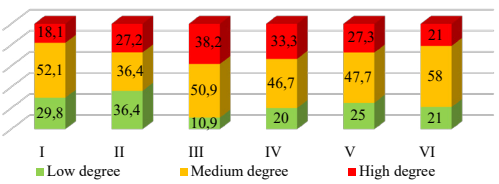

Fig. 4. Share of emotional exhaustion during exams/modules of medical students (female), %

In terms of emotional component, female students were the most vulnerable group and had the highest rates. For timely detection/prevention of SES, it is necessary to conduct regular surveys/testing in higher education institutions for timely detection, preventive measures to minimize them, overcome them and prevent the transition from pre-disease states to disease.

Based on the results of the survey of medical students, the following component "depersonalization" was identified. It is manifested in deterioration of relationships and increased frequency of conflicts in the student group in which the applicant studies, conflicts with university teachers.

The second component of emotional burnout, "depersonalization", is characterized by increased psychological distancing from anything: from learning and communication, a decrease in empathy and indifferent/cynical attitude towards friends/students, relatives, teachers, pessimistic thoughts about learning, life, which negatively affects the attitude towards one's own life, towards one's choice of a correctly chosen future profession.

According to the results of the study, "depersonalization" both before the start of studying and during exams/modules showed high rates of "low" and "medium" degree (Fig. 5 and 6).

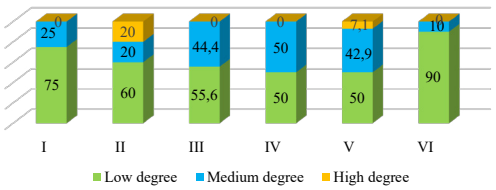

Fig. 5. The proportion of the degree of "depersonalization" before the start of training of male medical students,%

The "medium" degree of "depersonalization" according to the research results was observed to increase in second- and third-year students during exams/modules by 24.4% and 11.2%, respectively. The "high" degree of "depersonalization" among first-year students amounted to 6.8% among male students during exams and modules.

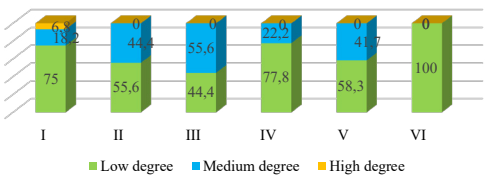

Fig. 6. The proportion of "depersonalization" grade during exams/modules for male medical students, %

A characteristic increase in the "depersonalization" component was observed among female students during exams/modules of all levels according to the questionnaires: "low", "medium", "high" (Fig. 7 and 8).

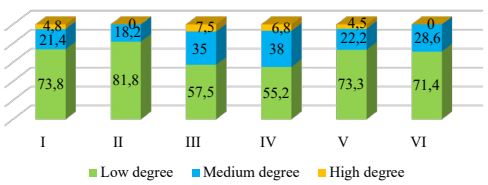

Fig. 7. The proportion of the degree of "depersonalization" before the start of training of female medical students, %

The increase in the "average" degree of "depersonalization" in female students of the VI year was 13.6%, 8.4% in the I year, 6.0% in the II year, and 5.3% in the IV year.

The "high" degree of "depersonalization" according to the survey results was 6.1% for female students of the second year and 5.3% for the sixth year.

The third component of emotional burnout, personal satisfaction, is characterized by negative self-assessment, the emergence of feelings of uncertainty,

incompetence in their professional field, awareness of failure in it, hesitation in choosing a professional activity, indifference to learning, friends, decreased motivation and self-esteem at a "low" level.

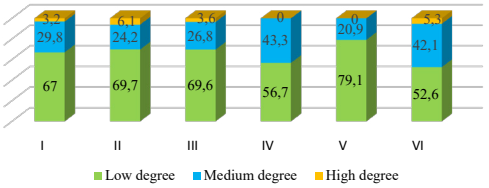

Fig. 8. The proportion of "depersonalization" degree during exams/modules of female medical students, %

This component of emotional burnout, personal satisfaction, can be manifested either in a tendency to negatively evaluate oneself, underestimate one's professional achievements and successes, negativity towards job responsibilities and opportunities, or positively evaluate one's own attributes.

According to the results of our study, among male applicants, the "average" degree of personal satisfaction during exams/modules was observed in the first year, second year, and fifth year of study, which increased compared to the indicators before the start of study (Fig. 9).

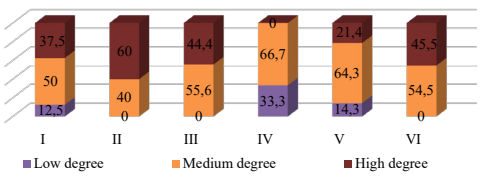

Fig. 9. Share of personal satisfaction before the start of training of male medical students, %

Before the start of training and before taking exams/modules, the degree of personal satisfaction in male students of the IV year increased by 55.6%, the VI year by 4.5% and the V year by 3.6%, which characterizes the positive dynamics of reducing emotional exhaustion. In male medical students of the III year, there was a negative trend in personal satisfaction, which can lead to more pronounced signs of transition from a pre-disease to a disease of psychological health (Fig. 10).

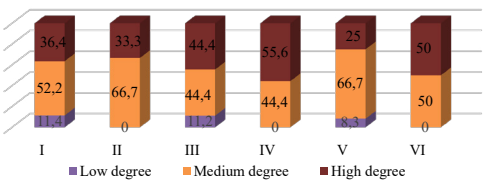

Fig. 10. Share of personal satisfaction during the exams/modules for male medical students, %

The indicators of personal satisfaction among female applicants were higher during the exams/modules of both "medium" and "high" degree, which characterizes the lowest vulnerability to emotional burnout on the part of the female gender and their better adaptability to academic workload (Fig. 11 and 12).

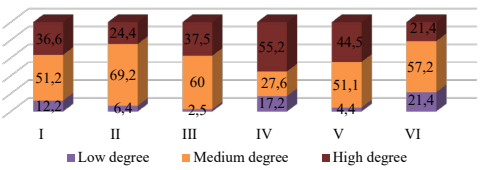

Fig. 11. The share of personal satisfaction before the start of training of female medical students, %

According to the questionnaire, the rate of "high" degree of personal satisfaction among female applicants increased by 27.1% – second year; 12.5% – third year; 10.2% – sixth year, indicating successful overcoming of the academic load.

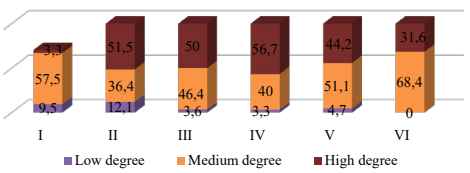

Fig. 12. Share of personal satisfaction during exams/modules for female medical students, %

Thus, it was found that female applicants are the most adapted to the educational and informational load, to passing exams/modules, which affects the psychosomatic state of future doctors. However, all students of higher education institutions need timely psychological assistance and correction.[8]

Discussion

EBS is a psychological syndrome that occurs as a long-term reaction to chronic professional stressors in the course of professional activity and education. EBS is a loss of energy, which is now a massive social and psychological phenomenon characterized by impaired productivity, fatigue, anxiety, poor health, insomnia, a pronounced predisposition to somatic diseases, and reduced quality of professional activity.[9]

The term "emotional burnout syndrome" was coined and introduced into the psychological field by the American psychiatrist Freudenberger in the 70s of the twentieth century.[10] The scientific literature later presented a slightly modified idea of burnout by the American psychologist Maslach in 1981, which was explained as a gradual increasing process of fatigue, cynicism and re-

duced commitment among social care professionals.[9,11] The turning point between the two definitions was the consideration of burnout as a syndrome, where a syndrome is understood as a set of symptoms and signs that exist simultaneously and clinically define a certain condition that is distinct from others.

The World Health Organization in 2019 proposed and included "burnout" as a syndrome as a professional phenomenon in the list of diseases of the 11th edition of the International Classification of Diseases (ICD-11). It was noted that this condition/status is exclusively related to certain features of a person's specialization and is not a medical disease. The syndrome, which occurs due to chronic stress in the workplace, is currently classified as premorbid manifestations: "Adaptation disorder" – F43; "Burnout" – Z73.0; "Neurasthenia" – F48.[12,13] It is a multidimensional phenomenon characterized by feelings of exhaustion, increased levels of negativity towards one's work, and decreased work performance and is inherent in individuals who have to work closely with other people, healthcare professionals, students, and service providers.

There are two categories of EBS factors that influence the development of predictors of EBS:

1. Individual – peculiarities of temperament and personality traits, i.e. psychophysiological processes of the human body;

2. External – communication between colleagues, working conditions, workload, financial situation, psychologically unhealthy team.[14]

EBS starts slowly, which leads to a complicated course of psychological exhaustion in the future. We have compiled a model for the development of EBS (Table 1).

The signs of EBS in each person have their own characteristics and peculiarities, which is explained by the different condition, degree of mental health disorder, level of stress resistance and specifics of the person's professional activity. Emotional burnout is now a massive socio-psychological phenomenon, an individual response to chronic work stress that develops progressively and, if untimely, can turn from a pre-disease into a disease, causing changes in psychosomatic health in people.[15]

The syndrome causes harm both at the cognitive (avoidance of communication with others, suicidal thoughts, difficulty performing everyday activities) and emotional (anxious thoughts about different situations) levels, which translates into negative behavior towards their professional duties, colleagues, patients, friends of students, teachers.[16]

Given the high rate of predictors of emotional burnout development among general medical students, the study of predictors of emotional burnout development among healthcare workers, its impact on their mental health, and the transition to pre-occupational disease states is relevant, requiring a thorough study and development of preventive countermeasures.[17]

Table 1. Model of development of PBS/EBS

Professional burnout syndrome		Emotional burnout syndrome	
Stages of development of the PBS	**Signs**	**Stages of EBS development**	**Signs**
1) Honeymoon	- filled with energy and strength, - satisfaction with the work performed, - commitment to the goals set, - positive dynamics of professional activity.	**1) Tension**	- dissatisfaction with yourself, - unable to resolve the situation constructively, - feeling of hopelessness, - increased fatigue and dissatisfaction with work, position.
2) Onset of stress	- periodic fatigue, - decreased productivity at work, - feeling of lack of strength and energy, - poor performance of professional duties, - work is less and less satisfying, - difficulty sleeping, - periodic manifestations of indifference.	**2) Strong feeling and experience of stress**	- anxiety and depressive psychosomatics, - non-perception of information and lack of concentration, - increased excitability and irritation, - excessive emotionality or vice versa apathy and isolation.
3) Chronic stress	- the above symptoms will appear on a permanent basis, - extreme depletion of internal resources, - poor quality and untimely performance of work, - closedness in communication, - postponement of tasks «for later», - tension between colleagues, - reduced body resistance to diseases.	**3) Physiological, affective-cognitive, behavioral reactions**	- ***physiological***: feeling of chronic fatigue, generalized asthenization, causeless headaches, weight loss or gain, shortness of breath, sleep problems, - ***affective and cognitive***: dissatisfaction with oneself, loss of interest in life, fixation on one's problems, cynical attitude towards others, decreased concentration, attention, demoralization, depersonalization, - ***behavioral***: maladjustment, distancing from professional duties, reduced motivation and work productivity
4) Burnout phase or crisis	- people are focused only on their own problems, - frequent development of chronic diseases of both the neurological and other internal systems of the body, - full or partial loss of ability to work, - changes become quite noticeable to others and loved ones.	**4) «Burning out» - physical and emotional exhaustion**	- complete exclusion of emotions, - psychosomatic and psycho-vegetative disorders, - personal detachment, - emotional alienation, - obsessive thoughts, - physical discomfort.
5) Habitual burnout or «hitting the wall»	- exacerbation of all problems, - development of dangerous diseases, - the emergence of extreme dissatisfaction with the quality of life, - the emergence of suicidal thoughts.		

EBS is defined as a state of psychological, emotional and physical stress that occurs under the constant in-

fluence of stressors.[18,19] Healthcare workers during the COVID-19 pandemic were constantly under stress.

According to epidemiological data, 5-7% of the world's population suffered from mental illness (before COVID-19). According to the Ministry of Health, during the pandemic in Ukraine, there were 625 new cases of psychological diseases per 100 thousand people. According to scientific studies around the world, the level of emotional burnout in healthcare facilities ranged from 31.4% to 85.8%. However, in Ukraine, this figure is somewhat higher and ranges from 73% to 89.3% During the COVID-19 pandemic, according to WHO, psychosomatic disorders increased to 50%.[20,21]

Globally, the COVID-19 pandemic has been a serious threat to global health and a challenge to health systems, and has highlighted healthcare strengths and weaknesses, emergency competence and preparedness, and the linkages between public and global health issues.

Scientific studies have shown that it may take the world more than a decade to recover from COVID-19 medically, socially, psychologically, and economically.[22,23] The new epidemiological features of COVID-19 associated with the rapid spread of the virus have not only emphasized the unpreparedness of many countries for such situations, but also generated anxiety, depression, stress, etc. According to Yavorovsky's research, psychological, physical, and chemical factors, as well as the lack of personal protective equipment (PPE), are more harmful than the virus itself. In Ukraine and in European countries, during the COVID-19 pandemic, there was a lack of specific protocols for the treatment and care of seriously ill patients, longer working hours, and a high workload for medical staff. The prevalence of high-level stress, depressive symptoms requiring treatment, and anxiety symptoms, depression requiring further examination among medical staff ranged from 3.7% to 17.7%.[24,25]

EBS are a common and growing problem among healthcare workers, especially those working in emergency settings: infection with the virus, skin diseases from prolonged use of PPE, exposure to toxic components of disinfectants, psychological distress, stigmatization, and chronic fatigue.[10,26]

During the COVID-19 pandemic, the need for communication, online consultations and remote work in the medical field has arisen due to social isolation, which has led to adaptations in medical activities to provide medical care to patients. Online consultations, online training courses, and the rapid need to exchange medical information have forced doctors to use telemedicine resources: to communicate with patients, to prescribe treatment, etc.

Dincer found that first-line healthcare workers, especially those involved in the diagnosis and treatment of patients with COVID-19, reported high levels of burnout associated with symptoms such as insomnia, depression, and anxiety.[27] Based on this, striking conclusions have been drawn that every doctor and medical student is also a patients[28], so it is important to identify early signs of burnout as predictors of future crises that contribute to exhaustion and develop preventive measures to overcome EBS.

EBS among medical students is confirmed by the latest data on the increase in the prevalence of burnout syndrome among students.[29] According to the results of the study by Navarro-Abal, it was found that burnout can affect both students and teachers of any level of education and institution.[30] Salmela-Aro found that SEB in students during their studies can lead to dramatic changes in mental health: depression, anxiety, suicide attempts, which increases the risk of suspension four times, while a passion for science can contribute to both life satisfaction and success in future educational achievements.[31]

There are internal and external factors that are associated with the increasing prevalence of EBS among medical students, including a perceived high workload, stress, and anxiety before modules/exams. EBS has a detrimental effect on the satisfaction with life and learning of future doctors, leading to poor academic performance and motivation.[32,33] The consequences of EBS have a negative impact on mental health, cardiovascular system, gastrointestinal tract, musculoskeletal and respiratory system, nervous system: depression, sleep disorders, alcohol abuse, suicidal thoughts, fatigue.[30,34]

The COVID-19 pandemic has led to a deviation from the usual regimen, forcing a shift from classical face-to-face education to distance learning. According to the results of the study by Fawaz, a decrease in student satisfaction with distance learning was found.[35,36] Online education includes the following negative factors: lack of physical presence in class, less informal discourse, and reduced social interaction between students and teachers.

They can lead to misunderstandings in communication, which can further manifest itself in the form of negative emotions and misconceptions, and behavior. Research has shown that social affinity is often associated with academic success in both face-to-face and distance learning settings, so it would be important to support student interaction in any learning environment.[37]

Models of learning demands are as follows: motivational, in which resources associated with distance education can lead to increased engagement in learning, and health deterioration, in which increased demands lead to tension, stress, and mental health problems, requiring medical students to quickly adapt to new digital learning practices and switch to social media platforms to maintain relationships with teachers and

other students.[38] As a result of EBS, medical students may be at high risk of developing depression and suicidal thoughts. Cross-sectional data from several higher education institutions showed that students experiencing burnout syndrome were 3 times more likely to have thought about suicide in the past. Students who previously reported burnout tend to recover from this condition and related suicidal thoughts.[8]

Thus, COVID-19 has created unique risks to psychological well-being, leading to increased stress, depressive disorders, and anxiety symptoms among students.[39] In order to overcome the negative impact of the development of EBS, it is necessary to identify resources that promote resilience during a psychological crisis.

The life of Ukrainians has changed dramatically during the full-scale invasion of Russia. Numerous human casualties, huge damage to the population, and the destruction of infrastructure – all these factors lead to serious mental health problems for Ukrainians, in addition to physical disorders.

Risks of the psychological impact of war: First, a war-related trauma is a traumatic event that threatens life or health because a person is directly exposed to psychological violence and witnesses' cruelty.[40] Direct exposure to war is a detrimental life event that can lead to long-term changes in mental well-being, can cause harm and mental health disorders such as post-traumatic stress disorder (PTSD), depression, anxiety, and EBS in adults and children.[2,41,42] The factors underlying wartime stress include both direct trauma and other psychosocial stressors, such as multiple human losses. To date, according to the United Nations (UN), 8,173 Ukrainian civilians alone have been killed and 13,620 wounded in the war. The war has had an impact on Ukraine's medical system, as 106 medical workers were killed during the year of war, 33 of them at their workplace. In addition, the Russians destroyed 174 medical institutions and severely damaged another 1,106.[39,43] These factors have an extremely detrimental impact on the development of EBS, both for the population and for healthcare workers and medical students who see their profession as the future and are prone to disappointment and a sense of uncertainty as a result of such developments.

Second, emotional suffering related to war can occur not only as a result of direct exposure, but also through indirect sources, such as watching violent scenes of war on television or social media. In particular, the social network "Telegram" has become a leader among other sources of information, and as noted, Ukrainians spend a lot of time in front of the gadget, reading news and following events. Outside of Ukraine, graphic images of the war on social media can also have a negative impact on people's psychological health. Through indirect exposure, people who are concerned about the war but live outside the war zone may also experience negative mental health consequences. Since the beginning of the war, more than 17 million people have left for European countries, and more than 9.1 million people have returned to Ukraine so far. As a result, two-thirds of Ukraine's children have become IDPs. More than 2.4 million Ukrainians have lost their homes and 406 educational institutions where students received their education. Each internally displaced family is constantly in despair, unable to cope with their negative obsessive thoughts and grief for their loved ones. According to the Ministry of Healthcare, about 15 million Ukrainians will need psychological support in the future, of which about 3-4 million will require medication.

Research shows that IDPs have higher levels of depression, anxiety, and SES than the general population that was not affected by the war.[44] Such people often develop survivor's syndrome, a feeling of guilt experienced by those who managed to get out of hot spots and go abroad, but after the adaptation period, they often experience a sense of betrayal and brokenness, stress from the fact that they have lost their loved ones and homes.

Thirdly, changes in the structure of society during the war lead to a decrease in the body's resistance, which leads to the development of depression, anxiety and diseases of the whole body.

First responders and volunteers in the combat zone are at particularly high risk of psychological morbidity due to the fact that they work at the limit of their physical and moral capabilities, often witness death, experience separation from loved ones and lack access to the most basic necessities. The fear and uncertainty created by the war is likely to have a lasting impact on the mental health of Ukrainians and people in other parts of the world. In addition, the war in Ukraine is the first war in history to be reported almost continuously by the media, with dramatic scenes and images available to virtually anyone with access to the Internet and television around the world. Risk factors associated with the development of depression, anxiety, stress, and EBS symptoms include self-rated health status, past psychiatric history, and avoidance of stress management.[45]

Timely diagnosis and prevention are essential in the case of EBS. We propose two approaches to overcoming EBS: individual and group approaches (Table 2).

Today, creative art therapy (CATs) is a very effective means of combating SEPD. It is the creative use of artistic means (art, music, dance) as a means of non-verbal or symbolic communication to achieve personal and/or social therapeutic goals that meet individual needs.[34,46]

In order to implement effective preventive measures to prevent and reduce the manifestation of EBS, the specific needs of people affected by war should be taken into account. Particular attention should be paid to internally displaced persons (IDPs). These people are under constant stress, experiencing increased fear

and anxiety, which manifests itself in uncertainty about their safety and future. In addition to providing material assistance, clothing, food, and accommodation, it is imperative to take care of providing psychological assistance to such people.[5]

Table 2. Preventive measures against EBS

Methods of prevention of EBS	Measures to overcome EBS
Individual recommendations for students/employees	
✓ improving communication skills through active learning methods (social and psychological training), ✓ studying effective communication styles and conflict resolution, ✓ trainings that stimulate motivation for self-development, personal and professional growth, ✓ observance of sleep, rest, nutrition and safety rules, ✓ mastering time management skills, ✓ physical activity and outdoor activities, ✓ creating an effective daily routine, with the possibility of realizing one's values, interests, dreams in order to enjoy the opportunities achieved (Work-life balance), ✓ methods of self-regulation (natural methods of self-regulation of the body).	✓ practical skills to be fully present «here and now», to notice habitual states of our consciousness, to control attention and behavior through concentration (Mindfulness), ✓ refusal to use the Internet and gadgets in communication for a certain period of time in order to take a break from the virtual world and switch attention to live communication (Digital Detox), ✓ personally oriented activities aimed at improving the ability to cope with stress, ✓ group/rational/cognitive-behavioral psychotherapy, ✓ autogenic training (self-hypnosis) - Schultz autogenic training and Jacobson progressive muscle relaxation; ✓ art therapy, ✓ search for new interests not related to academic activities.
Recommendations for heads of higher education and healthcare institutions	
✓ psychoeducation, ✓ trust in communication with students, ✓ continuous monitoring of students' emotional state, ✓ encouraging the creation of support groups, ✓ organizing and conducting trainings (balint groups), ✓ teaching students time management and various relaxation techniques, ✓ equal distribution of tasks among students, ✓ providing satisfactory learning and remuneration conditions, ✓ establishment of trusting, compliant relationships, ✓ social skills training, ✓ advisory assistance in adaptation to studies.	1. organization of systemic educational interventions, trainings, lectures, conferences on mental health and its disorders, development and implementation of educational topics with elements of medical ethics, morality and deontology aimed at developing stress resistance in students, 2. allocate a separate room in the home with appropriate equipment for psychological relief (comfort): a recreation/psychological relaxation area for group or individual psychotherapy/counseling; a gym area (with sports equipment) for breathing exercises (including various individual methods of relieving concussion), 3. develop a system of individual psychological counseling for students of higher education institutions at the main place of study who are experiencing academic stress to identify predictors of emotional burnout in students.

Psychologists advise that when providing assistance to IDPs, the basic rule is "LOOK, LISTEN, DIRECT". Guided by this principle, a specialist will be able to clearly understand the basis of a person's problem and find an approach to solving it. For IDPs, it is important to provide information on the activities of social and volunteer organizations, practical assistance centers, coaching on available resources in the material and psychological sphere, providing opportunities to contact family and friends, and referrals to psychological and medical centers to help those in need.

Psychocorrection techniques have been successfully proven to overcome PTSD and are currently being used quite successfully, such as EMDR (Eye Movement Desensitization and Reprocessing). This psychotherapy allows people to reduce or even get rid of the symptoms of emotional stress that result from disturbing life experiences.

Repeated studies show that with EMDR therapy, people can experience psychotherapy results that once took years to achieve. Some studies show that 84-90% of victims of single trauma no longer have post-traumatic stress disorder after just three 90-minute sessions. EMDR therapy includes eight phases of treatment. Eye movements (or other bilateral stimulation) are used for one part of the session. After the clinician has determined which memory to focus on first, he or she asks the patient to hold in mind different aspects of the event or thought and use the eyes to follow the clinician's hand as it moves back and forth in the field of vision. EMDR involves a patient suffering from post-traumatic stress disorder focusing on a traumatic image, thought, emotion, and bodily sensation while receiving bilateral stimulation, most often in the form of eye movements.[47,48]

Cognitive behavioral therapy, which was developed by Aaron Beck in the 1960s, is also widely used today, but its effectiveness has been scientifically proven only recently. It is a structured, didactic, and focused form of therapy. This approach is based on hands-on interaction, where the therapist and patient work in collaboration to change thinking and behavioral patterns to promote positive changes in the patient's mood and lifestyle. It is used to address a wide range of problems, and appropriate treatment protocols are applied depending on the diagnosis and problems faced by the patient, including PTSD caused by military actions and negative impact on psychological health.[49-51]

Summarizing the results of the questionnaire, it should be noted that female applicants are the most adapted to the educational and information load, to passing exams/modules, stress, which affects the psychosomatic state of future doctors.[52] Prospects for further research are the development and implementation of a program for the prevention of emotional burnout syndrome among medical students of higher education institutions.

Conclusion

Thus, it was found that female applicants are the most adapted to the educational and informational load, to passing exams/modules, which affects the psychosomatic state of future doctors. However, all students of higher education institutions need timely psychological assistance and correction.

Declarations

Funding

This research received no external funding.

Author contributions

Conceptualization, V.C. and H.S., M.S; Methodology, S.K.; Software, V.P., L.H.; Validation, V.C., Y.S., and M.S.; Formal Analysis, V.C., S.K.; Investigation, V.P.; Resources, L.H.; Data Curation, V.C.; Writing – Original Draft Preparation, V.C., Y.S. and M.S.; Writing – Review & Editing, H.S., M.S..; Visualization, H.S. and M.S.; Supervision, V.C.; Project Administration, V.C.; Funding Acquisition, V.P., L.H.

Conflicts of interest

The authors declare no competing interests.

Data availability

The datasets used and/or analyzed during the current study are open from the corresponding author on reasonable request.

Ethics approval

All subjects gave informed consent to the inclusion prior to participating in the study. The study has been approved by the Bioethics Committee at the University No 2023/09/01.

References

1. Chorna V, Makhniuk V, Pshuk N, Gumeniuk N, Shevchuk Y, Khliestova S. Burnout in mental health professionals and the measures to prevent it. *Georgian Med News*. 2021;(310):113-118.
2. Panagioti M, Gooding PA, Tarrier N. A meta-analysis of the association between posttraumatic stress disorder and suicidality: The role of comorbid depression. *Compr Psychiatry.* 2012;53:915-930. doi: 10.1016/j.comppsych.2012.02.009
3. Botha E, Gwin T, Purpora C. The effectiveness of mindfulness based programs in reducing stress experienced by nurses in adult hospital settings: a systematic review of quantitative evidence protocol. *JBI Database Sistem Rev Implement Rep.* 2015;13(10):21-29. doi: 10.11124/jbisrir-2015-2380
4. Rotenstein LS, Ramos MA, Torre M, et al. Prevalence of Depression, Depressive Symptoms, and Suicidal Ideation Among Medical Students: A Systematic Review and Meta-Analysis. *JAMA*. 2016;316(21):2214-2236. doi: 10.1001/jama.2016.17324.
5. Chorna VV. Psychical zdorov"ya of the vnutrishneperemischenikh persons and factors which influence on his changing. Materials of the II International Scientific and Practical Conference „Innovations and prospects in modern science", 13-15 лютого, 2023р, Sweden, (Stockholm, - SSPG Publish). 2023: 93-97.
6. Vandenbroeck S, Van Gerven E, De Witte H, Vanhaecht K, Godderis L. Burnout in Belgian physicians and nurses. *Occup Med (Lond*). 2017;67(7):546-554. doi: 10.1093/occmed/kgx126
7. Boni RADS, Paiva CE, de Oliveira MA, Lucchetti G, Fregnani JHTG, Paiva BSR. Burnout among medical students during the first years of undergraduate school: Prevalence and associated factors. *PLoS One*. 2018;13(3):e0191746. doi: 10.1371/journal.pone.0191746.
8. Chorna VV. Research of syndrome of the emotional burning down is among students-physicians : reasons of development and prophylaxis. Materials of the 30th International Scientific and Practical Conference „Modern aspects of modernization of science: status, problems, development trends", March 7, 2023, Hamburg (Germany). 2023:390-394.
9. Obregon M, Luo J, Shelton J, Blevins T, MacDowell M. Assessment of burnout in medical students using the Maslach Burnout Inventory-Student Survey: a cross-sectional data analysis. *BMC Med Educ*. 2020;20(1):376. doi: 10.1186/s12909-020-02274-3
10. Hillert A, Albrecht A, Voderholzer U. The Burnout Phenomenon: A Résumé After More Than 15,000. *Scientific Publications. Front Psychiatry*. 2020;11:519237. doi: 10.3389/fpsyt.2020.519237
11. Friganović A, Selič P, Ilić B, Sedić B. Stress and burnout syndrome and their associations with coping and job satisfaction in critical care nurses: a literature review. *Psychiatr Danub*. 2019;31(1):21-31.
12. Joshi VR, Younger JM, Das S, Goud BKM, Pramanik K. Factors influencing burnout in millennial medical students during the COVID-19 pandemic! *Ir J Med Sci*. 2022;4:1-7. doi: 10.1007/s11845-022-03016-8
13. World Health Organization WHO Coronavirus Disease (COVID-19) Dashboard. https://covid19.who.int/ (Accessed on 3 March 2021).
14. Amoafo E, Hanbali N, Patel A, Singh P. What are the significant factors associated with burnout in doctor? *Occup Med (Lond.* 2015; 65(2):117-121. doi: 10.1093/ocmed/kqu144
15. Edú-Valsania S, Laguía A, Moriano JA. Burnout: A Review of Theory and Measurement. *Int J Environ Res Public Health*. 2022;19(3):1780. doi: 10.3390/ijerph19031780
16. Bouza E, Gil-Monte PR, Palomo E. et al. Work-related burnout syndrome in physicians in Spain. *Rev Clin Esp (Barc)*. 2020;220(6):359-363. doi: 10.1016/j.rce.2020.02.002.
17. Chorna VV, Makhniuk VM, Mohylnyi SM, et al. The state of occupational stress in medical workers, counteraction and overcoming it. *Environment&Health*. 2022:2;53-62. doi: 10.32402/ dovkil2022.02.053
18. Al-Haddad A, Al-Omar F, Al-Khaleel A, Al-Khalaf A. Prevalence of burnout syndrome and its related risk factors among physicians working in primary health care centers of the Ministry of Health, Al Ahsa region, Saudi Arabia,

2018-2019. *J Family Med Prim Care*. 2020;9(2):571-579. doi: 10.4103/jfmpc.jfmpc_743_19

19. Elghazally SA, Alkarn AF, Elkhayat H, Ibrahim AK, Elkhayat MR. Burnout Impact of COVID-19 Pandemic on Health-Care Professionals at Assiut University Hospitals, 2020. *Int J Environ Res Public Health*. 2021;18(10):5368. doi: 10.3390/ijerph18105368
20. Kleinpell R, Moss M, Good VS, Gozal D, Sessler CN. The Critical Nature of Addressing Burnout Prevention: Results From the Critical Care Societies Collaborative's National Summit and Survey on Prevention and Management of Burnout in the ICU. *Crit Care Med*. 2020;48(2):249-253. doi: 10.1097/CCM.0000000000003964.
21. Suleiman-Martos N, Albendin-Garcia L, Gomez-Urguiza JL, et al. Prevalence and Predictors of Burnout in Midwives: F Systematic Review and Meta-Fnaiysis. *Int J Environ Res Public Health*. 2020;17(2):E641. doi:10.3390/ijerph17020641.
22. Chauhan S. Comprehensive review of coronavirus disease 2019 (COVID-19). *Biomed J*. 2020;43(4):334-340. doi: 10.1016/j.bj.2020.05.023.
23. Djalante R, Lassa J, Setiamarga D, et al. Review and analysis of current responses to COVID-19 in Indonesia: Period of January to March 2020. *Prog Disaster Sci*. 2020;6:100091. doi: 10.1016/j.pdisas.2020.100091
24. Guo J, Lianming L, Baoguo W. Psychological Effects of COVID-19 on Hospital Staff: A National Cross-Sectional Survey of China Mainland. *Soc Sci Electron. Publ*. 2020;4:6-11. doi: 10.2139/ssrn.3550050
25. Moss M, Good VS, Gozal D, Kleinpell R, Sessler CN. A Critical Care Societies Collaborative Statement: Burnout Syndrome in Critical Care Health-care Professionals. A Call for Action. *Am J Respir Crit Care Med*. 2016;194:106-113. doi: 10.1164/rccm.201604-0708ST.
26. Salyers MP, Bonfils KA, Luther L, et al. The Relationship Between Professional Burnout and Quality and Safety in Healthcare: A Meta-Analysis. *J Gen Intern Med*. 2017;32:475-482. doi: 10.1007/s11606-016-3886-9
27. Dincer B, Inangil D. The effect of Emotional Freedom Techniques on nurses' stress, anxiety, and burnout levels during the COVID-19 pandemic: a randomized controlled trial. *Explore*. 2021;17:109-114. doi: 10.1016/j.explore.2020.11.012
28. Chigwedere OC, Sadath A, Kabir Z, Arensman E. The impact of epidemics and pandemics on the mental health of healthcare workers: a systematic review. *Int J Environ Res Public Health*. 2021;18:1-35. doi: 10.3390/ijerph18136695
29. Salvagioni DAJ, Melanda FN, Mesas AE, González AD, Gabani FL, Andrade SM. Physical, psychological and occupational consequences of job burnout: A systematic review of prospective studies. *PLoS One*. 2017;12(10):e0185781. doi: 10.1371/journal.pone.0185781
30. Navarro-Abal Y, Gómez-Salgado J, López-López MJ, Climent-Rodríguez JA. Organisational Justice, Burnout, and Engagement in University Students: A Comparison between Stressful Aspects of Labour and University Organisation. *Int J Environ Res Public Health*. 2018;15(10):2116. doi: 10.3390/ijerph15102116
31. Salmela-Aro K, Upadyaya K, Vinni-Laakso J, Hietajärvi L. Adolescents' longitudinal school engagement and burnout before and during COVID-19: The role of socio-emotional skills. *J Res Adolesc J*. 2021;31:796-807.
32. Hernesniemi E, Räty H, Kasanen K, Cheng X, Hong J, Kuittinen M. Burnout among Finnish and Chinese university students. *Scand J Psychol*. 2017;58(5):400-408. doi: 10.1111/sjop.12380
33. Munjiza J, Britvic D, Radman M, Crawford MJ. Severe war-related trauma and personality pathology: a case-control study. *BMC Psychiatry*. 2017;17(1):100. doi: 10.1186/s12888-017-1269-3
34. Martin L, Oepen R, Bauer K, et al. Creative Arts Interventions for Stress Management and Prevention-A Systematic Review. *Behav Sci (Basel)*. 2018;8(2):28. doi: 10.3390/bs8020028
35. Debowska A, Horeczy B, Boduszek D, Dolinski D. A repeated cross-sectional survey assessing university students' stress, depression, anxiety, and suicidality in the early stages of the COVID-19 pandemic in Poland. *Psychol Med*. 2020:1-4. doi: 10.1017/S003329172000392X
36. Fawaz M, Samaha A. E-learning: Depression, anxiety, and stress symptomatology among Lebanese university students during COVID-19 quarantine. *Nurs Forum*. 2021;56(1):52-57. doi: 10.1111/nuf.12521
37. Tomás-Miquel JV, Expósito-Langa M, Nicolau-Juliá D. The influence of relationship networks on academic performance in higher education: A comparative study between students of a creative and a non-creative discipline. *Higher Education*. 2016;71(3):307-322. doi: 10.1007/s10734-015-9904-8
38. Händel M, Stephan M, Gläser-Zikuda,M, Kopp B, Bedenlier S, Ziegler A. Digital readiness and its effects on higher education students' socio-emotional perceptions in the context of the COVID-19 pandemic. *J Res Technol Educ*. 2020;54(2):267-280. doi: 10.1080/15391523.2020.1846147
39. UNESCO. Nurturing the social and emotional wellbeing of children and young people during crises. UNESCO COVID-19 https://unesdoc.unesco.org/ark:/48223/pf0000373271. Accessed March 20, 2023.
40. Jain N, Prasad S, Czárth ZC, et al. War Psychiatry: Identifying and Managing the Neuropsychiatric Consequences of Armed Conflicts. *J Prim Care Community Health*. 2022;13:21501319221106625. doi: 10.1177/21501319221106625
41. Marôco J, Assunção H, Harju-Luukkainen H, et al. Predictors of academic efficacy and dropout intention in university students: Can engagement suppress burnout? *PLoS One*. 2020;15(10):e0239816. doi: 10.1371/journal.pone.0239816
42. Smeeth D, McEwen FS, Popham CM, et al. War exposure, post-traumatic stress symptoms and hair cortisol

concentrations in Syrian refugee children. *Mol Psychiatry.* 2023;28(2):647-656. doi: 10.1038/s41380-022-01859-2

43. UNHR. Ukraine: civilian casualty update 26 December 2022. https://www.ohchr.org/en/news/2022/12/ukraine-civilian-casualty-update-26-december-2022. Accessed March 24, 2023.
44. Justino P. Poverty and violent conflict: A micro-level perspective on the causes and duration of warfare. *J. Peace Res.* 2009;46(3):315-333. doi: 10.1177/0022343309102655.
45. Kira IA, Templin T, Lewandowski L, Ramaswamy V, Ozkan B, Mohanesh J. The physical and mental health effects of iraq war media exposure on Iraqi refugees. *J Muslim Mental Health*. 2008;3(2):193-215. doi: 10.1080/15564900802487592
46. Ho AHY, Tan-Ho G, Ngo TA, et al. A novel mindful-compassion art therapy (MCAT) for reducing burnout and promoting resilience for end-of-life care professionals: a waitlist RCT protocol. *Trials.* 2019;20(1):406. doi: 10.1186/s13063-019-3533-y
47. Gettings RD, Kirtley J, Wilson-Menzfeld G, Oxburgh GE, Farrell D, Kiernan MD. Exploring the Role of Social Connection in Interventions With Military Veterans Diagnosed With Post-traumatic Stress Disorder: *Systematic Narrative Review. Front Psychol*. 2022;13:873885. doi: 10.3389/fpsyg.2022.873885
48. Novo Navarro P, Landin-Romero R, Guardiola-Wanden-Berghe R, et al. 25 years of Eye Movement Desensitization and Reprocessing (EMDR): The EMDR therapy protocol, hypotheses of its mechanism of action and a systematic review of its efficacy in the treatment of post-traumatic stress disorder. *Rev Psiquiatr Salud Ment (Engl Ed).* 2018;11(2):101-114. doi: 10.1016/j.rpsm.2015.12.002
49. Chand SP, Kuckel DP, Huecker MR. Cognitive Behavior Therapy. In: StatPearls [Internet]. Treasure Island (FL): *StatPearls Publishing*; 2022.
50. Keefe JR, Chambless DL, Barber JP, Milrod BL. Treatment of anxiety and mood comorbidities in cognitive-behavioral and psychodynamic therapies for panic disorder. *J Psychiatr Res*. 2019;114:34-40. doi: 10.1016/j.jpsychires.2019.04.009
51. Tolin DF, Wootton BM, Levy HC, et al. Efficacy and mediators of a group cognitive-behavioral therapy for hoarding disorder: A randomized trial. *J Consult Clin Psychol.* 2019;87(7):590-602. doi: 10.1037/ccp0000405
52. Chorna VV, Anhelska VY, Makhnyuk VV. Telemedical assistance of mental health centers of health care institutions. *I* Міжнародна науково-практична конференція "International scientific and practical conference" Scientific progress: innovations, achievements and prospects", October 9-11, 2022. Munich, Germany. 2022;559:29-36.

Wydawnictwo UR 2023
ISSN 2544-1361 (online)
doi: 10.15584/ejcem.2023.3.5

http://www.ejcem.ur.edu.pl
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2023; 21 (3): 476–482

ORIGINAL PAPER

The effect of foot baths on foot pain and leg edema of nursing students during clinical training

Sevim Çelik , Gülbahar Cerrah , Berşan Gürel , Cavide Sağlık , İrem Albayrak

Department of Nursing, Bartin University Faculty of Health Sciences, Bartin, Turkey

ABSTRACT

Introduction and aim. Edema and pain may occur in the lower extremities due to the long-term standing work of nursing students and the stress they experience. In this study, the aim was to determine the effect of foot baths applied after clinical practice on foot pain and leg edema in nursing students.

Material and methods. This randomized controlled study was carried out with second year nursing students. The intervention group received a foot bath (including immersion in water and massage). No intervention was performed on the control group. The data was collected using a survey form, edema follow-up form, visual analog scale, and foot bath equipment.

Results. Edema levels in the right tibia decreased statistically on the 1st and 2nd days after the foot bath compared to the values measured after nurses performed clinical practice, while the edema level in the left tibia decreased significantly in the first and fourth weeks ($p<0.05$). The right and left foot pain scores in the intervention group increased in the evening of the day after the clinical practice compared to before clinical practice, and pain scores decreased statistically significantly after foot bath ($p<0.001$). The foot pain scores expressed by the students in the first week increased continuously ($p<0.001$).

Conclusion. The study showed that a foot bath performed after clinical practice were effective for easing foot pain and leg edema.

Keywords. clinical training, foot pain, foot bath, leg edema, massage, nursing student

Introduction

Nursing education in our country includes theoretical and applied education. This nursing education covers at least four years or 4600 hours of theoretical and practical training. Students complete half of this training (2300 hours) under clinical settings.[1] They begin full-time clinical practice in order to use the knowledge they have acquired in order to gain the skills required for patient care of nursing education.[2,3]

Clinical settings are environments made up of a variety of physical, psychological, emotional and organizational factors that can affect student learning. In most of the clinical environments, the environments where students can rest are insufficient. The students experience physiological, psychological, social and environmental stress because they have to work for a long time in clinical learning environments and apply treatment and care practices for the first time in the clinical setting.[2-6]

Problems such as edema and pain may occur in the lower extremities due to the long-term standing work of the students and the stress they experience. Eyi and Eyi found that second-year students (62.9%) experienced statistically significantly more stress during clinical practice than third-year students (37%) ($p=0.002$).[7] Elyased stated that 71.1% of students experienced pain in the ankle or foot (43.9%) over the a 12 month period in their study investigating musculoskeletal disorders of students during their clinical training. In the same study, the prevalence

Corresponding author: Sevim Çelik, e-mail: sevimakcel@gmail.com

Received: 5.04.2023 / Revised: 5.05.2023 / Accepted: 9.05.2023 / Published: 30.09.2023

Çelik S, Cerrah G, Gürel B, Sağlık C, Albayrak İ. *The effect of foot baths on foot pain and leg edema of nursing students during clinical training.* Eur J Clin Exp Med. 2023;21(3):476–482. doi: 10.15584/ejcem.2023.3.5.

of musculoskeletal problems was found to be statistically significantly higher in students who performed clinical practice 2 days a week (p=0.05).[8] Similarly, Moodley et al. reported that 63.6% of nursing students experienced foot and ankle pain in the last 12 months.[9] Kamalruzaman et al. stated that clinical education is an important risk factor for students to experience musculoskeletal problems (p=0.047) (95%CI: 0.035, 5.526).[10]

Today, foot baths and foot massages are two popular applications because they are easy, applicable, inexpensive, safe, and non-invasive method of relieving foot pain and fatigue. Hot foot baths can increase peripheral temperature and peripheral blood flow without increasing body temperature and can be effective in relieving fatigue by reducing tension in the brain and muscles.[11] In foot massage, mental and psychological relaxation is provided by manipulation of soft tissues and muscles, thus increasing healing and relaxation.[12] In a study conducted by Lee et al. in previous years, it reported that edema, physical stress and fatigue in the lower extremities significantly reduced by immersing the feet in hot water at 40 degrees for 20 minutes 12 times a day in operating room nurses who stood for long period.[13] Oh and Gang also stated that a foot bath, including a 20-minute foot massage, is effective in reducing fatigue and stress.[14] Rahmani et al. reported that the sleep quality of the patients decreased significantly on the second and third nights with a 20-minute foot bath and massage.[12] In previous years, there were very few studies on the subject and these studies focused more on the effect of footbath on fatigue and sleep on the patients and nursing. Studies on nursing students are rare. In our country, there is no study on the subject among nurses and nursing students.

Aim

In this study, unlike other studies, the aim was to determine the effect of foot baths and foot massage applied after clinical applications on foot pain and leg edema in nursing students.

Materials and methods

Ethical approval

First, permission was obtained from the University's Social and Human Sciences Ethics Committee (decision no. 10, dated 18.03.2020). Written permission was obtained from the Rectorate of the university where the study conducted. Informed consent obtained from the students by stating that the purpose of the research, the data will used for scientific purposes, and that participation in the study was based on volunteerism.

Design and sample

The study adopted a randomized controlled study to determine the effect of foot baths applied after clinical applications on foot pain and leg edema in nursing students.

This study was carried out with second year students studying in the nursing department of the faculty of health sciences and practicing clinical for 12 hours a week in the 2021-2022 academic year. Forty-four students were included in the sample with a medium effect size of 0.25, a type 1 error of 0.5, and a power of 80%, which Cohen determined for repeated analysis of variance in the G power program. Since there may be students who can leave the study, the number of sample was increased by 50% and the sample group consisted of 66 students.

The block randomization method used in the study. Sixty-six students were enumerated and assigned to two block sizes, each of which determined by the researchers as the intervention and control group with a ratio of 1:1 (www.randomizer.org). The sample group was determined as 33 students in each block. The study completed with 27 intervention and 21 control group students (Fig. 1).

The inclusion criteria: (1) no hearing or vision problems, (2) continuing clinical training, (3) have no diagnosed leg problems, (4) have a smart phone and (5) wearing sports shoes, (6) studying in the second year, (7) agreed to participate.

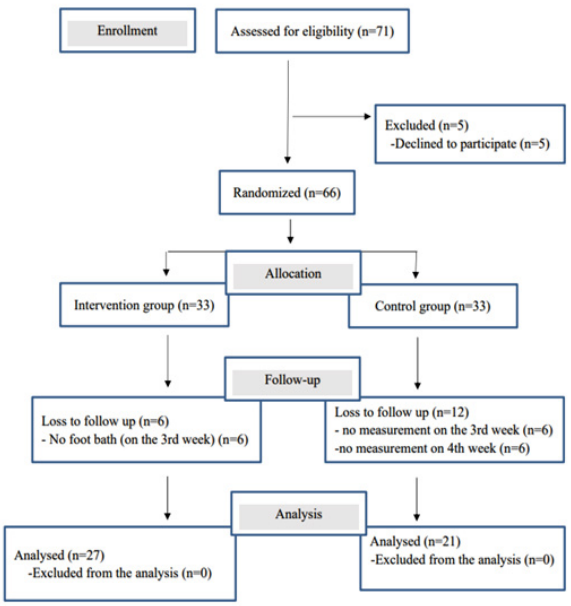

Fig. 1. CONSORT flow diagram

Instruments

The data collected using a survey form, edema follow-up form, visual analog scale, foot bath equipment, tape measure and smart phone.

The survey form

This form consist of items on students' age, gender, height, weight, daily sleep time, daily standing time, smoking and alcohol use, daily salt use, daily coffee consumption, general health definition status, foot health

definition status, working status, chronic disease, chronic disease, clinical setting, and exercise status.

Edema measurement and follow-up form
In the evaluation of edema, the patient's tibia bone is pressed with a finger for 3-5 seconds, and if there is a pit in the area, the depth of the pitting and how long it takes to come back are determined, and the edema is graded. If the godet's sign comes back in 2 mm and 15 sec, 1 positive, 4 mm and if it comes back in 15-30 sec, 2 positive, 6 mm and if it comes back in 30-45 sec, 3 positive, 8 mm and longer than 45 sec. If it comes back in time, it is expressed as 4 positive edema.[15]

Before going to the clinical practice every week, the students record the level of edema in their lower extremities on the edema follow-up form on the first evening of the clinical practice and the last evening of the clinical practice.

Pain follow up form
This form is a form in which students evaluate and record the level of pain in their lower extremities every week before going to clinical practice, on the first evening of clinical practice and on the last evening of clinical practice.

Visual analogue scale
It is a reliable and easily applicable scale used to measure the severity of pain in patients, accepted in the world literature. For pain intensity over a 10cm line, "no pain" is usually rated 0 points and "worst pain imaginable" 10 points.

Materials for the footbath and foot massage
A bucket used to put the water in the foot bath, a water thermometer to measure the temperature of the water, a foot towel, and pH neutral baby oil suitable for use on the skin used for foot massage.

Smartphone
It was used to send the training video prepared by the researchers to the students for the application of the footbath, the follow-up of the edema.

Data collection
Data was collected between 06.12.2021 and 31.12.2021. The intervention group was shown the application of foot bath in the skill laboratory, and follow-up of edema. In addition, the making of these applications videotaped, and the video recording sent to the students via smartphones so that they could watch the applications again. Data collection forms introduced to all students and given to completed after the applications, and they were collected from them at the end of a month.

Application of footbath
Application was performed by students in the intervention group in accordance with the application steps. The footbath done two hours before bedtime and lasted for 20 minutes. For this application, you should stand in a sitting position on a chair or bed, and the feet should hung down. First, the feet should kept for 6 minutes in a bowl filled with 40 degrees water up to 10 cm above the ankle. Then a foot massage should done for 8 minutes, and then kept in a bowl filled with 40 degrees water for 6 minutes.[6,13]

Application of foot massage
For foot massage, when the feet removed from the water, they should dried with a towel and the feet should extended by placing a towel under the feet in the bed. Massage should started by lubricating the hands with baby oil. For foot massage, firstly, the toes should rubbed with the help of the hands and stretched towards the fingertips. The feet should grasped by the heel and ankle and turned clockwise and counter clockwise once. Slight pressure should applied to the feet with circular movements from the sole of the foot to the toes. Then one hand should held on the ankle so that the other hand grasps the toes, the toes should pushed forward slowly. Foot massage should last 4 minutes for each foot. [6,13]

Statistical analysis
Descriptive statistics such as number, percentage, mean and standard deviation used in the evaluation of the data obtained. Whether the data showed normal distribution or not evaluated with the Kolmogorov-Smirnov (K-S) test. Data did not normally distributed. Data evaluated with Chi-square test, Fisher's exact test, Friedman test and Mann Whitney U test. Pairwise comparisons were examined using the Wilcoxon sign-rank test. A p value less than 0.05 considered statistically significant.

Results
There was no statistically significant difference in the intervention and control groups, and the groups were homogeneous (Table 1).

In table 2, right and left tibia edema levels of the students in the intervention group are compared before and after the clinical practice for four weeks. Accordingly, the edema levels measured from the right and left tibia of the students before and after the foot bath before and after the clinical practice varied between 0 and 2+. The edema levels measured from the right and left tibia before the evening foot bath on the 1st day after the clinical practice every week increased. In the follow-up for one month, the edema levels in the right tibia decreased statistically on the 1st and 2nd days after footbath compared to the values measured after the clinical practice, while the edema level in the left tibia decreased signifi-

cantly in the first and fourth weeks (p<0.05). In the four-week follow-up, the edema levels of the students in the right and left tibia showed a statistically significant difference (p<0.001).

Table 1. Comparison of the characteristics of the students[a]

Characteristic		Intervention Group	Control Group	Statistics test *
		Mean (SD)	Mean (SD)	
Age		20.59 (1.64)	19.95 (0.78)	Z=-1.328 p=0.184
Height (cm)		168.37 (7.94)	168.05 (7.18)	Z=-0.212 p=0.832
Weight (kg)		65.44 (23.99)	60.18 (10.20)	Z=-0.111 p=0.912
Sleep time (hour/day)		7.19 (1.03)	7.73 (1.07)	Z=-1.737 p=0.832
Standing time (hour/day)		8.04 (3.20)	8.91 (3.50)	Z=-0.587 p=0.557
		n (%)	n (%)	Statistics test
Gender	Female	21 (77.8)	16 (72.7)	**c^2=0.167
	Male	6(22.2)	6 (27.3)	p=0.747
Working status	working	1 (3.7)	1 (4.5)	**c^2=0.033
	not working	26 (96.3)	20(95.5)	p=1.000
Smoking	Yes	4 (14.8)	4 (19.0)	**c^2=0.152
	No	23(85.2)	17(81.0)	p=0.715
Salt consumption (daily)	More	3 (11.1)	-	***c^2=2.601
	Middle	16 (59.3)	13 (61.9)	p=0.272
	Little	8 (29.6)	8 (38.1)	
Coffee consumption (daily)	Not consume	11 (40.7)	13 (61.9)	***c^2=4.825
	One cup	10 (37.0)	2 (9.5)	p=0.090
	Two cups	6 (22.2)	6 (28.6)	
Perceived general health status	Excellent	1 (3.7)	-	***c^2=0.990
	Very good	9 (33.3)	6 (28.6)	p=0.609
	Good	17 (63.0)	15 (71.4)	
Perceived foot health status	Excellent	2 (7.4)	2 (9.5)	***c^2=1.665
	Very good	10 (37.0)	8 (38.1)	p=0.645
	Good	13 (48.1)	11 (52.4)	
	Poor	2 (7.4)	-	
Exercise status	Not doing	7 (25.9)	7 (33.3)	***c^2=2.581
	Irregular	17 (63.0)	14 (66.7)	p=0.275
	Regular	3 (11.1)	-	
Clinical learning setting	General services	16 (59.3)	19 (90.5)	***c^2=6.056
	Intensive care	2 (7.4)	-	p=0.048
	Private practice rooms	9 (33.3)	2 (9.5)	

[a] *Z – Mann Whitney U test; **c^2 – Fisher's Exact test; ***c^2 – Chi-square test

In pairwise comparisons performed using the Wilcoxon signed ranks test, the degree of edema in the right tibia significantly increased (p=0.034) before and after clinical practice on the evening of the first day without foot bath, in the first week (p=0.034), third week (p=0.034) and fourth week (p=0.008). However, there was no statistically significant difference in the evaluated edema degrees on the first and second evenings after clinical practice (p>0.05). The degree of edema in the left tibia was found to increase significantly (p=0.003) on the first day of the evening without performing a foot bath after clinical practice compared to before clinical practice in the first week, while there was no statistically significant difference between the degree of edema for other pairwise comparisons (p>0.05). These findings were clinically significant.

There was no significant difference between the degrees of edema in the right and left tibia in all comparisons made for the second, third, and fourth weeks (p>0.05). However, the degree of edema in the left tibia was found to increase significantly (p=0.003) between the fourth week before clinical practice and the first day of the evening before performing a foot bath, whereas it decreased significantly (p=0.008) after the foot bath on the same day. These findings were clinically significant.

Table 2. Comparison of right and left tibia edema levels of students in the intervention group before and after clinical practice [a]

	Measurement time	1st week	2nd week	3rd week	4th week	Statistics test
		Mean (SD)	Mean(SD)	Mean (SD)	Mean (SD)	
Right	Pre-clinical evening	0.67 (0.62)	0.70 (0.60)	0.70 (0.60)	0.70 (0.60)	
	Evening of the 1st day after clinical practice (before foot bath)	0.89 (0.75)	0.89 (0.75)	0.96 (0.80)	0.96 (0.80)	
	Evening of the 1st day after clinical practice (after foot bath)	0.70 (0.60)	0.70 (0.60)	0.70 (0.60)	0.74 (0.65)	c^2= 54.683 p<0.001
	Evening of the 2nd day after clinical practice (after foot bath)	0.70 (0.60)	0.74(0.65)	0.74 (0.65)	0.74 (0.65)	
	Statistics test	c^2= 11.000 p=0.012	c^2= 8.500 p=0.037	c^2= 12.356 p=0.006	c^2= 17.571 p=0.001	
Left	Pre-clinical evening	0.63 (0.56)	0.74 (0.65)	0.70 (0.60)	0.67 (0.55)	
	Evening of the 1st day after clinical practice (before foot bath)	0.96 (0.80)	0.89 (0.75)	0.89 (0.75)	1.00 (0.83)	
	Evening of the 1st day after clinical practice (after foot bath)	0.70 (0.60)	0.78 (0.75)	0.74 (0.65)	0.74 (0.65)	c^2=125.928 p<0.001
	Evening of the 2nd day after clinical practice (after foot bath)	0.70 (0.60)	0.74 (0.65)	0.74 (0.65)	0.74 (0.65)	
	Statistics test	c^2= 20.778 p<0.001	c^2= 5.824 p=0.121	c^2= 7.080 p=0.069	c^2= 20.778 p<0.001	

[a] c^2 – Friedman test; SD – standard deviation

In table 3, right and left tibia edema levels of the students in the control group compared before and after the clinical practice for four weeks. Accordingly, the edema levels measured by the students from the right and left tibia before clinical practice increased at the end of each week. However, the increase in edema levels in the right and left tibia only in the first week showed a statistically significant difference (p<0.05). In addition, the changes in the edema levels in the left tibia of the students during the follow-up for four weeks showed a statistically significant difference (p<0.05).

In pairwise comparisons, the degree of edema in both the right (p=0.046) and left (p=0.046) legs before clinical practice showed a statistically significant increase on the evening of the second day after clinical practice.

Table 3. Comparison of right and left tibia edema levels of students in the control group before and after clinical practice [a]

Measurement time		1st week	2nd week	3rd week	4th week	Statistics test
		Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	
Right	Pre-clinical evening	0.90 (0.30)	0.95 (0.38)	0.90 (0.30)	0.90 (0.30)	c^2= 19.417 p=0.054
	Evening of the 1st day after clinical practice	0.95 (0.38)	0.95 (0.38)	0.95 (0.38)	1.00 (0.44)	
	Evening of the 2nd day after clinical practice	1.10 (0.53)	1.05 (0.49)	1.00 (0.44)	1.00 (0.44)	
Statistics test		c^2= 6.500 p=0.039	c^2= 2.000 p=0.368	c^2= 3.000 p=0.223	c^2= 2.267 p=0.264	
Left	Pre-clinical evening	0.90 (0.30)	0.90 (0.30)	0.95 (0.38)	0.90 (0.30)	c^2= 21.342 p=0.030
	Evening of the 1st day after clinical practice	0.95 (0.38)	0.95 (0.38)	0.95 (0.38)	0.95 (0.38)	
	Evening of the 2nd day after clinical practice	1.10 (0.53)	1.05 (0.49)	1.05 (0.49)	1.00 (0.44)	
Statistics test		c^2= 6.500 p=0.039	c^2= 4.467 p=0.097	c^2= 2.000 p=0.368	c^2= 3.000 p=0.223	

[a] c^2 – Friedman test; SD – standard deviation

When table 4 was examined, the right and left foot pain scores in the intervention group increased in the evening of the day after the clinical practice compared to before clinical practice, and pain scores decreased statistically significantly after footbath (p<0.001). In addition, the changes in the pain scores of the students in the right and left legs showed a statistically significant difference in the follow-up for four weeks (p<0.001).

On the first day of the first week after clinical practice the level of pain in the right foot was found to increase significantly compared to before clinical practice both before (p=0.008) and after (p<0.001) the foot bath. However, there was no statistically significant difference in the level of pain in the right foot between before the foot bath on the first day and after the foot bath on the first and second days (p>0.05). All pairwise comparisons except for the level of pain in the right foot showed statistically significant differences during the second week, third week, and between before clinical practice and after the foot bath on the second day (p<0.05). The application of foot bath before and after the second day of clinical practice in the 4th week did not have a significant effect on the level of pain in the right foot (p>0.05). Similarly, there was no significant effect of foot bath applied after the first day of clinical practice and after the second day of clinical practice on the level of pain in the right foot (p>0.05). All other pairwise comparisons made on the right foot were statistically significant (p<0.05). In the pairwise comparisons conducted to evaluate the level of pain in the left foot, except for the foot bath performed on the first day after the clinical practice, all other pairwise comparisons had a significant effect on foot pain (p<0.05). These findings were clinically significant.

Table 4. Comparison of right and left foot pain levels of students in the intervention group before and after clinical practice [a]

Measurement time		1st week	2nd week	3rd week	4th week	Statistics test
		Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	
Right	Pre-clinical evening	1.00 (1.25)	0.92 (1.31)	0.88 (1.29)	1.16 (1.65)	c^2=152.410 p<0.001
	Evening of the 1st day after clinical practice (before foot bath)	3.84 (2.41)	3.58 (1.81)	3.54 (2.43)	3.16 (2.32)	
	Evening of the 1st day after clinical practice (after foot bath)	2.24 (2.20)	2.00 (2.04)	1.75 (1.64)	1.76 (1.66)	
	Evening of the 2nd day after clinical practice (after foot bath)	2.28 (2.33)	1.96 (1.62)	2.17 (2.05)	1.76 (1.73)	
	Statistics test	c^2= 46.070 p<0.001	c^2= 38.242 p<0.001	c^2= 33.292 p<0.001	c^2= 22.929 p<0.001	
Left	Pre-clinical evening	0.92 (1.24)	0.96 (1.57)	1.08 (1.55)	1.04 (1.39)	c^2= 84.194 p<0.001
	Evening of the 1st day after clinical practice (before foot bath)	3.63 (2.12)	3.63 (2.12)	3.48 (2.36)	2.92 (2.26)	
	Evening of the 1st day after clinical practice (after foot bath)	2.13 (1.54)	2.13 (1.54)	2.16 (2.01)	1.83 (1.68)	
	Evening of the 2nd day after clinical practice (after foot bath)	1.67 (1.43)	1.67 (1.43)	1.84 (1.88)	1.67 (1.81)	
	Statistics test	c^2= 39.192 p<0.001	c^2= 35.220 p<0.001	c^2= 29.145 p<0.001	c^2= 21.672 p<0.001	

[a] c^2 – Friedman test; SD – standard deviation

Table 5. Comparison of right and left foot pain levels of students in the control group before and after clinical practice [a]

Measurement time		1st week	2nd week	3rd week	4th week	Statistics test
		Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	
Right	Pre-clinical evening	0.86 (1.95)	1.05 (1.39)	1.10 (1.37)	0.90 (1.37)	c^2= 127.493 p<0.001
	Evening of the 1st day after clinical practice	3.62 (1.83)	3.90 (2.27)	4.29 (2.19)	3.71 (1.84)	
	Evening of the 2nd day after clinical practice	3.67 (1.90)	3.76 (1.92)	3.52 (2.18)	3.57 (1.93)	
	Statistics test	c^2= 25.241 p<0.001	c^2= 26.732 p<0.001	c^2= 28.587 p<0.001	c^2= 26.297 p<0.001	
Left	Pre-clinical evening	0.95 (1.35)	1.05 (1.35)	1.10 (1.22)	0.95 (1.20)	c^2= 131.322 p<0.001
	Evening of the 1st day after clinical practice	3.62 (1.43)	3.81 (2.25)	4.19 (2.01)	3.76 (1.75)	
	Evening of the 2nd day after clinical practice	3.90 (1.70)	3.57 (1.96)	3.81 (1.86)	3.57 (1.66)	
	Statistics test	c^2= 31.444 p<0.001	c^2= 21.658 p<0.001	c^2= 28.987 p<0.001	c^2= 27.029 p<0.001	

[a] c^2 – Friedman test; SD – standard deviation

In Table 5, the right and left foot pain scores of the students in the control group increased in the evening of the day after clinical practice compared to the day before

clinical practice. The pain scores expressed by the students in the right and left foot in the first week increased continuously, unlike the other weeks, and the scores that increased in the evening of the day after clinical practice in the other weeks decreased in the evening of the 2nd day compared to the 1st day ($p<0.001$). In the four-week follow-up, the changes in the pain scores of the students in the right and left foot showed a statistically significant difference ($p<0.001$). All pairwise comparisons made using the Wilcoxon signed ranks test showed statistically significant differences ($p<0.05$).

Discussion

In this study, the effect of foot bath and foot massage applied after clinical practices in nursing students on foot pain and leg edema was evaluated for four weeks before and after weekly clinical practice. It was determined that the level of tibia edema in the right and leg, which increased after clinical applications of the 20-minute foot bath and 8-minute (total for both feet) foot massage applied before going to bed by the nursing students decreased at the end of each week ($p<0.05$). Although tibia edema in the left leg decreased at the end of each week, this decrease was significant in the 1st and 4th weeks ($p<0.05$). This result showed that when foot bath and foot massage were applied together, it had a significant effect on lower extremity edema. It is seen that this finding is compatible with studies conducted in previous years. In the studies of Seo et al., they were reported that footbath performed for 20 minutes 3 times a week before going to bed was effective in reducing edema in both legs in nursing students ($p<0.001$).[6,11] Lee et al. reported that footbath applied for 20 minutes reduced tibia edema in operating room nurses who stood for long periods of time ($p<0.05$).[13] Oh and Yoon also found that leg massage applied by the nurses for 10 minutes reduced leg edema.[16]

In this current study, it was found that the footbath and foot massage applied by nursing students were effective in reducing the increasing foot pain after clinical practice ($p<0.001$). The foot pain score, which increased to an average of 3 points on the evening of the first clinical practice every week in the intervention group, fell below 3 points at the end of the week. The foot pain score, which increased after clinical practice in the control group, was still above 3 points at the end of the week ($p<0.001$). A single study was found to reduce the increased pain sensation after clinical practice of foot bath and foot massage in nurses or nursing students. In this study conducted by Oh and Yoon, it was reported that the foot pain score, which was 5 on average after working in the clinic, decreased to an average of 4 points after the leg massage performed by the nurses themselves, and this result was statistically significant.[16] Also, in the literature, it is emphasized that foot bathing by immersing the feet in water reduces foot pain by stimulating tactile sensations and reducing sympathetic nerve activity.[12,17]

Study limitations

This study had three main limitations. First, the study conducted only with second-year nursing students. Second, participants may not have filled out the forms correctly because they made the measurements themselves. Third, participants may not have watched or worked on the video adequately.

Conclusion

In this study, it was determined that nursing students experienced foot pain and leg edema after clinical practice. In addition, the study showed that foot bath and foot massage performed before bedtime after clinical practice were effective in reducing students' foot pain and leg edema. Foot baths may applied as an effective intervention to decrease leg edema and foot pain among nursing student during clinical training. The limited number of studies on the subject in previous years makes the results of this study important.

Acknowledgments

The authors would like to thank the nursing students who participated in the study.

Declarations

Funding

This study supported by the Scientific and Technological Research Council of Turkey (TUBITAK) within the scope of 2209-A - University Students Research Projects Support Programme.

Author contributions

Conceptualization, S.Ç. and G.C.; Methodology, S.Ç. and G.C.; Software, S.Ç.; Validation, S.Ç; Formal Analysis, S.Ç; Investigation, G.C., B.G., C.S., İ.A..; Resources, S.Ç. and G.C; Writing – Original Draft Preparation, S.Ç., G.C., B.G., C.S., İ.A.. Visualization, S.Ç. G.C.; Supervision, S.Ç. and G.C.; Project Administration, S.Ç. and G.C.; Funding Acquisition, S.Ç. and G.C.

Conflict of interest

The authors report no conflicts of interests.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Ethics approval

Study was approved by the University's Social and Human Sciences Ethics Committee (decision no. 10, dated 18.03.2020).

References

1. Bahçecik N, Alpar SE. Nursing education in Turkey: from past to present. *Nurse Educ Today*. 2009;29(7):698-703. doi:10.1016/j.nedt.2009.05.008
2. Arkan B, Ordin Y, Yılmaz D. Undergraduate nursing students' experience related to their clinical learning environment and factors affecting to their clinical learning process. *Nurse Educ Pract.* 2018;29:127-132. doi: 10.1016/j.nepr.2017.12.005
3. Bilgiç Ş, Çelikkalp Ü. Assessment of nursing students' stress levels and coping strategies during first clinical experience. *Clin Exp Health Sci.* 2021;11(3):437-443. doi: 10.33808/clinexphealthsci.777904
4. Bodys-Cupak I, Ścisło L, Kózka M. Psychosocial determinants of stress perceived among polish nursing students during their education in clinical practice. *Int J Environ Res Public Health.* 2022;19(6):3410. doi: 10.3390/ijerph19063410
5. Khater W, Akhu-Zaheya L, Shaban I. Sources of stress and coping behaviours in clinical practice among baccalaureate nursing students. *Int J Human Soc Sci Res*. 2014;4(6):194-202.
6. Seo S, Han H, Yeon S. Contribution of foot bath to fatigue relief measured by using smart phone applied questionnaires. *Int J Adv Sci Eng Inf Technol.* 2018;113:113-122. doi: 10.14257/ijast.2018.113.12
7. Eyi S, Eyi İ. Nursing students' occupational health and safety problems in surgical clinical practice. *SAGE Open.* 2020;10(1):2158244020901801.
8. Elsayed AA. Work-related musculoskeletal disorders among nursing students during clinical training. *Am J Nurs*. 2019;7(6):952-957. doi: 10.12691/ajnr-7-6-7
9. Moodley M, Ismail F, Kriel A. Work-related musculoskeletal disorders amongst undergraduate nursing students at the University of Johannesburg. *Health SA Gesondheid*. 2020;25:a1460. doi: 10.4102/hsag.v25i0.1460
10. Kamalruzaman NSA, Tengku Sabri TA, Isa SNI. Musculoskeletal disorders and quality of life among undergraduate health sciences students: A cross-sectional study. *Healthscope.* 2021;4(1):99-105.
11. Seo S, Yoon M, Yeon S. Effects of foot bath on leg edema and fatigue among college students. *J Korean Soc Sch Health.* 2017;30(1):21-28. doi: 10.15434/kssh.2017.30.1.21
12. Rahmani A, Naseri M, Salaree MM, Nehrir B. Comparing the effect of foot reflexology massage, foot bath and their combination on quality of sleep in patients with acute coronary syndrome. *J Caring Sci*. 2016;5(4):299-306. doi: 10.15171/jcs.2016.031
13. Lee SY, Kyung PH, Jea KH, Hee JY. Effects of foot therapy on operating room nurses' lower extremities edema, stress, and fatigue. *J Korean Acad Nurs*. 2014;20(1):102-112. doi: 10.22650/JKCNR.2014.20.1.102
14. Oh S, Gang GH. Effects of footbaths on stress and exahustion for high school senior students-a comparative study on the aroma oil and fermentation extract. *Journal of the Korea Academia-Industrial Cooperation Society.* 2010;11(1):402-408. doi:10.5762/KAIS.2010.11.1.402
15. Sert H, Olgun N. Yoğun bakımda ödem ve dehidratasyon. *Yoğun Bakım Hemşireliği Dergisi*. 2016; 20(1): 24-36.
16. Oh J, Yoon CM. Lower extremity edema and pain of nurses and the effect of self leg massage. *J Korean Acad Nurs*. 2008;38(2):278-286. doi: 10.4040/jkan.2008.38.2.278
17. Koraş Sözen K. [The effect of foot massage on postoperative pain level]. *Kahramanmaraş Sütçü İmam Üniversitesi Tıp Fakültesi Dergisi.* 2020;15(2):110-115. doi: 10.17517/ksutfd.67335

Wydawnictwo UR 2023
ISSN 2544-1361 (online)
doi: 10.15584/ejcem.2023.3.9

http://www.ejcem.ur.edu.pl
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2023; 21 (3): 483–491

ORIGINAL PAPER

Nutritional knowledge about the Mediterranean diet and its practical application among students in Poland

Sandra Karlik , Katarzyna Dereń

Institute of Health Sciences, College of Medical Sciences, University of Rzeszów, Poland

ABSTRACT

Introduction and aim. The popularity of the Mediterranean diet is increasing and following it has many health benefits, including improving mental well-being. The aim of the study was to assess the nutritional knowledge about the Mediterranean diet and its practical application among students in Poland.

Material and methods. The study assessed a group of 313 students in Poland. The research tool was a questionnaire that focused on knowledge about the Mediterranean diet.

Results. The study showed that in terms of knowledge of the Mediterranean diet, students scored on average 6.5±3.2 points (on a scale 0–11). When it comes to dietary compliance, the average score was 6.3±2.4 points (scale 1–13 points). The better the knowledge of the diet, the better the compliance with its rules. Statistically, the analysis showed that there is a significant relationship between the age and/or academic degree of the students and their higher level of knowledge. The greater compliance with Mediterranean eating patterns was influenced by the location, age, or education of the respondents.

Conclusion. The study shows that the surveyed group of students in Poland has only an average level of knowledge and compliance with the Mediterranean diet.

Keywords. Mediterranean diet, nutrition, students' knowledge

Introduction

Nowadays, there are many alternative ways of eating. One of them is the Mediterranean diet, which is often considered the healthiest model of nutrition in the world.[1] The Mediterranean diet is a typical way of eating people living in the Mediterranean. It is based mainly on products of plant origin, which provide large amounts of dietary fiber, antioxidants, vitamins, and minerals, which have a positive effect on health.[2] The Mediterranean diet has several nutritional recommendations. Olive oil should be consumed every day as the main source of fat in the diet. This fat contains monounsaturated fatty acids and antioxidants that sequester free radicals. The Mediterranean diet is based on eating two or more servings of vegetables, and fruits – one or two servings a day. These low-calorie products contain vitamins, minerals and flavonoids that have antioxidant properties. Whole grain products rich in dietary fiber and B vitamins should be consumed every day. Fish and seafood should be eaten 2 or 3 times a week, as they contain omega-3 acids that help prevent many diseases, have anti-inflammatory properties, and affect overall improvement of health and well-being. Legume seeds are a source of vegetable protein and dietary fiber, so they should be eaten often. In this diet pattern, dairy products (milk, cheese, yoghurt) should be chosen low in fat. This group of foods is important because it provides calcium and phosphorus. Nuts and seeds are rich in vitamin E and omega-3 fatty acids essential for maintaining proper health. In the Mediterranean diet, it is also important

Corresponding author: Sandra Karlik, e-mail: sandrak@dokt.ur.edu.pl

Received: 18.04.2023 / Revised: 24.05.2023 / Accepted: 26.05.2023 / Published: 30.09.2023

Karlik S, Dereń K. *Nutritional knowledge about the Mediterranean diet and its practical application among students in Poland*. Eur J Clin Exp Med. 2023;21(3):483–491. doi: 10.15584/ejcem.2023.3.9.

to use fresh herbs and spices every day – they support appetite and some of them are a source of antioxidants. Water is the primary drink. Additionally, wine is consumed in moderation (1 glass) mainly with a meal. It is recommended to limit the consumption of red meat due to its high saturated fat content. In the Mediterranean diet, it is recommended to choose seasonal and local products that are high in nutrients. Through the proper selection of food, the Mediterranean diet is characterized by a low consumption of saturated fatty acids and a high consumption of dietary fiber, vitamins, minerals, and mono- and polyunsaturated fats. Provides a high supply of antioxidants (polyphenols, lycopenes, and flavonoids), which come mainly from plant products. Furthermore, it has a positive effect on improving the lipid profile.[3,4] This diet has a positive effect on the prevention of cardiovascular disease, prevents obesity, and supports the treatment of cancer.[5,6]

The Mediterranean diet in 2023 was named the best diet of the year among 24 other popular eating patterns according to the US News & World Report. In addition, it was very well in the other categories, including diabetic, cardiological, plant-based diet, or the easiest to follow.[7] The Food and Agriculture Organization of the United Nations (FAO) has indicated the Mediterranean diet as an example of a diet that has a positive effect not only on health, but also on environmental resources through low greenhouse gas emissions.[8,9]

However, the researchers emphasize that a healthy lifestyle is responsible for the success of the Mediterranean diet. An important element of this diet, in addition to the use of local and fresh products, turns out to be eating meals in the company of loved ones or doing regular physical activity.[3] The use of the Mediterranean diet among young people, as well as the knowledge of its basic assumptions, can contribute significantly to improving health and inhibiting the development of many diseases of civilization at a later age.

Aim

The aim of the study was to assess the nutritional knowledge of the Mediterranean diet and its practical application by Polish students. The study was conducted to verify whether students understand the basic assumptions of the diet and how they implement them on their daily menus.

Material and methods

The study was conducted online among students in Poland using an anonymous survey developed by the authors. The survey consisted of 35 closed single or multiple-choice questions and two open questions. To conduct the study, the authors created a questionnaire consisting of two parts. The first was aimed at assessing the level of student knowledge of the Mediterranean diet and its practical application in their daily menu. The second part contained demographic questions about gender, age, place of residence, education, weight and height.

The inclusion criteria were voluntary participation in the survey. The exclusion criteria were: lack of consent to complete the questionnaire, withdrawal from completing the questionnaire. Finally, the study included a group of 313 volunteers.

Checking the level of knowledge of the respondents was examined through issues related to the products found in the Mediterranean diet and the impact of this way of eating on health. The method of implementing the basic assumptions of the diet was assessed using the frequency of consumption method.

An original scale was created, which awarded 1 point for giving the correct answer that qualifies for the use of Mediterranean diet patterns and having the appropriate level of knowledge. The range of adherence to the Mediterranean diet recommendations was determined, which corresponded to 1–5 points – low, 6–10 points. medium, above 10 points – high and the indicator of knowledge of its assumptions (1–4 points – low, 5–8 points – medium, above 8 points – high).

The respondents were asked about both the type and frequency of products consumed in their daily menu. The answers to the questions are presented by number (n) and frequency (%). Body mass index (BMI) was calculated based on height and weight given by respondents.

For statistical purposes, the following methods were used: Mann-Whitney U test, Kruskal-Wallis test, Spearman rank correlation coefficient significance test, Pearson's chi-square test, NW chi-square test, and the Fp test comparing k frequencies. Statistical analysis was performed using the EXCEL and STATISTICA 10 PL programs by Statsoft (Kraków, Poland). The value of $p<0.05$ was considered statistically significant.

Results

The vast majority of the respondents (85.9%) were women, and 14.1% were men (Table 1).

The BMI of the respondents ranged from 13.54 to 41.52 kg/m^2. Its average was 21.89±3.10 kg/m^2 (women 21.63±2.99 kg/m^2 and men 23.49±3.27 kg/m^2). In half of the subjects, this indicator did not exceed 21.45 kg/m^2.

The vast majority of students (76%) had normal body weight. Overweight was found in 13.7% of the respondents, and underweight in 8.9% of the respondents. The remaining respondents (1.3%) were obese.

Students in the field of knowledge about the Mediterranean diet obtained from 0 to 11 points, and their average was 6.5±3.2 points. Most of the students had an average level of knowledge about the Mediterranean diet (5–8 points) – 39.6% of the people (Fig. 1).

Table 1. General characteristics of the respondents

Parameter		n	%
Gender	Female	269	85.9
	Male	44	14.1
Age	18 – 21 years old	132	42.2
	22 – 25 years old	140	44.7
	26 years and older	41	13.1
Place of residence	City	207	66.1
	Village	106	33.9
Education	Secondary education	174	55.6
	Bachelor's degree	91	29.1
	Engineering degree	21	6.7
	Master degree	27	8.6

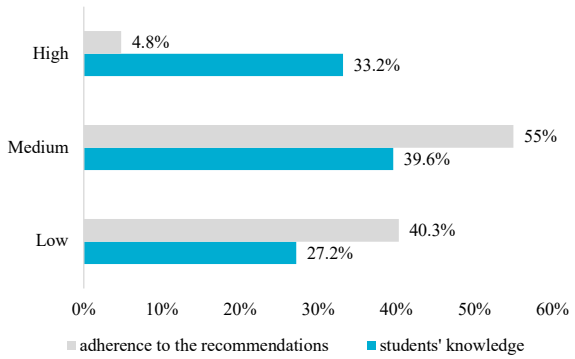

Fig. 1. Evaluation of adherence to the recommendations/ level of students' knowledge of the Mediterranean diet

Most of the students (78%) have heard of the Mediterranean diet. 22% of the respondents showed a lack of general knowledge of this way of eating. Most of the respondents (60.7%) correctly believed that the Mediterranean diet is one of the healthiest diets in the world. 6.4% of the respondents had a different opinion, while the remaining respondents (32.9%) did not know the answer to this question.

According to the majority of students (60.7%), an important element of the Mediterranean diet is a healthy lifestyle, including regular physical activity. 6.4% of the respondents disagreed with this statement. Some of the respondents (32.9%) did not know whether physical exercise is an important element of the Mediterranean model of nutrition. According to the vast majority of the respondents (80.2%), the Mediterranean diet can affect quality and length of life. 3 (1%) respondents disagreed with this statement. The remaining respondents did not know the answer to this question – 18.8%.

The opinion of the respondents on diseases that the Mediterranean diet may have a particularly positive impact was divided. Most often, the respondents chose obesity (87.2%), atherosclerosis (83.1%) or hypertension (82.4%) (Fig. 2).

The study did not confirm a statistically significant difference in knowledge level between women and men (p>0.05). Students' gender of the students had no effect on the knowledge of the Mediterranean diet.

The study observed a statistically significant difference in the level of knowledge between students of different ages (p<0.0001). The study showed a statistically significant difference in the level of knowledge between students with different education (p<0.0001). The average and median knowledge were higher in students with a bachelor's degree (Table 2).

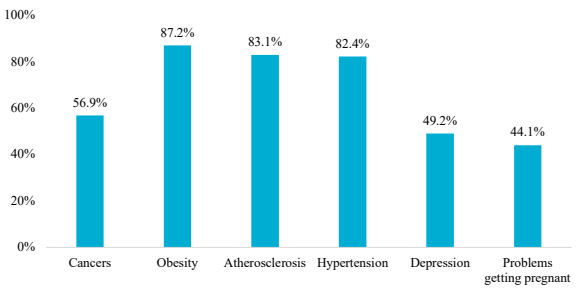

Fig. 2. Respondents' answers regarding diseases that can be particularly positively affected by the Mediterranean diet (multiple answers possible)

Table 2. Relationship between age/education of respondents and knowledge of the Mediterranean diet*

Parameter		n	Knowledge about the Mediterranean diet (points)					H	p
			Mean	SD	Median	Min.	Max.		
Age	18 – 21 years old	132	5.5	3.1	6	0	11	32.69	<0.0001
	22 – 25 years old	140	6.9	3.2	7	0	11		
	26 and older	41	8.4	2.5	9	1	11		
Education	Secondary education	174	5.7	3.1	6	0	11	31.12	
	Bachelor's degree	91	7.8	3.0	9	0	11		
	Engineer degree	21	6.7	3.6	8	0	11		
	Master	27	7.3	2.8	8	0	11		

* H – Kruskal-Wallis test value

There was no statistically significant difference in the level of knowledge between students living in the city and people living in the countryside (p>0.05). The residence of the students had no effect on the level of knowledge about the Mediterranean diet.

The study did not confirm a significant correlation between the BMI of the students and the level of knowledge of the Mediterranean diet (p>0.05). The students' BMI had no effect on their knowledge of the Mediterranean diet.

The study showed a significant correlation between the level of knowledge about the Mediterranean diet and the degree of adherence to the recommendations of this diet (p<0.0001). The higher the level of knowledge of

the students, the greater the degree of adherence to the recommendations of the Mediterranean way of eating (Table 3).

Table 3. The result of the Spearman rank correlation coefficient test of significance between the level of knowledge about the Mediterranean diet and the degree of adherence to the recommendations of this diet*

A pair of variables	n	Rs	p
Knowledge about the Mediterranean diet (points) & Degree of adherence to the recommendations of the Mediterranean diet (points)	313	0.409	<0.0001

* Rs – value of the Spearman rank correlation coefficient

For the degree of adherence to the Mediterranean diet recommendations, the students obtained 1 to 13 points. The average was 6.3±2.4 points. More than half of the students (55%) had an average level of adherence to the Mediterranean diet recommendations (6–10 points) (Fig. 1).

Whole grain products were consumed daily by 26.2% of the respondents, which is in line with the principles of the Mediterranean diet and 4.8% of respondents did not eat them at all. Dairy products were consumed every day by 42.8% of people. Olive oil was used daily by 13.4% of students and 64.8% of students used this type of fat too rarely. The nuts were eaten at least several times a week by 33.8% of the respondents and 8.6% of them did not consume nuts at all (Table 4).

Table 4. Frequency of consumption of selected products and meals by respondents

Products		I don't eat	Less than once a week	Once a week	Several times a week	Every day
Whole-grain products	n	15	42	47	127	82
	%	4.8	13.4	15	40.6	26.2
Dairy products	n	32	15	22	110	134
	%	10.2	4.8	7	35.1	42.8
Olive oil	n	68	72	26	105	42
	%	21.7	23.0	8.3	33.5	13.4
Nuts	n	27	131	49	68	38
	%	8.6	41.9	15.7	21.7	12.1
Fast-food meals	n	78	193	31	10	1
	%	24.9	61.7	9.9	3.2	0.3

Some students ate fruits too rarely (once a day) – 42.8% of them. The rest of the respondents did not eat fruit every day, 27.2% of the respondents. 63.2% of the students ate vegetables at least twice a day by 63.2% of the students, and 11.5% of them did not eat vegetables every day (Table 5).

Fish and seafood were consumed too rarely, and 74.7% of people consumed fish and seafood too rarely (Table 6).

Table 5. Frequency of fruit and vegetable consumption by respondents

Products		I don't eat every day	Once a day	2-3 times a days	4 or more times a day
Fruits	n	85	134	81	13
	%	27.2	42.8	25.9	4.2
Vegetables	n	36	79	156	42
	%	11.5	25.2	49.8	13.4

Table 6. Frequency of consumption of fish and seafood by students

Frequency of consumption of fish and seafood	n	%
I don't eat fish or seafood	55	17.6
Once a month or less	65	20.8
Several times a month	105	33.5
1 time a week	64	20.4
2 or more times a week	24	7.7

Wine is drunk at least several times a week by 7.3% of the students, including 0.3% every day. They were consumed too rarely by 68.1% of the people, and the remaining respondents did not drink wine at all, 24.6% of the respondents.

Students use different sources of fat in their diet. However, most of the time they choose: vegetable oil (62.9%), olive oil (60.4%) or butter (56.9%) (Fig. 3).

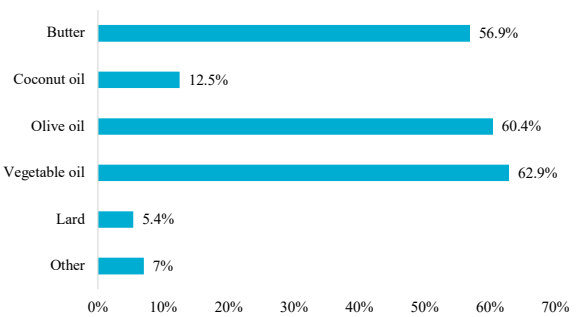

Fig. 3. Student responses on the use of a specific type of fat in the diet (multiple answers possible)

The vast majority of students used dried or fresh herbs and spices in their kitchen every day, 70.0% of the people. 28.8% of the respondents consumed them from time to time and the rest did not use them at all, 1.3% of the students.

There were no statistically significant differences in the level of adherence to the recommendations of the Mediterranean diet between women and men ($p>0.05$). The gender of the respondents had no effect on the degree of adherence to the recommendations of this diet.

The study showed a statistically significant difference in the level of adherence to the recommendations of the Mediterranean diet recommendations between respondents of different ages ($p=0.0001$). The study

results showed a statistically significant difference in the level of adherence to the Mediterranean diet recommendations between respondents living in the city and those living in the countryside (p=0.0005). The average and median degree of compliance with the recommendations of this diet was higher in people living in the city. The study showed a statistically significant difference in the level of adherence to the recommendations of the Mediterranean diet recommendations between respondents with different education (p<0.0001) (Table 7).

Table 7. The relationship between the age/place of residence/ education of the respondents and the degree of adherence to the recommendations of the Mediterranean diet*

			Degree of adherence to the recommendations of the Mediterranean diet (points)						
Parameter		n	Mean	SD	Median	Min.	Max.	H/Z	p
Age	18 – 21 years old	132	5.8	2.4	6	1	13	H = 19.37	0.0001
	22 – 25 years old	140	6.4	2.2	6	2	12		
	26 and older	41	7.8	2.8	9	2	13		
Place of residence	City	207	6.6	2.4	7	2	13	Z = 3.49	0.0005
	Village	106	5.7	2.3	5.5	1	13		
Education	Secondary Education	174	5.7	2.3	6	1	13	H = 27.75	<0.0001
	Bachelor's degree	91	7	2.4	7	2	12		
	Engineer degree	21	6.2	2.2	5	2	11		
	Master degree	27	7.7	2.3	8	4	13		

* H – Kruskal-Wallis test value; Z – Mann-Whitney U test value

There was no statistically significant correlation between the BMI of the respondents and the level of adherence to the Mediterranean diet (p>0.05). The students' BMI had no effect on adherence to Mediterranean dietary patterns. In the case of frequency of consumption, the answers were assigned rank values from 1 to 4/5, where 1 means no product consumption and 4 and 5 the most common consumption of the product. In this way, the frequency of consumption of the products was determined.

The study showed statistically significant correlations between the frequency of consumption and the level of knowledge about the Mediterranean diet for the following products: whole grain products, fruit, vegetables, fish and seafood, olive oil, nuts. Students who ate the products had a higher level of knowledge (Table 8).

The study showed a statistically significant difference in the level of knowledge between students with different frequency of legume consumption and different frequency of use of herbs and spices (p=0.0001). The average and median level of knowledge was higher in students who consumed legumes more than once a week. The average and median level of knowledge was higher in students who used herbs and spices daily.

Table 8. The results of the Spearman rank correlation coefficient test between the frequency of product consumption and the level of knowledge about the Mediterranean diet*

A pair of variables	n	Rs	p
Frequency of consumption of whole grain products & Knowledge about the Mediterranean diet (points)	313	0.292	<0.0001
Frequency of fruit consumption & Knowledge about the Mediterranean diet (points)	313	0.23	<0.0001
Frequency of vegetable consumption & Knowledge about the Mediterranean diet (points)	313	0.317	<0.0001
Frequency of fish and seafood consumption & Knowledge about the Mediterranean diet (points)	313	0.18	0.0014
Frequency of consumption of dairy products & Knowledge about the Mediterranean diet (points)	313	0.07	0.2156
Incidence of olive oil consumption & knowledge of the Mediterranean diet (points)	313	0.314	<0.0001
Frequency of nut consumption & knowledge of the Mediterranean diet (points)	313	0.318	<0.0001
Frequency of wine consumption & Knowledge of the Mediterranean diet (points)	313	0.06	0.289

* Rs – value of the Spearman rank correlation coefficient

No statistically significant correlations were observed between the frequency of consumption of recommended products in the Mediterranean diet and the BMI of the students.

Table 9. Relationship between knowledge of the positive impact of the Mediterranean diet on various diseases and the degree of adherence to the recommendations of this diet*

Diseases		n	Degree of adherence to the recommendations of the Mediterranean diet (points)					Z	p
			Mean	SD	Median	Min.	Max.		
Cancers	Yes	178	6.7	2.5	6.5	2	13	3.09	0.0020
	No	135	5.8	2.2	6	1	10		
Obesity	Yes	273	6.3	2.4	6	1	13	0.42	0.6722
	No	40	6.2	2.2	6	2	11		
Atherosclerosis	Yes	260	6.6	2.4	6	1	13	3.82	0.0001
	No	53	5.2	1.8	5	2	9		
Hypertension	Yes	258	6.6	2.4	7	1	13	4.18	<0.0001
	No	55	5.1	1.8	5	2	9		
Depression	Yes	154	6.8	2.5	7	2	13	2.81	0.0050
	No	159	5.9	2.2	6	1	11		
Problems of getting pregnant	Yes	138	6.7	2.6	7	2	13	2.11	0.0345
	No	175	6	2.2	6	1	11		

* Z – value of the Mann-Whitney U test

The study showed a statistically significant difference in the level of adherence to the Mediterranean diet recommendations between students who knew and those who did not know the positive impact of the Mediterranean diet on almost all diseases, except obesity ($p<0.05$). The average level of adherence to this diet was higher in students who indicated the following diseases (Table 9).

Discussion

Proper diet and lifestyle are factors that determine good health. It is particularly important to shape proper eating habits in people at a young age. Currently, among students, there is a growing interest in a healthy diet and the care of physical appearance. The Mediterranean diet is one of the most recommended nutrition models for people of all ages. Regular physical activity and a proper lifestyle are also the basic principles of this diet. In a study by La Fauci V et al. found a very high knowledge (90.5%) of the products present in this way of eating among young Italians. In the same group of respondents, despite a significant result of indicating typical food for this diet, only 11.4% of people were able to define what a Mediterranean diet is.[10] Analyzing the study by Bogacka et al., a low level of knowledge (30.3%) of people treated in cardiology clinics was shown about the Mediterranean diet. The reason was the low consumption of fruits and vegetables among respondents over 26 years of age.[11] The Mediterranean model of nutrition is one of the healthiest diets, which is why some people know its assumptions or have heard about it. Young people are increasingly interested in a healthy diet and lifestyle due to the growing fashion in this topic. Furthermore, they care more about their physical appearance than older people.

It should be noted that it is important not only to know the basic assumptions of the Mediterranean diet but also to apply it in practice in your daily menu. In the study by Garcia-Meseguer et al. it was observed that only 5.3% of Spanish students followed the Mediterranean diet model in their diet.[12] However, Bonaccorsi et al. proved that more than half of students in primary and secondary schools in Italy apply the assumptions of this way of eating and 24.8% of people adhere to it to a high degree. The nutritional status of children and adolescents or the degree of academics of parents did not significantly affect this relationship. Younger students and boys (33%) compared to girls (45%) showed less adherence to the Mediterranean diet recommendations. The researchers also pointed out that despite minor differences, excess body weight, including overweight or obesity, also resulted from a lower adherence to the recommendations of this model of nutrition.[13] Furthermore, it is also worth mentioning the study by Greiner et al., in which the authors proved that people with higher scientific degree and women more often follow the principles of the Mediterranean diet.[14] Looking at the age parameter, it can be concluded that older people are more aware of their food and choose healthier products as they grow up. Incorrect diet – high content of saturated fats in the diet or simple sugars – causes weight gain, which young people in particular cannot control. With age, students become more aware of their physical appearance and the parameters that are related to it. Better education or gender also affects the more frequent use of the Mediterranean diet. Women and people with a higher scientific degree pay more attention to what they eat and in what quantities to care for their figure or maintain proper health.

The type and frequency of consumption of particular food products have a significant impact on the quality of the diet. According to the Mediterranean model of nutrition, it is advisable to choose whole grain products every day. In the study by Kulesza et al., the authors showed that 27% of medical students ate whole-meal bread at least once a day and 1.0% of the respondents never chose it.[15] In turn, Lebiedzińska in her study showed that female students consume wholemeal bread more often than males. Only 14.8% of people consume this product every day and less than half of the students (42.4%) choose it several times a week. Moreover, the author showed that Polish students choose white bread much more often compared to whole grain bread.[16] The more frequent use of wholegrain products may be due to taste preferences or economic reasons, as they are usually cheaper than wholegrain products. In our own research, no significant differences were found between gender and the consumption of whole grain products, but other researchers indicated such a relationship. Women consume these products more often, because they usually pay more attention to the foods, they choose to take care of their figure.

Fruits and vegetables should be present in the Mediterranean menu in large quantities. They are a source of many vitamins, minerals, and antioxidants. López-Olivaresi et al. came to some conclusions about the consumption of fruits and vegetables in the student menu. in a study of Spanish students. Some of them (34.8%) ate at least three servings of fruit a day and more than half of the study participants (57.1%) chose vegetables at least twice a day.[17] In turn, in the study of Hadjimbei et al. showed that students from higher education institutions in Cyprus mostly consumed fruit every day (73.1%), but only some of them (31.6%) chose their second portion during the day. A similar dependence of the results was observed for vegetables - more than half of the people consumed them once a day, and only 29.5% of the respondents ate them at least twice a day.[18] In addition, the study by McLean-Meyinsse et al. should also be mentioned, in which the authors also proved a

similar correlation. A very small percentage of students (13%) chose fruits and vegetables more than once a day. It turns out that half of the respondents did not eat them every day (for fruits and vegetables, 50% and 52%, respectively).[19] Analyzing the above results, it can be concluded that students do not consume enough fruits and vegetables. It is recommended to introduce them to the daily menu due to their high content of vitamins, dietary fiber, and minerals.

Consumption of fish and seafood is important in the Mediterranean model of nutrition. These products are rich in omega-3 fatty acids and complete animal protein. Low consumption of fish was observed in the study of Kula and Śmiechowska. Their frequent consumption – more than two times a week – was found in a very small group of respondents (6%), and a complete lack of fish consumption was found in 9% of people. The authors of this article also presented the factors that influence these values. The students surveyed said that their frequency of buying fish is affected, among others, by the price (44%) or the ease of preparing this product at home (39%).[20] However, in another study, Kowalska noted a significant relationship between higher fish consumption by men compared to women.[21] Slightly different conclusions were reached by Mieziene et al. in their study with more than 3,000 people. The authors of the study showed that men consume fish much less often than women. Furthermore, the higher the level of education or the more frequent physical activity, the greater the consumption of fish and seafood among Lithuanian students.[22] Students consume too small amounts of fish, which means that they do not meet the basic recommendations of the Mediterranean diet, which indicates a minimum of 2–3 portions of this product a week. Low consumption is certainly due to the high price, taste, or eating habits developed in youth. Due to the high content of unsaturated fatty acids and a large amount of protein, they should definitely be eaten more often among people of all ages.

Choosing the right source of fat in the diet is very important. Excessive consumption of it can lead to: excessive body weight. According to the assumptions of the Mediterranean model of nutrition, it is recommended to use olive oil every day, preferably with every meal. It should be the main source of fat in your diet. The very high consumption of olive oil in the diet of Spanish students is highlighted by the study by López-Olivares et al., where most respondents (95.2%) used it in their diet.[17] However, in the study by Cobo-Cuenca et al., it has been shown that men and women in Spain – with a slight male predominance – use olive oil as the main source of fat to a similar extent (92.1% and 90.1%, respectively).[23] It is also worth analyzing the study by Mieziene et al., in which the nutritional patterns of Lithuanian students were examined. The study showed that only a small part of people (19.3%) chose olive oil as the basic fat in their daily diet. The students consumed an average of about 2.5 tablespoons each day, which is a small amount considering the recommended 4 tablespoons per day in the Mediterranean diet. The authors also noticed a certain correlation between the more frequent consumption of this source of fat and the gender and level of education of the respondents. Compared to men, women were much more likely to use olive oil in their cooking. The same was found among people with higher academic degrees compared to students with lower education. Furthermore, researchers have shown that Lithuanians who are more physically active consume this source of fat more frequently than sedentary people.[22] The low consumption of olive oil every day among the student population in Poland may be due to its material status or taste. The use of this fat in countries close to the Mediterranean Sea is quite popular due to its widespread availability and the lower price resulting, among others, from the lack of transport costs.

The number of scientific articles on the Mediterranean diet is constantly increasing. There is a lot of interest in this way of eating, which is why researchers are still trying to show new aspects regarding the positive impact of this diet on health and various entities of disease. According to current research, the Mediterranean model of nutrition is widely used in the prevention of many diseases. It is indicated for use at any age and also pays special attention to the right lifestyle. According to own research, students have a basic state of knowledge about this diet and implement its recommendations in their menu to an average degree. It is recommended to further educate and make each age group aware of the positive impact of the Mediterranean diet based on the latest scientific research on this issue.

The strengths of the study include the possibility of examining respondents from various universities throughout the country and the lack of time limits to answer. The weaknesses of the study include a non-standardized questionnaire, lack of direct contact with the respondents, the wrong understanding of the questions, or lack of complete answers. According to the recommendations of the Mediterranean diet, the frequency of consumption of different products varies, so it is recommended to use the same frequency of consumption scale in future studies.

Conclusion

Most of the Polish population students (39.6%) showed an average level of knowledge about the assumptions of the Mediterranean diet and adhere to its recommendations to the same extent (55% of the people). The higher the level of knowledge, the better the adherence to the Mediterranean diet recommendations among Pol-

ish students. Gender, place of residence, or BMI value are not related to the level of knowledge of students about the Mediterranean diet, while their age or level of education significantly affect this relationship. Adherence to Mediterranean diet patterns is related to the age, place of residence, and academic degree of college students. Gender or body mass index (BMI) do not significantly affect the level of adherence to the recommendations of the Mediterranean diet. Students who have more knowledge about the basic assumptions of this model of nutrition consume more often: wholegrain products, fruits, vegetables, fish and seafood, olive oil, legumes, nuts, and spices. The value of the BMI index does not affect the higher consumption of recommended products in the Mediterranean diet. Students have basic knowledge about the positive influence of the Mediterranean diet on various diseases. The greater the adherence to the recommendations of this way of eating, the greater the awareness of the prohealth effect of this diet on the occurrence of: cancer, atherosclerosis, hypertension, depression, or problems with getting pregnant.

Declarations

Funding
The author(s) declare no funding.

Author contributions
Conceptualization, S.K. and K.D.; Methodology, S.K. and K.D.; Formal analysis, S.K.; Investigation, S.K.; Resources, S.K; Writing – Original Draft Preparation, S.K.; Writing – Review & Editing, S.K. and K.D.; Supervision, K.D.

Conflicts of interest
The author(s) declare no competing interests.

Data availability
The data sets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval
All subjects gave their informed consent for inclusion before participating in the study. The study was carried out in accordance with the Declaration of Helsinki.

References

1. Morris L, Bhatnagar D. The Mediterranean diet. *Curr Opin Lipidol*. 2016;27(1):89-91. doi: 10.1097/MOL.0000000000000266
2. Martinez-Lacoba R, Pardo-Garcia I, Amo-Saus E, Escribano-Sotos F. Mediterranean diet and health outcomes: a systematic meta-review. *Eur J Public Health.* 2018:28(5):955-961. doi: 10.1093/eurpub/cky113
3. Bach-Faig A, Berry EM, Lairon D, et al. Mediterranean diet pyramid today. Science and cultural updates. *Public Health Nutr.* 2011;14(12A):2274-2284. doi: 10.1017/S1368980011002515
4. Zych P, Szostak-Węgierek D. Dieta paleolityczna. Część II. Porównanie z dietą śródziemnomorską. *Nowa Medycyna.* 2014;1:28-35.
5. Chudzińska M, Wołowiec Ł, Zukow W, Sinkiewicz W. Mediterranean diet recommended not only in cardiovascular diseases. *J Educ Health Sport.* 2017;7(6):732-74. doi: 10.5281/zenodo.999004
6. Dinu M, Pagliai G, Casini A, Sofi F. Mediterranean diet and multiple health outcomes: an umbrella review of meta-analyses of observational studies and randomised trials. *Eur J Clin Nutr.* 2018;72(1):30-43. doi: 10.1038/ejcn.2017.58
7. Best Diets Overall. U.S. News & World Report. https://health.usnews.com/best-diet/best-diets-overall/. Updated January 3, 2023. Accessed April 4, 2023.
8. Italy and FAO highlight the importance of the Mediterranean diet for the sustainable management of land and water resources. Food and Agriculture Organization of the United Nations. http://www.fao.org/news/story/en/item/1261467/icode/. Accessed April 4, 2023.
9. Grosso G. The Mediterranean diet as a sustainable dietetic model. *Eur J Public Health.* 2018;28:4. doi: 10.1093/eurpub/cky213.765
10. La Fauci V, Alessi V, Assefa DZ, et al. Mediterranean diet: knowledge and adherence in Italian young people. *Clin Ter.* 2020;171(5):e437-e443. doi: 10.7417/CT.2020.2254
11. Bogacka A, Nawrot M, Gryza E, Balejko E, Kucharska E. Ocena znajomości i przestrzegania zasad diety śródziemnomorskiej u pacjentów przychodni kardiologicznych. *Żywność. Nauka. Technologia. Jakość.* 2008;2(57):128-139.
12. García-Meseguer MJ, Burriel FC, García CV, Serrano-Urrea R. Adherence to Mediterranean diet in a Spanish university population [published correction appears in Appetite. 2014;80:271]. *Appetite.* 2014;78:156-164. doi: 10.1016/j.appet.2014.03.020
13. Bonaccorsi G, Furlan F, Scocuzza M, Lorini C. Adherence to Mediterranean Diet among Students from Primary and Middle School in the Province of Taranto, 2016-2018. *Int J Environ Res Public Health.* 2020;17(15):5437. doi: 10.3390/ijerph17155437
14. Greiner B, Wheeler D, Croff J, Miller B. Prior Knowledge of the Mediterranean Diet Is Associated With Dietary Adherence in Cardiac Patients. *J Am Osteopath Assoc.* 2019;119(3):183-188. doi: 10.7556/jaoa.2019.029
15. Kulesza K, Zujko M, Witkowska A. Ocena wybranych zwyczajów żywieniowych studentów Uniwersytetu Medycznego w Białymstoku. *Piel Zdr Publ.* 2019;9(1):33-39.
16. Lebiedzińska A. Wybrane produkty zbożowe jako elementy funkcjonalne diety – częstość spożycia produktów zbożowych wśród studentów. *Rocz Panstw Zakl Hig.* 2007;58(1):295-300.

17. López-Olivares M, Mohatar-Barba M, Fernández-Gómez E, Enrique-Mirón C. Mediterranean Diet and the Emotional Well-Being of Students of the Campus of Melilla (University of Granada). *Nutrients.* 2020;12(6):1826. doi: 10.3390/nu12061826
18. Hadjimbei E, Botsaris G, Gekas V, Panayiotou AG. Adherence to the Mediterranean Diet and Lifestyle Characteristics of University Students in Cyprus: A Cross-Sectional Survey. *J Nutr Metab.* 2016;2016:2742841. doi: 10.1155/2016/2742841
19. McLean-Meyinsse PE, Harris EG, Taylor SS, Gager JV. Examining College students' daily consumption of fresh fruits and vegetables. *J Food Distrib Res.* 2013;44(1):10-16.
20. Kula S, Śmiechowska M. The Assessment of Fish Consumption by University Students of Different Academic Profiles. *Int J Food Sci.* 2016;2(21):28-37. doi: 10.15611/nit.2016.2.02
21. Kowalska A. Zwyczaje żywieniowe studentów Uniwersytetu Ekonomicznego we Wrocławiu. *Roczn PZH.* 2010;61(3):277-282.
22. Mieziene B, Emeljanovas A, Fatkulina N, Stukas R. Dietary Pattern and Its Correlates among Lithuanian Young Adults: Mediterranean Diet Approach. *Nutrients.* 2020;12(7):2025. doi: 10.3390/nu12072025
23. Cobo-Cuenca AI, Garrido-Miguel M, Soriano-Cano A, Ferri-Morales A, Martínez-Vizcaíno V, Martín-Espinosa NM. Adherence to the Mediterranean Diet and Its Association with Body Composition and Physical Fitness in Spanish University Students. *Nutrients.* 2019;11(11):2830. doi: 10.3390/nu11112830

Wydawnictwo UR 2023
ISSN 2544-1361 (online)
doi: 10.15584/ejcem.2023.3.10

http://www.ejcem.ur.edu.pl
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2023; 21 (3): 492–499

ORIGINAL PAPER

The correlation between nursing students' levels of fear and stress related to the COVID-19 pandemic and their compliance with standard precautions

Fatma Yilmaz Kurt , Naile Alankaya , Selma Atay , Sevda Efil

Department of Nursing, Faculty of Health Sciences, Canakkale Onsekiz Mart University, Canakkale, Turkey

ABSTRACT

Introduction and aim. University students have been demonstrated in the literature to be a group particularly vulnerable to mental health problems during the COVID-19 pandemic. The aim of this study was to examine the correlation between the fear and anxiety levels of nursing students regarding the COVID-19 pandemic and their compliance with standard precautions.

Material and methods. The sample consisted of 509 volunteer students. Data were collected using a Personal Information Form, the Fear of COVID-19 Scale (FCV-19S), the COVID-19 Anxiety Scale (CAS) and the Compliance with Standard Precautions Scale (CSPS).

Results. The FCV-19S, CAS, and CSPS mean scores of the students were respectively 17.49±5.24 1.99±3.54 and 13.46±3.61. There was a moderate correlation ($p<0.05$) between the FCV-19S and CAS of the students, but no correlation was detected between CSPS and FCV-19S ($p>0.05$) and CAS ($p>0.05$).

Conclusion. It was determined that students had a moderate fear of COVID-19 and a low level of anxiety over COVID-19. However, fear of COVID-19 and COVID-19 anxiety levels were not correlated with compliance with standard precautions.

Keywords. anxiety, COVID-19, fear, nursing, pandemic, standard precautions

Introduction

The coronavirus infection (Coronavirus disease 2019, COVID-19) has affected the entire world, and has continued to have a physiological and psychological impact on health.[1] Satıcı et al., determined that the fear of COVID-19 negatively affected life satisfaction, and this may be associated with depression, anxiety, and stress.[2] In the literature, it is reported that the fear of COVID-19 decreased life satisfaction[3], had a negative effect on an individual's well-being, and caused negative mental health conditions, such as stress, depression, and anxiety.[4-6] Being physically healthy or not infected with coronavirus does not prevent a person from suffering unfavorable psychological emotions caused by COVID-19.[7] Anxiety and fear can be caused by the severity of the disease, its contribution to mortality, and the risk of transmission.[8-10] Addition of uncertainty into psychological problems may impair individual well-being and lead to maladaptive behaviors.[11,12] Korukçu et al. determined that COVID-19 infection caused a high level of fear in individuals living in Turkey.[13] Another study found that nurses were fearful of contracting an infection or unintentionally infecting others.[14]

Psychological and emotional factors experienced during the pandemic may adversely affect fighting against it and make it more difficult to exhibit healthy

Corresponding author: Sevda Efil, e-mail: sevda_efil@hotmail.com, drefl.sevda@yahoo.com

Received: 18.04.2023 / Revised: 15.05.2023 / Accepted: 30.05.2023 / Published: 30.09.2023

Kurt FY, Alankaya N, Atay S, Efil S. *The correlation between nursing students' levels of fear and stress related to the COVID-19 pandemic and their compliance with standard precautions*. Eur J Clin Exp Med. 2023;21(3):492–499. doi: 10.15584/ejcem.2023.3.10.

lifestyle behaviors.[15,16] University students have been demonstrated in the literature to be a group which has been particularly vulnerable to mental health problems during the COVID-19 pandemic.[17-20] The number of psychological disorders observed in university students during the pandemic has increased compared to previous years. Fear can be increased by concerns about the risk of being infected. It is important to determine the fear of contracting COVID-19 infection and to understand the associated risks.[21] One study reported that there was a high level of COVID-19 anxiety and fear among nursing students.[22] Cici and Yılmazel found that nursing students had high levels of anxiety due to the COVID-19 pandemic and their professional perspectives were adversely affected by the pandemic.[23] Huang et al. determined that nurses and nursing students' anxiety, fear, sadness, anger, and coping strategies were affected during the COVID-19 pandemic.[24]

Nursing students are exposed to patient contact during their clinical practices to gain professional experience. This poses a risk of infection unless infection control precautions are taken.[25] During a pandemic, applying standard preventive measures is the most important factor in reducing cross-transmission between health workers and patients.[26] It has been reported that during the COVID-19 pandemic, nurses' conformity to standard measures was inadequate.[27,28] Therefore, it is important to comply with standard precautions in the fight against COVID-19, which persists as a global threat to health.[7] Controlling infection is critical for patient and employee safety. It is also an indicator of the delivery of high-quality nursing care. Compliance with standard precautions is important in preventing infection from being transmitted. Standard precautions are universally recognized guidelines for infection control in health care practices.[29] Topçu and Emlek found that nursing students' conformity to standard preventive measures during the pandemic was at an optimal level.[30] It was stated in a study in China that health students' personal preventive measures and maintenance of social distancing was better during the pandemic.[31] It has been found that there was no correlation between nurses' fear of COVID-19 and their attitudes to patient safety, and that nurses at a younger age had negative attitudes to patient safety.[32] The fear and stress associated with COVID-19 may make it difficult for students who are future nurses to comply with standard precautions. To the best of our knowledge, there is no study in the literature that investigates the correlation between the level of fear and anxiety among nursing students during the COVID-19 pandemic and their compliance with standard precautions. For this reason, the correlation between nursing students' fear and stress levels relating to the COVID-19 pandemic and their conformity to standard preventive measures was examined in this study.

Aim

The aim of the study was to examine the correlation between the fear and anxiety levels of nursing students during the COVID-19 pandemic and their compliance with standard precautions.

Research questions

- What are the levels of fear in nursing students during the COVID-19 pandemic?
- What are the levels of anxiety in nursing students during the COVID-19 pandemic?
- What is the level of compliance of nursing students with standard precautions during the COVID-19 pandemic?
- Is there a correlation between the nursing students' levels of fear of COVID-19 and compliance with standard precautions?
- Is there a correlation between the nursing students' levels of anxiety of COVID-19 and their compliance with standard precautions?

Material and methods

Ethics approval

Ethical approval was obtained from the Scientific Research Ethics Committee of the relevant university (decision no: E-84026528-050.01.04-2100216470, dated 4 November 2021). Written approval (dated 9 November 2021) was obtained from the COVID-19 Scientific Research Assessment Commission under the Republic of Turkey Ministry of Health. In accordance with the Declaration of Helsinki, the objectives and content of the study were explained to the nursing students in the sample. Written permission was obtained by e-mail from the researchers to use the scales in the collection of data. They were informed that their participation was entirely voluntary, and their personal information and privacy would be protected. The data was created using Google Drive during the pandemic, and the survey invitation was given an online survey link via WhatsApp, making it clear that the participation was voluntary. Informed consent was obtained from all participants.

Study design and participants

The study was conducted as descriptive and correlational research. Data collection was performed between 10 November and 31 December 2021 using an online Google questionnaire. The population of the study was comprised of nursing students who attended a university in Turkey providing nursing education during the fall term of the academic year 2021-2022. The study was conducted at the Faculty of Health Sciences. The program Epi Info™ version 7.2.3.1, USA: American Centers for Disease Control and Prevention (CDC) 2022, was used for sample size calculation. Since there was no similar study, the expected and observed values were accepted

as 50% respectively. The sample size was calculated using the sample formula with an unknown population. According to this formula, the number of students required be included in the study was calculated as 384 with a Type I error (alpha=.05) and power of 95%.[33] The study included second, third and fourth year nursing students who accessed the questionnaire sent via the internet, and who volunteered to participate in the study, fully completed the questionnaire, and were actively studying during the time of the study. Those who were not active students in classes at the time of the study and also first-year students were excluded from in the study. Students perform clinical practice in Medical Nursing in the second year, Gynecology and Obstetrics Nursing in the third year, and Psychiatric Nursing in the fourth year during the fall semester. The first-year students begin their first hospital practice in the spring semester, and therefore, they were excluded from the study. The study was completed with 509 students.

Data collection tools

Four main questionnaires were used for the current study.

Personal information form

This form consisted of nine questions about the socio-demographic characteristics of students who participated in the study.[22,34,35]

Fear of COVID-19 scale (FCV-19S)

The Turkish language validity and reliability of the FCV-19S, which was developed by Ahorsu et al., was tested by Ladikli et al.[8,36] FCV-19S has a single dimension and seven items. The scale is scored as a five-point Likert type scale ranging between 5= "strongly agree" and 1= "strongly disagree". The total score of the scale ranges from 7 to 35. Scores at or above the cut-off point of the scale signify that individuals have a fear of COVID-19. The internal consistency coefficient of the scale was found to be 0.82 in the study by Ahorsu and 0.86 in the study by Ladikli et al. The internal consistency alpha coefficient of the scale was found to be 0.76 in this study.

COVID-19 anxiety scale (CAS)

Lee developed the COVID-19 Anxiety Scale to determine the levels of anxiety associated with the crises created by the coronavirus.[37] Evren et al. conducted the Turkish validity and reliability study of the scale.[38] The CAS, a five-point Likert-type scale, has one dimension and five items. The items are rated as 0: "Never", 1: "rare, less than one or two days", 2: "a few days", 3: "more than seven days", and 4: "almost every day in the last two weeks". A total CAS score of ≥ 9 indicates dysfunctional anxiety associated with coronavirus. An increase in the total CAS score indicates that individuals have a high level of anxiety, whereas a decrease in the total CAS score implies that individuals have a low level of anxiety. The internal consistency coefficient of the scale was found to be 0.93 in the study by Lee, and 0.80 in the study by Evren et al. The internal consistency alpha coefficient of the scale was 0.93 in this study.

Compliance with standard precautions scale (CSPS)

The CSPS is a scale intended to reveal compliance with standard preventive precautions, assessed within the scope of precautions that healthcare professionals must take for infection control. In other words, it is a self-assessment tool used by healthcare professionals to determine whether or not they exhibit protective and safe behaviors in infection control and prevention. In 2011, Simon Ching Lam developed the scale, based on international preventive precautions published by the CDC.[39] Samur et al. assessed the Turkish validity and reliability study of the scale.[40] A four-point Likert-type scale with one dimension is rated as 1: "Never", 2: "rarely", 3: "sometimes", and 4: "always". In the assessment of the scale, the "always" response to positively scored items (items 1, 3, 5, 7, 8, 9, 10, 11, 12, 13, 14, 16, 17, 18, 19, and 20) is coded with "1" and the other responses are "0". The "never" response to negatively scored items (items 2, 4, 6, and 15) is coded as "1," while the remaining responses are coded as "0" on the scale. Total score ranges from 0 to 20. Higher scores signify that compliance with standard precautions increases. The internal coefficient of consistency of the scale was found to be 0.79 in the study by Lam, and 0.84 in the study by Samur et al. The internal consistency alpha coefficient of the scale was 0.76 in this study.

Data collection

The data were collected between 10 November 2021 and 31 December 2021 from students who met the inclusion criteria. The students were reached through a closed online communications program that they used to communicate with each other, and a survey link to the study was shared. The first page of the online survey includes an informed consent form that explains the objective of the study, that participation in the study voluntary, and that all personal data will be kept confidential. The data collection form was resumed after acquiring the consent of the students who volunteered to participate in this study. It took roughly 8-10 minutes to complete the survey electronically. Each student was only allowed to fill out the survey once.

Statistical analysis

The data in this study were analyzed using SPSS 22.0 (IBM Corp., Released 2013, IBM SPSS Statistics for Windows, Version 22.0, Armonk, NY: IBM Corp.). The

data were presented as mean ± SD deviation, median, and minimum and maximum values for variables determined by measuring, and as numbers and percentages for variables determined by counting. The Kolmogorov Smirnov Test was used to analyze whether or not the sample had normal distribution in comparative statistics. The Mann Whitney U Test was used to compare continuous data with independent variables with two groups, while the Kruskal Wallis Analysis of Variance was used to compare continuous data with more than two groups. The Kruskal Wallis Analysis Multiple Comparison Test was used to analyze which group or groups caused the difference. The correlation between the scores of scales was determined using Spearman's Correlation Analysis. The statistical significance level was accepted as $p<0.05$.

Results

It was determined that 78.4% of the students were female and 49.8% were second-year students. 82.2% of the students reported that they had not been diagnosed with COVID-19 and 98% stated that they had the COVID-19 vaccine. Also, 58.5% of the students stated that there was an infection prevention procedure in the clinic where they completed their internship, and 56.8% stated that they had previously attended a seminar/training for infection control and prevention. Furthermore, 80.1% of the students stated that they had been trained for standard precautions before starting to work in the clinic.

The students who participated in the study had a moderate fear of COVID-19 according to their FCV-19S mean scores (17.49±5.24), they had a low level of dysfunctional anxiety according to their CAS mean score (1.99±3.54), and their compliance with standard precautions was high according to their CSPS mean scores (13.46±3.61) (Table 1).

Table 1. Mean scores of the students on FCV-19S, CAS, and CSPS*

	Number of items	Mean± SD	Min-Max
FCV-19S	7	17.49±5.24	8–33
CAS	5	1.99±3.54	0–20
CSPS	20	13.46±3.61	0–20

* FCV-19S – fear of COVID-19 scale; CAS – COVID-19 anxiety scale; CSPS – compliance with standard precautions scale

When characteristics of the students were compared with their FCV-19S, CAS and CSPS mean scores, it was observed that the mean scores of female students for all three scales (18.03±5.26; 2.20±3.78; 13.57±3.44) were higher than the mean scores of male students (15.57±4.70; 1.23±2.42; 13.03±4.18 respectively). However, while there was a statistically significant difference in FCV-19S and CAS mean scores between female and male students, there was no statistically significant difference between their CSPS mean scores. Among the other variables, there was no difference between the students' grades, statuses of having COVID-19, statuses of being vaccinated with COVID-19 vaccine, previous training on infection control and prevention, or mean scores of FCV-19S, CAS, and CSPS (Table 2).

Table 2. Comparison of students' characteristics with their FCV-19S, CAS and CSPS mean scores[a]

	FCV-19S	CAS	CSPS
	Mean ± SD	Mean ± SD	Mean ± SD
Gender			
Female	18.03±5.26	2.20±3.78	13.57±3.44
Male	15.57±4.70	1.23±2.42	13.03±4.18
Test & p	15549.0[b] 0.000*	17973.0[b] 0.003*	20693.0[b] 0.517
Year			
2nd Year	17.37±5.23	1.88±3.39	13.64±3.63
3rd Year	18.16±5.58	2.36±4.15	13.34±3.54
4th Year	16.84±4.66	1.74±2.94	13.19±3.68
Test & p	3.216[c] 0.200	0.255[c] 0.880	2.440[c] 0.295
Diagnosis of COVID 19			
Yes	16.86±5.08	2.14±3.26	14.04±3.44
No	17.62±5.27	1.96±9.61	13.33±3.65
Test & p	16708.5[b] 0.188	17258.0[b] 0.340	16095.5[b] 0.069
Yes	17.54±5.27	2.02±3.57	13.50±3.58
No	14.70±3.49	0.60±1.26	11.20±4.63
Test & p	1680.5[b] 0.085	1934.0[b] 0.204	1625.5[b] 0.065
Seminar/training for infection control and prevention			
Yes	17.09±4.74	1.70±3.09	13.47±3.67
No	18.00±5.79	2.36±4.05	13.43±3.55
Test & p	28619.0[b] 0.152	28719.5[b] 0.133	30538.0[b] 0.810

[a] FCV-19S – fear of COVID-19 scale; CAS – COVID-19 anxiety scale; CSPS – compliance with standard precautions scale; c – Kruskal Wallis; b – Mann Whitney U; *– $p<0.05$

When the correlation between FCV-19S, CAS and CSPS was examined, a moderately significant correlation was found between FCV-19S and CAS ($p<0.05$). No correlation was found between CSPS and FCV-19S ($p>0.05$) and CAS ($p>0.05$) (Table 3).

Table 3. Correlation between FCV-19S, CAS, and CSPS[a]

		FCV-19 S	CAS	CSPS
FCV-19S	R	1.000	0.487*	0.075
	p	…..	0.000	0.09
CAS	R	0.487*	1.000	-0.073
	p	0.000	…..	0.100
CSPS	R	0.075	- 0.073	1.000
	p	0.092	0.100	……

[a] FCV-19S – fear of COVID-19 scale; CAS – COVID-19 anxiety scale; CSPS – compliance with standard precautions scale; * – Spearman's correlation analysis

Discussion

The rapid spread and pandemic nature of COVID-19 caused anxiety, depression and fear in individuals.[8] Nurses in general experience stress in relation to COVID-19, but it is seen that younger nurses have greater difficulty in coping with the pandemic.[22,41,42] Also, the negative psychological effect on them may constitute a risk to their care behaviors.[41] This study was conducted to examine the correlation between the fear and anxiety levels of nursing students during the COVID-19 pandemic and their compliance with standard precautions. Based on their FCV-19S mean score (17.49±5.24), it can be asserted that the nursing students who participated in the study had a moderate fear of COVID-19. This result of the study was thought to be associated with the fact that the data was collected in the later period of the pandemic. The students may have been informed about COVID-19 and its repercussions, prevention methods, and its impacts on life during this period. In the literature, there are studies indicating that nursing students have a moderate or high level of fear of COVID-19.[14,43] The results of studies conducted in Turkey, Spain and Israel have indicated that the fear of COVID-19 is high among nursing students.[19,22,43]

While it is believed that fear and anxiety share similar features, they are essentially two concepts that exist independently of one another but have an effect on one another. Some studies have reported that the pandemic has considerably affected both the fear and anxiety levels of students.[17,22] Although nursing students had a moderate fear of COVID-19 and a low level of dysfunctional anxiety in this study, a moderately significant correlation was found between FCV-19S and CAS, and as the FCV-19S mean scores of the students increased, the mean scores of CAS also increased. These results were associated with the fact that nursing students did not go to the hospital for clinical practices, they avoided transmission of hospital-acquired infection and they stayed with their families during the pandemic. In contrast to the findings of the present study, a study by Kuru Alici et al. found that the anxiety levels of students were high, and stated that this could be attributed to the high rate of COVID-19 transmission, the fear of death, and the lack of knowledge of the students in terms of preventative precautions and behaviors.[22] Huang et al. stated that the fear of contracting COVID-19 increased anxiety during the pandemic.[24] On the other hand, Yuan et al. asserted that active use of social media by students and sharing knowledge or experiences with one another were effective in reducing anxiety.[44]

Nurses' level of knowledge of and compliance with standard precautions are very important for improving health services, reducing care costs and increasing patient satisfaction. The CSPS mean score of nursing students was found to be high in the study. It is important for nursing students to avoid the risk of catching COVID-19 during clinical practice.[25] The students get training on standard precautions and procedures such as infection prevention, hand washing, and the use of personal protective equipment beginning in their first year of nursing undergraduate school in Turkey. It can be argued that it is also effective for the students to attend applied nursing courses and some elective courses (Communicable Diseases Nursing, Hospital-Acquired Infections, etc.) to familiarize themselves with subjects related to standard precautions prior to beginning clinical practice. Studies conducted in different parts of the world have reported that nursing students have different levels of compliance with standard precautions. The level of compliance with standard precautions was reported to be relatively high in studies conducted with nursing students in China and Jordan.[25,45] A large-scale study including 4439 healthcare professionals at 34 hospitals in France revealed that the compliance of nursing students with standard precautions was higher than that of other working healthcare professionals.[46]

The FCV-19S and CAS mean scores of female students in the study were found to be higher than the scores of the male students. It can be argued that this was associated with gender traits or the fact that women are more emotionally vulnerable and sensitive than men. Cheng et al. found that male students experienced a lower level of stress compared to female students.[31] Studies by Kuru Alici et al. and Savitsky et al. also yielded similar results.[22,43] However, in a study conducted by Sun et al. (2020) in China, higher levels of fear and anxiety were found in men.[47] Some studies conducted after other epidemics (SARS, Ebola, MERS) stated that women were more negatively affected by the epidemic than men, and that they suffered from higher levels of anxiety and depression.[15,48] Sun et al. stated that women stayed at home during the pandemic and cleaned the house, met the needs of family members, cooked and fulfilled other household chores, all of which increased the burden on women, thus resulting in increasing fear and anxiety.[47] The fact that the majority of the nursing students were female is compatible with these results.

Yasemin et al. found that the COVID-19 pandemic affected nursing students' mental health and eating habits.[49] It is also known that nursing students' academic success is affected by the fear which they experience, their sleep quality is affected and they experience the intention to leave the nursing school.[50,51] The fact that nurses work in a risky environment in the pandemic and experience deficiencies in professional knowledge and skill may be the reason for this. Therefore, the correlation between the stress and fear experienced by nursing students and their conformity to standard protective measures was considered, and it was found that there was no correlation between the COVID-19 fear

and stress which they experienced and their conformity to standard protective measures. Understanding these issues is necessary because nursing students are expected to play a larger role in the current crisis than lay people by volunteering, educating the public and increasing awareness of the COVID-19 pandemic.[52]

Study limitations

The research was conducted during the pandemic period and all responses were collected online. For this reason, the data obtained from the study is limited to students who had internet access and could answer and agree to participate in the research.

Conclusion

In conclusion, it was determined that the levels of fear of COVID-19 of the nursing students were moderate, their levels of anxiety concerning COVID-19 were low, and female students had higher levels of fear and anxiety than male students. Also, the students' levels of compliance with standard precautions were relatively high. The correlation between FCV-19S and CAS was moderately significant. No correlation was found between CSPS and FCV-19S and CAS. Given these findings, it is recommended that students attend courses on coping with fear and anxiety during epidemics, psychological awareness, and resilience during their nursing undergraduate education.

Acknowledgments

The authors thank all students who participated in the study.

Declarations

Funding

This research received no external funding.

Author contributions

Conceptualization, F.Y.K., N.A., S.A. and S.E.; Methodology, F.Y.K., N.A., S.A. and S.E; Software, F.Y.K., N.A., S.A. and S.E; Validation, F.Y.K., N.A., S.A. and S.E; Formal Analysis, F.Y.K., N.A., S.A. and S.E; Investigation, F.Y.K., N.A., S.A. and S.E; Resources, F.Y.K., N.A., S.A. and S.E; Data Curation, F.Y.K., N.A., S.A. and S.E; Writing Original Draft Preparation, F.Y.K., N.A., S.A. and S.E; Writing – Review & Editing, F.Y.K., N.A., S.A. and S.E Visualization, F.Y.K., N.A., S.A. and S.E; Supervision, F.Y.K., and S.E; Project Administration, F.Y.K., N.A., S.A. and S.E.

Conflicts of interest

All authors declare that they have no conflicts of interest.

Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval

Ethical approval was obtained from the Scientific Research Ethics Committee of the relevant university (decision no: E-84026528-050.01.04-2100216470). Written approval (dated 09.11.2021) was obtained from the COVID-19 Scientific Research Assessment Commission under the Republic of Turkey Ministry of Health

References

1. Saladino V, Algeri D, Auriemma V. The Psychological and social impact of Covid-19: New perspectives of well-being. *Front Psychol.* 2020;11:577684. doi: 10.3389/fpsyg.2020.577684
2. Satici B, Gocet-Tekin E, Deniz ME, et al. Adaptation of the Fear of COVID-19 Scale: Its association with psychological distress and life satisfaction in Turkey. *Int J Ment Health Addic.* 2021;19(6):1980-1988. doi: 10.1007/s11469-020-00294-0
3. Krok D, Zarzycka B, Telka E. Risk of contracting covid-19, personal resources and subjective well-being among healthcare workers: The mediating role of stress and meaning-making. *J Clin Med.* 2021;10(1):132. doi: 10.3390/jcm10010132
4. Harper CA, Satchell L, Fido D, et al. Functional fear predicts public health compliance in the COVID-19 pandemic. *Int J Ment Health Addict.* 2021;19(5):1875-1888. doi: 10.1007/s11469-020-00281-5
5. Catton H. Global challenges in health and health care for nurses and midwives everywhere. *Int Nurs Re.* 2020;67(1):4-6. doi: 10.1111/inr.12578
6. Mo Y, Deng L, Zhang L, et al. Work stress among Chinese nurses to support Wuhan in fighting against COVID-19 epidemic. *J Nurs Manag.* 2020;28(5):1002-1009. doi: 10.1111/jonm.13014
7. Dymecka J, Gerymski R, Machnik-Czerwik A, et al. Fear of COVID-19 and life satisfaction: The role of the health-related hardiness and sense of coherence. *Front Psychiatry.* 2021;12:712103. doi: 10.3389/fpsyt.2021.712103
8. Ahorsu DK, Lin CY, Imani V, et al. The fear of COVID-19 scale: Development and initial validation. *Int J Ment Health Addict.* 2020;27:1-9. doi:10.1007/s11469-020-00270-8
9. Arpaci I, Karataş K, Baloğlu M. The development and initial tests for the psychometric properties of the COVID-19 Phobia Scale (C19P-S). *Pers Individ Dif.* 2020;164:110108. doi: 10.1016/j.paid.2020.110108
10. Erdoğdu Y, Koçoğlu F, Sevim C. An investigation of the psychosocial and demographic determinants of anxiety and hopelessness during COVID-19 pandemic. *J Clin Psy.* 2020;23(1): 24-37. doi:10.5505/kpd.2020.35403
11. Holmes EA, O'Connor RC, Perry VH, et al. Mental illness during COVID-19. *Lancet Psychiatry*. 2020;7(6):547-560. doi: 10.1016/S2215-0366(20)30168-1
12. Li Y, Wang Y, Jiang J, et al. Psychological distress among health professional students during the COVID-19 outbreak. *Psychol Med.* 2021;51(11):1952-1954. doi: 10.1017/S0033291720001555

13. Korukcu O, Ozkaya M, Faruk Boran O, et al. The effect of the COVID-19 pandemic on community mental health: A psychometric and prevalence study in Turkey. *Health Soc Care Community.* 2021;29(5):e204-e213. doi: 10.1111/hsc.13270
14. Labrague LJ, de los Santos JAA. Fear of COVID-19, psychological distress, work satisfaction and turnover intention among frontline nurses. *J Nurs Manag.* 2021;29(3):395-403. doi: 10.1111/jonm.13168
15. Martínez-Lorca M, Martínez-Lorca A, Criado-Álvarez JJ, et al. The fear of COVID-19 scale: Validation in spanish university students. *Psychiatry Res.* 2020;293:113350. doi: 10.1016/j.psychres.2020.113350
16. Kuśnierz C, Rogowska AM, Kwaśnicka A, et al. The mediating role of orthorexia in the relationship between physical activity and fear of covid-19 among university students in Poland. *J Clin Med.* 2021;10(21):5061. doi: 10.3390/jcm10215061
17. Aslan I, Ochnik D, Çınar O. Exploring perceived stress among students in Turkey during the covid-19 pandemic. *Int J Environ Res Public Health.* 2020;17(23):8961. doi: 10.3390/ijerph17238961
18. Campos R, Pinto V, Alves D, et al. Impact of COVID-19 on the mental health of medical students in Portugal. *J Pers Med.* 2021;11(10):986. doi: 10.3390/jpm11100986
19. Muyor-Rodríguez J, Caravaca-Sánchez F, Fernández-Prados JS. Covid-19 fear, resilience, social support, anxiety, and suicide among college students in Spain. *Int J Environ Res Public Health.* 2021;18(15):8156. doi: 10.3390/ijerph18158156
20. Ochnik D, Rogowska AM, Kuśnierz C, et al. A comparison of depression and anxiety among university students in nine countries during the covid-19 pandemic. *J Clin Med.* 202129;10(13):2882. doi: 10.3390/jcm10132882
21. Deng J, Zhou F, Hou W, et al. The prevalence of depressive symptoms, anxiety symptoms and sleep disturbance in higher education students during the COVID-19 pandemic: A systematic review and meta-analysis. *Psychiatry Res.* 2021;301:113863. doi: 10.1016/j.psychres.2021.113863
22. Kuru Alici N, Ozturk Copur E. Anxiety and fear of COVID-19 among nursing students during the COVID-19 pandemic: A descriptive correlation study. *Perspect Psychiatr Care.* 2022;58(1):141-148. doi: 10.1111/ppc.12851
23. Cici R, Yilmazel G. Determination of anxiety levels and perspectives on the nursing profession among candidate nurses with relation to the COVID-19 pandemic. *Perspect Psychiatr Care*. 2021;57(1):358-362. doi: 10.1111/ppc.12601
24. Huang L, Lei W, Xu F, et al. Emotional responses and coping strategies in nurses and nursing students during Covid-19 outbreak: A comparative study. *PLoS One.* 2020;15(8):e0237303. doi: 10.1371/journal.pone.0237303
25. Swift A, Banks L, Baleswaran A, et al. COVID-19 and student nurses: A view from England. *J Clin Nurs.* 2020;29(17-18):3111-3114. doi: 10.1111/jocn.15298
26. Wong EL, Ho KF, Dong D, et al. Compliance with Standard Precautions and Its Relationship with Views on Infection Control and Prevention Policy among Healthcare Workers during COVID-19 Pandemic. *Int J Environ Res Public Health.* 2021;25;18(7):3420. doi: 10.3390/ijerph18073420
27. Berdida DJE. Nursing staff compliance and adherence to standard precautions during the COVID-19 pandemic: A cross-sectional study. *Nurs Health Sci.* 2023;25(1):108-119. doi: 10.1111/nhs.12998
28. Dobrina R, Donati D, Giangreco M, et al. Nurses' compliance to standard precautions prior to and during COVID-19. *Int Nurs Rev.* 2023;25. doi: 10.1111/inr.12830
29. Cheung K, Chan CK, Chang MY, et al. Predictors for compliance of standard precautions among nursing students. *Am J Infect Control.* 2015;43(7):729-734. doi: 10.1016/j.ajic.2015.03.007
30. Topçu S, Emlek Sert Z. Turkish nursing students' compliance to standard precautions during the COVID-19 pandemic. *Peer J.* 2023;16;11:e15056. doi: 10.7717/peerj.15056
31. Cheng WLS, Kwong EWY, Lee RLT, et al. Compliance with Standard Precaution and Its Relationship with Views on Infection Control and Prevention Policy among Chinese University Students during the COVID-19 Pandemic. *Int J Environ Res Public Health.* 2022;27;19(9):5327. doi: 10.3390/ijerph19095327
32. Efil S, Türen S, Demir S. Nurses' COVID-19 fears and patient safety attitudes in the pandemic. *Eur J Clin Exp Med.* 2023;21(1):19-26. doi: 10.15584/ejcem.2023.1.3
33. Erdogan S, Nahcıvan N, Esin MN. *Research in Nursing: Process, Practice, Critical*. Istanbul: Nobel Tıp Kitabevleri; 2014:186
34. Alsolais A, Alquwez N, Alotaibi KA, et al. Risk perceptions, fear, depression, anxiety, stress and coping among Saudi nursing students during the COVID-19 pandemic. *J Ment Health.* 2021;30(2):194-201. doi: 10.1080/09638237.2021.1922636
35. van Gulik N, Bouchoucha S, Apivanich S, et al. Factors influencing self-reported adherence to standard precautions among Thai nursing students: A cross sectional study. *Nurse Educ Pract.* 2021;57:103232. doi: 10.1016/j.nepr.2021.103232
36. Ladikli N, Bahadır E, Yumuşak FN, et al. The reliability and validity of Turkish version of coronavirus anxiety scale. *International Journal of Social Sciences.* 2020;3(2):71-80.
37. Lee SA. Coronavirus Anxiety Scale: A brief mental health screener for COVID-19 related anxiety. *Death Stud.* 2020;44(7):393-401. doi: 10.1080/07481187.2020.1748481
38. Evren C, Evren B, Dalbudak E, et al. Measuring anxiety related to COVID-19: A Turkish validation study of the Coronavirus Anxiety Scale. *Death Stud*. 2020:46(5):1052-1058. doi: 10.1080/07481187.2020.1774969
39. Lam SC. Universal to standard precautions in disease prevention: Preliminary development of compliance scale for clinical nursing. *Int J Nurs Stud.* 2011;48(12):1533-1539. doi: 10.1016/j.ijnurstu.2011.06.009

40. Samur M, Seren Intepeler S, Lam SC. Adaptation and validation of the Compliance with Standard Precautions Scale amongst nurses in Turkey. *Int J Nurs Pract.* 2020;26(3):e12839. doi: 10.1111/ijn.12839
41. Efıl S, Turen S, Yıldız Ayvaz M, et al. Burnout levels and care behaviours in intensive care nurses: A cross-sectional, multicentre study. *Intensive Crit Care Nurs.* 2022;71:103246. doi: 10.1016/j.iccn.2022.103246
42. Wang C, Pan R, Wan X, et al. A longitudinal study on the mental health of general population during the COVID-19 epidemic in China. *Brain Behav Immun.* 2020;87:40-48. doi: 10.1016/j.bbi.2020.04.028
43. Savitsky B, Findling Y, Ereli A, et al. Anxiety and coping strategies among nursing students during the covid-19 pandemic. *Nurse Educ Pract.* 2020;46:102809. doi: 10.1016/j.nepr.2020.102809
44. Yuan T, Liu H, Li XD, et al. Factors affecting infection control behaviors to prevent COVID-19: An online survey of nursing students in Anhui, China in March and April 2020. *Med Sci Monit.* 2020;26:e925877. doi: 10.12659/MSM.925877
45. AL-Rawajfah OM, Tubaishat A. Nursing students' knowledge and practices of standard precautions: A Jordanian web-based survey. *Nurse Educ Today.* 2015;*35*(12):1175-1180. doi: 10.1016/j.nedt.2015.05.011
46. Atif ML, Brenet A, Hageaux S, et al. Awareness of standard precautions for 4439 healthcare professionals in 34 institutions in France. *Med Mal Infect.* 2013;43(1):10-16. doi: 10.1016/j.medmal.2012.11.004
47. Sun Y, Wang D, Han Z, et al. Disease prevention knowledge, anxiety, and professional identity during COVID-19 pandemic in nursing students in Zhengzhou, China. *J Korean Acad Nurs.* 2020;50(4):533-540. doi: 10.4040/jkan.20125
48. Lehmann M, Bruenahl CA, Löwe B, et al. Ebola and psychological stress of health care professionals. *Emerg Infect Dis.* 2015;21(5):913-914. doi: 10.3201/eid2105.141988
49. Kalkan Uğurlu Y, Mataracı Degirmenci D, Durgun H, et al. The examination of the relationship between nursing students' depression, anxiety and stress levels and restrictive, emotional, and external eating behaviors in COVID-19 social isolation process. *Perspect Psychiatr Care.* 2021;57(2):507-516. doi: 10.1111/ppc.12703
50. Okwuikpo M, Abazie O. Adetunji A, et al. Coronavirus: nursing students' knowledge and risk perception of clinical practice during the pandemic. *Nurs Midwifery Stud.* 2021;10(2):107-113. doi: 10.4103/nms.nms_76_20
51. De Los Santos JAA, Labrague LJ, Falguera CC. Fear of COVID-19, poor quality of sleep, irritability and intention to quit school among nursing students: a cross-sectional study. *Perspect Psychiatr Care.* 2022;58(1):71-78. doi: 10.1111/ppc.12781
52. Mustafa RM, Alrabadi NN, Alshali RZ, et al. Knowledge, attitude, behavior and stress related to COVID-19 among undergraduate health care students in Jordan. *Eur J Dent.* 2020;14:S50-S55. doi: 10.1055/s-0040-1719212

Wydawnictwo UR 2023
ISSN 2544-1361 (online)
doi: 10.15584/ejcem.2023.3.11

http://www.ejcem.ur.edu.pl
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2023; 21 (3): 500–505

ORIGINAL PAPER

Estimation of age by mental foramen using CBCT in central India

Sourav Bose [1], Jaideep Sur [1], Fatima Khan [1], Deeplaxmi Dewangan [1], Sushmita Paul [2], Ekta Sawriya [1], Ayesha Roul [1]

[1] Department of Oral Medicine and Radiology, Rungta College of Dental Sciences and Research, Bhilai, Chattisgarh, India
[2] Institute of Dental Sciences and Research, SOA University, Odisha, India

ABSTRACT

Introduction and aim. This study aims to estimate the age of a population in Central India by analyzing the mental foramen using cone beam computed tomography (CBCT). The objectives of the study are to determine the parameters for age determination and study bilateral variations in mental foramen dimensions using CBCT.

Material and methods. One hundred and twenty CBCT scans, HD LED monitor, GALAXIS GALLIEOS viewer.

Results. Results from the study show significant values for various parameters such as the upper and lower borders of the mandible and mental foramen. Bilateral variations were also observed. The age regression model indicates a significant correlation between estimated and original ages for individuals aged between 31 to 50 years. While the study only analyzed five parameters of the mental foramen, it suggests that a more comprehensive assessment of mental foramen parameters with a larger sample size can yield more definitive results for age determination.

Conclusion. As only five parameters of mental foramen had been assessed in this retrospective study, so a comprehensive assessment of various other parameters of mental foramen with an increased sample size may be done for more definitive results for gender and age determination.

Keywords. cone-beam computed tomography, forensic science, mental foramina

Introduction

In the field of forensic dentistry, determining the age of severely deteriorated and obscure skeletal remains play an extremely significant role in the recognizable proof. Age can be resolved from various parts of the facial skeleton; mandible is one of them.[1-3] A more durable nature of this bone makes it a reasonable tool for age estimation. Mandible continues in an all-around protected state longer than some other bone because of the presence of a dense layer of cortical bone.[3-5] As a result, forensic anthropologists and criminological dental specialists primarily use the morphological features of the mandible to determine age. There are various mandibular criteria that can be used to determine the age of the obscure skeleton. Mental foramen is one of the mandibular components that can be quite helpful in determining age. Radiographs can be used to survey the morphological components of the mental foramen in both living and deceased people. Panoramic imaging, readily demonstrates the mental foramen.[4,5] Straight (linear), perpendicular, and anterior loop (AL) patterns have been observed in the mental foramen.[4-6] The existence of AL has been mentioned in the literature on rare occasions. CBCT is widely regarded as the gold standard in radiological imaging. Because CBCT can produce high-resolution three-dimensional images, it can detect accessory canals with small diameters and those that bifurcate in any direction.[6-8] Many researches have been published in panoramic imaging about age as-

Corresponding author: Sourav Bose, e-mail: souravbose89@gmail.com

Received: 15.04.2023 / Revised: 28.05.2023 / Accepted: 30.05.2023 / Published: 30.09.2023

Bose S, Sur J, Khan F, Dewangan D, Paul S, Sawriya E, Roul A. *Estimation of age by mental foramen using CBCT in central India.* Eur J Clin Exp Med. 2023;21(3):500–505. doi: 10.15584/ejcem.2023.3.11.

sessment using the mental foramen, and a lesser number of studies have been reported in CBCT, thus utilizing a 3D imaging approach will help to find the exact position of the mental foramen.[8,9] Therefore, there is a need for the forensic experts and anthropologists to have a thorough knowledge about the utilization of the CBCT and the accuracy it provides in determining the exact location of mental foramen. As a result, using CBCT, this study was carried out to determine age based on the location and size of mental foramen.[9,10]

Aim

This study aims to determine the age of a population in Central India by analyzing the mental foramen using CBCT.

Material and methods

The present study was conducted in the department of Oral Medicine, Diagnosis and Radiology, Rungta College of Dental Sciences and Research, Kohka Kurud, Bhilai, Chhattisgarh. Prior to the commencement of the study, ethical clearance was obtained from the institutional ethical committee (RCDSR/IEC/MDS/2018/01).

Source of data

For this retrospective study, a total of 120 CBCT scans were collected from the Oral Radiology Department, Rungta College of Dental Sciences and Research from November 2018 to December 2019.The sample size was determined using G-Power software (Version 3.1.9.7) with the power of the test kept at 80%. The scans of the subjects were randomly collected from the available data. The patient's identification number, sex, date of birth was kept confidential and a unique study case number was allotted. Analysis of the reformatted images was performed using SIDEXIS software using GALAXIS GALLIEOS viewer.

A CBCT scans of mandible were grouped under the following category:

- Group I comprising of age 21–30 years – 30 subjects
- Group II comprising of age 31–40 years –30 subjects
- Group III comprising of age 41–50 years –30 subjects
- Group IV comprising of age 51–60 years –30 subjects

Inclusion criteria

- the subjects should be of age from 21–60 years,
- all teeth may or may not present adjacent to mental foramen, from canine to first molar, on both sides.

Exclusion criteria

- presence of any imaging artifact of mandible,
- presence of any radiolucent or radio-opaque lesion, obscuring the MF region,
- presence of any fracture in the parasympyseal region.

A CBCT apparatus, the SIRONA ORTHOPHOS XG 3D (Dentsply Sirona, Germany) was used with the following parameters: a tube voltage of 85 kV, a tube current of 6 mA and an exposure cycle of 14.4s of 8X5 FOV. Contiguous sectional images in three directions, parallel section (parallel to the dental arch), cross-section (perpendicular to the dental arch) and horizontal section images, were reconstructed from the projection data with a slice width of 1 mm. Viewing the contiguous sectional images using dedicated SIDEXIS software (GALAXIS GALLIEOS viewer), the images were evaluated in each section on a high-definition light emitting diode (HD LED) monitor.

Evaluation of scans

Two subject experts from the Department of Oral Medicine and Radiology, Rungta College of Dental Sciences and Research, with an experience of 10 years and 7 years respectively calibrated the principal investigator for the evaluation of various parameters required in the study. The Kappa (k) value obtained was 0.86. The required measurement (Fig. 1) of CBCT scan were done in SIDEXIS Software by a single observer on these following parameters:

- upper border of crest of mandible to upper border of mental foramen (Fig. 2),
- upper border to lower border of mandible (Fig. 3),
- upper border of mental foramen to lower border of mandible (Fig. 4),
- lower border of mandible to lower border of mental foramen (Fig. 5).

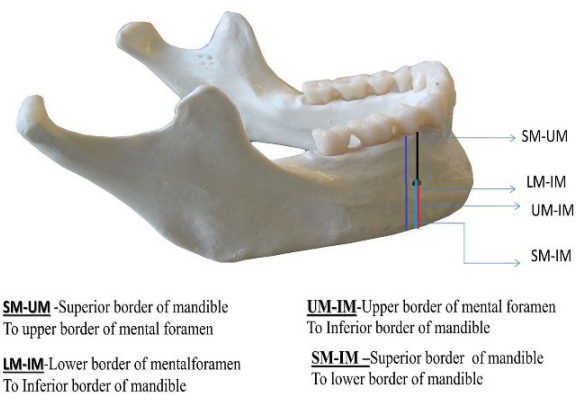

Fig. 1. Graphic representation of the measurements done for the mental foramen

Statistical analysis

The data so collected was entered into the MICROSOFT OFFICE EXCEL SPREAD SHEET which was then transferred to SPSS version 16.0 IBMT for statistical analysis. Descriptive statistics with mean and standard deviation was calculated for various parameters. Student unpaired t- test and Chi square test was used to

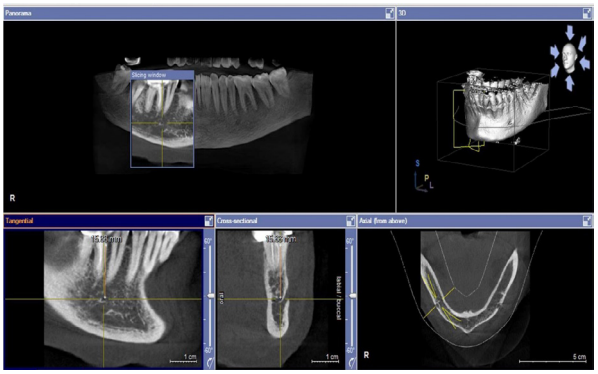

Fig. 2. Measurement from the crest of alveolar ridge to upper border of mental foramen

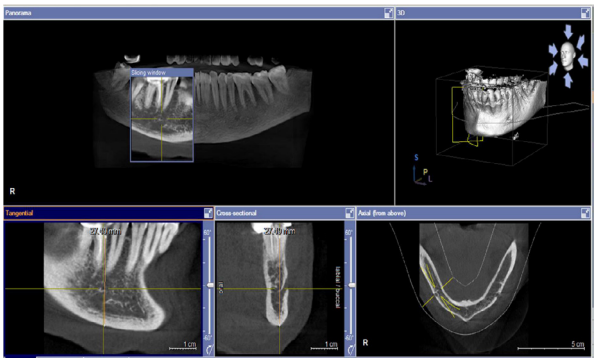

Fig. 3. Measurement from the upper border of mandible to lower border of mandible

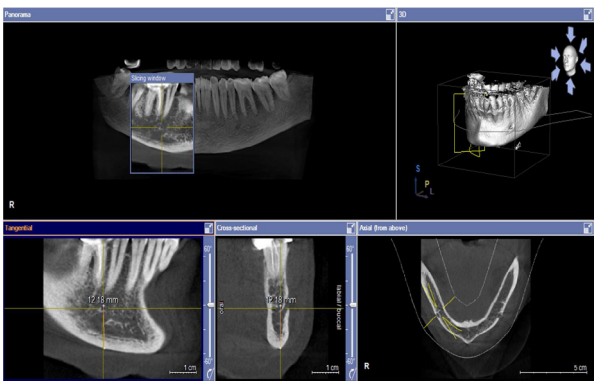

Fig. 4. Measurement from the upper border of mental foramen to lower border of mandible

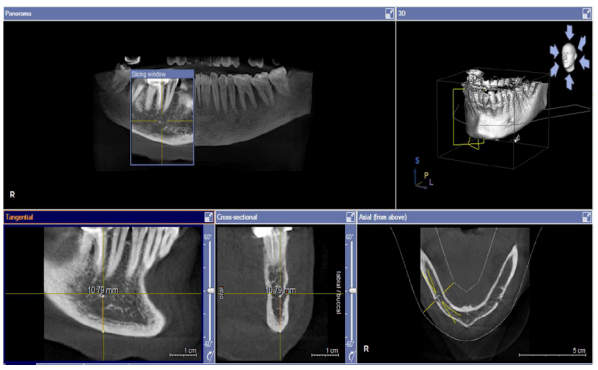

Fig. 5. Measurement from the lower border of mental foramen to lower border of mandible

analyze the data. The confidence Interval (CI) was kept at 95% and p value < 0.05 was statistically significant.

Results

The mean measurement recorded for the right side for 31–40 years the mean measurement among males and females were 29.95±1.75 and 27.01±2.41 with a mean difference of 2.94, which was statistically significant (p≤0.05) whereas for the left side it was 30.3±2.1 among males and 27.64±2.02 with a mean difference of 2.65 which was highly significant. The age group of 41-50 years the mean measured among males and females was 29.05±0.96 and 27.33±0.71 with a mean difference of 1.72 and found to be statistically significant (p≤0.05) whereas for the left side it was 28.99±0.93 among males and 27.28±0.71 with a mean difference of 1.71 (p≤0.05). The final age group of 51–60 years the mean measurement among males and females recorded were 28.69±2.44 and 27.02±1.62 respectively with a mean difference of 1.66 and found to be statistically significant (p≤0.05) whereas for the left side it was 28.27±2.21 among males and 26.35±2.15 with a mean difference of 1.91 also found to be significant as shown in Table 1.

Table 1. Mean comparison of measurement of right and left side upper border to lower border of mandible: among male and female

Age	Gender	n	Mean±SD		Mean Difference		T value		p	
			Right	Left	Right	Left	Right	Left	Right	Left
21–30	Male	15	26.49±1.68	26.81±1.87	0.41	-0.127	0.643	-0.193	0.52	0.849
	Female	15	26.90±1.81	26.94±1.74						
31–40	Male	15	29.95±1.75	30.30±2.10	2.94	2.65	3.805	3.51	0.001	0.002
	Female	15	27.01±2.41	27.64±2.02						
41–50	Male	15	29.05±0.96	28.99±0.93	1.72	1.71	5.48	5.62	0.001	0.001
	Female	15	27.33±0.71	27.28±0.71						
51–60	Male	15	28.69±2.44	28.27±2.21	1.66	1.91	2.19	2.40	0.03	0.023
	Female	15	27.02±1.62	26.35±2.15						

Table 2. Mean comparison of measurement for right and left upper border of crest of mandible to upper border of mental foramen

Age	Gender	n	Mean±SD		Mean Difference		T value		p	
			Right	Left	Right	Left	Right	Left	Right	Left
21–30	Male	15	13.89±1.34	13.91±0.82	-0.74	-0.47	-1.44	-0.998	0.157	0.327
	Female	15	14.63±1.45	14.38±1.60						
31–40	Male	15	15.91±1.96	15.75±1.70	1.86	0.63	2.15	0.864	0.018	0.395
	Female	15	14.05±2.09	15.12±2.26						
41–50	Male	15	13.49±0.88	13.56±0.84	-1.42	1.08	-4.46	-3.421	0.001	0.002
	Female	15	14.91±0.86	14.65±0.89						
51–60	Male	15	15.43±1.91	14.66±1.63	0.71	0.44	1.19	0.621	0.256	0.540
	Female	15	14.71±1.47	14.23±2.81						

For 31–40 years the mean measurement among males and females were 15.91±1.96 and 14.05±2.09

respectively with a mean difference of 1.86, which was statistically significant (p≤0.05) whereas for the left side it was 15.75±1.70 among males and 15.12±2.26 with a mean difference of 0.63 (p≤0.05). The age group of 51–60 years the mean measurement among males and females recorded was 15.43±1.91 and 14.71±1.47 respectively with a mean difference of 0.71 this was found to be statistically significant (p≤0.05), whereas for the left side it was 14.66±1.63 among males and 14.23±2.81 with a mean difference of 0.44 also found to be significant as shown in Table 2.

The mean comparison of measurement of right and left lower border of mandible to lower border of mental foramen; of both male and female. For 31–40 years the mean measurement among males and females were 12.63±1.38 and 11.25±1.15 respectively with a mean difference of 1.37600, which was statistically significant (p≤0.05), whereas for the left side it was 12.55±1.33 among males and 11.06±1.23 with a mean difference of 1.58933 which was highly significant. The age group of 41–50 years the mean measured among males and females was 13.86±1.53 and 10.56±0.41respectively with a mean difference of 3.29800, this was found to be statistically significant (p≤0.05) whereas for the left side it was 14.01±1.52 among males and 10.81±0.69 with a mean difference of 3.19 (p≤0.05) as shown in Table 3.

Table 3. Mean comparison of measurement of right and left lower border of mandible to lower border of mental foramen

Age	Gender	n	Mean±SD		Mean Difference		T value		p	
			Right	Left	Right	Left	Right	Left	Right	Left
21–30	Male	15	10.45±1.08	10.55±0.97	-0.16	-0.41	-0.370	-1.181	0.755	0.248
	Female	15	10.60±1.22	10.95±0.91						
31–40	Male	15	12.63±1.38	12.55±1.33	1.37	1.49	2.952	3.17	0.006	0.004
	Female	15	11.25±1.15	11.06±1.23						
41–50	Male	15	13.86±1.53	14.01±1.52	3.29	3.19	8.059	7.39	0.001	0.001
	Female	15	10.56±0.41	10.81±0.69						
51–60	Male	15	11.07±1.31	10.89±1.13	0.17	0.22	0.377	0.535	0.709	0.597
	Female	15	10.90±1.21	10.67±1.07						

The mean comparison of the measurement of right and left upper border of mental foramen to lower border of mandible of both male and female among all age groups were determined. For 31–40 years the mean measurement among males and females was 16.29±1.58 and 13.54±0.87 with a mean difference of 2.747, this was statistically significant (p≤0.05). whereas for the left side it was 16.25±1.85 among males and 13.76±1.1 with a mean difference of 2.48 (p≤0.05). The age group of 41–50 years the mean measured among males and females was 15.97±1.49 and 12.86±0.82 with a mean difference of 3.105, this was found to be statistically significant (p≤0.05) whereas for the left side it was 15.96±1.54 among males and 13.6±1.09 with a mean difference of 2.79 (p≤0.05) as shown in Table 4.

Table 4. Mean comparison of measurement of right and left upper border of mental foramen to lower border of mandible among male and female

Age	Gender	n	Mean±SD		Mean Difference		T value		p	
			Right	Left	Right	Left	Right	Left	Right	Left
21–30	Male	15	14.15±1.03	14.19±0.76	0.41	0.04	0.99	0.085	0.327	0.93
	Female	15	13.74±1.21	14.15±1.52						
31–40	Male	15	16.29±1.58	16.25±1.85	2.75	2.48	5.88	4.455	0.001	0.001
	Female	15	13.54±0.87	13.76±1.10						
41–50	Male	15	15.97±1.49	15.96±1.54	3.11	2.79	7.064	5.705	0.001	0.001
	Female	15	12.86±0.82	13.16±1.09						
51–60	Male	15	13.54±1.86	13.85±1.50	0.31	1.29	0.437	2.218	0.665	0.03
	Female	15	13.23±1.96	12.56±1.67						

The mean comparison of measurement for right and left dimension of mental foramen of both male and female among all age. For 31-40 years the mean measurement for the left side it was 3.70±0.930 among males and 2.70±0.878 with a mean difference of 0.99667 which was highly significant. The age group of 41-50 years the mean measured for the left side was 1.95±1.560 among males and 2.35±1.252 among females with a mean difference of -0.40133 (p≤0.05) The final age group of 51-60 years the mean measurement among males and females recorded was 2.46±1.008 and 2.33±1.482 respectively with a mean difference of 0.13133 this was found to be statistically significant (p≤0.05). whereas for the left side it was 2.96±0.934 among males and 1.89±1.396 with a mean difference of 1.07400 also found to be significant as shown in Table 5.

Table 5. Mean comparison of measurement for right and left dimension of mental foramen among male and female

Age	Gender	n	Mean±SD		Mean Difference		T value		p	
			Right	Left	Right	Left	Right	Left	Right	Left
21–30	Male	15	3.70±1.146	3.64±0.737	0.57	0.45	1.253	1.191	0.221	0.244
	Female	15	3.13±1.329	3.19±1.248						
31–40	Male	15	3.66±0.905	3.70±0.930	1.37	0.99	4.342	3.018	0.001	0.005
	Female	15	2.29±0.822	2.70±0.878						
41–50	Male	15	2.10±1.130	1.95±1.560	-0.19	-0.40	-0.473	-0.777	0.640	0.444
	Female	15	2.30±1.102	2.35±1.252						
51–60	Male	15	2.46±1.008	2.96±0.934	0.13	1.07	0.284	2.476	0.779	0.020
	Female	15	2.33±1.482	1.89±1.396						

Using all the measurement of all the five parameters of the mandible and mental foramen formula for estimation of age was derived using simple linear regression model (p<0.05, CI= 95%).

Age estimate equation for right side= 24.030+ (1.763* UB.to.LB.right) +(-0.933* UBCM.To. UBMF. Right) + (-0.819* LBM.to.LBMF. Right) + (-3.447* Dimension.MF.Right)

Age estimate equation for left side = 45.988+ (2.617* UB.to.LB.Left) +(-2.880* UBCM.To. UBMF.Left) +(-2.200*LBM.to.LBMF. Left) +(-4.002* Dimension. MF.Left)

Discussion

Forensic odonatologists can play a role in the identification of gender using facial bones especially mandible, which is a stronger bone.[9] Mental foramen is one of the stable landmarks of mandible.[10] It is located on lateral surface of body of mandible below the apices of first and second premolars. Wical and Swoope reported that there is no effect of alveolar bone resorption on the distance between mental foramen and lower border of the mandible. It remains relatively constant throughout life.[11] Lindh et al. and Guler et al. also agreed with the results suggested by Wical and Swoope.[12,13] All these studies demonstrated that linear measurements on radiographs can be a sound indicator of the alveolar bone resorption with age. CBCT is a relatively accurate technology, introduced to dentistry in 1998, which is used for the three-dimensional imaging. Currently, high resolution CBCT is the most promising and accurate technology available for quantitatively determining the position of MF.[14,15] The vertical measures computed from the superior edge of the mental foramen to the crest of the alveolar ridge were found to be larger in edentulous males than in women in this study. Ajmal et al. reported similar findings, noting that the measurement dropped considerably with age.[16] Bhardwaj et al. studied 300 digital panoramic radiographs for predicting age in various age groups and were divided into 3 groups of 25–34 years, 35–44 years, and 45–54 years using five parameters collectively, which were: gonial angle, ante gonial angle, mental foramen, mandibular canal, mandibular foramen.[17] Among all the data studied, changes in the mandibular canal and mandibular foramen were shown to be very significant (p=0.05) as age progressed, however in our study, the analysis was limited to the factors linked to the mental foramen using CBCT.[18-20]

Similar to our study Gungor et al. studied the locations of mental foramen (MF) from age 10-70 years using CBCT found that the vertical size of the MF was similar on right and left side (p=0.785) whereas in our study it was only similar and highly significant in the age group of 41–50 years i.e., 0.001,0.005 (p<0.05).[21] According to the study's findings, the vertical position of the MF changes with age. In younger age groups, it is closer to the lower border of the mandible; in middle age groups, it is equidistant from the alveolar crest and the lower border of the mandible; and in older age groups, it is closer to the alveolar crest. These findings agreed with those of Ahmed et al., who found that only the distance between the inferior edge and the lower border of the mandible is statistically significant (p=0.01).[22] The position of the mental foramen also influences the distance between the superior edge of the foramen and the alveolar crest.

Conclusion

Mental foramen is one of the most important anatomical landmarks present in the mandible. As CBCT is considered a gold standard in radiological imaging, so the mandibular foramen was assessed using the 3D imaging technique which helped to determine the exact parameters of the mental foramen without any radiological errors. The current study concluded that the distance between the upper border of the body of the mandible and the upper border of the mental foramen, the height from the superior border of the mandible to the lower border of the mandible, and the distance from the lower border of the mental foramen to the lower border of the mandible all play an important role in determining age. This study demonstrated that mental foramen could potentially serve as a useful forensic criterion due to its clinical importance. The age assessment was found to be highly significant for the age groups of 31–40 years and 41–50 years (p<0.05). Same was also noticed in case for gender estimation for the same age groups. Least significance was found in the age group of 51–60 years. The dimensions of the right and left sides of the mental foramen varied bilaterally. Using the obtained parameters, the predicted age using the age regression formula was shown to be very significant in the age groups 21–30, 31–40, and 41–50 years. This study can also serve as a guide for orthognathic operations, implant placement, and pre-prosthetic surgery, as well as help in more accurate identification of the dead and deceased, which is critical in forensic investigations.

Declarations

Funding

No funding has been received for the study.

Author contributions

Conceptualization, S.B. and J.S.; Methodology, S.B.; Software, S.B.; Validation, J.S., F.K. and D.D.; Formal Analysis, J.S.; Investigation, S.B.; Resources, S.P.; Data Curation, S.B.; Writing – Original Draft Preparation, S.P.; Writing – Review & Editing, E.S.; Visualization, A.R.; Supervision, J.S.; Project Administration, F.K.; Funding Acquisition, D.D.

Conflicts of interest

The authors declare no competing interests.

Data availability

The datasets used and/or analyzed during the current study are open from the corresponding author on reasonable request.

Ethics approval

Research protocols and procedures were approved according to the ethical standards of the Helsinki Declaration 2013 and by the Ethics Committee of Rungta College of Dental Sciences & Research (ethical approval RCDSR/IEC/MDS/2018/01).

References

1. Standring S, Neil R Borley H. *Gray's Anatomy: The Anatomical Basis of Clinical Practice*. USA: Elsevier; 2008.
2. Chaurasia BD. *BD Chaurasia's Human Anatomy Regional and Applied Dissection and Clinical: Vol. 3: Head-Neck Brain*. CBS Publishers & Distributors; 2013.
3. Phulari RGS, Dave EJ. Evolution of dental age estimation methods in adults over the years from occlusal wear to more sophisticated recent techniques. *Egypt J Forensic Sci*. 2021;11(1):36. doi: 10.1186/s41935-021-00250-6
4. Sujatha S, Azmi SR, Devi B, Shwetha V, Kumar TP. CBCT--the newfangled in forensic radiology. *Journal of Dental and Orofacial Research*. 2017;13(2):47-55.
5. Ghali S, Katti G, Shahbaz S, Katti C. Cone beam computed tomography: A boon for maxillofacial imaging. *J Indian Acad Oral Med Radiol*. 2017;29(1):30. doi: 10.4103/jiaomr.jiaomr_89_16
6. Pelé A, Berry PA, Evanno C, Jordana F. Evaluation of mental foramen with cone beam computed tomography: A systematic review of literature. *Radiol Res Pract*. 2021;2021:8897275. doi: 10.1155/2021/8897275
7. Santos Oliveira D, Coutinho R, Panzarella K. Morphometric Analysis of the Mental Foramen Using Cone-Beam Computed Tomography. *Int J Dent*. 2018.
8. Breeland G, Aktar A, Patel BC. Anatomy, Head and Neck, Mandible. In: *StatPearls*. Treasure Island (FL): StatPearls Publishing; June 11, 2022.
9. Saxena S, Sharma P, Gupta N. Experimental studies of forensic odontology to aid in the identification process. *J Forensic Dent Sci*. 2010;2(2):69-76. doi: 10.4103/0975-1475.81285
10. Shah J, Asrani V. Mental foramen: A predictor of age and gender and guide for various procedures. *J Forens Sci Med*. 2018;4(2):76. doi: 10.4103/jfsm.jfsm_2_18.
11. Wical KE, Swoope CC. Studies of residual ridge resorption. Part 1. Use of panoramic radiographs for evaluation and classification of mandibular resorption. *J Prosthet Dent*. 1974;32:7-12.
12. Lindh C, Petersson A, Klinge B. Measurements of distances related to the mandibular canal in radiographs. *Clin Oral Implants Res*. 1995;6(2):96-103. doi: 10.1034/j.1600--0501.1995.060205.x
13. Güler AU, Sumer M, Sumer P, Biçer I. The evaluation of vertical heights of maxillary and mandibular bones and the location of anatomic landmarks in panoramic radiographs of edentulous patients for implant dentistry. *J Oral Rehabil*. 2005;32(10):741-746. doi: 10.1111/j.1365--2842.2005.01499.x
14. Raju G, Sairam V, Vikas S. Evaluation of mental foramen - A CBCT assessment. *IP Int J Maxillofac Imaging*. 2018;4(1):23-26.
15. Sheikhi M, Kheir MK. CBCT assessment of mental foramen position relative to anatomical landmarks. *Int J Dent*. 2016;2016:5821048. doi: 10.1155/2016/5821048.
16. Ajmal M. Evaluation of mental foramen position from panoramic dental radiographs. *J Contemp Dent Pract*. 2014;15(4):399-402. doi: 10.5005/jp-journals-10024-1551
17. Bhardwaj D, Kumar JS, Mohan V. Radiographic evaluation of mandible to predict the gender and age. *J Clin Diagn Res*. 2014;8(10):ZC66-9. doi: 10.7860/JCDR/2014/9497.5045
18. Sheikhi M, Karbasi Kheir M, Hekmatian E. Cone-beam computed tomography evaluation of mental foramen variations: A preliminary study. *Radiol Res Pract*. 2015;2015:124635. doi: 10.1155/2015/124635
19. Lee YH, Auh QS, Chun YH, An JS. Age-related radiomorphometric changes on panoramic radiographs. *Clin Exp Dent Res*. 2021;7(4):539-551. doi: 10.1002/cre2.375
20. Shalash M, Khallaf ME, Ali AR. Position and dimensions of the mental foramen and presence of the anterior loop in the Egyptian population: a retrospective CBCT study. *Bull Natl Res Cent*. 2020;44(1):110. doi: 10.1186/s42269-020-00364-2
21. Guven S, Gungor E, Aglarci OS, Unal M, Dogan MS. Evaluation of mental foramen location in the 10–70 years age range using cone-beam computed tomography. *Niger J Clin Pract*. 2017;20(1):88. doi: 10.4103/1119-3077.178915
22. Ahmed AA, Ahmed RM, Jamleh A, Spagnuolo G. Morphometric analysis of the mandibular canal, anterior loop, and mental foramen: A cone-beam computed tomography evaluation. *Int J Environ Res Public Health*. 2021;18(7):3365. doi: 10.3390/ijerph18073365

Wydawnictwo UR 2023
ISSN 2544-1361 (online)
doi: 10.15584/ejcem.2023.3.12

http://www.ejcem.ur.edu.pl
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2023; 21 (3): 506–512

ORIGINAL PAPER

Factors affecting work-life balance and psychological resilience levels of nurses working in internal clinics during COVID-19

Tuba Korkmaz Aslan [1], Rukiye Burucu [2], Yunus Akdoğan [3]

[1] Psychiatric Nursing Education, Seydişehir Kamil Akkanat Faculty of Health Sciences, Necmettin Erbakan University, Konya, Turkey
[2] Medical Diseases Nursing Education, Seydişehir Kamil Akkanat Faculty of Health Sciences, Necmettin Erbakan University, Konya, Turkey
[3] Department of Statistics, Selcuk University Faculty of Science, Konya, Turkey

ABSTRACT

Introduction and aim. COVID-19 intensity has affected both the psychology of the nurses and the balance established between their work and social lives. In this study, it was aimed to examine the work-life balance and psychological resilience levels of nurses.

Material and methods. Four Hundred and seventy two nurses, working in various hospitals and internal units in Turkey, voluntarily participated in the study. Data was collected online using the Work-Life Balance Scale, Brief Psychological Resilience Scale, and the Personal Information Form.

Results. The sub-dimensions of the work-life balance scale include allocation of time for yourself (β=1.892; p<0.001), life merely being based on work (β=-0.513; p<0.05) and work-life balance; affect the psychological resilience score (β=-0.364; p<0.05). Based on this, devoting time for yourself has a positive effect on psychological resilience, while others have a negative effect. The total score of the nurses on work-life balance is 51.51±7.22 and their psychological resilience score is 15.27±3.93.

Conclusion. Psychological resilience is affected by educational status, marital status, having children, and working schedule; work-life balance is affected by educational status, marital status, having children, income levels, and working schedule; while work-life balance sub-dimensions are affected by education, marital status, having children, income levels, place of work and working schedule. Both work-life balance and its sub-dimensions affect the psychological resilience of nurses.

Keywords. COVID-19, nursing, psychological resilience, work-life balance

Introduction

Establishing a balance between the work-life and private life of working individuals has an important effect on both their satisfaction and happiness. Because this situation affects not only the employee but also the family and therefore the whole society.[1] Work-life balance, which can be defined in different ways; is a term that explains the balance between both self-time with family, the activities in social life such as hobbies and arts; and the activities in business life.[2] Psychological resilience is explained as "psychological strengthening" or "self-recovery strength".[3] According to this, if the individual can find a balance between his work and social life, they will have managed to have a work-life balance.[2]

COVID-19 only started as pneumonia but then continued as a pandemic.[4] It was thought that it would be sufficient to take standard measures in social life to prevent infection to end the pandemic.[5] However, the epidemic has progressed rapidly with each passing day and this process has increased the workload of nurses. In addition to

Corresponding author: Tuba Korkmaz Aslan, e-mail: tkorkmazaslan@erbakan.edu.tr

Received: 12.04.2023 / Revised: 30.05.2023 / Accepted: 30.05.2023 / Published: 30.09.2023

Korkmaz Aslan K, Burucu R, Akdoğan Y. *Factors affecting work-life balance and psychological resilience levels of nurses working in internal clinics during COVID-19.* Eur J Clin Exp Med. 2023;21(3):506–512. doi: 10.15584/ejcem.2023.3.12.

the increase in patient density in the hospitals; the closure of some clinics in the hospital and the opening of new and different clinics, insufficient number of personnel,[6] and working overtime negatively affected the work-life balance of nurses.[7] The psychological resilience of nurses whose work-life balance is disrupted has also started to deteriorate.[8,9] It was stated that during the COVID-19 process, patients were frequently hospitalized in internal clinics due to respiratory problems, and nurses, especially young and inexperienced ones, were the most affected group, and their stress and anxiety levels were higher.[10] COVID-19 patients frequently received inpatient treatment in internal clinics and internal intensive care units. For this reason, it is important to evaluate the conditions of the nurses working in the clinics in question.

Aim

In this study, it is aimed to determine the factors affecting the work-life balance and psychological endurance levels of nurses working in internal clinics.

The research questions prepared for this purpose are given below: Is there a difference in psychological resilience according to the workplace, working hours, and sociodemographic characteristics? Is there a difference in work-life balance and sub-dimensions according to the workplace, working hours, and sociodemographic characteristics? Does work-life balance and sub-dimensions affect their psychological resilience?

Material and methods

Type of research and sample and tools

It is descriptive and relational research.[11] Power analysis was done with the G power package program. It was determined that the sample should be at least 310 people with 0.5 alpha margin of error, 95% power and 0.42 effect size, and 472 people formed the sample. Personal information form, work-life balance scale (WLBS) and brief psychological resilience scale (BPRS) were used.

Personal information form

The form was prepared by the researchers based on the literature.[6,9,12-14] In the information form; there are 17 questions about demographic variables such as information about the unit and working hours, gender, age, income status, number of children.

Work-life balance scale

The scale was developed by Apaydın, and it is a five-point Likert scale consisting of 20 items. The 6th, 7th, 8th, 9th, 17th, and 19th items constitute the work-life compatibility sub-dimension of the scale. The 1st, 2nd, 4th, 5th, 10th, and 11th items constitute the neglect of life sub-dimension of the scale. The 12th, 13th, 18th, and 20th items constitute the sub-dimension of self-time allocation of the scale. The 3rd, 14th, 15th, and 16th items constitute the life consisting of work sub-dimension of the scale. The total internal consistency Cronbach Alpha coefficient of the scale is 0.91, and it is specified in the order in the sub-dimensions as 0.88; 0.81; 0.77; 0.79. The scale does not have a cut-off score, the total score ranges between 20 and 100. On the scale, an increase in work-life compatibility and self-time allocation scores creates a positive perception, while an increase in scores in other dimensions indicates negativity.[15]

Brief psychological resilience scale (BPRS)

The scale was developed by Smith et al. and its validity and reliability in Turkish was carried out by Doğan.[16] It is a five-point Likert scale with six items based on self-report. Items two, four, and six are coded in reverse, and high scores from the scale indicate that individuals perceive themselves as psychologically sound. Scale total score ranges between 6-30. The Cronbach Alpha coefficient of the scale is 0.83.[17]

Research variables

Continuous variables

Work-life balance scale score, brief psychological resilience scale score, age, and professional experience, weekly/monthly working hours, total working time.

Categorical variables

Categorical variables involve questions about sociodemographic characteristics, the work unit, and the state of thinking about resigning.

Universe and sampling

The universe of the research was created by nurses working in Turkey. The sample was prepared using the data of a similar study. In the study in question, it was explained that there is a difference between the psychological decencies of those who have to leave the house during the pandemic and those who do not have to leave the house (who have to leave the house: 285.03, who do not have to leave: 237.47, $\chi 2$: 6.232, p:0.044).[12] Power analysis was performed in the G-power 3.1.9.4. package using the data of this study; the error margin of 0.05 alpha, the number of people who need to be reached with 85% power was determined as 436. The study was completed with 471 people. The posthoc power of the study is 0.88.

The study included individuals working in internal clinics as nurses, continuing to work during the COVID 19 process, accessible online, and *excluded* nurses working in another country other than Turkey.

Collection of data

Data were collected between March 2021 and June 2021. The surveys were prepared online. In order to ensure the reliability of the data, the surveys were organized

in such a way that each participant answered only once. The questionnaires that were arranged were filled out online for 20 people before the application and the necessary arrangements were made. These 20 people were not included in the study. The prepared questionnaires were transmitted to the nurses online both individually and through nurses' associations.

Ethics approval

For research; Research permission was obtained from the Ministry of Health Scientific Research Platform (2021-02-17T20_50_25), Necmettin Erbakan University Health Sciences Scientific Research Ethics Committee (03.03.2021, Decision Number: 06). Permission to use was obtained from the scale owners and written consent was obtained from the participants. The study has loyally been carried out to the Declaration of Helsinki. It is prepared, implemented, and reported by the Observational Research Reporting Criteria (STROBE).[18]

Structural equation modelling

In order to test the hypotheses of the study, the structural equation modelling, created between the sub-dimensions of the WLB scale such as work-life compatibility (WLC), neglecting life (NL), self-time allocation (STA), life-based on work (LBW), and BPRS, is shown in Figure 1.

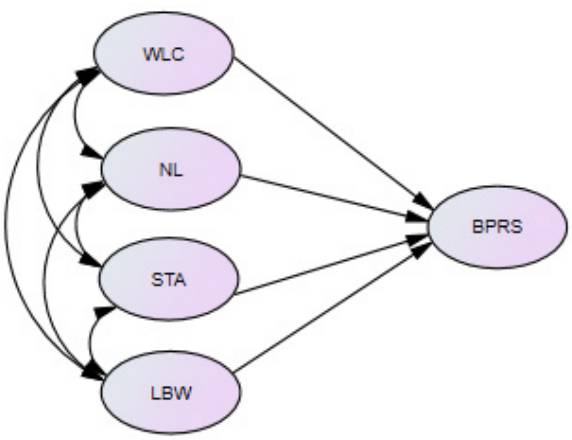

Fig. 1. Structural equation modelling

Statistical analysis

Statistics were made by using the Statistical Package for Social Sciences (SPSS, IBM, v. 22, Armonk, NY, USA) and SPSS AMOS 22 program. For the normal distribution of data; the Kolmogorov-Smirnov test and Levene test results were examined, and it was observed that WLBS and its sub-dimensions and BPRS scores were not normally distributed. For this reason, Mann-Whitney U Test was used for two independent groups from non-parametric tests for analysis. Spearman Correlation coefficient was calculated for the relationship between scores and values. Single-factor confirmatory factor analysis (CFA) and structural equation modelling (SEM) were performed for the used scales (Fig. 1). All results were evaluated at a significance level of 0.05.

Results

To test the validity of the used scales with the SPSS AMOS 22 program; first-order multifactorial confirmatory factor analysis was performed for WLBS and a single-factor confirmatory factor analysis was performed for the BPRS (Table 1).

Table 1. Factor loads of scales and Cronbach-alpha coefficients[a]

Questions	Factor loads		Item reliability	Cronbach Alpha reliability coefficient
	Binding loads	p		
Work-life compatibility				0.816
I can strike a balance between my work and my personal life.	0.843	–	0.768	
I think that I distribute my time in 4 ways, both in my business and personal life.	0.794	***	0.746	
I can do all my work by planning my life in 2.	0.784	***	0.772	
I do activities that I enjoy in my work and private life.	0.720	***	0.815	
I decide what my priorities are in my business life and act accordingly.	0.648	***	0.795	
Neglecting other life activities				0.775
I can't even find time for simple things in the day	0.744	–	0.725	
Although I think my life reflects the ideal lifestyle, I live with the thought that I am missing something.	0.735	***	0.732	
I cannot keep up with the intensity of my work.	0.729	***	0.730	
I see myself as someone who just knows how to work, but doesn't live the rest of life.	0.725	***	0.738	
Because I try to do a lot of work at the same time, I sacrifice basic life activities such as sleep, regular nutrition, and movement.	0.702	***	0.743	
Allocation of Self-Time				0.713
Tensions arising from my job affect my private life negatively.	0.786	–	0.616	
On an ordinary day, I make unhealthy decisions about what jobs to put my time and energy into.	0.785	***	0.615	
"If I had to do things that would make me happy, maybe I would be happier".	0.784	***	0.618	
I am having difficulties in my job because I do not compromise my personal life.	0.563	***	0.735	
A life merely being based on work				0.715
I often leave work late.	0.813	–	0.650	
I continue to work non-stop at the weekends.	0.801	***	0.598	
I miss non-work activities because of the time I spend on my job.	0.780	***	0.622	
Brief Psychological Resilience				0.822
I can do all my work by planning my life.	0.845	–	0.776	
I think I'm watching life from behind.	0.812	***	0.146	
I see myself as someone who just knows how to work, but doesn't live the rest of life.	0.766	***	0.147	
I can't even find time for simple things in the day	0,747	***	0.191	
Because I try to do a lot of work at the same time, I sacrifice basic life activities such as sleep, regular nutrition, and movement.	0.649	***	0.275	

[a] *** – p< 0.001

From Work-Life Balance Scale (WLBS); 3, 4, and 9. questions, from Brief Psychological Resilience Scale (BPRS) 3. questions were removed without verification, since the fit values (X2/df = 3.038, GFI=0.923, CFI=0.954, RMSEA=0.066) produced by the measurement models were within acceptable limits, the structures of the scales used in the study were confirmed.

Since the model fit values (X^{2nd}/df=2,804, GFI=0.902, CFI=0.953, RMSEA=0.062) were within acceptable limits are shown in Table 2. No statistically significant effect was found between variables in the relationship between BPRS and work-life compatibility and neglect of life (p>0.05). The self-time allocation (β=1.892; p<0.001), life merely being based on work (β=-0,513; p<0.05) and work-life balance affect the brief psychological resilience score (β=-0.364; p<0.05). When the self-time allocation score increases by one unit, the BPRS score increases by 1.892 units. However, life-based on work and work-life balance affect BPRS negatively. Accordingly, when the score of life-based on work increases one unit, the BPRS score decreases by 0.513 units.

Table 2. Structural equation modelling coefficients[a]

Model	Coefficients (β)	S.E.	p	Confidence intervals for 95%
Work–life compatibility – Brief Psychological Resilience	0.022	0.106	0.834	(-0.172, 0.156)
Neglecting life activities – Brief Psychological Resilience	-0.067	0.230	0.771	(-0.453, 0.319)
Self–Time Allocation – Brief Psychological Resilience	1.892	0.456	***	(1.126, 2.657)
Life–based on work – Brief Psychological Resilience	-0.513	0.171	*	(-0.800, 0.226)
Work–life balance–Brief Psychological Resilience	-0.364	0.023	***	(-0.544, -0.152)

[a] *** – p<0.001; * – p<0.05

Information about scale scores is given in Table 3.

Table 3. Descriptive analyses for WLBS and BPRS Scores

Scale scores	Min	Max	Mean ± SD
Work-life compatibility	5	25	14.34±4.04
Neglecting other life activities	5	25	16.74±3.99
Self-harm	4	20	11.58±3.55
A life merely being based on work	3	15	8.86±2.96
Total WLBS	32	80	51.51±7.22
Total BPRS	5	25	15.27±3.93

Descriptive data are given in Table 4.

Spearman correlation analysis was applied to the variables. In evaluations; There is a negatively "weak" relationship between, "Neglect of life" (-0.564), "self-harm" (-0.514) and the "work-life compatibility" sub-dimension, there is a "very weak" relationship between "A life merely being based on work" (-0.430), the total of WLBS (-0.232), and the "work-life compatibility" sub-dimension", There is a positively "weak" relationship between the BPRS (0.423) and the "work-life compatibility" sub-dimension. There is a positively weak relationship between, "Self-harm" (0.586) and "life merely being based on work" (0.574) and the "neglecting life" sub-dimension, there is a negatively very weak relationship between WLBS (-0.373) and the "neglecting life" sub-dimension, there is a positively medium relationship between WLBS (0.759) and the "neglecting life" sub-dimension. There is a positively weak relationship between, "Life merely based on work" (0.577), WLBS (0.556) and the "self-harm" sub-dimension, there is a positively medium relationship between the total WLBS (0.768) and the "self-harm" sub-dimension. There is a

Table 4. Analysis of the differences between sociodemographic characteristics and the scales of WLB and BPR*

Sociodemographic Characteristics		n	Work-life compatibility p	Neglecting other life activities p	Self-harm p	A life merely being based on work p	Total WLBS p	BPRS p
Educational Status	High School	94	4.664[a] 0.097[a]	7.139[a] 0.028[a]	12.299[a] 0.002[a]	6.372[a] 0.041[a]	8.783[a] 0.012[a]	16.637[a] 0.000[a]
	University	321						
	Postgraduate	56						
Marital Status	Single	180	5.574[a] 0.062[a]	4.205[a] 0.122[a]	8.788[a] 0.012[a]	9.662[a] 0.008[a]	6.375[a] 0.041[a]	9,981[a] 0.007[a]
	Married	278						
	Other	13						
Having children	Yes	271	-1.633[b] 0.102[b]	-0.320[b] 0.749[b]	-2.725[b] 0.006[b]	-4.563[b] 0.000[b]	-2.365[b] 0.018[b]	-2.899[b] 0.004[b]
	No	200						
Income status	Income is more than expenses	61	6.780[a] 0.034[a]	11.765[a] 0.003[a]	3.765[a] 0.152[a]	8.112[a] 0.017[a]	7.882[a] 0.019[a]	0.395[a] 0.821[a]
	Income is equivalent to expenses	229						
	Income is less than expenses	181						
Working Unit	Adult internal clinics	224	5.223[a] 0.156[a]	3.018[a] 0.389[a]	5.213[a] 0.157[a]	10.919[a] 0.012[a]	3.891[a] 0.273[a]	3.284[a] 0.350[a]
	Pediatrics internal clinics	24						
	Intensive care	167						
	Emergency Department	56						
Requesting Flexible Work	Yes	406	-3.064[b] 0.002[b]	-4.031[b] 0.000[b]	-6.093[b] 0.000[b]	-4.538[b] 0.000[b]	-4.991[b] 0.000[b]	-6.046[b] 0.000[b]
	No	65						

* a – test statistics and p-value according to the result of the Kruskal-Wallis test; b – test statistics and p-value according to the Mann-Whitney U test result

negatively ''very weak" relationship between, WLBS (-0.266) and the "Life merely based on work" sub-dimension, there is a positively medium relationship between total WLBS (0.772) and the "Life merely based on work" sub-dimension, On the other hand, a negative and weak relationship (-0.352) was found between total BPRS and total WLBS (Table 5).

Table 5. The relationship analyses between WLBS together with its sub-dimensions and BPRS scores[a]

		Work-life compatibility	Neglecting other life activities	Self-harm	A life merely being based on work	BPRS	Total WLBS
Work-life compatibility	Correlation	1.000					
Neglecting other life activities	Correlation	-0.564**	1.000				
Self-harm	Correlation	-0.514**	0.586**	1.000			
A life merely being based on work	Correlation	-0.430**	0.574**	0.577**	1.000		
BPRS	Correlation	0.423**	-0.373**	0.556**	-0.266**	1.000	
Total WLBS	Correlation	-0.232**	0.759**	0.768**	0.772**	-0.352**	1.000

[a] ** – Spearman correlation analysis

Discussion

In this study, the work life balance of nurses is affected by their educational status, marital status, childbearing, income status and the working hours they work. It was found that their psychological stability was affected by educational status, marital status, having children and the working hours they worked. The work-life balance of nurses affects their psychological well-being. The results were discussed in the literature. Participants in this study are all women, mostly young adults, married and have children, and their income is equivalent to their expenses. Considering the studies conducted both in Turkey and other countries, it is seen that is mostly in the young adult age group, mostly women, and individuals who do not have income problems.[9,12,19-21] The fact that all the participants are women may make us think that women value such academic studies more, and the fact that the majority of those who practice nursing are women may be another factor.[22]

In this study, the average score of the nurses' work life balance scale was just above the average. It was found that the group had the highest average score in the "neglecting life" sub-dimension. It is similar in the study of Yayla and Eskici.[23] One of the factors that negatively affects the work life balance is the working order. Working more than 40 hours a week, keeping more night shifts, not being able to work with flexible overtime is an undesirable condition for work-life balance.[7] Working at unusual times also negatively affects the work life balance of nurses.[6] One study reported that married women have a higher work-life balance score.[24] Because the social support/family support offered to the working individual contributes to the achievement of work-life balance.[6] Especially in married and working women, spousal support positively affects a woman's work life balance.[25] The problems experienced by nurses related to their work affect their lives.[26] One of the problems experienced is domestic conflicts. It has been explained that these conflicts have reasons such as shift work, increased workload, etc.[20] In this study, it was seen that the work life balance scores of those who were single were higher and those who had children were lower. It can be said that those who have children from Turkish nurses are married and their spouses do not offer adequate support, nurses are trying to continue their lives without neglecting life despite everything.

Working with patients whose general condition is not good causes nurses to show depressive symptoms.[9] In a study conducted with nurses working in a pulmonology clinic during the COVID-19 process, it was explained that nurses at a young age and with little professional experience had higher anxiety and depression scores during this process.[10] A higher psychological well-being score was reported for those who worked in internal clinics. However, the score is lower in those who work in intensive care.[23] During the COVID-19 process, patients were generally treated as inpatients in internal clinics and those whose condition deteriorated in intensive care units. The fact that nurses work with patients whose general condition is not good and who need constant follow-up and treatment may have negatively affected their psychological well-being.

Psychological well-being increases as the level of education increases and it has been determined that it is better for married people than singles.[23] It is explained that the nurses who continue to work at home and whose income level is not good and in poor health, are feeling more depressed, and their concerns about COVID-19 infection are higher, both for themselves and their family members.[9] Individuals' anxiety negatively affects their psychological resilience. Economic anxiety seems to decrease psychological resilience while increasing anxiety scores in women.[12] Increasing education level also positively affects psychological resilience.[27] In this study, the BPRS score is found to be higher for those who are married, have children, and want to work flexibly, and lower for those with postgraduate education. The fact that all the participants here are women and that they work intensively both at home and in the hospital can be an important factor affecting their psychological resilience.

While nurses' work affects their family lives, their family lives also affect their psychological resilience. The pandemic process increases the stress of nurses and stress will magnify existing problems.[6,28] It is emphasized that unresolved problems will lead nurses to

resign.[29] Nurses, it is stated that they wanted to resign due to workload and responsibilities.[30] While half of the nurses do not intend to resign, the other half considers it, but most of them want to work flexible shift.[7,31] It is stated that working with flexible working hours is an important predictor of the quality of work life of nurses, it is difficult for them to keep their work and family lives in balance, and flexible working hours can be an alternative to facilitate this.[7,31] In this study, nurses likely wanted to work flexibly to fulfil their family roles because they were all women, married, and had children. It is a problem that a nurse's life is based only on work, they have no social life, and their psychological resilience is negative. However, the effective execution of a nurse's job is closely related to their psychological resilience.[32] This study found that psychological resilience and work-life balance affect each other in different sub-dimensions.

Conclusion

As a result, the psychological resilience of nurses is affected by education status, marital status, having children, and working order. Nurses' work-life balance is affected by education status, marital status, having children, income status, and working order. Work-life balance sub-dimensions are affected by education, marital status, having children, income level, place of work, and working order. Both work-life balance and its sub-dimensions affect the psychological resilience of nurses. In institutions, it can be suggested to determine the factors that negatively affect nurses' work-life balance and psychological resilience, ensuring adequate social support and establish a supportive institutional culture.

Acknowledgments

We thank all the nurses who participated in the study.

Declarations

Funding

No fund support has been received from any institution/organization.

Author contributions

Conceptualization, T.K.A. and R.B.; Methodology, R.B.; Software, T.K.A.; Validation, T.K.A., R.B. and Y.A.; Formal Analysis, Y.A.; Investigation, T.K.A.; Resources, T.K.A., R.B. and Y.A.; Data Curation, T.K.A.; Writing - Original Draft Preparation, T.K.A. and R.B.; Writing - Review and Editing, T.K.A., R.B. and Y.A.; Visualization, T.K.A.; Supervision, R.B.; Project Administration, T.K.A. and R.B.; Funding Acquisition, T.K.A., R.B. and Y.A.

Conflicts of interest

There is no conflict of interest between the authors.

Data availability

Data available on request from the authors.

Ethics approval

Ethical approval and informed consent were obtained (2021-02-17T20_50_25 and 03.03.2021, Decision Number: 06).

References

1. Uysal N, Çayır Yılmaz M. Determination Of Work-Life Balance And The Opinions Of Remote Working In Academicians. *Int J Soc Sci Stud*. 2020;4(2):26-37.
2. Hıdır Özcan A. Bayarçelik E. Job Satisfaction, Burnout and Work Life Balance According to Generations. *International Journal of Management and Administration*. 2020;4(7):54-70. doi: 10.29064/ijma.665688
3. Kimter N. Examining the Psychological Resilience Levels of Individuals in the Days of COVID-19 in Terms of Some Variables. *IBAD Journal of Social Sciences*. 2020;574-605. doi: 10.21733/ibad.805481
4. WHO. Coronavirus disease (COVID-19) pandemic. (2020); https://www.who.int/emergencies/diseases/novel-coronavirus-2019. Accessed April 30, 2021.
5. Yuen KS, Ye ZW, Fung SY, Chan CP, Jin DY. SARS-CoV-2 and COVID-19: The most important research questions. *Cell Biosci*. 2020;10(40):40. doi: 10.1186/s13578-020-00404-4
6. Heath C, Sommerfield A, von Ungern-Sternberg BS. Resilience strategies to manage psychological distress among healthcare workers during the COVID-19 pandemic: a narrative review. *Anaesthesia*. 2020;75(10):1364-1371. doi: 10.1111/anae.15180
7. Mi Sook J, Hyun-Li K, Yoonjeong L. Factors Influencing Work-life Balance in Korean Registered Nurses. *Korean Journal of Occupational Health Nursing*. 2017;26(2):114-123. doi: 10.5807/kjohn.2017.26.2.114
8. Louise Duncan D. What the COVID-19 pandemic tells us about the need to develop resilience in the nursing workforce. *Nurs Manag (Harrow)*. 2020;27(3):22-27. doi: 10.7748/nm.2020.e1933
9. Yörük S, Güler D. The relationship between psychological resilience, burnout, stress, and sociodemographic factors with depression in nurses and midwives during the COVID-19 pandemic: A cross-sectional study in Turkey. *Perspect Psychiatr Care*. 2021;57(1):390-398. doi: 10.1111/ppc.12659
10. Roberts NJ, McAloney-Kocaman K, Lippiett K, Ray E, Welch L, Kelly C. Levels of resilience, anxiety and depression in nurses working in respiratory clinical areas during the COVID pandemic. *Respir Med*. 2021;176:106219. doi: 10.1016/j.rmed.2020.106219
11. Karaman F, Yerebakan AN, Çakmak S. Choosing nursing as a profession during the Covid-19 pandemic. *Genel Sağlık Bilimleri Dergisi*. 2022;4(1):29-37.
12. Artan T, Atak I, Karaman M, Cebeci F. Relationship Between Sociodemographic Characteristics, Psychological Resilience and Anxiety Levels in the Coronavirus (CO-

VID-19) Outbreak. *J Turk Stud*. 2020;15(6):79-94. doi: 10.7827/TurkishStudies.43882
13. Badu E, O'Brien AP, Mitchell R, et al. Workplace stress and resilience in the Australian nursing workforce: A comprehensive integrative review. *Int J Ment Health Nurs*. 2020;29(1):5-34. doi: 10.1111/inm.12662
14. Hiçdurmaz D, Üzar Y. Protection of COVID-19 Frontline Nurses' Mental Health and Prevention of Psychological Trauma. *Journal of Hacettepe University Faculty of Nursing*. 2020;1-7. doi: 10.31125/hunhemsire.775531
15. Apaydın Ç. Relationship Between Workaholism Levels of Faculty Members and Work-Life Balance and Work-Family Life Balance. *Graduate School of Educational Sciences Educational Administration and Policy Department Doctoral Dissertation.* Ankara; 2011.
16. Smith BW, Dalen J, Wiggins K, Tooley E, Christopher P, Bernard J. The brief resilience scale: assessing the ability to bounce back. *Int J Behav Med*. 2008;15:194-200. doi: 10.1080/10705500802222972
17. Doğan T. Adaptation of the Brief Resilience Scale into Turkish: A validity and reliability study. *The Journal of Happiness & Well-Being*. 2015;3(1):93-102.
18. Karaçam Z. STROBE Writing Standardization in Observational Researches. Ankara. *Ankara Nobel Tıp Kitabevi*; 2018:35-45.
19. Güçlü A, Kurşun Ş. Quality of Work Life and Related Factors of Nurses Working in Surgical Clinics. *Florence Nightingale Journal of Nursing*. 2018;26(3):187-197. doi: 10.26650/fnjn285947
20. Suresh S, Kodikal R. SEM approach to explore Work Life Balance: A study among nurses of Multispecialty Hospitals. *SJOM Journal of Management*. 2017;1(1):1-17.
21. Özdemir N. The Relationship Between Social Intelligence, Self-Esteem with Psychological Resilience for Healthcare Professionals and Affecting Factors. *J Psychiatr Nurs*. 2020;12(1):18-28. doi: 10.14744/phd.2020.96658
22. Gürel S, Özsoy S, Dönmez RÖ. Affecting Factors of Work--Family Conflict Level of Married Nurses Working in Family Health Centers. *ACU Sağlık Bil Derg*. 2017;3:150-156.
23. Yayla A, Eskici İlgin V. The relationship of nurses' psychological well-being with their coronaphobia and work–life balance during the COVID-19 pandemic: A cross-sectional study. *J Clin Nurs*. 2021;30(21-22):3153-3162. doi: 10.1111/jocn.15783
24. Mathew RV, Panchanatham N. An exploratory study on the work-life balance of women entrepreneurs in South India. *Asian Academy of Management Journal*. 2011;16(2).
25. Alarifi A, Basahal A. S. The Role of Spousal Support in Working Mothers Work-Life Balance. *International Business Research*. 2023;16(4):1-39. https://doi.org/10.5539/ibr.v16n4p39
26. Felix Claret E, Umamaheswari R, Venkateswaran P. Linkage between the Overall Work Life and Life Satisfaction of Nurses at Hospitals in Coimbatore District at COVID – 19. *Indian Journal of Natural Sciences.* 2020;10(61):27493-27501.
27. Ren Y, Zhou Y, Wang S, Luo T, Huang M, Zeng Y. Exploratory study on resilience and its influencing factors among hospital nurses in Guangzhou, China. *Int J Nurs Sci*. 2018;5(1):57-62. doi: 10.1016/j.ijnss.2017.11.001
28. Alharbi J, Jackson D, Usher K. The potential for COVID-19 to contribute to compassion fatigue in critical care nurses. *J Clin Nurs*. 2020;29(15-16):2762-2764. doi: 10.1111/jocn.15314
29. Matsuo M, Suzuki E, Takayama Y, Shibata S, Sato K. Influence of Striving for Work-Life Balance and Sense of Coherence on Intention to Leave Among Nurses: A 6-Month Prospective Survey. *Inquiry*. 2021;58:1-9. doi: 10.1177/00469580211005192
30. Ulcay D, Bahçecik N. Psychological Contract Levels of Nurses and Affecting Factors. *Journal of Academic Research in Nursing.* 2020;6(2):324-330. doi: 10.5222/jaren.2020.93063
31. Kossek E, Lee K. The coronavirus & work–life inequality: Three evidence-based initiatives to update U.S. work–life employment policies. *Behavioral Science & Policy.* 2020:1-9.
32. Şenol L, Üzüm B. Psychological Resilience And Alienation Levels of Private Health Institutions Employees. *Journal of Business Research - Turk*. 2019;11(2):1092-1102. doi: 10.20491/isarder.2019.658

Wydawnictwo UR 2023
ISSN 2544-1361 (online)
doi: 10.15584/ejcem.2023.3.13

http://www.ejcem.ur.edu.pl
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2023; 21 (3): 513–518

ORIGINAL PAPER

Are our sub-centers prepared enough to tackle high-risk pregnancies? A cross-sectional survey from Southern Rajasthan, India

Bharti Paliwal [1], Rupa Sharma [1], Rajath Rao [2]

[1] Department of Community Medicine, RNT Medical College, Udaipur, India
[2] Department of Community Medicine, Kasturba Medical College, Mangalore, Manipal Academy of Higher Education, Manipal, Karnataka, India

ABSTRACT

Introduction and aim. Sub-centers (SC) are the first contact point with the community with auxiliary nurse midwife (ANM) as the instrument, delivering all the primary health care services. The SCs are under constant criticism for their inability to deliver quality services. This study assessed the preparation of facilities available at the SC to manage high-risk pregnancies (HRP) and to compare the same between rural and tribal blocks of the selected district.

Material and methods. This health facility-based cross-sectional observational study was done for 6 months among 276 rural and tribal SC of the Udaipur district by a two-stage random sampling method using an observational checklist to assess the infrastructure and logistics of SCs. Data were analyzed using SPSS 20.

Results. The study covered 264 (95.7%) non-24x7 SCs and 12 (4.3%) 24x7 SCs. Only one-third SCs, 93 (33.6%) were situated at the center of the village. Only 151 (54.7%) SCs had attached ANM quarters. All 24x7 SCs and 78.4% of non-24x7 SCs had adequate equipment and infrastructure.

Conclusion. Most of the subcentres' infrastructure and functional equipment was equipped to tackle HRP. Rural SC adhered more than tribal. Most HRPs were tracked and referred to higher centers. Unless we emphasize strengthening SCs, the dream of a healthy nation will remain obscure.

Keywords. auxiliary nurse-midwifery, high-risk pregnancy, primary health care, subcentres

Introduction

The sub-centers (SC)are the geographically closest first contact point with the community with auxiliary nurse midwife (ANM) as the instrument. The health planners in India have visualized the subcentres (SCs) as the proper structural units to provide health services to the rural population.[1] A well-functioning SC providing quality and timely outdoor health care services is important for successfully implementing all health care programs and maternal and child Health (MCH). As per population norms, there shall be one SC established for every 5000 population in plain areas and for every 3000 population in hilly/tribal/desert areas.[2] The ANM has to finish class 12 and have a training of 2 years in midwifery given by Indian Nursing Council to get the awarded with the title ANM and start serving the community.[3] ANM along with a multipurpose worker male (MPW-M) are present in one sub-centre. The ANM is given training under various compoments of primary health care along with maternal and child health care. Different training programs for Induction, skill building and leadership, new programs and if required, refresher training are given to ANM.[4]

Corresponding author: Rajath Rao, e-mail: urrr16@gmail.com

Received: 24.02.2023 / Revised: 23.05.2023 / Accepted: 4.06.2023 / Published: 30.09.2023

Paliwal B, Sharma R, Rao R. *Are our sub-centers prepared enough to tackle high-risk pregnancies? A cross-sectional survey from Southern Rajasthan, India.* Eur J Clin Exp Med. 2023;21(3):513–518. doi: 10.15584/ejcem.2023.3.13.

The number of functioning Sub Centres in Rajasthan was 14408 as of 31st March 2016. It accounted for 9.25% of the total number of functioning Sub Centres in India as on 31st March 2016.[5]

Mothers who are in the HRP group include those who have a history of chronic disease (diabetes, hypertension, heart disease, etc) or those with a history of previous pregnancy problems (abortion and stillbirth). Multiple pregnancies, gestational age under 18 years or over 35 years, pregnancy more than 4 times (the fifth and beyond), and the interval between pregnancies less than one year. All of these can be considered high-risk pregnancies.[6] Out of all pregnancies, 20-30% belong to the high-risk category.[7]

According to AHS 2012-2013, the Maternal Mortality Rate (MMR) of India was 174 and Rajasthan is 208.[8] Rajasthan is the state with the second highest number in maternal mortality in India. High MMR is one of the major areas of concern for the state. Timely identification and management of HRP can prevent most maternal deaths. This can only be possible if the complete range of the required services is accessible to the pregnant women at grass root level at the geographically closest health center, that is SC.

The SCs are under constant criticism for their inability to deliver quality services, The main reasons are the non-availability of health workers, inadequate infrastructure and facilities, and insufficient supply of drugs, and equipment.[9] In many a place, SCs do not have buildings for providing services to the beneficiaries.[10] Basic amenities like water and electricity were also found deficient at SCs, as suggested by previous studies.[5,10,11]

Identification of HRP is a prerequisite for ensuring maternal health. This will ensure safe delivery, and timely and adequate referral and will also play a major role in reducing premature deliveries and infant mortality. Besides, the perinatal outcome can be changed significantly by early detection followed by special intensive care for HRP. Worldwide it has been observed that delays at three levels are the reasons for the deaths of pregnant mothers. Most of the deaths of pregnant mothers can be averted by addressing these delays. The third delay occurs at the facility level, when a pregnant lady reaches at facility either trained manpower, equipment, or drugs are not available. Hence initiation of treatment is delayed to address all these delays and problems faced by a pregnant lady.[12]

Aim

This study was undertaken to assess the preparation of facilities, availability of functional equipment and logistics for the identification and tracking of HRP at the sub-centers and to compare the same among the rural and tribal subcentres. Also, to describe the different high-risk pregnancies registered in the selected blocks of the district.

Material and methods

Ethics approval

The study was approved by the institutional ethical committee, RNT Medical college, Udaipur, Rajasthan (RNT/Stat/IEC/2017/167). Permission to collect data was obtained from the district chief medical and health officer, Udaipur. We adhered to the principles of ethics thereafter throughout the study.

Study design and duration

This was a health facility-based observational cross-sectional study done for 6 months (July 2018-Dec 2018)

Study setting

This study was done among rural and tribal SC of the Udaipur district.

Udaipur district is the southernmost district of the state of Rajasthan, India, and a predominantly rural district with a population of 3,068,420 (Census 2011, India) and having 12 subdivisions and 629 subcentres catering to the population of these areas.

Sample size estimation and sampling technique

According to Manas PR et al., 56.2% of SC were operational and met the Indian Public Health Standards (IPHS) norms in their study.[13] So, the minimum sample size required for this study was 266 rounding off to 276 at 95% confidence intervals,80% power, and 6% absolute precision. The sample size was calculated using Statulator (an online sample size calculator).

We used a two-stage random sampling technique. There were a total of 12 Blocks in the Udaipur district out of which six were tribal, five were rural, and one urban.[14]

In stage one, 50% of the tribal (three blocks) and 50% of the rural (rounded to three blocks) blocks were selected randomly by the lottery method. The blocks thus selected were Gogunda, Jhadol (Phalasia), Sarada, Salumber, Bhinder, and Badgaon. In stage two, all the 305 SC among these selected Blocks[15] were line listed and 276 SC were randomly selected to arrive at the sample size.

Study tools and techniques

An observational checklist, each for infrastructure and logistics and skills of ANM like antenatal examination, measuring blood pressure, and laboratory testing for hemoglobin, urine albumin, and sugar were made and records at the subcentre were checked for common high-risk pregnancies (HRP) noted in each SC. SC has scored accordingly. SC with >60% scores was considered as 'SC with adequate functional equipment' for tackling HRP and further SC were categorized as 'good (8-10 functional equipment)', 'average (5-7 functional equipment)', and 'below average (<5 functional equipment)' based on the availability of functional equipment. For antenatal care (ANC), the equipments like sphygmo-

manometer, measuring tapes, weighing scale, haemoglobinometer, urine dip sticks for glucose & protein, thermometer, stethoscope, essential medicines including iron and folic acid, Inj. Tetanus toxoid, record register and for labor room, equipments like partograph, sphygmomanometer, stethoscope, fetoscope were assessed. The skills of ANM towards essential ANC was assessed based on checklist having skills like history taking, examination, lab investigation, treatment (Iron and Folate distribution, Inj. Tetatnus toxoid), counselling and referral of antenatal mothers. The skills were considered adequate if ANM scored ≥60% for all essential parts of skill assessed on checklist. Prior permission was taken from the district Chief Medical and Health officer for the visits and block meeting days were excluded after discussing with the respective block chief medical officer, SCs were visited and the ANMs were not given any prior information about the visit.

Data analysis

Data was coded and entered in a Microsoft excel sheet and analyzed on SPSS version 20 (SPSS Inc., Chicago, IL, USA). Results were expressed as tables and figures wherever necessary. Categorical variables like type of SC, basic facilities at SC, adequacy of availability of functional equipment at SC, and common HRP registered as frequency and proportions. The chi-square test of association was applied to compare characteristics across 24x7 and non-24x7 SC and rural-tribal SC and the difference was ascertained as significant when the p value was <0.05.

Results

General characteristics of SC

Most of the SCs, 264 (95.7%) in the study were of non-24x7 type. Only 12 (4.3%) SCs functioned 24x7. Tribal areas had more, 8 (5.7%) 24x7 SCs as compared to rural areas [4 (3%)].

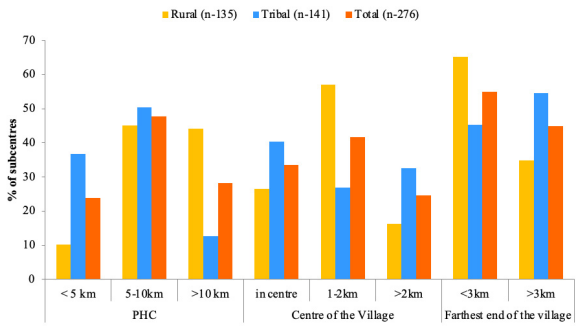

Fig 1. Location of the sub centres from different setups (n=276)

Only one-fourth, 66 (23.9%) SCs were situated within five km from the nearest PHC. 52(36.8%) being tribal and 14 (10.3%) being rural. Only one third, 93(33.6%) were situated in the centre of the village, 57 (40.4%) in tribal and 36 (26.6%) rural SCs. The majority, 152 (55%) of SCs were within three km from the farthest end of the village, 88 (65.1%) were rural, and 64 (45.3%) were tribal SCs (Fig. 1).

Basic facilities at SC

All SCs had names and facilities displayed prominently and had color-coded waste bins. Most, (≥80%) SCs fulfilled other requirements of infrastructure. Only 151 (54.7%) had attached ANM quarters. Separate space for the laboratory was available at only 126 (45.7%) SCs. All 24x7 SCs in both rural and tribal areas fulfilled most of the infrastructure norms. None of the SCs had a registered telephone line. 24-hour electricity backup was available only at the 24x7 SCs (Table 1).

Table 1. Distribution of subcentres according to availability of basic facilities required (n=276)*

Basic facilities	Non 24x7 sub-centers (n=264)		24x7 sub-centers (n=12)		Total (%)
	Rural (n=131,%)	Tribal (n=133,%)	Rural (n=4,%)	Tribal (n=8,%)	
Name and facilities displayed prominently	131 (100)	133 (100)	4 (100)	8 (100)	276 (100)
Display of HRP related IEC Material	106 (80.9)	102 (76.7)	4 (100)	8 (100)	220 (79.7)
Dedicated Room for ANC	115 (87.8)	109 (82)	4 (100)	8 (100)	236 (85.5)
Privacy is ensured in examination room	124 (94.7)	117 (88)	4 (100)	8 (100)	253 (91.7)
Separate space for laboratory	52 (39.7)	64 (48.1)	4 (100)	6 (75)	126 (45.7)
Color coded Waste bins	131 (100)	133 (100)	4 (100)	8 (100)	276 (100)
Attached ANM Quarters	75 (57.3)	64 (48.1)	4 (100)	8 (100)	151 (54.7)
Registered telephone line	0 (0)	0 (0)	0 (0)	0 (0)	0(0)
Separate Labor Room with Attached Toilet	0 (0)	0 (0)	4 (100)	8 (100)	12 (4.3)
24 hours electricity back up	0 (0)	0 (0)	4 (100)	8 (100)	12 (4.3)

* ANC – Antenatal care; HRP – high risk pregnancy; IEC – information education communication

Logistics and functional equipment at SC for tackling HRP

All (100%) 24x7 SCs in both rural and tribal areas had adequate functional equipment related to ANC and labor Room. Most (≥85%) non-24x7 SCs had adequate functional equipment (Table 2).

Most, 207 (78.4%) of non-24x7 Sub Centres had good availability of functional equipment. Only 87 (65.4%) tribal as compared to 120 (91.6%) rural non-24x7 Sub Centres had good availability of functional equipment. The difference was statistically significant.

(p<0.001). All 12 (100%) 24x7 SC had good availability of functional equipment (Table 3).

Table 2. Number of subcentres having adequate functional equipment (n=276)*

S.N.	*Adequate functional equipment	Non 24x7 sub centres (n=264)		24x7 sub centres (n=12)		Total SCs (n=276)
		Rural (n=131)	Tribal (n=133)	Rural (n=4)	Tribal (n=8)	
1	ANC related functional equipment available	125 (95.4)	113 (85)	4 (100)	8 (100)	250 (90.6)
2	Labor room related functional equipment available	NA	NA	4 (100)	8 (100)	117 (42.4)

*Score >60% of total equipment; ANC – antenatal care

Table 3. Level of availability of functional equipment in Sub Centres (n=276)

Rating	Non 24x7 sub-centres			24x7 sub-centres		
	Rural (n=131)	Tribal (n=133)	Total (n=264)	Rural (n=4)	Tribal (n=8)	Total (n=12)
Good *	120 (91.6)	87 (65.4)	207 (78.4)	4 (100)	8 (100)	12 (100)
Average**	5 (3.8)	26 (19.5)	31 (11.7)	0 (0)	0 (0)	0 (0)
Below Average***	6 (4.6)	20 (15)	26 (9.8)	0 (0)	0 (0)	0 (0)
Total	131 (100)	133 (100)	264 (100)	4 (100)	8 (100)	12 (100)
*8-10, **5-7, ***< 5 Functional equipment						
Chi-square (χ^2) =27.012, df= 2, p<0.001#						

#p<0.05 is significant

Skills of ANM on different components of essential antenatal checkup

Almost all, 266 (96.3%) out of 276 ANM displayed adequate skills of prescribing treatment for ANC but only half had adequate skills of history taking (50.7%) and counselling (50%). Almost less than half were skilled adequately for examination (47.8%), referral (46%) and lab investigation (44.6%).

Table 4. Types of HRP cases identified and registered at the sub centre (in last 3 months)*

Type of HRP	Rural	Tribal	Total
Anaemia	393 (73.04)	500 (83.89)	893 (100)
APH	36 (6.6)	12 (2.01)	48 (100)
Malpresentation	42 (7.8)	34 (5.7)	76 (100)
Pre-eclampsia	67 (12.4)	50 (8.3)	117 (100)
Other	0 (0)	0 (0)	0 (0)
Total	538 (47.4)	596 (52.5)	1134 (100)

* HRP – high risk pregnancy

Common HRP registered at SC

In the last three months of the study, 1134 high risk antenatal pregnancies (HRAP) were identified and registered at the selected SCs. 596 (52.6%) of these at tribal and 538 (47.4%) at rural SCs. 990 (87.3%) of these were tracked and referred to higher health centers. None of the HRAP were delivered at the SC.

Anaemia [893, 78.7%] was the most commonly associated risk factor among the HRAP identified followed by pre-eclampsia [117 (10.3%)]. More HRAP were identified at tribal 596 (52.5%) as compared to rural SCs 538 (47.4%). Anaemia was associated as a risk factor in more tribal ANC cases 500 (83.89%) as compared to rural ANC cases 393 (73.04%) (Table 4).

Discussion

Out of the 276 SCs covered in the study only 12 (4.3%) were 24x7 SCs, all of which had the infrastructure and functional equipment required to tackle HRP. Only 87 (65.4%) tribal as compared to 120 (91.6%) rural non-24x7 Sub Centres had good availability of functional equipment (p<0.001). A study from Bihar has also reported a huge gap of more than 50% in the required v/s functional SCs.[16] The SCs were in poor condition that lacked even basic furniture. There was the unavailability of equipment for ANC, like BP instrument, haemoglobinometer, stethoscope, weighing scale with the ANMs, and irregular supply of iron and folic acid tablets and tetanus toxoid injections.

According to IPHS guidelines for Sub-Centers, the SC is to be located within the village for providing easy access to the people and safety of the ANM, as far as possible no person has to travel more than 3 km to reach the SC.[17] But in this study, it was observed that most of the ANC sessions are being conducted at Anganwadi centers (AWC) and not at SC despite the availability of infrastructures and ANC check-up facilities at the SCs. This was because AWCs are located at the center of the village and are easily approachable for the beneficiaries as compared to SCs. In our study, only one-third SCs 93 (33.6%) were situated at the center of the village while more than half, 152 (55%) were within three km from the farthest end of the village. 72.8% were situated within 10 km of the nearest PHC.

The majority 151 (54.7%) of SCs had attached ANM quarters but it was observed that only one-fourth, 66 (23.9%) subjects were living in the quarters attached to the sub-center. The SCs being located far from the village and lack of effective supervision and monitoring might be the reasons behind it. The unavailability of ANM at the SC adversely affects the quality of identification and management of HRP ANC. In contrast, Rural Health Statistics 2014-15 states that in Rajasthan only 47.2% of SCs had attached ANM quarters and 89.7% SCs were with ANM living in sub-center quarters.[18]

Out of the 1134 HRP ANCs identified in the last three months, 990 (87.3%) were tracked and referred to higher health centers. None of the HRP ANCs were delivered at the SC. In Indian families, the husband and mother–in–laws play an important and dominant role in making the decision that is crucial to women's health. Certain symptoms in pregnancy though are indications for referral and or hospitalization, are mostly not report-

ed to even ANM or Accredited Social Health Activisit as the decision-makers do not realize their gravity. The follow-up visits of the HRP antenatal care are also decided by them. In Rajasthan, about one-third of total deliveries (28.4%) are still conducted at home and in the Udaipur district this is 41.7%.[8] This marks the need of a robust system to tackle such HRPs in the district.

Recommendation

The inadequacy of equipment, drugs, and infrastructure should be assessed through facility surveys and the deficits to be filled up urgently and they should be able to meet the standard norms. Also, there should be a registered telephone line and a locally available and readily accessible vehicle at all sub-centers. Lastly, the identification of potential SCs for conducting deliveries and upgrading them according to the standards is necessary.

Study limitations

Only one district is covered and hence results cannot be generalized to the whole of the state. The sampling would have been better if not for simple random sampling. Also, the study has more of local value and the health care system of one country (India).

Conclusion

The infrastructure, logistics, and availability of functional equipment at most of the subcentres were sufficient enough to identify, track and tackle high-risk pregnancies. Rural SC scored better than tribal. All 24x7 SCs in both rural and tribal areas fulfilled most of the infrastructure norms. All 24x7 SCs and most Non 24x7 SCs in both rural and tribal areas had adequate functional equipment and good availability of functional equipment related to ANC and labor room. Anaemia was the most common HRP identified in the selected SCs. Although National Rural Health Mission (now known as National Health Mission) was launched with a holistic approach, many basic issues including the availability of suitable infrastructure support at peripheral health centers are still required to be addressed. Unless we emphasize strengthening SCs, the dream of a healthy nation will remain obscure as no population can improve the basic parameters of health, based only on tertiary health care. Hence, primary health care should be the priority of health reform.

Acknowledgments

We would like to thank the district Chief Medical and Health officer, Udaipur, and the block Chief medical officers of selected blocks of Udaipur District, Rajasthan, India for permitting us to carry out the study. Also, we are thankful to the Head of the department, Department of Community Medicine, RNT Medical College, Udaipur for constant support.

Declarations

Funding

No funding was received for this study.

Author contributions

Conceptualization, B.P. and R.S.; Methodology, R.S.; Software, B.P. and R.R.; Validation, B.P. and R.R.; Formal Analysis, B.P. and R.R.; Writing – Original Draft Preparation, R.R.; Writing – Review & Editing, B.P, R.S and R.R.; Visualization, R.R.; Supervision, R.S.; Project Administration, R.S.

Conflicts of interest

None to declare

Data availability

Data is available on request to corresponding author

Ethics approval

Institutional Ethical Committee, RNT Medical College and MB General Hospital has been taken for the study (RNT/Stat/IEC/2017/167).

References

1. Patil SK, Shivaswamy SM. Assessment of sub-centres of Belagavi district according to Indian public health standards 2012 guidelines: a cross sectional study. *Int J Community Med Public Health*. 2017;4(6):1938-1942. doi: 10.18203/2394-6040.ijcmph20172153
2. National Health Mission. https://nhm.gov.in/images/pdf/guidelines/iphs/iphs-revised-guidlines-2012/sub-centers.pdf. Accessed April 12, 2022.
3. Indian Nursing Council. Guidelines and minimum requirements to establish new auxiliary nurse and midwives school of nursing. https://indiannursingcouncil.org/uploads/pdf/167818116369773447364070 32b70397.pdf. Accessed April 12, 2022.
4. National Health Mission. http://nhm.gov.in/images/pdf/guidelines. Accessed August 22, 2022.
5. Open Government Data (OGD) Platform India. Open Government Data (OGD) Platform India. https://data.gov.in. Published January 21, 2022. Accessed April 12, 2022.
6. Chaman R, Yunesian M, Golestan B, Holakouie Naieni K. Evaluation of the Effects of High-Risk Pregnancy on Neonatal Mortality in a Rural Area of Iran: A Nested Case--Control Study. *Iran J Epidemiol*. 2008;3(3):1-6.
7. Dutta DC. Textbook of obstetrics. In: *Textbook of Obstetrics*. 6th ed. New Central Book Agency(P) Ltd.; 2004:631.
8. Census of India: Annual Health Survey 2011 - 12 Fact Sheet. https://www.censusindia.gov.in/vital_statistics/ahsbulletins/ahs_factsheets_2011_12.html. Accessed April 12, 2022.
9. Reddy NB, Prabhu GR, Sai TSR. Study on the availability of physical infrastructure and manpower facilities in sub--centers of Chittoor district of Andhra Pradesh. *Indian*

J Public Health. 2012;56(4):290-292. doi: 10.4103/0019-557X.106417
10. Bajpai N, Sachs JD, Dholakia RH. Improving Access, Service Delivery and Efficiency of the Public Health System in Rural India. 2009. doi: 10.7916/D8NK3DCT
11. Pal DK, Tiwari R, Kasar PK, et al. Functioning of the Sub Health Centers (SHCs) in Mandla District. 2009;8:183-190.
12. Concepts of NRHM. http://nrhmrajasthan.nic.in/healthfacalities.htm#Healthdelivery. Accessed April 12, 2022.
13. Roy MP, Mohan U, Singh S, Singh V, Shrivastava A. Sub Centre Support, Need of the Hour: A Comparative Study from Lucknow. *People's Journal of Scientific Research*. 2014;7(2):5.
14. Blocks/Tehsils/Panchayats. https://udaipur.rajasthan.gov.in/content/raj/udaipur/en/about-udaipur/blocks-tehsils--panchayats.html. Accessed July 20, 2021.
15. http://nrhmrajasthan.nic.in/Subcenter%20of%20pdf/Udaipur.pdf. Accessed April 12, 2022.
16. State Health Society, Bihar. Bihar State Gap Analysis. http://statehealthsocietybihar.org/qa-monitoring.html. Accessed April 12, 2022.
17. http://www.sihfwrajasthan.com/ppts/full/IPHS.pdf. Accessed April 12, 2022.
18. Rural Health Statistics 2014-15, Ministry of Health and Family Welfare Statistics Division | Ministry of Women & Child Development. https://wcd.nic.in/acts/rural-health-statistics-2014-15-ministry-health-and-family-welfare-statistics-division. Accessed April 12, 2022.

Wydawnictwo UR 2023
ISSN 2544-1361 (online)
doi: 10.15584/ejcem.2023.3.14

http://www.ejcem.ur.edu.pl
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2023; 21 (3): 519–528

ORIGINAL PAPER

The effect of uniform wearing and candle lighting ceremony on nursing students' perspective on the profession – a qualitative study

Özge Buldan [1], Pinar Harmanci [2]

[1] Faculty of Health Sciences, Tokat Gaziosmanpasa University, Erbaa, Turkey
[2] Faculty of Health Sciences, Kahramanmaraş Istiklal University, Kahramanmaraş, Turkey

ABSTRACT

Introduction and aim. The aim of this study was to investigate the effect of the Uniform Wearing Ceremony and the Candle Lighting on nursing students' perceptions of the profession.

Material and methods. A qualitative, phenomenological method was used to design the study. The standardised criteria for reporting qualitative research with 32 elements were used for the research. The study was conducted with 60 students who volunteered to participate in the study by not selecting a sample of first year nursing students from a university in Northern Turkey. Interviews were conducted 3 times for approximately 20-40 minutes.

Results. In this study, the rate of students who perceived the Uniform Wearing Ceremony as positive was as high as (n=42), the rate of those who thought that the uniform had an effect on the image of the nursing profession was 88.3%, and the rate of those who considered nursing as a respected profession was (23%).

Conclusion. Nursing students who participated in the study stated that the effect of the Uniform Wearing Ceremony and the Candle Lighting Ceremony positively influenced their perception of the profession and that the uniform had an impact on the image of the nursing profession.

Keywords. candle lighting, ceremony, nursing student, professional perception, uniform

Introduction

Nursing is generally perceived by society as a white uniformed profession, the epitome of love, compassion and kindness.[1,2] As an indispensable member of the health care team, nursing has important responsibilities such as the protection and promotion of health and the healing of illness.[1] These responsibilities highlight the importance of nursing education. The educational process aims to ensure that the student nurse has a positive outlook on the profession, is open to development and has a modern professional nursing identity.[2]

The first steps towards entering the profession and becoming a professional are taken during the student years and continue throughout professional life.[3] For nursing students, the perception of the profession begins with the perspective of the environment in which they live and continues to develop and mature throughout the educational process.[4,5] There are many studies in the literature to determine nursing students' perceptions of the nursing profession.[6-8]

Nightingale emphasised the importance of nursing education, drawing attention to the importance of formal education by opening the first nursing school and

Corresponding author: Özge Buldan, e-mail: ozgebuldan1@gmail.com

Received: 12.04.2023 / Revised: 31.05.2023 / Accepted: 12.06.2023 / Published: 30.09.2023

Buldan Ö, Harmanci P. *The effect of uniform wearing and candle lighting ceremony on nursing students' perspective on the profession – a qualitative study.* Eur J Clin Exp Med. 2023;21(3):519–528. doi: 10.15584/ejcem.2023.3.14.

emphasising the importance of training qualified nurses to meet the ever-changing health needs of society.[9,10] The literature states that nursing began in the mid-19th century under the leadership of Florence Nightingale during the Crimean War.[9,11] Florence Nightingale was a manager, educator and statistician who took reformist initiatives in nursing care to improve hospital conditions and the wounded around the world.[10] Florence Nightingale introduced nursing reform initiatives to improve hospital conditions and wounded people in all countries of the world.[10] She was a manager, educator and statistician who dedicated nursing to the care of human life in a practical and scientific way.[11]

During the Crimean War, the gas lamp carried by Nightingale helped to spread goodness, love and help from Istanbul to the whole world.[12] As Nightingale examined the sick and wounded in the wards at night with a kerosene lamp in her hand, she also dispelled the dark atmosphere that dominated the inner world of the patients with her lamp that illuminated the darkness of the night.[12-14] The soldiers, both touched and comforted by her boundless source of compassion, called F. Nightingale, who was very popular with her lamp, the "Lady with the Lamp".[15,16.]

This lamp, which has been carried at graduation ceremonies and other academic rituals in educational institutions since 1920, is still used today as a symbol of the nursing profession.[16] In the lighting ceremonies held in our country, Safiye Hüseyin (Elbi), who is considered the Florance Nightingale of the Turks and the pioneer of modern nursing, is commemorated along with F. Nightingale, and first-year students experience this justified pride as they take their first step into the profession.

Nursing is the only profession in which the ritual of candle lighting is performed. The Candle Lighting Ceremony is a blessed time in every nurse's life.[17] The representative candlelight symbolises the effort to help others and not to feel alone in times of pain and suffering, as well as knowledge that dispels ignorance, enlightenment, experience, hope, love and comfort.[17,18]

The 'Uniform Wearing Ceremony', which was performed together with the 'Candle Lighting Ceremony' ritual in the first year of nursing, has begun to become a ritual adopted by various health professions. It is practised by various health professions, including dentistry, pharmacy, medicine, physiotherapy and nursing.[19]

The Uniform Wearing Ceremony is a symbol of transformation that marks the transition from a vocational student to a health professional.[20] In a review of studies conducted abroad, it is stated that this ceremony is a welcome greeting to students who are members of the health profession, and the essence of the ceremony emphasises humanism, excellence and compassion.[21] These ceremonies are recognised as a practice that increases professional commitment to a profession and acceptance of professional values.[22] In addition, it is stated that the Uniform Wearing Ceremony contributes to students experiencing a sense of pride and shows what professional appearance and presentation means for nursing.[19]

Nevertheless, controversial opinions about the Uniform Wearing Ceremony persist.[19] In the literature review on nursing symbols and rituals, some authors state that these ceremonies have no meaning or purpose today, and suggest that these ceremonies should not be discarded, but should be reconnected with the values of contemporary professional nursing.[23]

Traditional ceremonies reflect the emotional impact and moral significance of professional commitment. However, there are those who argue that it would be better for students to take the oath as an honourable students, promising to abide by the academic rules of the school and fulfil the duties they have undertaken, rather than in the name of the symbols and rituals of a profession they know nothing about.[24]

Another view was that it was not possible for the uniform ceremony to represent both a welcome to the profession and professional advancement, arguing that it was not understood why nursing needed a 'Uniform Wearing Ceremony' to remind students that they should have compassion and empathy when nursing has a long history of caring. It was also pointed out that this could lead to role confusion with other health professionals.[25]

It is stated that it is important for nursing students to recognise, love and consciously choose their profession in terms of professionalism and quality health service delivery.[26]

Looking at the studies, there are mostly studies with medical and pharmacy students with the Uniform Wearing Ceremony, but there is limited research on the Candle Lighting Ceremony with nursing students.[22,27]

Aim

The purpose of this study was to determine the professional perceptions of the Uniform Wearing and The Candle Lighting Ceremony from the perspective of nursing students.

Material and methods

Ethics approval

Ethical permission for the study was obtained from Tokat Gaziosmanpaşa University Scientific Research and Publication Ethics with permission number 01-42 dated 27.05.2022. Prior to this permission, written research permission was also obtained from the School of Nursing Students where the study would be conducted. Participants were informed verbally and the Declaration of Helsinki was read to each participant. Informed consent was signed.

Study design

A qualitative, phenomenological method was used in the design of the study. This method was chosen to elicit nursing students' opinions about the impact of the uniform and light ceremony on nursing students' views of the profession, which is the aim of the study. Consolidated criteria for reporting qualitative research with 32 elements were used for the study.[28]

Population and sample

First-year nursing students at a university in northern Turkey were included in the study. All first year students constitute the population of the study and also the sample. There are 60 nursing students in the study (Fig. 1). As part of the research, the students were asked questions about what the Uniform Wearing and Candle Lighting Ceremony, which has professional themes, made them think about the nursing profession. All 60 students were basically interviewed 3 times. At the end of the basic interviews within the sample (simultaneously), the data reached saturation. The criterion of data saturation was therefore met.

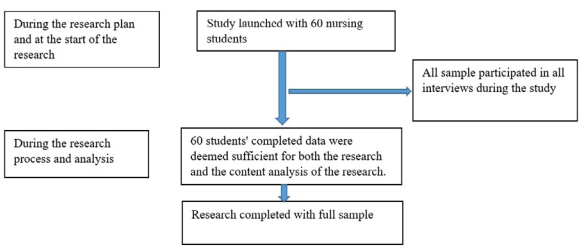

Fig. 1. Flow chart of the study

Data collection

Two separate forms were designed for the purpose of the study and according to the sample. A basic form consisting of 8 questions including age, gender, marital status, place of residence, whether anyone in the family works as a nurse, reasons why students prefer nursing, whether the uniform has an effect on the image of the nursing profession, and whether they like the nursing uniform they will wear in the clinic. The other form (form 2, 8 questions) is the second form that contains open-ended interview questions to obtain the data of the qualitative research to be conducted. Form 2, developed as part of the research, was designed to assess the nursing students' thoughts about the profession after the Uniform Wearing and Candle Lighting Ceremony (Table 1). Form 2, which was designed to collect the research data in a scientific manner and in accordance with the phenomenological design, was created by taking the opinions of three different experts who have studies in the same methodology in the field. Within the framework of the research, the 2nd interview plan was made with the data obtained after the 1st interview with the nursing students. According to the data obtained in the 2nd interview, the 3rd interview plan was made and the data reached saturation. Consent for the study was obtained from the student participants and recorded in writing. The interviews were conducted in a calm, quiet, well-ventilated environment where the interviewer could answer the questions well. The interviews were conducted in a suitable classroom of a faculty where the nursing department is located and lasted approximately 20-40 minutes. The flow chart of the research process is shown in Figure 2.

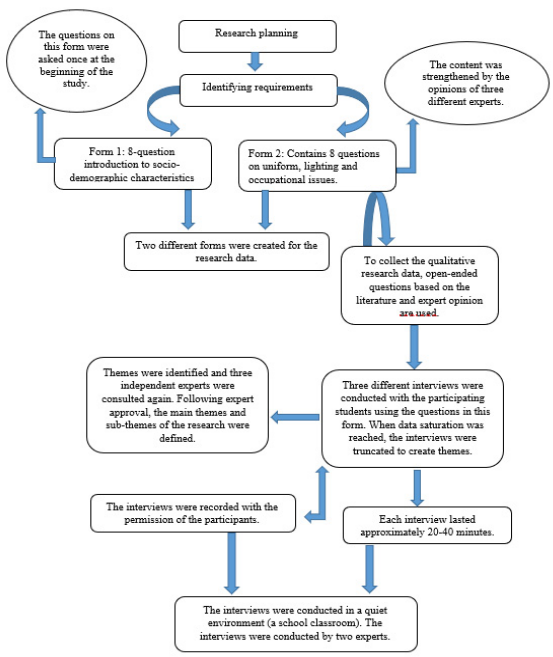

Fig. 2. Flowchart of the research process

Information about the uniform wearing and candle lighting ceremony

All first year nursing students attended the ceremony in their uniforms. A selected nursing student gave a speech about their feelings and thoughts about the ceremony. Then, one by one, the students were called up to the stage and received their lights from the lantern in the hands of a nursing instructor. The ceremony ended with the recitation of the nursing oath.

Rigor

Four main criteria were identified in the study to ensure details in terms of reliability, confirmability and transferability.[29] To ensure reliability, the researcher tried to maintain a long-term relationship with the nursing students who participated in the study and to establish a friendly and trusting relationship with them. The researcher has a long and significant experience in nursing education. The researcher explained to the nursing students that they could express themselves freely and that every opinion was very valuable/important. All interviews were

carried out by two experienced researchers. During data analysis, repeated listening, comparisons and repetition of questions helped to ensure the reliability of the findings. Three different experts experienced in qualitative research were involved in the coding and theme development process in a controlled and comparative way. In addition, the Consolidated Criteria for Qualitative Research Reporting checklist was used to ensure that the study report was transparent and reliable.[28] Preliminary interviews were conducted prior to the study and experts were consulted on an ongoing basis. This increased the validity and reliability of the research. However, as with all qualitative studies, not all statements can be included in the data transfer, so an attempt has been made to present the most important and generally reflective data.

Table 1. Semi-structured open-ended questions used in the interview

Subject	Key questions
Professional ceremonies related to nursing	What do you think of the Uniform Wearing Ceremony?
	What do you think of the Candle Lighting Ceremony?
	What was your first reaction when you heard that the ceremony was going to take place?
	Do you think the ceremony of Uniform Wearing and Candle Lighting is necessary for the nursing profession?
Perceptions of the nursing profession and the impact of professional ceremonies	Do you think that the perception of the nursing profession and the ceremony performed are related?
	How did you feel about your profession during the ceremony?
	How did you feel after the ceremony?
Perception of relationship between nursing profession and uniform	What do you think the nursing uniform means for the profession?

Data analysis

The data obtained from the interviews were analysed using the content analysis method. The qualitative content analysis method is a method that aims to describe and evaluate the phenomena that appear on the subject by dividing them into main topics and themes.[30] The data were listened to, written, read and evaluated several times by the researcher. The first codes were developed as part of this evaluation. After the coding process, which was carried out by reading the data in a controlled and comparative way, the researcher developed themes.[31] The themes were not predetermined but were derived from the data obtained. No computerised algorithms were used in the qualitative data analysis process.

Results

This study was carried out to investigate student nurses' perspectives on the nursing profession from the students' point of view during the Uniform Wearing and Candle Lighting Ceremony. Of the 60 participants in this study, 38 (63.3%) were female and 34 (56.6%) were aged 18-20 years (Table 2).

23% of the students stated that they chose nursing because they considered it to be a respectable profession. 88.3% of nursing students reported that the uniform had an effect on the image of nursing (Table 2).

Table 2. Descriptive characteristics of participants

Descriptive Feature Question		n	Percentage (%)
Age	18-20	34	56.6
	21-23	23	38.4
	24 and over	3	5
Total		60	100
Gender	Female	38	63.3
	Male	22	36.7
Total		60	100
Place of residence	City	40	66.6
	District	15	25
	Village	5	8.4
Total		60	100
Do you have a nurse in your family?	Yes	0	0
	No	60	100
Total		60	100
What is your reason for choosing nursing?	Because my parents wanted it.	11	19
	Because I can be easily appointed	12	20
	Because I can make good money	14	23
	Because I see it as a respectable profession.	14	23
	Because I love the job	9	15
Total		60	100
Do you think the uniform affects the image of the nursing profession in our country?	Yes	53	88.3
	No	7	11.7
Total		60	100
Do you like the uniform you will wear in the clinic during your training?	Yes	41	68.3
	No	19	31.7
Total		60	100

The effect of professional ceremonies on students' thoughts

This section presents data on the impact of professional ceremonies on students' thoughts.

The effect of ceremonies on students' ideas and thoughts about the profession

Although it was seen that the students generally had the same opinion about the Uniform Wearing and the Candle Lighting Ceremony in relation to the nursing profession, it was seen that 18 of the students (n=18) thought that the Candle Lighting Ceremony was "not suitable for our culture".

''I felt very important and like a real nurse. During the ceremony, I also wanted to research the theoretical nurses I had heard about from my professors. Hearing that it is a very labour-intensive profession brought me closer to the profession."

(Female - 11th student)

''When our names were read out one by one with all my friends, it was a great honour for me to be one of them. With the nurse's uniform I wore for the first time, it was as if we were saying to the professionals from different fields who were watching us at the ceremony, "We will develop and improve your health"... It was great."

(Male – 23rd Student)

"The ceremony was very nice. I felt very good when my name was read out, but the lighting ceremony was more like other cultures. I will still investigate this situation."

(Female – 21st Student)

The impact of professional ceremonies on students' perceptions of the nursing profession

This section contains data that assesses the students' previous experiences, how they viewed the profession before enrolling on the nursing programme, and how they felt after the ceremony.

The effect of the Candle Lighting Ceremony on students' professional perceptions

''I had heard about the woman with the lamp because my cousin was a nurse. But I didn't know it was such a powerful story in the profession. Especially during the Lighting Ceremony, I felt that our teacher had paved the way for us. I also felt a great sense of responsibility. To be honest, this sense of responsibility was not what I had thought before. It was like growing up..."

(Male – 37th Student)

''I had never heard of the Candle Lighting Ceremony. Even if it was mentioned, it would not sound like a ceremony in Turkish culture. Besides, when I won the nursing department, I did not think it was a professional ceremony. When we lit the candle lit by our teacher in the Candle Lighting Ceremony in our own hands one by one, I said to myself: 'I am a nurse now'."

(Female – 41st Student)

The effect of the Uniform Wearing Ceremony on students' professional perceptions

In this section, we found that student nurses often use uniform and professionalism together. Students attribute meanings to the Uniform Wearing Ceremony such as ''taking the first step towards becoming a professional.

''The uniform was always the thing I most wanted to see on me. I have already written this chapter very fondly. During the Uniform Wearing Ceremony I felt like a professional, as if I already had the spirit to become a nurse."

(Female – 8th Student)

''The Uniform Wearing Ceremony is a ceremony that must be held in all future years because the student walks like a nurse during the ceremony. Honestly, when my name was called, I felt like a great protector and healer of life. From now on I will be more careful when studying. I also enjoyed being with my classmates at the Uniform Wearing Ceremony. It was like we were all there for a purpose, like a bonding ceremony."

(Male – 58th Student)

The effect of the uniform on students' perception of the profession

This section presents the students' data on how the uniform affects their perceptions of the nursing profession and how important it is to the profession, regardless of the ceremonies.

The effect of the uniform on students' perceptions of the nursing profession

''The nurse's uniform is important as far as I know. Because before we went to the clinic, even before our uniforms were sewn, our teachers told us that a uniform should never be worn outside the clinic (on the way home or in the street). I think this is wrong in many ways. The uniform is not worn everywhere. It also gives you a lot of responsibility in the clinic. It made me feel that I would be doing a very important job and that I should never be separated from equality."

(Male – 43th Student)

''I think a nurse should choose her uniform very carefully and carefully. I think the uniform is like a flag that describes all the duties of a profession... It increases respect and responsibility. In the past, when I saw nurses wearing these uniforms, all I could think of was that they would give me an injection and hurt me. Now I can't wait to learn and practice."

(Female – 34th Student)

The main themes and subcategories developed in the study are presented in Table 3.

Table 3. Main themes and subcategories developed in the study

Main topics	Subcategories
The impact of professional ceremonies on students' thinking	The impact of ceremonies on students' ideas and thoughts about the profession
The effect of professional ceremonies on students' perceptions of the nursing profession	The impact of the Candle Lighting Ceremony on students' professional perceptions
	The impact of the Uniform Wearing Ceremony on students' professional perceptions
The impact of uniforms on students' perceptions of the profession	The effect of uniform on students' perceptions of the nursing profession

Discussion

This study was undertaken to investigate the effect of the Uniform Wearing and Candle Lighting Ceremony on nursing students' views of the profession. Of the 60 participants in this study, 38 (63.3%) were female and 34 (56.6%) were aged 18-20 years.

Traditions have the power to convey important beliefs and values held by professional disciplines.[23,32] Professional rituals are activities that can increase students' desire to learn, create a sense of belonging, and transform the classroom from an individualistic space into a community.[33] It is known that professional ceremonies have been important since ancient times as a "welcoming" ritual to grasp the basic philosophy of the profession and to adhere to basic ethical rules.[34] There are other studies in the literature that explain the impact of professional ceremonies on the ideas and thoughts of participating students. In a study of 77 nursing students who participated in the Uniform Wearing Ceremony, it was reported that the ceremony was valued by the participants, that it was perceived as a symbolic transition into professional nursing education, and that such ceremonies should be continued.[19] Another study reported that the "Welcome to the Profession" ceremony and the uniform worn at the ceremonies, conducted with 69 participants consisting of third and fourth year nursing students, improved the sense of professional identity in 92.6% of the third year students who wore the uniform and 76.5% of the fourth year students who did not wear the uniform.[34] An author who participated in the passing of the Candle Lighting Ceremony for medical students reported that all participants were deeply moved, satisfied and praised the ceremony in a small sample in which he, as an outside observer, asked the students after the ceremony what they thought of the ceremony.[22]

In the White Coat Ceremony for pharmacy students, it was reported that the majority of participants saw the White Coat Ceremony as a welcome to the profession (46%), while a smaller proportion saw the ceremony as a way to develop the student's professional identity (12%).[35] According to the findings of this study, based on the students' statements about the ceremony, although the participants generally reported positive statements, some students (n=18) also reported that the 'Candle Lighting' ceremony was not suitable for our culture. The fact that the students who felt that it was not appropriate to our culture may be due to the fact that they come from different traditions and that they had not previously received information about this ceremony from their environment and the media.

Professional ceremonies are activities that prepare the ground for student nurses to form a professional identity and to take pride in their profession.[36] The Candle Lighting Ceremony is one of the activities that enables students to understand the characteristics of the nursing profession and to embrace their profession. The Candle Lighting that students receive during the ceremony also represents the ethical commitment that will continue throughout their careers.[18] There are studies in the literature that explain the effect of professional and Candle Lighting Ceremonies on students' perceptions of the nursing profession. In Canada, it was reported that 92.6% of third-year nursing students and 76.5% of fourth-year nursing students made positive statements that the ceremony had improved (or would improve) their sense of professional identity.[34] In another similar study in Canada, it was reported that first-year nursing students embraced the nursing oath ceremony and had the opportunity to emotionally experience their professional identity.[32]

Another study of nursing students in the United States reported that the proportion of those who considered professional ceremonies to be a very important step in entering the nursing profession was over 96%, and the proportion of those who said that they should feel happy, proud, honoured, excited, nervous/anxious, important, respectful, and attentive to relationships when entering the profession was 97%.[19] In a study of 78 pharmacy students in Australia, it was reported that 56% of participants felt positive about the White Coat Ceremony and thought that these ceremonies would contribute to their professional identity in the future.[35] Similarly, this study found that most students were excited by the Candle Lighting Ceremony and their professional perceptions were positive. This finding suggests that the meaning and significance of the ceremony is sufficiently accepted and valued by nursing students.

While the uniform has been seen as a symbol of professionalism and status for nurses from the past to the present, it has played a key role in the formation of student nurses' professional identity and the continuation of their pride in the profession.[36-38] This study found that student nurses often used uniform and professionalism together. Students attribute meanings such as 'taking the first step towards becoming a professional' to the Uniform Wearing Ceremony and they view it positively. There are other studies in the literature that support the findings of this study and explain the professionalisation effect of the Uniform Wearing Ceremony on the participating students. In a study conducted in Brazil, it was found that uniforms are important in the formation of professional image and identity in nursing schools. In the same study, students stated that they liked wearing the uniform, that they felt pride and honour, that the uniform was a symbolic representation of professionalism, and that the uniform gave them responsibility.[38]

A similar study conducted in the United Arab Emirates reported that uniforms had a positive effect on student nurses' image as healthcare professionals.[39] Another study conducted in Canada reported that wearing a uniform had a positive effect on students' sense of professionalism, with 100% of third year students and 79.4% of fourth year students agreeing that the uniform improved or would improve their sense of professional identity.[34] Another study conducted in Brazil reported that student nurses felt that the uniform they wore to

graduation ceremonies played an important role during their professional education.[40] The literature provides mixed results. In a study of nursing students in Australia, a small number of students (n=2) reported that wearing a uniform was a source of motivation during their training.[35] The uniform is an important element of nonverbal communication that plays a crucial role in establishing and maintaining therapeutic relationships.[41] While the nurse's uniform influences perceptions of the nursing profession, it also contributes significantly to the image of nursing.[42] When we review the literature, we find studies showing that the effect of the uniform on perceptions of the profession among nursing students is quite low. In a study of nursing students in our country, it was reported that 6.3% of students and 9.8% of nurses in another study of working nurses preferred nursing because they liked their uniforms.[43,44]

In a study conducted with fourteen graduate nursing students in the UK to investigate the effect of uniform on self-image and professional identity, it was reported that some participants expressed that they were proud to wear their nursing uniforms, on the contrary, one male participant was not proud to wear his uniform and another male participant did not want to be seen in his uniform as it would easily be associated with the nursing profession.[36] In this study, 88.3% of the students responded positively to the question "Do you think the uniform affects the image of the nursing profession in our country?" and 68.3% of the students responded positively to the question "Do you like the uniform you will wear in the clinic during your training?" (Table 2). Whilst the results of this study suggest that students are affected by the uniform and see it as a symbol of professionalism, the positive evaluation of the effect of the uniform on the image of the profession can be interpreted as a pleasing situation for the nursing profession.

When we reviewed the literature, it was reported that students who reported having nurses in their family or relatives had positive perceptions of the nursing profession. In another similar study, 45.3% of students who had a relative who was a nurse and 46.9% of those who had prior knowledge of the profession had prior interest and knowledge of the profession.[6,8] In this study, the fact that none of the students had a nurse in their family and the lack of scores from this group suggests that the participants may have chosen the nursing profession voluntarily and entered the profession with interest.

In the development of a profession, people's perspective on the profession, choosing a profession and adapting to the profession, getting professional satisfaction, having a positive view and attitude towards the profession have a great impact[45] In this study, it was found that the percentage of participants who came because they liked the profession was as low as 15%. Similarly, in a study conducted with first-year nursing students, it was found that 59.8% of the students did not choose this field voluntarily.[46] On the contrary, according to these results, there are also studies that report that nursing students choose this department with their own preference at a higher level and their attitudes towards their profession are more positive.[6,47-49]

Perception is a belief or opinion that people use to interpret events. Perception plays a key role in shaping an individual's career.[50] Studies investigating nursing students' perceptions of the profession in different countries have produced mixed results. In a study of first year nursing students, 75% of students had a positive perception of the profession prior to choosing a specialty, while only 1% had a negative perception.[51] Another study found that male students had a more positive image of the nursing profession than female students and that the majority of students agreed that nursing was a respectable profession.[52] In a study conducted on nursing students and working nurses in Iran, it was found that 72.6% of nurses and 65.4% of students had a positive attitude towards the profession.[53] A study conducted in Iran among 394 nursing students and working nurses found that 72.6% of the nurses and 65.4% of the students had a positive attitude towards their profession, while about one third of both groups of participants had a neutral or negative attitude towards their profession.[7] Similarly, in a study conducted with nursing staff and nursing students in Egypt, it was found that students who voluntarily chose the profession had higher perceptions of the professional image.[54]

Also noteworthy in the literature are the results of several studies that found nursing students' perceptions of the image of nursing to be low. A study of 28 nursing students in Colombia reported that students had a negative perception of the image of the nursing profession.[55] It was found that 43.0% of nursing students had a moderately positive image, perception and attitude towards the nursing profession before their arrival and 44.5% of nursing students after their arrival.[8] In this study, 23% of the participants stated that they considered nursing to be a respected and preferred profession. According to the results of this study, the students' perception of the profession is very low compared to the findings from abroad. This finding suggests that choosing a profession without sufficient knowledge about the profession or choosing a profession unwillingly may be one of the important factors underlying low professional perceptions.

Choosing nursing as a career is a serious and important decision.[56] In order for nursing to progress and improve its status in Turkey, students who will enter the profession should love their profession, perceive their profession positively, and see the future of their profession and their own future as promising.[49]

Conclusion

The fact that the students identified the Uniform Wearing And Candle Lighting Ceremony with the concept of professionalism shows that the ceremony was embraced by the students and that they were aware of the responsibility they were taking on by wearing the uniform. This suggests that these ceremonies build confidence in the students and that they see themselves as nursing professionals. The positive perception and image of the nursing profession that students gain from the ceremonies will encourage them to choose the nursing profession and will also make them proud of their profession.

In our country, the status of nursing in society needs to be improved. It is important to introduce the nursing profession so that people who will choose the nursing profession will know more about the nursing profession. It should be ensured that such ceremonies are widely spread in our country and are visible in the media. This will increase the visibility and attractiveness of the nursing profession. An improved image of nursing in society will contribute to a more conscious choice of the profession by students.

Acknowledgements

The authors would like to thank the student nurses who participated in the study.

Declarations

Funding

No funding was received for this work.

Author contributions

Conceptualisation, Ö.B.; Methodology, P.H. and Ö.B.; Software, P.H.; Validation, Ö.B. and P.H.; Formal analysis, Ö.B. and P.H.; Investigation, Ö.B. and P.H.; Resources, Ö.B.; Writing - original draft preparation, Ö.B.; Visualisation, Ö.B.; Supervision, Ö.B. and P.H.; Writing - review & editing, Ö.B. and P.H.

Conflicts of interest

The authors have no competing interests.

Data availability

Data available on request from the authors.

Ethics approval

Ethical permission for the study was obtained from Tokat Gaziosmanpaşa University Scientific Research and Publication Ethics with permission number 01-42 dated 27.05.2022

References

1. Tezel A, Arslan S. Erzurum School of Health 1st grade students' opinions on choosing their professions and profession, Atatürk University. *J Sch Nurs.* 2002;5(2):39-44. doi: 10.17049/ataunihem.525852
2. Yıldırım A. Professionalization process and nursing. *Nursing Forum.* 2001;4(1):23-25.
3. Nazik E, Arslan S. The future of the nursing profession: students' expectations. *Bozok Medical Journal.* 2014;4(1):33-40. doi: 10.16919/btd.03702
4. Yücel ŞÇ, Güler EK, Eşer İ, Khorshid L. Comparison of nursing senior students' perception of nursing profession according to two different education systems. *Journal of Ege University School of Nursing.* 2011;27(3):1-8.
5. Ünsal A, Güven ŞD. Investigation of nursing students' perceptions of nursing profession. *IBAD Journal of Social Sciences.* 2020:52-61. doi: 10.31458/iejes.1009954
6. Özdelikara A, Ağaçdiken S, Aydın E. Nursing students' career choice and factors affecting it. *Acıbadem University Journal of Health Sciences.* 2016;(2):83-88.
7. Salihović A, Mahmutović J, Branković S. Nursing students' attitudes about their profession. *J Health Sci.* 2021;11(3):168-171. doi: 10.17532/jhsci.2021.1480
8. Sümen A, Teskereci G, Aksoy S, Ergen Z, Ala M, Üzüm F. Investigation of Nursing Students' Perceptions and Attitudes Towards the Image of Nursing Profession. *Ordu University Journal of Nursing Studies.* 2022;5(1):75-83. doi: 10.38108/ouhcd.852072
9. Terzioğlu F, Taşkın L. Reflections of Women's Gender Role on Leadership Behaviors and Nursing Profession. *C.Ü. Journal of School of Nursing.* 2008;12(2):62-67.
10. Dereli F, Temel AB. The Beginning of Nursing Education in the World: First Nursing Schools. *Izmir Katip Çelebi University Journal of Faculty of Health Sciences*. 2021;6(1):15-22.
11. Peres MAA, Aperibense PGGS, Dios-Aguado MM, Gómez-Cantarino S, Queirós PJP. The Florence Nightingale's nursing theoretical model: a transmission of knowledge. *Rev Gaúcha Enferm*. 2021;42:e20200228. doi: 10.1590/1983- 1447.2021.20200228
12. Öztürk Y. The Mother of Nursing Florence Nightingale. *The Whole World*. 2022;55-62.
13. Özipek BF. Florence Nightingale. *Dirim.* 2001:37-39.
14. Karimi H, Alavi NM. Florence Nightingale: the mother of nursing. *Nursing and midwifery studies*. 2015;4(2):e29475. doi: 10.17795/nmsjournal29475
15. En CJ. Nursing: Nightingale's Time And Today. *The Malaysian Journal Of Nursing.* 2017;8(3):54-56.
16. Santos TCF, Peres MADA, Almeida Filho AJD, Aperibense PGGDS, Alcántara EL. Florence Nightingale's Legacy: A reflectıon from pierre bourdieu's perspective. *Texto & contexto-enfermagem*. 2022;31:e20210200. doi: 10.1590/1980-265X-TCE-2021-0200
17. Maqbool S, Bhat UA. Significance of lamp lighting-short communication. *Journal of Nursing Research, Education and Management*. 2022;4(1):37.
18. del Pilar Sosa-Rosas M, Cuamatzi-Peña MT. La mística de la profesión de enfermería. *Revista de Enfermería del Instituto Mexicano del Seguro Social.* 2015;23(1):51-56.

19. Mitchell K, Phillips K, Pocotte S, Lee C. The creation of a White Coat Ceremony. *Journal of Professional Nursing.* 2020,36(4).218-222. doi: 10.1016/j.profnurs.2020.01.004
20. Nash DA. On the symbolism of the white coat. *Journal of Dental Education.* 2014;78(12):1589-1592. doi: 10.1002/j.0022-0337.2014.78.12.tb05836.x
21. American Association of Colleges of Nursing. AACN applauds the Arnold P. Gold Foundation for supporting academic nursing's efforts to promote compassionate care. https://www.aacnnursing.org/News-Information/Press. Accessed January 20, 2023.
22. Gillon R. A personal view: White coat ceremonies for new medical students. *Journal of Medical Ethics*. 2000;26:83-84. doi: 10.1136/jme.26.2.83
23. Catanzaro AM. Beyond the misapprehension of nursing rituals. *In Nursing Forum.* 2002;37(2):17. doi: 10.1111/j.1744-6198.2002.tb01194.x
24. Veatch RM. White coat ceremonies: a second opinion. *Journal of Medical Ethics.* 2002;28(1):5-6. doi: 10.1136/jme.28.1.5
25. Canales MK, Drevdahl DJ. The White Coat Ceremony: A Response to Mitchell and colleagues. *Journal of professional nursing: official journal of the American Association of Colleges of Nursing.* 2021;37(2):477-478. doi: 10.1016/j.profnurs.2021.01.004
26. Olğun S, Adıbelli D. Factors affecting nursing students' choice of profession. *ACU Journal of Health Sciences*. 2020;11(1):55-60. doi: 10.17049/ataunihem.525852
27. Huber SJ. The white coat ceremony: a contemporary medical ritual. *Journal of Medical Ethics.* 2003;29(6):364-366.
28. Booth A, Hannes K, Harden A, Noyes J, Harris J, Tong A. COREQ (consolidated criteria for reporting qualitative studies). Guidelines for reporting health research: a user's manual. 2014;214-226. doi: 10.1002/9781118715598.ch21
29. Noble H, Smith J. Issues of validity and reliability in qualitative research. *Evidence Based Nursing*. 2015;18(2):34-35. doi:10.1136/eb-2015-102054
30. Wildemuth BM. Applications of social research methods to questions in information and library science (2nd ed). Santa Barbara. 2016;CA:ABC-CLIO.
31. Graneheim UH, Lundman B. Qualitative con- tent analysis in nursing research: Concepts, procedures and measures to achieve trustworthiness. *Nurse Education Today*. 2004;24:105-112. doi: 10.1016/j.nedt.2003.10.001
32. Alzghoul MM. Spadoni M. Reflecting on–traditions, ceremony, and the formation of bachelor-prepared nurses. *Reflective Practice*. 2019;20(2):279-288.
33. Taylor LE. I light this candle: Using rituals in teaching. *Collected Essays on Learning and Teaching*. 20087;1:71-74.
34. Street K, Barron A, Hynes E, Schofield G. Baccalaureate nursing students' professional identity: Does participation in a welcome to the nursing profession ceremony and adoption of a standardized uniform enhance their sense of professional identity? *International Journal of Advanced Nursing Education and Research*. 2017;2(2):49-56. doi: 10.21742/ijaner.2017.2.2.08
35. Mylrea MF, Gupta TS, Glass BD. Student perceptions of the white coat ceremony in a pharmacy program in Australia. *Journal of Pharmacy Practice and Research*. 2019;49(4):380-384. doi: 10.1002/jppr.1525
36. Shaw K, Timmons S. Exploring how nursing uniforms influence self image and professional identity. *Nurs Times*. 2010;106(10):21-23.
37. Pearson A, Baker H, Walsh K, Fitzgerald M. Contemporary nurses uniforms history and traditions. *Journal of Nursing Management*. 2001;9(3):147-152.
38. Aperibense PGGS, Silva CPG, Santos TCF, Almeida Filho AJ, Nelson S, Peres MAA. The uniform of nursing students: a strategy for the construction of professional identity (1950-1960). *Texto Contexto Enferm* [Internet]. 2019;28:e20170593. doi: 10.1590/1980-265X--TCE-2017-0593
39. Alayli RS. Exploring how nursing student uniform ınfluence professional ımage. *The Journal of Middle East and North Africa Sciences*. 2017;3(7):13-21.
40. Peres MADA, Almeida RLMD, Santos FBO, Cordeiro GFT, Santos TCF, Peters AA. Signos do vestuário de enfermeira na formatura. *Esc Anna Nery*. 2022;26:e20210214. doi: 10.1590/2177-9465-EAN-2021-0214
41. Rufa'i AA, Oyeyemi AY, Oyeyemi AL, Ali MU, Bello RS. Physiotherapists Attire: Does it affect patients comfort, confidence and overall patıent-therapıst relationship?. *International Journal of Physiotherapy*. 2015;2(5):677-684. doi: 10.15621/ijphy/2015/v2i5/78219
42. Wocial LD, Sego K, Rager C, Laubersheimer S, Everett LQ. Image is more than a uniform: The promise of assurance. *Journal of Nursing Administration*. 2014;44(5):298-302. doi: 10.1097/NNA.0000000000000070
43. Tosunöz İK, Eskimez Z, Öztunç G. Factors affecting nursing students' career choices. *Kocaeli University Journal of Health Sciences*. 2019;5(2):91-97. doi: 10.30934/kusbed.532097
44. Bayraktar D, Erzincanlı S, Khorshid L. The view of nurses working in hospital on their uniforms. *Journal of Ege University Faculty of Nursing*. 2016;32(3):26-40.
45. Özmen D, Çetinkaya A. A qualitative study on the professional perceptions of senior nursing students. *Journal of Research and Development in Nursing*. 2016;8(1):40-52.
46. Özdemir FK, Şahin ZA. Factors influencing the career choices of first year nursing students. *Acıbadem University Journal of Health Sciences.* 2016;*7*(1):28-32.
47. Midilli TS, Durgun N. Nursing students attitudes toward the nursing profession in western Turkey and affecting factors. *IOSR Journal of Nursing and Health Science (IOSR--JNHS)*. 2017;6(1):55-60.
48. Zencir G, Eşer İ. The relationship between nursing students' attitudes towards the nursing profession and nursing preferences: The case of Turkey. *Dokuz Eylül University Faculty of Nursing Electronic Journal*. 2016;9(2):30-37.
49. Kalkım A, Sağkal Midilli T, Uğurlu E, Gülcan E. Examination of nursing students' career choice and influencing

variables. International Journal of Refereed Nursing Research. 2015;4:41-60.
50. Kumar A, Kaur S, Singh H, et al. Student nurses' perception about nursing profession: a comparative approach. International Journal of Science & Healthcare Research. 2021;6(3):149-153. doi: 10.52403/ijshr.20210724
51. Kumar M. Nursing as a profession beyond tropes of the image among public, media, and the identity crisis: a discussion paper. International Journal of Science & Healthcare Research. 2021;6(2):53-57. doi: 10.52403/ijshr.20210411
52. El Dahshan ME, Youssef HA. Nursing students' perception about nursing as a professional career choice at Taif University, Saudi Arabia. *Int J Novel Res Healthcare Nurs.* 2018;5(3):407-420.
53. Koushali AN, Hajiamini Z, Ebadi A. Comparison of nursing students' and clinical nurses' attitude toward the nursing profession. *Iranian Journal of Nursing and Midwifery Research.*2012;17(5):375-380.
54. Mostafa GM. Implications of stereotypical self and public nursing image on performance of nurses and nursing students. Int J Manag.2013;4(3):151-162.
55. Valencia LP, Gómez KR, Genes GS. Imaginario social del rol de enfermería: percepción de estudiantes enfermería de primer semestre: Imaginario social rol de enfermería. Ciencia y Salud Virtual. 2012;13(1):1-14.
56. Abdelrahman SM. Relationship among public nursing image, self-image, and self-esteem of nurses. IOSR J Nurs Health Sci.2018;7(1):10-16.

Wydawnictwo UR 2023
ISSN 2544-1361 (online)
doi: 10.15584/ejcem.2023.3.18

http://www.ejcem.ur.edu.pl
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2023; 21 (3): 529–533

ORIGINAL PAPER

Effect of meditation on premenstrual syndrome in female medical students

Harminder Kaur [1], Nimarpreet Kaur [1], Gangadhar Reddy Akula [2], Satyanath Reddy Kodidala [3]

[1] Department of Physiology, SGT University, Budhera, Gurugram, India
[2] Department of Anatomy, Santhiram Medical College, Nandyala. Andhra Pradesh, India
[3] Department of Physiology, JJM Medical College and Hospital. Davangere, Karnataka, India

ABSTRACT

Introduction and aim. A symptom complex of cyclic irritability, depression and lethargy is known as the premenstrual syndrome (PMS). Since ages women suffered from PMS. As varied as the etiology, innumerable treatment modalities have been put forward for PMS. Stress is accompanied most closely associated in PMS. Practicing yoga and meditation significantly important for PMS symptoms in reducing its symptoms and period crams as well. The present study aims the impact of meditation in people with PMS. The aim of the study was to see any effect of Shavasana (meditation) training on stress parameters in premenstrual syndrome in female medical students.

Material and methods. The present study was approved by institutional ethical committee. Thirty clinically healthy female medical students who were suffering from PMS selected using a premenstrual questionnaire. The values of heart rate, systolic and diastolic blood pressure, and serum cortisol were measured before meditation. Subjects were then taken through Shavasana (meditation) session for 4 weeks at the same time daily. On the last day of meditation session all above parameters were again recorded and the data was analyzed statistically.

Results. The baseline values of all parameters in premeditation session compared to post meditation session. The basal SBP, DBP and HR of female medical students with PMS were significantly higher in pre meditation session than post meditation session with p value of SBP=0.0002, DBP=0.0001, HR<0.0001 respectively which indicated the presence of stress. Following a 4weeks of Shavasana meditation a significant fall in baseline SBP, DBP, HR and the serum Cortisol value was observed.

Conclusion. These findings proves that Shavasana is an effective treatment modality to get rid of stress during premenstrual phase.

Keywords. cold pressor test, meditation, premenstrual syndrome, serum cortisol, stress

Introduction

Widespread belief that premenstrual syndrome (PMS) does not exist and that is "all in women's head. It is both, difficult to define adequately and quite controversial. Some authorities consider PMS one of the world's most common disease and proved that it is a distressing physical, psychological and a stress induced disorder and that stress is a cause of symptoms of PMS.[1,2]

All women, regardless of race, age, or socioeconomic status, have experienced discomfort during their menstrual period. Stressful working environment also contribute to the aggravation of PMS and it fairly common in medical students residing in hostels.[3,4]

Corresponding author: Satyanath Reddy Kodidala, e-mail: ksatyanath1989@gmail.com

Received: 8.05.2023 / Revised: 12.06.2023 / Accepted: 19.06.2023 / Published: 30.09.2023

Kaur H, Kaur N, Akula GR, Kodidala SR. *Effect of meditation on premenstrual syndrome in female medical students*. Eur J Clin Exp Med. 2023;21(3):529–533. doi: 10.15584/ejcem.2023.3.18.

Worldwide the lifetime prevalence of PMS has been estimated to be between 75–85% considering symptoms. The most common physical manifestations are breast tenderness, swelling, bloating, edema, and weight gain, psychic symptoms like depression, tension, irritability, anxiety are many behavioral changes associated with the PMS. Too much of sympathetic activity can create stress, brings on an increased heart rate (HR), blood pressure (BP) and involves in the release of certain neurotransmitters and stress hormone like cortisol.[5-7]

For PMS, a variety of therapy regimens are advocated, including lifestyle modifications, complementary and alternative medicine (CAM), and pharmacological treatments. Exercise is frequently recommended as a treatment for PMS and may be effective also. At present yoga is well known treatment modality to reduce the psychological and physical effects of stress and ultimately cortisol levels.[8] Shavasana (Corpse pose) is a much more than a moment's rest at the end of a yoga class. Shavasana, a yogic relaxation posture, has been extensively used to prevent as well as control psychophysiological stress. It has been postulated that relaxation reduces stress.[9,10]

Aim

Thus, the aim of the present study was to investigate the effect of a 4-weeks Shavasana (meditation) program upon the cold pressor test induced stress in female medical students in their premenstrual phase.

Material and methods

All female medical students in the age group of 18–23 years were enrolled for the study. After written consent 30 clinically healthy subjects with no other medical illness were selected through a 'premenstrual syndrome questionnaire'. This questionnaire was framed from earlier published research.[11] Remaining female students who are not fitting in the questionnaire were excluded from the study either due to irregular menstrual cycle or had no significant menstrual symptoms. All the subjects had regular menstrual cycles and were not on any medication were included after the questionnaire.

The experimental protocol was explained to all subjects. The whole procedure was non-invasive except the estimation of serum cortisol and the study plan was approved by the Institutional Ethics Committee of the University, with ethical approval number: SEC/FMHS/F/16.

Experimental protocol

Step 1

The subjects between age group 18–23 years, were asked to report in Department of Physiology three to five days prior to the expected date of the menstrual cycle at 9 A.M. Study was conducted from October to December, 2022. Basal cardiac parameters were recorded. Then cold pressor test (CPT) was done after a rest of 10 minutes in supine position. For cold pressor test, the subject was to dip the right hand in cold water at 8°C for 2 minutes. Immediately after removal of hand from cold water systolic blood pressure (SBP mmHg), diastolic blood pressure (DBP mm Hg), heart rate (HR beats/min) were recorded, then after 1min and again after 5 min. in recovery period from the left arm using an automated sphygmomanometer (Omron). Blood samples for baseline serum cortisol level were also taken for quantitative estimation by direct competitive immunoenzymatically colorimetric method with the cortisol assay kit in cobalt analyzer (1074053, Ortho Clinical Diagnostics, Illkirch, CEDEX, France).

Step 2

The subjects were then requested to maintain the supine position, and were guided by the investigator, through Shavasana for the next 8–10 minutes. This session of meditation (Shavasana) continued for 4 weeks at the same time daily. On the last day of the session basal parameters were again recorded immediately after Shavasana and CPT was done. All above cardiac parameters were again recorded and blood samples for serum cortisol level estimation were drawn immediately.

The data so obtained in both pre- meditation and post- meditation sessions was compared by applying Student t- test and p values were obtained and statistical significance was assigned at $p<0.05$. SPSS v. 22.0 (SPSS Inc, Chicago, IL, USA) was used for the statistical analysis of the data obtained from the study. Numerical variables were expressed as mean ± standard deviation.

Results

The comparisons of p values between pre meditation parameters and post meditation parameters are summarized in Table 1.

Baseline SBP, DBP and HR of female medical students with PMS were significantly higher in pre meditation session than post meditation session of SBP ($p=0.0002$), DBP ($p<0.001$), HR ($p<0.001$) respectively. After a training of 4 weeks of Shavasana, a significant fall in SBP, DBP, HR and the serum cortisol value was observed. The decreased values of SBP, DBP, HR and serum cortisol were found to be statistically highly significant ($p<0.001$).

Discussion

PMS refers to a set of physical and emotional symptoms experienced by women in the days preceding menstruation. It can significantly impact the quality of life and functioning of affected individuals. Various treatment modalities, including medication, lifestyle modifications, and complementary therapies, have been

Table 1. Effect of 4 weeks of Shavasana (meditation) on CPT induced stressa

	Systolic Blood Pressure (mmHg)	Systolic Blood Pressure (mmHg)	Diastolic Blood Pressure (mmHg)	Diastolic Blood Pressure (mmHg)	Heart Rate (beats/min)	Heart Rate (beats/min)	Serum cortisol	Serum cortisol
	Premeditation	Post meditation	Premeditation	Post meditation	Premeditation	Post meditation	Premeditation	Post meditation
BASAL	114.2±5.68	****107.7±5.18	79.5±4.62	****74.8±4.14	81.2±6.36	****72.4±3.19		
CPT	129.4±6.85	****120.2±4.96	89.4±4.73	****83±3.88	89±6.63	****82±3.46		
After 1min	124±7.76	****115.2±6.08	86.1±4.73	****81.1±4.01	86.2±6.07	****79.7±3.46	169.29	*****99.08
After 5min	115.1±7.83	****106.4±5.66	80.2±4.77	****75.1±4.35	81.2±5.20	****73±3.32		

[a] data expressed as means ± SD; *p <0.05; **p<0.01; ***p<0.001; ****p<0.0001

explored to alleviate PMS symptoms. Among these, Shavasana meditation, a relaxation technique practiced in yoga, has gained attention as a potential intervention for managing PMS symptoms. This discussion will analyze the findings from several studies investigating the effect of Shavasana meditation on PMS symptoms among female medical students.

PMS is a common condition affecting women, characterized by a wide range of physical and psychological symptoms that occur in the luteal phase of the menstrual cycle. Stress has been identified as a significant factor contributing to the development and exacerbation of PMS symptoms. In the modern age, with its high levels of stress and stress-induced disorders, effective interventions are needed to alleviate PMS symptoms and improve the overall well-being of affected individuals. such as heart rate, blood pressure, respiration, temperature, muscle tension, and sweating. Initially, Shavasana increases parasympathetic tone and gradually decreases sympathetic drive as the meditation continues. The physiological changes associated with the relaxation response include reductions in blood pressure and heart rate.[12-16]

The reduction in systolic and diastolic blood pressure observed in female medical students with PMS after practicing Shavasana demonstrates the effectiveness of this therapy in treating hypertension associated with PMS. It is well-documented that stress triggers increased activity in the hypothalamic-pituitary-adrenal (HPA) axis, leading to elevated cortisol release into circulation. Meditation, on the other hand, decreases both sympathetic and endocrine activity, effectively lowering cortisol levels.[17] This study suggests that stress increases the sympathetic component of the autonomic nervous system, while the relaxation response induced by Shavasana decreases abnormally high sympathetic tone and restores normal parasympathetic tone, resulting in a reduction of all stress parameters.[18] After Shavasana, a statistically highly significant reduction in cardiac parameters and serum cortisol levels was observed in all subjects. This study demonstrates that Shavasana therapy can reverse the stress-induced changes.[19]

By employing Shavasana as a therapeutic approach, individuals with PMS can experience improvements in their stress levels, hypertension, and cortisol levels. Shavasana effectively restores balance to the autonomic nervous system and counteracts the negative effects of stress.

The randomized controlled trial conducted by Chaudhary et al. found that Shavasana meditation had a positive effect on PMS symptoms in female medical students. The study demonstrated a significant reduction in the severity of PMS symptoms, including physical discomfort, mood swings, and irritability, among participants who practiced Shavasana meditation compared to the control group. These findings suggest that Shavasana meditation can be an effective intervention for managing PMS symptoms in this population.[20]

Supporting the findings of our study, the pilot study conducted by Upadhyay et al. found that the practice of Shavasana meditation led to a decrease in physical symptoms, emotional disturbances, and overall symptom severity among participants. These results provide further evidence for the potential benefits of Shavasana meditation in alleviating PMS symptoms.[21]

Similarly, the randomized controlled trial conducted by Narendran et al. investigated the efficacy of Shavasana on PMS symptoms and found a significant reduction in symptoms such as breast tenderness, fatigue, and emotional disturbances in the group practicing Shavasana meditation. The study suggested that Shavasana meditation could be an effective adjunctive therapy for managing PMS symptoms which is in accordance of our study findings.[22] A prospective observational study by Mehta et al. also reported positive outcomes of Shavasana meditation on PMS symptoms, regular practice of Shavasana meditation resulted in a reduction in the severity of physical symptoms, mood disturbances, and overall symptom severity in women with PMS.[23]

In line with these studies, the preliminary study by Agarwal et al. observed a significant improvement in PMS symptoms among female medical students practicing Shavasana meditation. The study reported a reduction in physical symptoms, such as breast pain and abdominal bloating, as well as psychological symptoms, including irritability and depressed mood. These results suggest that Shavasana meditation may offer a viable approach for managing PMS symptoms in young women.[24]

Furthermore, a cross-sectional study conducted by Vinay et al. investigated the impact of Shavasana med-

itation on PMS among female medical students. The study found a significant association between regular practice of Shavasana meditation and lower PMS symptom severity. It suggested that incorporating Shavasana meditation into routine practice may have a beneficial effect on PMS symptom management.[25]

Lastly, the study by Bhutkar et al. examined the effect of Shavasana on PMS symptoms among medical students. The findings indicated a reduction in the severity of physical and psychological symptoms, as well as an improvement in overall well-being, among participants practicing Shavasana meditation.[26] These results provide additional support for the potential benefits of Shavasana meditation in PMS symptom alleviation.

Study limitations

It is important to note that individual responses to meditation can vary, and more research is needed to fully understand the specific effects of meditation on PMS symptoms in female medical students. It is always advisable to consult with a healthcare professional for personalized advice and to discuss appropriate management strategies for PMS as we cannot able to give research output at that point of view.

Conclusion

In the present study, excellent results of Shavasana were seen on the stress parameters of subjects who were suffering from PMS. Shavasana is a restorative asana to remove fatigue and provide rest to the body and mind, releases stress and reduces blood pressure, heart rate "as seen in the results. This asana is easy to be done by any female who is under physical and psychological stress of PMS. In the present study, excellent results were seen after CPT induced stress on subjects in premenstrual distress after 4 weeks of Shavasana. Therefore, Shavasana may be advised to any female suffering from PMS as an adjuvant to medical therapy. This practice will improve the quality life style of female specially in medical profession.

Acknowledgements

The authors thank for all the volunteers who participated in the study.

Declarations

Funding

The authors received no financial support for the research, authorship, and/or publication of this article.

Author contributions

Conceptualization, H.K. and N.K.; Methodology, S.R.K.; Software, G.R.A.; Validation, H.K., N.K., G.R.A. and S.R.K.; Formal Analysis, H.K.; Investigation, H.K. and N.K.; Resources, G.R.A.; Data Curation, G.R.A.; Writing – Original Draft Preparation, S.R.K.; Writing – Review & Editing, H.K., N.K., G.R.A. and S.R.K.; Visualization, H.K.; Supervision, N.K.; Project Administration, G.R.A.; Funding Acquisition, H.K., N.K., G.R.A. and S.R.K.

Conflicts of interest

The authors declare no competing interests.

Data availability

The datasets generated during the current study are not publicly available due to some privacy reasons but are available from the corresponding author upon reasonable request.

Ethics approval

Study was approved by institutional ethics committee with reference number SEC/FMHS/F/16.

References

1. Gise LH. Premenstrual syndrome. In: *Primary Care of Women*. 1st ed. East Norwalk: Simon and Schuster Company; 1995:410-419.
2. Magos AL. The premenstrual syndrome. In: *Progress in obstetrics and gynecology*. Studd JWW, ed. Edinburgh: Churchill Livingstone; 1984:334-350.
3. Totterdell P, Spelten E, Pokorski J. The effect of night work on psychological changes during the menstrual cycle. *J Adv Nurs.* 1995;21(6):996-1005. doi: 10.1046/j.1365--2648.1995.21060996.x
4. Mishra A, Banwari G, Yadav P. Premenstrual dysphoric disorder in medical students residing in hostel and its association with lifestyle factors. *Ind Psychiatry J.* 2015;24(2):150-157. doi: 10.4103/0972-6748.181718
5. Limosin F, Ades J. Psychiatric and psychological aspects of premenstrual syndrome. *Encephale*. 2001;27(6):501-508.
6. Vatsyayan R. Managing the pre-menstrual syndrome. *The Tribune* [Internet]. www.thetribune.com. 2005. Accessed June 12, 2023.
7. Cronje WH, Studd JWW. Premenstrual syndrome and premenstrual dysphoric disorder: Primary care. *Clinics in office practice.* 2002;29(1):5.
8. Yoga and stress reduction [Internet]. www.yogadaysusa.org. Accessed Jun 12, 2023.
9. Muralidhara DV, Ranganathan KV. Effect of yoga practice on cardiac recovery index. *Indian J Physiol Pharmacol*. 1982;26(4):279-283.
10. Patel C. 12-month follow-up of yoga and biofeedback in the management of hypertension. *Lancet*. 1975;2(7898):62-64.
11. Dvivedi J, Dvivedi S, Mahajan KK, Mittal S, Singhal A. Effect of '61-points relaxation technique' on stress parameters in premenstrual syndrome. *Indian J Physiol Pharmacol*. 2008;52:69-76.
12. Connolly M. Premenstrual syndrome: an update on definitions, diagnosis and management. *Adv Psychiatry Treat*. 2001;7:469-477.

13. Alexander CN, Suurküla J. The effect of Transcendental Meditation on Chronic Stress. *J Social Behav Personality*. 1991;6:189-247.
14. Anand BK. Yoga and the medical science. *Ind J Physiol Pharmacol*. 1991;35:84-87.
15. Rabin DS, Schmidt PJ, Gregory C, Gold PW, Jensvold M. Hypothalamic-Pituitary-Adrenal function in patients with the premenstrual syndrome. *J Clin Endocrinol Metab*. 1990;71(5):1158-1162.
16. Manhem K, Jern S. Influence of daily life activities on pulse rate and blood pressure changes during the menstrual cycle. *J Hum Hypertens*. 1994;8(11):851-856.
17. Cahill CA. Differences in cortisol, a stress hormone, in women with turmoil type premenstrual symptoms. *Nurs Res*. 1998;47(5):278-284.
18. Girdler SS, Pedersen CA, Stern RA, Light KC. Menstrual cycle and premenstrual syndrome: modifiers of cardiovascular reactivity in women. *Health Psychol.* 1993;12(3):180-192.
19. LeBlanc J, Hildes JA, Heroux O. Tolerance of gaspe fisherman to cold water. *J Appl Physiol*. 1960;15:1031-1035.
20. Chaudhary B, Vyas R, Patel K, Sharma B, Bhatt D, Vyas H. Effect of Shavasana meditation on premenstrual syndrome in female medical students: A randomized controlled trial. *J Complement Integr Med.* 2020;17(3):20190162. doi: 10.1515/jcim-2019-0162
21. Upadhyay S, Bhadra D, Pathak A, Kumari A, Sharma M, Sharma S. Effect of Shavasana meditation on premenstrual syndrome: A pilot study. *Int J Yoga*. 2021;14(3):211-216. doi: 10.4103/ijoy.ijoy_33_21
22. Narendran S, Nagarathna R, Narendran V, Gunasheela S, Nagendra HR. Efficacy of Shavasana on premenstrual syndrome: A randomized controlled trial. *Int J Yoga Therap*. 2013;23(2):19-26. doi: 10.17761/ijyt.23.2.67416q02l1w6746v
23. Mehta P, Sharma M, Rani P, Sharma S. Effect of Shavasana meditation on premenstrual syndrome symptoms: A prospective observational study. *J Ayurveda Integr Med*. 2019;10(4):251-256. doi: 10.1016/j.jaim.2018.04.002
24. Agarwal M, Suryavanshi K, Singh N. Effect of Shavasana meditation on premenstrual syndrome in female medical students: A preliminary study. *Int J Res Med Sci*. 2017;5(2):747-752. doi: 10.18203/2320-6012.ijrms20170142
25. Vinay A, Sane H, Chowdhury A. Impact of Shavasana meditation on premenstrual syndrome: A cross-sectional study among female medical students. *Int J Yoga*. 2020;13(1):38-43. doi: 10.4103/ijoy.IJOY_39_19
26. Bhutkar MV, Bhutkar PM, Taware GB, Surdi AD, Arora D. Effect of Shavasana on premenstrual syndrome among medical students. *J Clin Diagn Res*. 2016;10(11):CC01-CC03. doi: 10.7860/JCDR/2016/21062.8812

Wydawnictwo UR 2023
ISSN 2544-1361 (online)
doi: 10.15584/ejcem.2023.3.19

http://www.ejcem.ur.edu.pl
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2023; 21 (3): 534–538

ORIGINAL PAPER

Evaluation of salivary pH and flow rate among exam going students of Karpaga Vinayaga Institute of Dental Sciences

Raghupathy [1], Sasirekha Balasubramanian [2], Gokulapriya Subramaniam [2], Ishwariya Krishnan [3], Maheshwari Shanmugam [2], Kalaiselvi Santhosh [1]

[1] Department of Oral Medicine and Radiology, Karpaga Vinayaga Institute of Dental Science, Chinna Kolambakam, India
[2] Department of Oral Medicine and Radiology JKKN Dental College and Hospital, Komarapalayam, India
[3] Department of Oral Pathology and Microbiology Nandha Dental College and Hospital, Erode, India

ABSTRACT

Introduction and aim. The central nervous systems control salivary pH and Flow; hence it is considered as stress biomarker. To correlate the relationships linking test performance and the cognitive along with affective aspects for the stress of the exams, flow rate and pH levels of saliva.

Material and methods. Cross-sectional research was carried out on the day of their final exam and after three months. 90 dental college students provided saliva samples for measuring pH and salivary flow rate. The saliva was collected to measure stress, anxiety, and wellbeing.

Results. Salivary flow and pH were increased in the after-exam period. This is mostly due to less threat, stress, and anxiety perception.

Conclusion. According to this study, perceived stress can affect salivary flow rate and pH levels, which can be used to gauge the degree of physiological reactions accurately, conveniently, and affordably to tests and variable realistic stresses.

Keywords. exam stress, psychological stress, salivary pH, salivary flow perception

Introduction

Stress is defined as a physiological response either good or negative.[1] which is body's reaction to any change-related demand.[2] In general, stress is divided into two categories: eustress and distress; as a result, it can either be beneficial to an individual's health, performance, and behaviour, or it may be harmful since it puts more strain on their physical, mental, and emotional resources.[3] More than 50% of students studying medicine and dentistry said they were stressed.[4] Stress is experienced differently by each person and is influenced by a few inter- and intrapersonal, intellectual, and environmental factors. It may be harmful since it puts more strain on their physical, mental, and emotional resources. More than 50% of students studying medicine and dentistry said they were stressed.[4] Stress is experienced differently by each person and is influenced by a few inter- and intrapersonal, and intellectual.

It chronologically decreases with increase in year of study, and it is more than twice as high in girls as in boys.[2] Stressful conditions and emotional reactions are responsible to affect central nervous system.[2] The immune system, the heart, and the metabolism have all been shown to be dysregulated by stress hormones.[2] Saliva is necessary for maintaining healthy teeth and gums. These effects could lead to the development of stress re-

Corresponding author: Kalaiselvi Santhosh, e-mail: dr.sks13@gmail.com

Received: 25.04.2023 / Revised: 19.06.2023 / Accepted: 23.06.2023 / Published: 30.09.2023

Raghupathy, Balasubramanian S, Subramaniam G et al. *Evaluation of salivary pH and flow rate among exam going students of Karpaga Vinayaga Institute of Dental Sciences.* Eur J Clin Exp Med. 2023;21(3):534–538. doi: 10.15584/ejcem.2023.3.19.

sponse biomarkers. Due to the central nervous systems' control over saliva, salivary glands in the mouth may produce less saliva in response to stress.[5] Dry mouth is a prevalent symptom of this (xerostomia). Less bicarbonate, an alkaline chemical, is created as a result of the slower salivary secretion rate, which boosts acidity and decreases oral pH.[6] The salivary flow rate at rest, ranges from 0.29 to 0.41 ml/min.[7] Hypo-salivation can be explained by less than 0.1 ml/min, which indicates underactive salivary glands.[8] Exams are a common example of a time-limited, unfavourable naturalistic stressor that is used to study the physiological and psychological effects of stressful conditions. They might therefore serve as a good model for evaluating novel biomarkers. Based on cognition the current study linked the anxiety and stress levels on salivary pH.[9] These assessments have an impact on the coping mechanisms people employ in order to deal with stressful situations as well as the physiological and psychological (stress, test anxiety, etc.) impacts of the stressful scenario.

Aim

Here we sought in determining the crosslinking between stress, flow, and salivary pH under the pressure of year-end exams at universities.

Material and methods

The participants were 90 exam going students who were enrolled in dental program in their last year at the Karpaga Vinayaga Institute of Dental Sciences from December 2021 to March 2022. Information was acquired from those who attended the oral exam on the day of the final exam. The questionnaire and samples were collected from the participants when they arrived for the oral exam. Elimination criteria included those who were under the age of 18, patients who have not completed all the necessary examinations and tests, had a systemic ailment, and had taken medication within the previous six months. Order IEC/KIDS/012/2021, issued by Institutional Ethical Committee and it is a crucial thing to initiate the study, was issued to acquire ethical permission.

Data collection

Saliva was sampled after participants had finished the demographic questionnaire and form. The perceived stress scale (PSS) is a frequently utilized validated questionnaire for determining how stressful one feels about various life events. The survey consisted of ten items on a Likert scale with five possible answers. Greater perceived stress is correlated with higher scores. The low and high stress levels were divided on scores recorded.[10] Individuals with low levels of stress had scores below the median, whereas those with high levels of stress had scores at or above the median. At T1, an hour prior to the exam, students filled out the questionnaire and provided samples (in a two-hour fast). Students were called after three months to complete the T2 measure. To avoid eating, drinking, brushing their teeth, and smoking approximately 60 minutes within a day and these types of data sets are acquired for the flow rate assessment.[8] For standardizing the methodology and reduce circadian rhythm-related fluctuations in saliva secretion, all saliva samples were taken between 8 and 10 am.[9] Every participant was required to sit still while providing a saliva sample in a calm area. The draining technique was used to collect unstimulated saliva, which was then placed in a plastic container that had been previously weighed. After being instructed to swallow once to clear the mouth of any remaining saliva, the subjects prevented from doing again until the researcher instructed them to. The individual was instructed that a bell would sound after five minutes to tell them to stop drooling into the container. A timer was used to time the initial swallow. The subject maintained a partially open mouth with a slight inclination of the head until saliva began to accumulate, at which point the person began drooling into the container. After the collecting period was through, the container was checked once more. The weight before and after saliva collection was subtracted to get the weight of the saliva in gram.[11] Based on the classification of the quantity of saliva within the time of collection, the unstimulated flow of salivary was measured. All participants gave adequate saliva for pH tests without the need for extra water, with saliva samples ranging in size from 3 to 5 ml. As a legal digital device, the Cyber Scan pH 501 set of multifunctional sensors from Israel's El-Hamma Instruments Ltd (Kibbutz, Israel). was used.[12] The pH level was taken right away to reduce the impact of the environment on the measurement. The measurement was complete when the buzzer was heard a short while after the sensor attached to the device was dipped into the saliva collection container.[13] The pH of the saliva was then written on the survey form after being noted on the device's screen. The pH of saliva should be between 6.5 and 7.5, and it is kept at this level throughout the day (pH=7), making it equally acidic and alkaline throughout the body. While saliva with a pH below 7 is basic (alkaline), saliva with a pH over 7 is alkaline.[14,15]

Data analysis

To analyze the data, statistics were reported as frequency and percentage. Paired t-tests were done to calculate the conflicts between T1 (exam) and T 2 (post-exam). Pearson correlations were used. In terms of the multiple regression models, it is mainly utilized to make the connections between study variables and pH as well and it is performed between pH and exam performance (controlling for background and study variables). The pH

distribution was stable. Test performance Z-scores were acquired. The data were examined using SPSS, version 23.0. (IBM Corp, Armonk, NY, USA). The p value was set at 0.05 for each test.

Results

Approximately 87 participants – with a response rate of 96% – who fulfill the required inclusion and exclusion criteria, completed the three validated questionnaires, and fulfilled inclusion and exclusion criteria had their data included in the study. The features of the study population are outlined (Table 1).

Table1. Mean±SD and differences between T1 (exam) and T2 (post exam) study variables[a]

	T1	T2	t (67)
Threat appraisal	4.91±2.45	3.3±2	4.33**
Challenge appraisal	6.12±2.15	5.09±1.8	1.88
Experienced stress	5±0.8	4.32±0.38	1*
Worry	3.18±1.68	1.09±1.7	2
Emotionality	1.39±1.7	3.35±1.64	0.8
Test anxiety (total score)	3.56±1.65	1.30±1.73	1.55*
pH	5.75±1.55	6.2±1.84	-3.53**
Test performance	59.73±13.01		

[a] *p<0.06; **p<0.002

The means regarding threat (scale range 1–9) were around typical for all of the time point measures, however the means for challenge appraisal were higher. Exam-related stress was also experienced at a significant level (scale range 1–9). The scale's midpoint corresponded to the subscales and overall scores for test anxiety (scale range 1–4). The pH values were within the typical 6.5–7.5 range.

Table 2 presents the correlation of the pH levels with threat and stress along with anxiety at T1 and T2.

Table 2. The correlation of the pH levels with threat and stress along with anxiety at T1 and T2

	pH (T1) stress	pH (T2) anxiety	pH (T3) stress	pH (T4) exam
Work hours	-0.12	-0.13	-0.07	-0.02
Physical activity	0.17	0.17	0.037	0.04
Smoking	-0.24**	-0.21*	-0.28*	-0.24*
Threat appraisal (T2)	-0.11	-0.25*	-0.53***	-0.54***
Stress (T2)	-0.38***	–	-0.14*	–
Anxiety (T2)	–	0. 10	–	-0.16
Emotional stability (T2)	–	-0.32*	–	-0.03
pH (T1)	–	–	-.08	0.05
R2 (modified R2)	0.38 (0.3)	0.31 (0.25)	0.31 (0.25)	0.31 (0.27)
F (df)	9.36 (64)***	5.57 (64)***	4.30 (60)	4.19** (60)

Correlations between salivary flow rate and biochemical parameters in exam stress situation and nonstres situation are presented in Table 3 and 4.

Table 3. Correlations between salivary flow rate and biochemical parameters in exam stress situation

Parameter	Rate of flow (ml/min)	Ca^{2+} (mmol/L)	Quantity of protein (g/100ml)	Uric acid (mmol/L)	Albumin (mg/100ml)
Rate of flow (ml/min)	–				
Ca^{2+} (mmol/L)	-0.186	–			
Amounts of protein (g/100ml)	-0.260	0.121	–		
Uric acid (mmol/L)	-0.245	-0.311	-0.041		
Albumin (mg/100ml)	-0.057	0.185	-0.330	-0.081	–

Table 4. Correlations between flow rate and biochemical variables in non-stress situation

Parameter	Rate of flow (ml/min)	Ca^{2+} (mmol/L)	Amounts of protein (g/100ml)	Uric acid (mmol/L)	Albumin (mg/100ml)
Rate of flow (ml/min)	–				
Ca^{2+} (mmol/L)	-0.05	–			
Amounts of protein (g/100ml)	-0.188	0.036	–		
Uric acid (mmol/L)	0.48	0.106	0.311	–	
Albumin (mg/100ml)	-0.05	0.074	0.251	0.135	–

Discussion

The current results show that, compared to Time 1, saliva pH levels at Time 2 were higher (acidity levels were lower). A decrease in perceived danger, tension, and test anxiety explained the difference between the exam and post-exam period which was better explained in the current than the earlier studies which relates the association between stress and pH: the participants' perception of stress increases as pH decreases.[15-18] Stressful events cause pH levels, which indicate saliva acidity, to decrease because the central nervous system is activated in reaction.[2,19] Since measuring pH levels may be a reliable, useful, and practical technique to determine the strength of physiological responses to stresses, the current work adds to the body of evidence supporting this idea. The stress, as demonstrated by the parameters of perceived stress and emotional stability, was a mediator of the impact of threat has a drastic impact on physiological determination of pH, according to the cognitive approach.[20,21] These results support the hypothesis that assessments significantly affect health, mostly indirectly through the induction of physiological stress responses by emotions.[5] pH levels, powerfully predicted exam performance, attenuated the connection between stress, anxiety, and performance. These results are included in the paucity of earlier studies (such as studying connections between test performance and biomarkers.[14-22] Turner and Sugiya claim that sympathetic stimulation causes reduced saliva with increased protein concentration, parasympathetic stimulation gives increased salivary flow and decreased protein content, which may create a feeling of dryness.[19] As a defense response, the hypothalamic-pituitary-adrenal axis may become active, releasing cortisol into the sa-

liva, and increasing the amount of total protein and secretory immunoglobulin A. Increased sympathetic tone and catecholamine output and stimulation of the hypothalamic-pituitary-adrenal axis and salivary cortisol redemption are these two potential explanations of this spike.[2,4,25-27] Changes in the concentration of specific salivary ingredients have an impact on the kinetics of the mechanisms that aid in creating a new equilibrium between tooth demineralization and remineralization.[6,28,29] Recent studies have emphasized how different salivary glands create different types of saliva and how flow velocity affects the concentration of various salivary constituents.[17,18] The present study's edge over previous studies is defines that potential design along with the evaluation of performance is considered a prominent outcome variable. Researchers typically stress the requirement to utilize accurate metrics that are less likely to introduce bias into research than questionnaires.[30,31] One of the major flaws of the study is extremely small sample size, which limits the generalizability of the results. Additional pH and flow rate measurements may provide fresh information on the relationships between pH and the stress of the exam. An important notable restriction is the one-time pH readings in saliva. As pH levels vary with time mainly in reaction to various stimuli and an associated flow index, variation is present in the value of pH, and it may be predicted. In this study, all assessments were made between 8 and 10 am, at least two hours after ceasing to eat or drink, and without smoking. However, it is possible that multiple assessments made on a day or on some other days in a row could provide a better control for pH variable effects.[10,32]

Conclusion

The final test of this study illustrates how stress affects numerous important salivary components. The results suggest that acute psychological stress affects salivary pH and composition. Furthermore, each group experienced a shift in salivary pH towards acidity which in turn raises the possibility of caries and related problems. This highlights the significance of saliva as most important dynamic biological fluid which helps in preserving the process for oral health.

Acknowledgements

The authors are showing their gratitude to the Karpaga Vinayaga Institute of Dental Sciences, and Karpaga Vinayaga Institute of Medical Sciences and Research Center, for the spontaneous support regarding this entire research study.

Declarations

Funding

Authors have clearly stated that they do not have any commercial interest and financial interest. The research costs were easily covered by the researchers.

Author contributions

Conceptualization, L.P.R and S.B.; Methodology, L.P.R, S.B., G.S., I.K S.M and K.S.; Validation, I.K. and K.S.; Resources, G.S and S.M.; Writing – Original Draft Preparation, L.P.R, S.B and G.S ; Writing—Review & Editing, I.K, S.M and K.S.; Supervision, L.P.R., S.B. and K.S.; Project Administration, L.P.R. and K.S.

Conflicts of interest

All authors clearly stated that they do not have any conflicts of interests.

Data availability

Usually, the sets of data are created during and/or analyzed throughout the entire study and are available from the corresponding author on reasonable request.

Ethics approval

The ethical approval was acquired from institutional ethical committee of Karpaga Vinayaga Institute of Dental Sciences KIDS/012/2021/II.

References

1. Selye H. *The stress of life*. New York: McGraw-Hill; 1956.
2. Kiecolt-Glaser JK, McGuire L, Robles TF, Glaser R. Psychoneuroimmunology and psychosomatic medicine: back to the future. *Psychosom Med.* 2002;64(1):15-28. doi: 10.1097/00006842-200201000-00004
3. Macaluso M, Preskorn SH. How biomarkers will change psychiatry: from clinical trials to practice. Part I: introduction. *J Psychiatr Pract.* 2012;18(2):118-121. doi: 10.1097/01.pra.0000413277.11091.25
4. Cohen M, Meir T, Klein E, et al. Cytokine levels as potential biomarkers for predicting the development of posttraumatic stress symptoms in casualties of accidents. *Int J Psychiatry Med.* 2011;42(2):117-131. doi: 10.2190/PM.42.2.b
5. Matos-Gomes N, Katsurayama M, Makimoto FH, et al. Psychological stress and its influence on salivary flow rate, total protein concentration and IgA, IgG and IgM titers. *Neuroimmunomodulation*. 2010;17:396-404. doi: 10.1159/000292064
6. Hellhammer DH, Wüst S, Kudielka BM. Salivary cortisol as a biomarker in stress research. Psychoneuroendocrinology. 2009;34(2):163-171. doi: 10.1016/j.psyneuen.2008.10.026
7. Marques AH, Silverman MN, Sternberg EM. Evaluation of stress systems by applying noninvasive methodologies: measurements of neuroimmune biomarkers in the sweat, heart rate variability and salivary cortisol. *Neuroimmunomodulation.* 2010;17(3):205-208. doi: 10.1159/000258725
8. Diep MT, Jensen JL, Skudutyte-Rysstad R, et al. Xerostomia and hyposalivation among a 65-yr-old population living in Oslo, Norway. *Eur J Oral Sci.* 2021;129(1):e12757. doi: 10.1111/eos.12757

9. Bosch JA, Brand HS, Ligtenberg TM, et al. Psychological stress as a determinant of protein levels and salivary-induced aggregation of Streptococcus gordonii in human whole saliva. *Psychosom Med.* 1996;58(4):374-382. doi: 10.1097/00006842-199607000-00010
10. Bosch JA, Veerman EC, de Geus EJ. α-Amylase as a reliable and convenient measure of sympathetic activity: don't start salivating just yet! *Psychoneuroendocrinology.* 2011;36(4):449-453. doi:10.1016/j.psyneuen.2010.12.019
11. Levine A, Zagoory-Sharon O, Feldman R et al. Measuring cortisol in human psychobiological studies. *Physiol Behav.* 2007;90(1):43-53. doi: 10.1016/j.physbeh.2006.08.025
12. Teeuw W, Bosch JA, Veerman EC. Neuroendocrine regulation of salivary IgA synthesis and secretion: implications for oral health. *Biol Chem.* 2004;385(12):1137-1146. doi: 10.1515/BC.2004.147
13. Bosch JA, de Geus EE, Ring C, Nieuw Amerongen AV, Stowell JR. Academic examinations and immunity: academic stress or examination stress? *Psychosom Med.* 2004;66(4):625-627. doi:10.1097/01.psy.0000133254.46947.ac
14. Nater UM, Rohleder N, Gaab J, et al . Human salivary alpha-amylase reactivity in a psychosocial stress paradigm. *Int J Psychophysiol.* 2005;55(3):333-342. doi: 10.1016/j.ijpsycho.2004.09.009
15. Khalaila R, Cohen M, Zidan J. Is salivary pH a marker of depression among older spousal caregivers for cancer patients? *Behav Med.* 2014;40(2):71-80. doi: 10.1080/08964289.2013.861794
16. Kiecolt-Glaser JK, Garner W, Speicher C, et al. Psychosocial modifiers of immunocompetence in medical students. *Psychosom Med.* 1984;46(1):7-14. doi: 10.1097/00006842-198401000-00003
17. Ng V, Koh D, Mok BY, Chia SE, Lim LP. Salivary biomarkers associated with academic assessment stress among dental undergraduates. *J Dent Educ.* 2003;67(10):1091-1094.
18. Cohen M, Khalaila R. Saliva pH as a biomarker of exam stress and a predictor of exam performance. *J Psychosom Res.* 2014;77(5):420-425. doi: 10.1016/j.jpsychores.2014.07.003
19. Turner RJ, Sugiya H. Understanding salivary fluid and protein secretion. *Oral Dis.* 2002;8(1):3-11. doi: 10.1034/j.1601-0825.2002.10815.x
20. Chiappelli F, Iribarren FJ, Prolo P, et al. Salivary biomarkers in psychobiological medicine. *Bioinformation.* 2006;1(8):331-334. doi: 10.6026/97320630001331
21. Surdacka A, Strzykata K, Rydzews-ka A. Changeability of oral cavity environment. *Eur J Dent*. 2007;1(1):14-17.
22. Golden SH, Wand GS, Malhotra S, et al. Reliability of hypothalamic–pituitary–adrenal axis assessment methods for use in population-based studies. *Eur J Epidemiol.* 2011;26(7):511-525. doi: 10.1007/s10654-011-9585-2
23. Lawrence HP. Salivary markers of systemic disease: noninvasive diagnosis of disease and monitoring of general health. *J Can Dent Assoc.* 2002;68(3):170-174.
24. Bergdahl M, Bergdahl J. Low unstimulated salivary flow and subjective oral dryness: association with medication, anxiety, depression, and stress. *J Dent Res.* 2000;79(9):1652-1658. doi: 10.1177/00220345000790090301
25. Sculley DV, Langley-Evans SC. Periodontal disease is associated with lower antioxidant capacity in whole saliva and evidence of increased protein oxidation. *Clin Sci (Lond).* 2003;105(2):167-172. doi: 10.1042/CS20030031
26. Bayazit V. Evaluation of cortisol and stress in captive animals. *Aust J Basic Appl Sci.* 2009;3(2):1022-1031.
27. Edgar WM, Higham SM, Manning RH. Saliva stimulation and caries prevention. *Adv Dent Res.* 1994;8(2):239-245. doi: 10.1177/08959374940080021701
28. Navazesh M. Method for collecting saliva. *Ann Ny Acad Sci.* 1993;694:72-77.
29. Burtis CA, Ash wood ER. *Tietz Textbook of clinical chemistry.* 3rd ed. Philadelphia*:* WB Saunders Co; 1999:477- 530, 1245-1250, 1395-1406.
30. Carlson GW. The salivary glands: embryology, anatomy, and surgical applications. *Surg Clin North Am.* 2000;80(1):261-xii. doi: 10.1016/s0039-6109(05)70405-9
31. Carey CM, Vogel GL. Measurement of calcium activity in oral fluids by ion selective electrode: method evaluation and simplified calculation of ion activity products. *J Res Natl Inst Stand Technol.* 2000;105(2):267-273. doi: 10.6028/jres.105.030
32. Sewón LA, Karjalainen SM, Söderling E, et al. Associations between salivary calcium and oral health. *J Clin Periodontol.* 1998;25(11 Pt 1):915-919. doi: 10.1111/j.1600-051x.1998.tb02390.x

Wydawnictwo UR 2023
ISSN 2544-1361 (online)
doi: 10.15584/ejcem.2023.3.20

http://www.ejcem.ur.edu.pl
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2023; 21 (3): 539–545

ORIGINAL PAPER

A study of knowledge attitude practices and identification of perceived barriers towards screening for diabetic retinopathy amongst diabetics in an industrial area in western Maharashtra

Prachi Bakare [1], Sejal Sethi [2], Iqra Mushtaq [3], Rupali Maheshgauri [1], Renu Magdum [1], Neha Kamalkishor Modani [1], Parikshit Gogate [1,4]

[1] Department of Ophthalmology, PCMC PGI YCM Hospital, Pimpri, Pune, India
[2] Dr DY Patil Medical College Hospital and Research Centre, Pimpri, Pune, India
[3] Department of Ophthalmology, Dr DY Patil Medical College, Pimpri, Pune, India
[4] Community Eye Care Foundation, Dr Gogate's Eye Clinic, Pimpri, Pune, India

ABSTRACT

Introduction and aim. Diabetic retinopathy (DR) screening is first step in prevention of diabetic retinopathy related ocular morbidity. The aim of the study was to assess the gaps in the knowledge and awareness and evaluate its effects on the attitude and practice in known diabetic patients and to assess any anticipated barriers and its association with knowledge, attitude and practices.

Material and methods. A closed-ended questionnaire was given to 112 diabetics who met the inclusion criteria and visited a tertiary eye hospital in western Maharashtra. The purpose of the questionnaire was to assess knowledge, attitude and practices and perceived barriers to the study of DR. The statistical analysis was done on SPSS software.

Results. In our study, 73.4% did not know about DR, suggesting poor awareness about the disease, 59.5% did not feel the need for regular eye checkup. 67 (61.1%) had no knowledge about blood sugar control level and lipid control for effective control of DR, 89 (79.5%) of participants did not know about effective treatment options. Moreover, 74 (66.1%) participants were advised for DR screening by physician, while 60 (53.5%) were willing to get screened, negative attitude was not getting screened when vision is good seen in 87 (79.1%) participants. Furthermore, 84 (75%) patient think eye checkup are expensive, 66% people believe availability of doctor is an issue. 60 (54.5%) think that eye checkups are time consuming.

Conclusion. Our study demonstrated, that Spearman's correlation coefficient between knowledge and awareness and based on attitude and practice, is 0.54, and that this is statistically significant ($p<0.01$). Our study has shown poor awareness and knowledge about the disease thus affecting attitude and practices, eye screening is considered expensive has been pointed out a major barrier in this study.

Keywords. awareness, barriers to diabetic retinopathy screening, diabetic retinopathy screening, knowledge

Introduction

Diabetes (diabetes mellitus) is a metabolic disorder characterized by high blood glucose levels, which may eventually result in multi-systemic complications. In India, 77 million individuals were affected with in 2019, and that number will increase to 134 million by 2045.[1] An alarmingly high prevalence of diabetic retinopathy, i.e 3.5% was found in Sindhudurg district of the Maharashtra.[2]

In India, diabetes has become a common cause of blindness and ocular morbidity. The prevalence of di-

Corresponding author: Prachi Bakare, e-mail: prachi.b7@gmail.com

Received: 3.05.2023 / Revised: 19.06.2023 / Accepted: 23.06.2023 / Published: 30.09.2023

Bakare P, Sethi S, Mushtaq I et al. *A study of knowledge attitude practices and identification of perceived barriers towards screening for diabetic retinopathy amongst diabetics in an industrial area in western Maharashtra*. Eur J Clin Exp Med. 2023;21(3):539–545. doi: 10.15584/ejcem.2023.3.20.

abetic retinopathy (DR) in individuals with diabetes is expected to be around one-fifth.[3] The WHO-NPCB surveys reveals that during the past 20 years, it has emerged as at number 6 from number 17 among the list of causes for blindness in India making it more crucial than ever to look into this issue.[4]

The diabetic retinopathy cannot be prevented from progression, but its impairing effects can be postponed. In accordance to two large randomised clinical trials early treatment Diabetic Retinopathy Study (ETDRS) and Diabetic Retinopathy Study, it was stated that with the help of LASER photocoagulation, about 50% of patients prevented their visual loss with proliferative diabetic retinopathy and macular oedema.[5,6]

However, the benefits of those treatments can only be enjoyed with early detection and timely referral.

Epidemiological data states that prior to the development of visual deterioration, retinopathy may be well advanced. Patients are generally symptom-free when they should receive preventive treatment which gives a strong argument for establishment of more effective screening programmes. Screening programmes would be successful if diabetic population has knowledge and awareness about the disease. However, there have been reports of suboptimal attendance and significant demographic disparities. It is estimated that one-third of diabetic population never have an ocular examination.[7] Pointing out to the need for the development of quality improvement strategies and to increase the screening attendance, which is crucial for the understanding of theoretical determinants, such as obstacles, which is expected to be achieved after this study.[8] Industrial areas are economic development Hubs in our country with easy access to health care services, However getting screened becomes the first step of prevention in DR even after easy availability of health care services.

Aim

The aim of the study was to assess the gaps in the knowledge and awareness and evaluate its effects on the attitude and practice in known diabetic patients and to assess barriers to diabetic retinopathy screening in an Industrial area.

Material and methods

It is a cross sectional study conducted at urban health center of a tertiary medical college in an industrial area. This study was carried out in walk in patients in medicine outpatient department aged above 40 years, and diagnosed with insulin dependent diabetes mellitus and non-insulin dependent diabetes mellitus. Patients unwilling to participate in the study were excluded. The study was carried out from June 2019 to December 2019 after institutional ethical clearance (Dr DY Patil Medical College and Research Center Institutional Ethics Sub-committee, Ref No.I.E.S.C/C-42/18). Total of 112 patients were enrolled during this period after taking a written consent, all the patients included in the study were enrolled. The purpose of this research study was informed and explained to them.

Data collection

Patients were presented with a detailed questionnaire based on knowledge, attitude and practice (KAP) survey model to assess the awareness of diabetic retinopathy and its sight threatening complication and barriers were assessed which prevented them from screening for diabetic retinopathy.

Questionnaire was prepared by the author on basis of literature available.[9] The content of questionnaire was validated by experts in the field of ophthalmology. Questions were framed in English and then translated in patient's regional language before presenting it to them. In presence of one of the investigator, patients were advised to fill in the questionnaire, Investigator recorded the response for illiterate patients without any interference or any prompting of relatives.

The questionnaire was divided in 3 Parts. The contents included were:

Part A: demographic data including age, gender, literacy, socioeconomic status based modified Kupuswamy classification, residential address were collected.[10] Primary education was considered till class IV, secondary till class 12, graduation was completion of degree, and duration of diabetes was noted;

Part B: included questions based to asses knowledge and awareness, attitude and practices related to the disease and complications, anticipated barriers to screening for diabetic retinopathy. While understanding the disease is knowledge, simply knowing about it is awareness.[11] Attitude was defined as what is thought and practices was defined as what is done.[12]

Desirable answers were marked beforehand and were compared to response received knowledge was considered excellent if more than 60% of questionnaire were answered correctly, moderate between less than 60 to 30% and less than 30% were considered poor,

The answers will be later classified into good or poor awareness, positive and negative attitude, healthy and unhealthy practices. The barriers to screening were recorded. Barriers would be graded extreme if more than 50% of participants accepted it as an obstacle for DR screening. Patients with poor awareness and unhealthy practices will be briefed about the importance of good control of diabetes and regular health check-ups.

Data analysis

Descriptive statistics were summarized using frequencies, percentages, medians, and ranges. Continuous

data are presented as mean (SD) and medians with Inter quartile range.

Comparison between two groups were done using non-parametric Mann-Whitney U test. Whereas, comparison between more two groups were done using non-parametric Kruskal-Wallis test. All tests were 2 tailed and significance set at $p<0.05$.

Scoring procedure

Summary score were calculated using sum of all right answers. Level domain categories (knowledge and awareness, attitude and practices, anticipation of barriers) were defined as total score divided by 3. All analyses were performed using SPSS version 25 (IBM, Armonk, NY, USA)

Results

Total 112 patients were included in the study, 59 (52.68%) were females, 67 (59.82%) belonged to low income group while 67(59.82%) had primary education, while 49 (43.75%) had diabetes mellitus less than 1 year (Table 1).

Table 1. Demographic data of participants

	n (%)
Gender	
Female	59 (52.68%)
Male	53 (47.32%)
Total	112 (100%)
Economic status	
Low	67 (59.82%)
Moderate	42 (37.5%)
High	3 (3.23%)
Education Levels	
Primary	67 (59.82%)
Secondary	29 (25.89%)
Graduation and higher	12 (10.71%)
Illiterate	4 (3.57%)
Duration of diabetes	
Less than 1 year	49 (43.75%)
1–5 years	24 (21.43%)
6–9 years	18 (16.07%)
10–14 years	13 (11.61%)
More than 15 years	8 (7.14%)
How long on medication	
Less than 1 year	40 (35.71%)
1–5 years	30 (26.92%)
6–9 years	8 (7.14%)
10–14 years	8 (7.14%)
More than 15 years	7 (6.25%)
No med/No response	19 (16.96%)

Based on knowledge 47 (42%) had poor knowledge and awareness, Unhealthy practices were noted amongst 74 (66.1%), while 54 (48.2%) considered diabetic retinopathy screening had some barriers (Table 2).

Table 2. Domain scores descriptive statistics table

	n (%)
Based on knowledge and awareness	
Excellent	18 (16.1%)
Moderate	47 (42%)
Poor	47 (42%)
Based on attitude and practice	
Strongly agree (healthy, positive practice)	11 (9.8%)
Slightly agree	27 (24.1%)
Disagree (unhealthy, negative practice)	74 (66.1%)
Based on anticipation of barriers	
Extreme barrier	48 (42.9%)
Somewhat of a barrier	54 (48.2%)
Not a barrier	10 (8.9%)

Table 3 showed that association of demographic factors and with based on knowledge and awareness. We found education is associated with levels of knowledge and awareness (p=0.014). Healthy attitude and practices (p=0.001). Other demographic variables included age, gender, economic status, duration of diabetic were and duration of medication were not significantly associated with levels of knowledge and awareness, attitude and practices and anticipation of barriers.

Table 3. Comparison of demographic factors with domains on knowledge and awareness

Factors		Based on knowledge and awareness			p
		Excellent	Moderate	Poor	
Age, median (IQR)		61.5 (44.75–70.5)	60 (50–66)	52 (44.5–61.5)	>0.05
Sex	Female	6 (33.3%)	24 (53.3%)	26 (57.8%)	0.208
	Male	12 (66.7%)	21 (46.7%)	19 (42.2%)	
Economic status	Low	5 (38.5%)	20 (54.1%)	30 (69.8%)	0.271
	Medium	7 (53.8%)	16 (43.2%)	12 (27.9%)	
	High	1 (7.7%)	1 (2.7%)	1 (2.3%)	
Education level	Primary	7 (43.8%)	19 (47.5%)	24 (55.8%)	0.014
	Secondary	7 (43.8%)	15 (37.5%)	7 (16.3%)	
	Graduation and higher	2 (12.5%)	6 (15%)	4 (9.3%)	
	Illiterate	0 (0%)	0 (0%)	8 (18.6%)	
Duration of diabetes	Less than 1 years	6 (33.3%)	20 (43.5%)	25 (56.8%)	0.337
	1–5 years	5 (27.8%)	10 (21.7%)	8 (18.2%)	
	6–9 years	4 (22.2%)	9 (19.6%)	4 (9.1%)	
	10–14 years	3 (16.7%)	3 (6.5%)	6 (13.6%)	
	More than 15 years	0 (0%)	4 (8.7%)	1 (2.3%)	
Years of medication	Less than 5 years	9 (64.3%)	14 (51.9%)	15 (65.2%)	0.278
	6 to 9 years	3 (21.4%)	10 (37%)	6 (26.1%)	
	More than 9 years	2 (14.3%)	3 (11.1%)	2 (8.7%)	

The results are presented in a matrix such that, as can be seen the correlations are replicated. Our study resulted, that spearman's correlation coefficient between knowledge

Table 4. Knowledge, awareness, attitude and practices regarding diabetic retinopathy

Knowledge and awareness	Frequency	Percentage
Do you think diabetes can affect multiple organ systems?		
Yes	39	34.8
No	72	64.3
No Idea	1	0.9
Which organs do you think would be affected?		
Stomach	1	0.8
Eyes	1	0.8
Kidneys	9	8
Lungs	4	3.6
Eyes and kidneys	6	5.4
No idea	91	81.3
Do you think diabetes patients require regular eye checkups?		
Yes	45	40.5
No	67	59.5
Do you feel timely treatment can help prevent damage to the eyes in diabetic patients?		
Yes	67	59.8
No	45	40.2
Whom do you thinks need to be consulted in case of eye problems?		
Ophthalmologist	54	48.2
Any specialist	5	4.5
Optometrist	2	1.8
General practitioner	51	45.5
When do you think a diabetic patient should visit the ophthalmologist?		
When blood sugar is well controlled	1	0.9
When blood sugar is poorly controlled	23	20.2
Don't know	88	78.9
Do you know about diabetic retinopathy?		
Yes	29	25.89
No	83	74.1
How did you come to know about diabetic retinopathy?		
Doctor/Nurse/ophthalmologist	16	14.3
TV/Newspaper/Radio	1	0.9
Family members/friends/relatives with diabetes	3	2.7
Other sources		
Not answered	92	82.1
What do you feel can help treat diabetic retinopathy effectively?		
Control of diabetes	9	8
Only medication	1	0.9
LASER	13	11.6
Don't know	89	79.5
Is retina the main part that gets damaged?		
Yes	26	23.2
No	83	74.1
Don't know	3	2.7
Do you think that the eye doctor will have special equipments to check for the effects of diabetes on eyes?		
Yes	57	50.9
No	55	49.1
Does eye treatment become more effective with controlled blood sugar and lipids?		
Yes	67	59.82
No	45	40.18
Do you think one eye can be affected before the other eye in diabetes?		
Yes	29	36.37
No	82	73.21
Don't know	1	0.9
Do you feel children who have diabetes also have a risk of developing eye complications?		
Yes	46	41.07
No	64	57.14
Don't know	2	1.79
If vision is damaged due to diabetes, use of 'low vision aids' helps in daily work?		
Yes	58	52.7
No	49	44.5
Don't know	3	4.46
On successfully being treated with LASER, that eye does not require LASER treatment again		
Yes	54	50.5
No	48	44.9
Don't know	10	8.93
Attitude and Practice		
Has the physician advised you to visit the eye doctor?		
Yes	74	66.1
No	38	33.9
If Yes, will you go?		
Yes	60	53.6
No	16	14.3
Not decided	1	0.9
Not answered	35	31.2
If the patient has their diabetes under control, do you feel there is a need to visit the ophthalmologist		
Yes	31	28.4
No	76	69.7
Don't Know	1	0.9
Not answered	4	3.57
How often do you get your eyes examined?		
Monthly	2	1.8
Half yearly	14	12.5
Annually	13	11.6
Need Basis	48	42.9
This is the first time	35	31.3
How regularly do you check your blood sugar?		
Daily	8	7.14
Weekly	9	8.04
Monthly	45	40.18
Yearly	50	44.64
Does having good vision mean that your eyes are not affected?		
Yes	87	77.67
No	22	19.64
Don't know	3	2.68
Does taking eye treatment mean that there is no further need for you to control your blood sugar and lipid?		
Yes	53	47.32
No	54	46.42
Don't know	7	6.25

and awareness and based on attitude and practice, is 0.54, and that this is statistically significant ($p<0.01$).

A Spearman's rank-order correlation was run to determine the relationship between based on knowledge

and awareness based on attitude and practice. There was a moderate, positive correlation between both domains, which was statistically significant. Moreover, a Spearman's rank-order correlation was run to determine the relationship between based on anticipation of barriers and based on knowledge and awareness. There was an almost low negative correlation between based on anticipation of barriers and based on knowledge and awareness which was not statistically significant ($p>0.05$). Almost low negative correlation was found between Based on anticipation of barrier and attitude and practices.

In this table, only 40.5% agreed knew that regular eye checkup is needed, maximum participants 45. 66 (59.5%) did not know about regular eye checkup for DR screening, 80 (73.4%) had no idea about DR.

74 (66.1%) participants were advised for DR screening by physician, while 60 (53.6%) were willing to get screened, negative attitude was not getting screened when vision is good in 87 (77.67%) of participants (Table 4).

Table 5. Anticipated barriers towards DR screening

Barriers	Frequency	Percentage
Based on anticipation of barriers		
Do you feel undergoing LASER treatment may be painful?		
Yes	63	56.25
No	42	37.5
Don't know	7	6.25
Do you think eye check-ups are expensive?		
Yes	84	75
No	28	25
Do you think travelling the distance to go for eye checkups is worth the effort?		
Yes	66	58.9
No	46	41.1
Do you have anyone to accompany you during eye checkups?		
Yes	80	71.43
No	32	28.57
Do you feel information on eye problems due to diabetes should be given by eye doctor ONLY?		
Yes	55	50.5
No	54	49.5
Is the availability of doctor an issue in your neighborhood?		
Yes	66	58.93
No	46	41.07
Do you feel patients with diabetes waste their time in eye checkups?		
Yes	60	54.6
No	49	44.5
Don't know	3	2.68

The survey revealed that a significant percentage of respondents perceived LASER treatment as potentially painful (56.25%) and eye checkups as expensive (75%). A majority believed traveling for eye checkups was worthwhile (58.9%) and had someone accompany them (71.43%). Opinions were divided on the source of information (50.5% preferred eye doctors), availability of doctors (58.93% considered it an issue), and time wasted during checkups (54.6% felt it was wasted).

Discussion

DR if diagnosed early can prevent irreversible visual loss. This depends on the overcoming anticipated barriers along with a good level of knowledge awareness and attitude of patients diagnosed with diabetes. Secondly, along with lowering the personal suffering caused due to blindness and visual impairment, providing ophthalmic care to patients with diabetes at the right time can result in significant cost savings for eye care programmes.[13]

There are fewer studies about KAP from industrial area of Maharashtra majority of awareness studies are done in South India. In our study, 73.4 % were unaware about DR, when leading question was asked timely screening for DR prevents damage to eye, 67 (59.5%) answered positively. Too many questions on the identical topic, participants could have answered correctly. In study done by Srinivasan et al. 49 patients (17.01%) were aware of DR as an ocular complication of diabetes.[14] Study done in South India by Babu et al., only 28% of the population was 'aware' of diabetes, while 5.4% answered that eye or kidneys may be affected in diabetes.[15] Namperumalsamy et al. showed that person from the community were aware that diabetes could affect all 5 major systems listed.[16]

61.1% of pariticpants had no knowledge about blood sugar control and lipid control level for effective control of DR, while 79.5% of participants did not know about effective treatment options. We found poor awareness about DR resulting in low grade knowledge about treatment option. Probably a question asked about lasers 48 (44.9%) answered correctly could be due to no other modality of treatment mentioned. Though this is in comparison with other study done in Oman which have better literacy rates. We found higher level of education is associated with healthy attitude and positive practices ($p=0.001$). Screening for DR is of the utmost importance considering it a preventable visual loss if diagnosed early, All strategies developed for sight saving would be beneficial only if knowledge about the disease is well perceived amongst diabetics.

Discussing about attitude and practices, 66.1% participants were advised for DR screening by physician, while 53.5% were willing to get screened, negative attitude was not getting screened when vision is good seen in 79.1% participants, additionally, patients often become complacent if they have control over their blood sugar levels without realizing that their chance of having DR increases with the length of their diabetes. Majority get blood sugar level checked regularly again suggesting good awareness about diabetes but not about its complications. Shubha Kumar et al. determined that getting such eye care was unnecessary in the absence of any symptoms reported.[17]

We found barriers to DR screening, 75% of patients think eye check-ups are expensive, while 66% people be-

lieve availability of doctor is an issue. Moreover, more than half of participants think that eye checkups are time consuming. Graham et al. found that the early detection and treatment can prevent 50% to 70% of DR-related visual impairments.[18]

Strength of the study is a qualitative study based on responses from patients and their perspective, limitation were unavoidable leading questions. A larger sample size may have provided more insight.

Our study pointed out positive attitude and practices were noted amongst diabetics who were aware about the disease which was statistically significant (0.54), however a negative correlation was noted between anticipation of barriers and knowledge and awareness also with attitude and barriers. Similar results were seen in study done by Srinivasan et al. where positive attitude and good practice patterns had a significant association with the knowledge about the disease.[14]

There is wider gap between health policies, its perception about the disease complication and its utilization, till date majority attention has been directed towards cataract. Diabetes being more complex requires frequent follow up which is only possible if patients are aware about the disease process.

Compulsory referral for DR screening should be made mandatory at each time contact with health care workers to catch diabetic retinopathy early. Outreach camps and telemedicine can help in bridging the gap. The information gained from our study reveals significant association between of knowledge awareness, and its impact on attitude and practices however we did not find any correlation between knowledge awareness and anticipation of barriers.

In spite of rapid technological advances in screening and management of DR, primary prevention still remains to be the only feasible approach in many developing countries with competing demands.[19,20]

Conclusion

Health education in form of booklets, advertisements about it benefits, creating community leaders, strong referral systems and health care policy implementation for improving of awareness amongst diabetics should be set earliest to prevent a pandemic of diabetic retinopathy. Knowledge and awareness about the disease will result is healthy attitude and practices. Overcoming barriers by incorporation of telemedicine, cost and time effective approach like organizing local camps for DR screening should be fruitful in early diagnosis and timely treatment.

Declarations

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Author contributions

Conceptualization, P.B., S.S., Ru.M. and Re.M.; Methodology, P.B. and I.M.; Software, N.K.M. and P.G.; Validation, Ru.M., Re.M. and P.B.; Formal Analysis, P.B.; S.S.; Resources, S.S.; Data Curation, P.B.; Writing – Original Draft Preparation, P.B.; Writing – Review & Editing, P.B. and P.G.; Visualization, P.B.; Supervision, P.B.; Project Administration, R.M.

Conflicts of interest

There are no conflict of interest.

Data availability

The author confirm that data supporting the finding are available within the article. Raw data supporting the findings of this study are available from the corresponding author, on reasonable request.

Ethics approval

Study design was approved by Dr DY Patil Medical College and Research Center Institutional Ethics Sub-committee, Ref No.I.E.S.C/C-42/18.

References

1. Saeedi P, Petersohn I, Salpea P, et al. Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas, 9th edition. *Diabetes Res Clin Pract*. 2019;157:107843. doi: 10.1016/j.diabres.2019.107843
2. Patil S, Gogate P, Vora S, et al. Prevalence, causes of blindness, visual impairment and cataract surgical services in Sindhudurg district on the western coastal strip of India. *Indian J Ophthalmol.* 2014;62(2):240-245. doi: 10.4103/0301-4738.128633
3. Ramachandran A, Jali MV, Mohan V, Snehalatha C, Viswanathan M. High prevalence of diabetes in an urban population in south India. *BMJ*. 1988;297(6648):587-590. doi: 10.1136/bmj.297.6648.587
4. Murthy GV, Gupta SK, Bachani D, Jose R, John N. Current estimates of blindness in India. *Br J Ophthalmol.* 2005;89(3):257-260. doi: 10.1136/bjo.2004.056937
5. Photocoagulation treatment of proliferative diabetic retinopathy. Clinical application of Diabetic Retinopathy Study (DRS) findings, DRS Report Number 8. The Diabetic Retinopathy Study Research Group. *Ophthalmology.* 1981;88(7):583-600.
6. Early photocoagulation for diabetic retinopathy. ETDRS report number 9. Early Treatment Diabetic Retinopathy Study Research Group. *Ophthalmology.* 1991;98(5): 766-785.
7. Mohan D, Raj D, Shanthirani CS, et al. Awareness and knowledge of diabetes in Chennai--the Chennai Urban Rural Epidemiology Study [CURES-9]. *J Assoc Physicians India.* 2005;53:283-287.

8. Graham-Rowe E, Lorencatto F, Lawrenson JG, et al. Barriers and enablers to diabetic retinopathy screening attendance: Protocol for a systematic review. *Syst Rev.* 2016;5(1):134. doi: 10.1186/s13643-016-0309-2
9. Khandekar R, Harby SA, Harthy HA, Lawatti JA. Knowledge, attitude and practice regarding eye complications and care among Omani persons with diabetes - A cross sectional study. *Oman J Ophthalmol.* 2010;3(2):60-65. doi: 10.4103/0974-620X.64228
10. Singh T, Sharma S, Nagesh S. Socio-economic status scales updated for 2017, international Research in medical sciences. *Int J Res Med Sci*. 2017;5(7):3264-3267.
11. Mahesh G, Elias A, Sandhya N, et al. Chengamanad Diabetic Retinopathy Awareness Study (CDRAS). *Kerala Journal of Ophthalmology.* 2006;28:14-21.
12. World Health Organization. https://www.who.int/cholera/vaccines/kap_protocol.pdf. Accessed 12 February, 2023.
13. Javitt JC, Aiello LP, Chiang Y, Ferris FL, Canner JK, Greenfield S. Preventive eye care in people with diabetes is cost-saving to the federal government. Implications for health-care reform. *Diabetes Care.* 1994;17(8):909-917. doi: 10.2337/diacare.17.8.909
14. Srinivasan NK, John D, Rebekah G, et al. Diabetes and Diabetic Retinopathy: Knowledge, Attitude, Practice (KAP) among Diabetic Patients in A Tertiary Eye Care Centre. *J Clin Diagn Res*. 2017;11(7):NC01-NC07. doi: 10.7860/JCDR/2017/27027.10174
15. Babu N, Kim, Ramchandani B, et al. Diabetes and Diabetic Retinopathy: Knowledge, Attitude, Practice (KAP) Among Paramedical Personnel (PMPS) and Community Members (CMS) In Southern India. *AIOC Proceedings.* 2009: 150-153.
16. Namperumalsamy P, Kim R, Vignesh TP, et al. Prevalence and risk factors for diabetic retinopathy: a population-based assessment from Theni District, south India. *Br J Ophthalmol*. 2009;93(4):429-434. doi: 10.1136/bjo.2008.147934
17. Kumar S, Kumar G, Velu S, et al. Patient and provider perspectives on barriers to screening for diabetic retinopathy: an exploratory study from southern India. *BMJ Open*. 2020;10(12):e037277. doi: 10.1136/bmjopen-2020-037277
18. Graham-Rowe E, Lorencatto F, Lawrenson JG, et al. Barriers to and enablers of diabetic retinopathy screening attendance: a systematic review of published and grey literature. *Diabet Med.* 2018;35(10):1308-1319. doi: 10.1111/dme.13686
19. Lin KY, Hsih WH, Lin YB, Wen CY, Chang TJ. Update in the epidemiology, risk factors, screening, and treatment of diabetic retinopathy. *J Diabetes Investig.* 2021;12(8):1322-1325. doi: 10.1111/jdi.13480
20. Tan TE, Wong TY. Diabetic retinopathy: Looking forward to 2030. *Front Endocrinol (Lausanne).* 2023;13:1077669. doi: 10.3389/fendo.2022.1077669

Wydawnictwo UR 2023
ISSN 2544-1361 (online)
doi: 10.15584/ejcem.2023.3.21

http://www.ejcem.ur.edu.pl
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2023; 21 (3): 546–552

ORIGINAL PAPER

Cancer patients' attitudes towards holistic complementary and alternative medicine in the management of sleep problems

Dilek Baykal [1], Burcu Dedeoğlu Demir [2], Dilek Yildirim [3]

[1] Department of Nursing, Faculty of Health Sciences, Istanbul Atlas University, Istanbul, Turkey
[2] Department of Nursing, Faculty of Health Sciences, Istanbul Arel University, Istanbul, Turkey
[3] Department of Nursing, Faculty of Health Sciences, Istanbul Aydin University, Istanbul, Turkey

ABSTRACT

Introduction and aim. Sleep problems are frequently experienced in cancer patients and complementary and alternative medicine (CAM) is solve the problem. However, patients' attitudes towards this practice are crucial. This study was purpose to investigate the attitudes of cancer patients towards CAM practices in the management of sleep problems.

Material and methods. This cross-sectional. and descriptive study was conducted between February-June 2020, on 140 cancer patients in oncology/haematology clinics, three different hospitals in Istanbul. Data were collected by using the Information Form, "Richard Campbell Sleep Questionnaire and The Attitudes towards Holistic Complementary and Alternative Medicine scale".

Results. It was found that the patients had problems with sleep in general 42.74±21.31, mostly in the aspect of the quality of sleep 36.28±26.1. It was determined that stage IV cancer, education and income level of patients affect sleep problems. While female holistic health attitudes were more negative and, their attitudes towards CAM were positive. It was found in the analysis of variance that was performed to determine the difference between the mean scores towards sleep problems of patients in different stages ($F=3.062$; $p<0.05$).

Conclusion. It was found that cancer patients usually had sleep problems, and their attitudes towards CAM practices were positive.

Keywords. cancer, attitude, complementary therapies, sleep

The list of abbreviations:

CAM – complementary and alternative medicine, **NCCIH** – National Center for Complementary and Integrative Health, **RCSQ** – Richard Campbell sleep questionnaire, **HCAMS** – the attitudes towards holistic complementary and alternative medicine scale, **HH** – holistic health

Introduction

Complementary. and alternative medicine (CAM) is defined by the National Center for Complementary and Integrative Health (NCCIH) as non-mainstream that used in conjunction with traditional medical treatments.[1] NCCIH classifies complementary health practice as natural products (vitamin and minerals, probiotics etc.), mind and body practices (acupuncture, chiropractic, yoga, osteopathic manipulation and meditation etc.) and other complementary therapies (such as Traditional Chinese medicine, naturopathy and ayurvedic medicine).[2] CAM is used to reduce the side effects of treatment in cancer patients.[1] It is appeared that CAM practices in cancer patients are used to reduce fatigue, pain, sleep problems and menopausal symptoms, and to improve the quality of life.[3-7] In Turkey, it was reported that patients use CAM to order to fight with cancer, and the prevalence of usage is 22.1–84.1%.[8]

Corresponding author: Dilek Baykal, e-mail: dilek.baykal@atlas.edu.tr

Received: 23.05.2023 / Revised: 18.06.2023 / Accepted: 27.06.2023 / Published: 30.09.2023

Baykal D, Demir BD, Yildirim D. *Cancer patients' attitudes towards holistic complementary and alternative medicine in the management of sleep problems.* Eur J Clin Exp Med. 2023;21(3):546–552. doi: 10.15584/ejcem.2023.3.21.

Patients apply to CAM use, especially when they think that they are not able to benefit from medical treatment sufficiently in chronic diseases.[9] Other reasons of CAM usage are how much patient is disturbed by the symptoms, the severity of the disease (such as cancer) and whether they perceive their health as a whole (mind, body and spirit in health).[10] Cancer is a life-threatening disease upon the stage of diagnosis. In addition, sleep problems are frequently encountered depending on the disease and the side effects of the treatments applied. In the meta-analysis conducted with patients with head or neck cancer, it was reported the prevalence of sleep problems was observed as 29% before treatment, 45% during treatment and 40% after treatment.[11] It was also stated that 42.8% of patients with newly diagnosed with breast cancer have insomnia.[12] Sleep disorders, which are frequently seen from the time of diagnosis and continue at the end of the treatment, can affect the quality of life of patients.[13] Patients who do not use or prefer not to use medical methods to cope with their sleep problems can benefit from CAM, which is more accessible.

However, as in every alternative treatment method, the attitudes and behaviors of the patients are important in CAM. Studies on the use of CAM in sleep disorders, which are frequently seen from the time of diagnosis and continue to be seen at the end of treatment, are quite limited. However, in the studies conducted in the world and in Turkey, the results of study that evaluating the attitudes of patients towards holistic CAM in the management of the sleep problems have not been found.[14-16]

Aim

In this study, it was purpose to determine the attitudes of cancer patients towards CAM practices in the management of the sleep problems.

Material and methods

Ethical approval

Written informed consent was obtained from the participants who met the inclusion criteria after the purpose of the study was explained. During the study, the Helsinki Declaration was adhered to. Ethics committee approval was obtained from the Haliç University Non-Interventional Ethics Committee on (2020/decision number: 06) for this study.

Study design

This study was design as a descriptive, cross-sectional study to evaluate attitudes of cancer patients towards CAM practices in the management of the sleep problems. After the purpose of the research was explained, face-to-face interviews were conducted with the participants who agreed.

This study was conducted between February-June 2020, in oncology and haematology clinics, 3 different hospitals in Istanbul, Turkey.

Inclusion criteria

Patients who were diagnosed with cancer at least 1 month ago, 18 years and older, who could speak and understand Turkish and voluntary to participate in the study were included. After explaining the purpose of the study, the patients who answered yes to the question of "Do you have any problems with your sleep" were included in the study.

Exclusion criteria

The patients who answered no to the question of "Do you have any problems with your sleep" were excluded in the study.

Sampling and sample size

According to the analysis performed in the G-Power statistical software, the sample size was calculated as 0.05 and 86 participants with a power significance level of 95% (G*Power Version 3.1.9.2 statistical software). The study was completed with 140 patients.

Data collection

The Socio-demographic information form, "Richard Campbell sleep questionnaire" and the "Attitudes towards holistic complementary and alternative medicine" scales were used in data collection.

The socio-demographic information form

In this study, data were collected with questionnaire prepared by the researchers based on studies. In the form, socio-demographic characteristics such as age, gender, marital and economic levels; and disease-treatment features such as disease stage, treatment type and duration were questioned.[4-13]

Richard Campbell sleep questionnaire (RCSQ)

The Turkish validity and reliability of the scale were made by Ozlu and Ozer and consist of 6 items that evaluate the sleep depth, sleep latency, number of awakenings, efficiency (percentage of time awake), sleep quality and noise level of the environment. The 6th item evaluating the noise level of environment does not count in the calculation of the total score. The Cronbach's alpha value of the original scale is 0.82. Increasing the total score of the scale means an increase in the sleep quality of the patients. Each item consists of a visual analogue scale technique chart between 0-100, and if the total score is between 0-25, it means very poor sleep and between 76-100, it means very nice sleep. The Cronbach's alpha value is 0.92.[17]

The attitudes towards holistic complementary and alternative medicine scale (HCAMS)

Its validity and reliability for our country was made by Erci in 2003. The Cronbach's alpha value, which is the reliability coefficient of the scale, is 0.72. The Cronbach's

alpha value was calculated as 0.76 for this study. The scale has two sub-scales CAM and holistic health (HH). This scale is a Likert-type scale consisting of 11 items. Minimum 11, maximum 66 points can be obtained from the scale. Positive attitude towards CAM increases as the score of the scale decreases.[18]

Statistical analysis

SPSS packaged software was used to evaluate the data (Statistical Package for Social Sciences, version 25.0, SPSS Inc; Chicago, IL, USA). In the analysis of the data, variables were defined as mean, standard deviation (SD), median, frequency, and percentages, and the Chi-Square test were used for comparisons. Normality analysis of data Shapiro-Wilk test was used it. One-way ANOVA and independent samples t test were used for comparisons. Pearson correlation test was used to determine the relationship between RCSQ and the HCAMQ. All results were considered meaningful at $p<0.05$ and a confidence interval of 95%.

Results

The mean age of patients was 61.38±10.45 years, 74.28% of them were male, 87.14% of them were single, 52.14% of them were ≤8 years school graduate, 88.57% of them were not working, and the income of 7.85% is equal to their expenses (Table 1).

Table 1. Socio-demographic characteristics of patients

Characteristics		min–max	X± SD
Age		28–92	61.38±10.45
Others		n	%
Gender	Female	36	25.72
	Male	104	74.28
Marital status	Single	122	87.14
	Married	18	12.86
Education	No formal education	7	5.0
	≤ 8 years	73	52.14
	High school	22	15.71
	University	38	27.14
Employment status	Working	16	11.43
	Not working	124	88.57
Income	Income>Expenses	69	49.28
	Income<Expenses	60	42.85
	Income=Expenses	11	7.85

39.30% of patients were diagnosed with lung cancer, 44.30% of them were in Stage II, 51.42% were receiving chemotherapy treatment and the average duration of the diagnosis was 25.63±21.24 months (Table 2).

They received a total of 26.92±4.42 points from the HCAMQ, 15.43±1.76 from the CAM subscale and 13.44±2.78 points from the HH subscale (Table 3). It was understood that patients' attitudes towards CAM were positive.

Table 2. Characteristics of patients diagnosis and treatment

Characteristics		min–max	X± SD
Diagnosis duration (month)		2–72	25.63±21.24
Others		n	%
Disease Diagnosis	Lung cancer	55	39.30
	Laryngeal cancer	20	14.28
	Breast cancer	32	22.85
	Multiple myeloma	21	15.0
	Prostate cancer	12	8.57
Stage of Disease	Stage I	35	25.0
	Stage II	62	44.30
	Stage III	32	22.85
	Stage IV	11	7.85
Treatment	Chemotherapy	72	51.43
	Radyotherapy	44	31.43
	Chemotherapy+radiotherapy	24	17.14

It was found that they got a total of 38.74±23.64 points from the RCSQ; from the subscales, 42.71±27.72 points from sleep depth, 42.66±27.53 points from sleep latency, 38.18±27.26 points from the number of awakenings, 39.46±26.19 points from percentage of time awake and 36.28±26.16 points from quality of sleep (Table 3). It was determined that the patients had sleep problems according to their total score obtained from the scale. It was also found that most common problem was the quality of sleep.

Table 3. "The Attitudes towards HCAMS and RCSQ" mean total and subscale points of patients*

Scale and Subscale Points		$\bar{x}$± SD
HCAMS (Total)		26.92±4.42
Subscales	Complementary and Alternative Medicine	15.43±1.76
	Holistic Health	12.44±2.78
RCSQ (Total)		38.74±23.64
Subscales	Sleep depth	42.71±27.72
	Falling asleep	42.66±27.53
	Frequency of awakening	38.18±27.26
	Percentage of time awake	39.46±26.19
	Quality of sleep	36.28±26.16

* HCAMS – attitudes towards holistic complementary and alternative medicine scale; RCSQ – Richard Campbell sleep questionnaire

There was no significant relationship between Richard Campbell sleep questionnaire, CAM (R=-0.052; p=0.542), HH subscale (R=-0.084; p=0.327), HCAMS (R=-0.088; p=0.304) and total attitudes towards CAM (p>0.05) (Table 4).

When the relationship between RCSQ in terms of the characteristics of the patients was examined, it was found that there was no relationship according to the treatment method, age, disease duration, employment, marital status and gender (p>0.05), on the other hand, there was

a relationship between the disease stage and sleep scale (p<0.05) (Table 5). When the relationship between HH, HCAMS and gender (p<0.05) (Table 5). It was determined that Stage IV patients had more sleep problems.

Table 4. The relationship between RCSQ and attitudes towards HCAMS*

Scale	CAM		Holistic Health		HCAMS (Total)	
	R	p	R	p	R	p
RCSQ (Total)	-0.052	0.542	-0.084	0.327	-0.088	0.304

* Pearson correlation analysis was used, p<0.05;
HCAMS – attitudes towards holistic complementary and alternative medicine scale; RCSQ – Richard Campbell sleep questionnaire; CAM – complementary and alternative medicine

Discussion

In this study, it was figured out that cancer patients' attitudes towards to CAM were positive. Moreover, it was found that patients had sleep problems, and they had problems with sleep quality at most. It was also spotted that sleep problems were most common in Stage IV cancer patients.

It was found that the majority of patients participating in the study were male and have lung cancer. In the studies with lung cancer patients, it was reported to be more common in males. Since men smoke more than women, they are more likely to get lung cancer.[19] For similar reasons, it was considered that men participating in this study were diagnosed with lung cancer more.

Lung cancer is mostly asymptomatic, which may lead to delays in diagnosis. Initiating pharmacological treatments for patients with symptoms such as chest pain, cough and dyspnoea, and not being directed for further examination causes this delay.[20] However, thanks to radiological developments such as low-density CT, much smaller lesions can be detected, and it enables early diagnosis.[21] Furthermore, studies on the formation of tobacco-related lung cancer in Turkey since the 2010s have led to develop awareness.[22] Through all these reasons, it was considered that the patients participating in the study were found in Stage II by diagnosing earlier.

Among other symptoms, sleep problems are frequently encountered in cancer.[11] Moreover, sleep problems in patients can occur in different ways such as difficulty in falling asleep, frequent night awakening, early morning awakening and difficulty in getting out the bed.[23] Similar to the literature, it was found that patients in this study experience sleep problems. Moreover, it was also determined that they experienced sleep problems mostly in the sleep quality. In the literature, it was reported that in the studies with breast and advanced cancer patients, sleep quality was decreased.[24,25] It was also found that patients diagnosed as Stage IV in the study group experienced sleep problems more. Sleep

Table 5. The relationship of RCSQ and attitudes towards HCAMS with patient characteristics

Characteristics		RCSQ (Total)			CAM			Holistic Health			HCAMS (Total)		
		$\bar{x} \pm SD$	p	S.V*	$\bar{x} \pm SD$	p	S.V*	$\bar{x} \pm SD$	p	S.V*	$\bar{x} \pm SD$	p	S.V*
Gender	Female	38.06±25.55	0.402	0.841[2]	14.88±2.39	0.13	-1.524[2]	12.91±2.66	0.004	-2.926[2]	26.30±4.14	0.004	-2.892[2]
	Male	38.74±19.23			15.68±3.04			11.42±2.93			28.60±4.34		
Marital status	Single	34.37±21.94	0.243	1.172[1]	17.00±2.61	0.128	-1.532[1]	10.87±3.90	0.08	1.715[1]	27.87±5.19	0.915	0.107[1]
	Married	43.49±21.29			15.40±2.86			12.64±2.74			28.04±4.38		
Education	No formal	29.00±6.51	<0.001	15.565[1]	15.38±2.61	0.212	1.420[1]	12.52±2.90	0.309	1.203[1]	27.91±4.28	0.35	1.128[1]
	≤8 years	30.50±9.81			14.75±2.62			16.00±1.41			28.75±1.5		
	High school	59.85±21.98			15.00±2.64			12.71±3.08			27.71±4.38		
	University	56.00±21.46			17.18±3.76			12.31±2.82			29.50±4.4		
Employment	Working	29.90±13.76	0.2	-1.28[2]	15.20±1.93	0.94	0.06[2]	13.90±3.07	0.12	1.55[2]	29.1±4.14	0.29	1.05[2]
	Not working	37.88±19.00			15.14±2.71			12.44±2.79			27.58±4.32		
Income	Inc.>Exp.	55.79±21.73			15.57±3.17			12.19±2.62			27.76±4.65		
	Inc.<Exp.	29.69±10.49	<0.001	39.56[1]	15.31±2.6	0.87	0.14[1]	12.77±2.97	0.763	0.468[1]	28.09±4.13	0.873	0.136[1]
	Inc.=Exp.	29.54±12.02			15.25±1.89			12.00±3.82			27.25±5.31		
Stage of disease	Stage I	43.37±24.71			15.31±2.27	0.150	1.801[1]	11.81±2.9	0.479	0.831[1]	27.12±4.24	0.259	1.355[1]
	Stage II	41.31±23.35	0.03	3.062[1]	15.61±2.73			12.80±3.14			28.41±4.6		
	Stage III	38.38±22.02			14.61±2.88			12.48±2.44			27.09±4.42		
	Stage IV	24.85±21.45			16.45±3.79			12.55±2.28			29.00±3.89		
Treatment	CT	37.80±24.26			15.14±2.6			12.04±2.7	0.577	0.661[1]	27.18±4.02		
	RT	39.41±23.43	0.883	0.124[1]	15.50±3.2			12.84±3.01			28.45±5.06	0.530	0.739[1]
	CT+RT	40.36±23.01			15.42±2.76			12.50±2.8			27.92±4.28		
Age (r/p)		R= -0.131 p=0.126			R=-0.05 p=0.557			R=0.08 p=0.352			R=0.018 p=0.832		
Dis. Duration(r/p)		R=0.046 p=0.589			R=-0.001 p=0.991			R=0.033 p=0.699			R=0.021 p=0.81		

* [1] – One way Anova test was used, p<0.05; [2] – independent samples t test; Inc – income; Exp – expenses; CT – chemotherapy; RT – radiotherapy

problems increase with aggravating symptoms of dyspnoea and pain in the later stages of the disease.[26]

CAM are used to support modern medicine. Patients usually receive these treatments when they think conventional medicine is inadequate in chronic diseases.[27] In addition to many problems experienced by cancer patients, CAM practices are also used in the solution of sleep problems.[28] With the frequently usage of CAM practices in recent years, awareness is also increasing in the patients. When the HH subscale mean scores and the CAM subscale mean scores of patients participating in this research were evaluated, it was determined that their attitudes were positive. Similar to the results of this study, it was reported that the attitudes towards CAM practices were positive in the studies conducted in cancer patients.[17,29]

In the study found that female scored significantly higher on the HH and subscales. It studies conducted in different countries, it has been reported that female prefer to use CAM more likely in cancer patients. In a study conducted in the USA, it was reported that being female and having a high level of education and income increased the preference for CAM use.[30] In the study carried out in Europe, young and higher-education levels females mostly refer to the use of CAM.[31] In another study, it is reported that being female, young, living in the city, and having a high social status increases the use CAM.[32] In the study conducted in Turkey, it is reported that female use CAM more.[33] The authors attributed the preference for more CAM use by female, those living in the city center and those with high social status, to easier access to information and CAM. In this study, it is thought that female prefer the use of CAM more for similar reasons. Another finding in this study is that people with low education levels have more problems and high-income levels have less sleep problems. Similar to these results, it was reported that female with low education levels experienced more sleep problems in a study conducted with patients with breast cancer.[34] However, there is a study stating that those with higher education and high-income levels have more sleep problems.[35] On the other hand, contrary to this study, it is reported that low-income level negatively affects sleep quality.[36] The reason for this suggests that cancer patients with high-income levels cope with their problems more easily, and therefore their sleep quality is higher. In the literature, it is reported that having a high-income level is a factor that increases coping and quality of life in cancer patients.[37]

Study limitations

This study has few limitations. The important one is that the acquired data were obtained with scales. In qualitative research, it may be possible to obtain detailed information about the positive attitudes of patients about CAM usage and the underlying causes of sleep problems. Another limitation is that the data collected from three different oncology-haematology clinics in Istanbul, Turkey. This makes difficult to generalize the result to Turkey. Nevertheless, it was considered that information about general population can be provided with this study data since Istanbul is the most populous city in Turkey.

Conclusion

In conclusion, cancer patients experience sleep problems at every stage of their illness. However, advanced cancer patients are particularly affected by the sleep problems. In addition, among sleep problems, quality of sleep is affected most.

Nurses' awareness of the decrease in sleep quality of cancer patients will enable them to focus on the solution to these problems. Nursing practices aimed to increase sleep quality from the early stages of cancer may enable patients to experience fewer problems in the later stages.

In future studies, it may be recommended that researchers do it in a larger sample and multicentre. In addition, it is thought that investigating other factors affecting the use of CAM in sleep problems in cancer patients will provide a different perspective.

Acknowledgements

The author thanks to participants for their contributions.

Declarations

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Author contributions

Conceptualization, D.B., B.D.D. and D.Y.; Methodology, D.B., B.D.D. and D.Y.; Software, D.B., B.D.D.; Validation, D.B., B.D.D.; Formal Analysis, D.B.; Investigation, D.B., B.D.D.; Resources, D.B., B.D.D. and D.Y.; Data Curation, D.B.; Writing Original Draft Preparation, D.B., B.D.D. and D.Y.; Writing-Review & Editing, D.B., B.D.D. and D.Y.; Visualization, D.B., B.D.D. and D.Y.; Supervision, D.B., B.D.D.; Project Administration, D.B., B.D.D.

Conflict of interest

All authors declare that they have no conflicts of interest.

Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval

Written consents were obtained from the participants. During the study, the Helsinki Declaration was adhered to. Ethics committee approval was obtained from the Haliç University Non-Interventional Ethics Committee on (2020/decision number: 06) for this study.

References

1. National Center for Complementary and Integrative Health. https://nccih.nih.gov/health/integrative-health. Accessed 18 May, 2023.
2. Bonacchi A, Toccafondi A, Mambrini A, et al. Complementary. needs behind complementary therapies in cancer patients. *Psychooncology* 2015;24(9):1124-1130. doi: 10.1002/pon.3773
3. Duong N, Davis H, Robinson PD, et al. Mind and body practices for fatigue reduction in patients with cancer and hematopoietic stem cell transplant recipients: a systematic review and meta-analysis. *Crit Rev Oncol Hematol.* 2017;120:210-216. doi: 10.1016/j.critrevonc.2017.11.011
4. Lee SH, Kim JY, Yeo S, Kim SH, Lim S. Meta-analysis of massage therapy on cancer pain. *Integr Cancer Ther.* 2015;14:4:297-304. doi:10.1177/1534735415572885
5. Kreutz C, Schmidt ME, Steindorff K. Effects of physical and mind–body exercise on sleep problems during and after breast cancer treatment: a systematic review and meta-analysis. *Breast Cancer Res Treat.* 2019;176(1):1-15. doi: 10.1007/s10549-019-05217-9
6. Chien TJ, Hsu CH, Liu CY, Fang CJ. Effect of acupuncture on hot flush and menopause symptoms in breast cancer-A systematic review and meta-analysis. *PLoS One.* 2017;12(8):e0180918. doi: 10.1371/journal.pone.0180918
7. Tao W, Luo X, Cui B, et al. Practice of traditional Chinese medicine for psycho-behavioral intervention improves quality of life in cancer patients: a systematic review and meta-analysis. *Oncotarget.* 2015;6(37):39725. doi: 10.18632/oncotarget.5388
8. Kav S, Hanoğlu Z, Algier L. Use of complementary and alternative medicine by cancer patients in Turkey: A literature review. *International Journal of Hematology and Oncology* 2008;28:4:032-038.
9. Saldana, Sandra B. Perceptions of Complementary and Alternative Medicine in Western Society: A Focus Study on Switzerland. *Independent Study Project (ISP) Collection.* 2810. https://digitalcollections.sit.edu/isp_collection/2810. Accessed 18 May, 2023.
10. Salamonsen A, Wiesener S. "Then I went to a hospital abroad": acknowledging implications of stakeholders 'differing risk understandings related. to use of complementary and alternative medicine in European health care contexts. *BMC Complement Altern Med.* 2019;19(1):93. doi: 10.1186/s12906-019-2499-3
11. Santoso AM, Jansen F, de Vries R, et al. Prevalence of sleep disturbances. among head and neck cancer patients: A systematic review and meta-analysis. *Sleep Medicine Reviews.* 2019;47:62-73. doi: 10.1016/j.smrv.2019.06.003
12. Hoang HTX, Molassiotis A, Chan CW, et al. New-onset insomnia among cancer. patients undergoing chemotherapy: prevalence, risk factors, and its correlation with other symptoms. *Sleep and Breath.* 2020;24(1):241-251. doi: 10.1007/s11325-019-01839-x
13. Zhao C, Grubbs A, Barber EL. Sleep and gynecological cancer outcomes: opportunities to improve quality of life and survival. *Int J Gynecol Cancer.* 2022;32(5):669-675. doi: 10.1136/ijgc-2022-003404
14. Ozturk R, Satir DG, Sevil U. Use of complementary and alternative medicine and attitudes in patients with gynecological cancers. *Gaziantep Medical Journal.* 2016;22:3:141-147. doi: 10.5152/EurJTher.2016.006
15. Yildirim Y, Tinar S, Yorgun S, et al. The use of complementary and alternative medicine (CAM) therapies by Turkish women with gynecological cancer. *Eur J Gynaecol Oncol.* 2006;27(1):81-85.
16. Algier LA, Hanoglu Z, Özden G, Kara F. The use complementary and alternative (non-conventional) medicine in cancer patients in Turkey. *Eur J Oncol Nurs.* 2005;9(2):138-146. doi: 10.1016/j.ejon.2005.03.010
17. Ozlu ZK, Ozer N. Richard-Campbell Sleep Questionnaire validity and reliability study. *Journal of Turkish Sleep Medicine.* 2015;2:29-32. doi: 10.4274/jtsm.02.008
18. Erci B. Attitudes towards holistic complementary and alternative medicine: a sample of healthy people in Turkey. *J Clin Nurs.* 2007;16:4:761-768. doi: 10.1111/j.1365-2702.2006.01655.x
19. Fitzmaurice C, Dicker D, Pain A, et al. Global burden of disease cancer collaboration. The global burden of cancer 2013. JAMA Oncology. 2015;1:4:505-527. doi: 10.1001/jamaoncol.2015.0735
20. Chhabra N, Kennedy J. A review of cancer immunotherapy toxicity: immune checkpoint inhibitors. *Journal of Medical Toxicology.* 2021;17(4):411-424. doi: 10.1007/s13181-021-00833-8
21. Vliegenthart R, Fouras A, Jacobs C, Papanikolaou N. Innovations in thoracic imaging: CT, radiomics, AI and x-ray velocimetry. *Respirology* 2022;27(10):818-833. doi: 10.1111/resp.14344
22. The process of combating tobacco in Turkey. https://havanikoru.saglik.gov.tr/surec.html. Accessed May 20, 2023.
23. Strollo SE, Fallon EA, Gapstur SM, Tenbroeck GS. Cancer-related problem, sleep quality, and sleep disturbance among long-term cancer survivors at 9-years post diagnosis. *Sleep Medicine.* 2020;65:177-185. doi:10.1016/j.sleep.2019.10.008
24. Imanian M, Imanian M, Karimyar M. Sleep quality. and fatigue among breast cancer patients undergoing chemotherapy. *Int J Hematol Oncol Stem Cell Res.* 2019;13(4):196-200.
25. Chen Q, Terhost L, Lowery-Allison A, et al. Sleep problems. in advanced cancer patients and their caregivers:

Who is disturbing whom? *J Behav Med.* 2020;43(4):614-622. doi: 10.1007/s10865-019-00088-3

26. Currow DC, Davis W, Connolly A, et al. Sleeping-related. distress in a palliative care population: A national, prospective, consecutive cohort. *Palliative Medicine*. 2021;35:9:1663-1670. doi: 10.1177/0269216321998558.
27. National Center for Complementary and Integrative Health. The use of complementary and alternative medicine in the United States. https://www.nccih.nih.gov/research/statistics/nhis/2017 Accessed April 26, 2023.
28. Ng JY, Parakh ND. A systematic review and quality assessment of complementary and alternative medicine recommendations in insomnia clinical practice guidelines. *BMC Complement Med Ther.* 2021;21(1):1-11. doi: 10.1186/s12906-021-03223-3
29. Akeeb AA, King SM, Olaku O, White JD. Communication between cancer patients and physicians about complementary and alternative medicine: A systematic review. *J Altern Complement Med.* 2023;29(2):80-98. doi: 10.1089/jicm.2022.0516
30. Gansler T, Kaw C, Crammer C, Smith T. A population-based study of prevalence of complementary methods use by cancer survivors: A report from the American Cancer Society's studies of cancer survivors. *Cancer.* 2008;113(5):1048-1057. doi:10.1002/cncr.23659
31. Molassiotis A, Ortega P, Put D, Ozden G, et al. Use of complementary and alternative medicine in cancer patients: a European survey. *Ann Oncol.* 2005;16(4):655-663. doi:10.1093/annonc/mdi110
32. Pedersen CG, Christensen S, Jensen AB, Zachariae R. Prevalence, socio-demographic and clinical predictors of post-diagnostic utilisation of different types of complementary and alternative medicine (CAM) in a nationwide cohort of Danish women treated for primary breast cancer. *Eur J Cancer.* 2009;45(18):3172-3181. doi: 10.1016/j.ejca.2009.09.005
33. Ustundağ S, Demir Zencirci A. Complementary and alternative medicine use among cancer patients and determination of affecting factors A questionnaire study. *Holist Nurs Practice* 2015;29(6):357-369. doi: 10.1097/HNP.0000000000000113
34. Colagiuri B, Christensen S, Jensen AB, et al. Prevalence and predictors of sleep difficulty in a national cohort of women with primary breast cancer three to four months post-surgery. *J Pain Symptom Manag.* 2011;42(5):710-720. doi: 10.1016/j.jpainsymman.2011.02.012
35. Zhou ES, Clark K, Recklitis CJ, et al. Sleepless from the Get-go: Sleep problems prior to initiating cancer treatment. *Int J Behav Med.* 2018;25:502-516. doi: 10.1007/s12529-018-9715-2
36. Ratcliff CG, Zepeda SG, Hall MH, et al. Patient characteristics associated with sleep disturbance in breast cancer survivors. *Support Care Cancer.* 2021;29:2601-2611. doi: 10.1007/s00520-020-05777-3
37. Andrykowski MA, Aarts MJ, van de Poll-Franse LV, Mols F, Slooter GD, Thong MS. Low socioeconomic status and mental health outcomes in colorectal cancer survivors: disadvantage? advantage?... or both? *Psychooncology* .2013;22(11):2462-2469. doi: 10.1002/pon.3309

Wydawnictwo UR 2023
ISSN 2544-1361 (online)
doi: 10.15584/ejcem.2023.3.22

http://www.ejcem.ur.edu.pl
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2023; 21 (3): 553–560

ORIGINAL PAPER

Evaluation of the relationship between job satisfaction and professional behaviors in pediatric nurses

Fatma Dinç [1], Dilek Yıldız [2]

[1] Department of Nursing, Faculty of Health Sciences, Bartın University, Bartın, Turkey
[2] Department of Pediatric Nursing, Gulhane Faculty of Nursing, University of Health Sciences, Ankara, Turkey

ABSTRACT

Introduction and aim. Pediatric nurses play an important role in ensuring quality healthcare through job satisfaction and professional behaviors. This study aims to examine the relationship between job satisfaction and professional behaviors in pediatric nurses.

Material and methods. This was a descriptive and cross-sectional study.The sample of the study consisted of 244 pediatric nurses who worked in tertiary university hospitals in Turkey and agreed to participate in the study. Data were obtained through face-to-face interviews using a personal information form, the job satisfaction scale for nurses (JSSN), and the behavioral inventory form for professionalism in nursing (BIPN). Data were analyzed using the SPSS 22.0 software and evaluated using descriptive statistics, independent t-tests, one-way ANOVA, Pearson correlation, and simple linear regression analysis.

Results. The nurses' JSSN and BIPN means scores were 3.42±0.59 and 5.69±4.05, respectively. A statistically significant moderate positive relationship was found between the pediatric nurses' professional behaviors and job satisfaction (R=0.342; p<0.001). The regression analysis revealed that professional behaviors significantly and positively influenced job satisfaction in pediatric nurses (β=0.050; p<0.001).

Conclusion. This study found that pediatric nurses had a moderate level of job satisfaction and a low level of professional behaviors, and as their professional behavior increased, their job satisfaction also increased.

Keywords. job satisfaction, nursing professionalism, pediatric nurses

Introduction

According to the State of the World's Nursing 2020 report by the World Health Organization (WHO), nurses are the largest professional group in the healthcare sector worldwide, numbering 27.9 million.[1] However, nursing shortages continue to be a significant problem in global health services. The WHO estimates that an additional nine million nurses will be needed worldwide by 2030.[2]

Pediatric nurses work with children who have different biopsychosocial characteristics and are unable to fully express themselves verbally and cognitively.[3] Job satisfaction is defined as positive emotions and expectations from work resulting from one' evaluation of their job and influences one's intention to leave their job.[4] Job satisfaction in health professionals has a significant impact on patient care and treatment.[5] Job satisfaction in pediatric nurses also affects the quality of care they provide to children.[6,7] Therefore, it is important to examine job satisfaction and related factors in pediatric nurses.

Professionalism is considered a process associated with acquiring professional knowledge, skills, values, norms, and behaviors, and fulfilling both roles and responsibilities of a specific profession.[8] Professionalism is necessary and important for establishing nursing stan-

Corresponding author: Fatma Dinç, e-mail: fatmadinccocuk@hotmail.com

Received: 30.05.2023 / Revised: 26.06.2023 / Accepted: 27.06.2023 / Published: 30.09.2023

Dinç F, Yıldız D. *Evaluation of the relationship between job satisfaction and professional behaviors in pediatric nurses.* Eur J Clin Exp Med. 2023;21(3):553–560. doi: 10.15584/ejcem.2023.3.22.

dards and providing quality services.[9] Nowadays, it is not enough for nurses to have experience; they should also have high compatible professional behaviors in line with the changes and developments in the healthcare sector.[10] To foster the development of nursing profession, there is a need for professional nurses who embrace professional identity, consider ethical values, have a high level of autonomy and problem-solving skills, follow scientific advancements and publications, and engage in continuous development.[11,12] The Wheel of Professionalism in Nursing Model, developed by Miller provides a framework for understanding professional behaviors among nurses.[13] Professional behaviors are important for pediatric nurses in elevating the significance and values of nursing profession and establishing a framework for providing quality and ethical care.[14,15]

Recent studies have suggested a relationship between nurses' job satisfaction and several concepts such as professional behaviors and professional values. However, evidence-based research is needed to further elaborate this relationship.[15,16] This study aims to provide comprehensive data in this field and lay the groundwork for future research. Various practices can be implemented to enhance job satisfaction among pediatric nurses, and one of them is increasing their professional behaviors.[11,14] Increased professional behavior can enhance the knowledge, skills, autonomy, and empowerment of pediatric nurses, which in turn can lead to job satisfaction.[17] When nurses are highly committed to their profession, they can derive more satisfaction from their work.[18] Therefore, higher levels of professional behaviors are considered important to retain nurses in the profession.[11,14] There are only a few studies in the literature that examine nurses' job satisfaction and professional behaviors. Most of these studies have focused on determining the job satisfaction and professional behaviors of nurses working outside pediatric clinics.[10,11,17,18] Job satisfaction among pediatric nurses has been examined in relation to concepts such as burnout and compassion fatigue.[15,16,19-22] However, to the best of our knowledge, there is no study specifically on the impact of professional behaviors on job satisfaction among pediatric nurses. Therefore, this study aims to determine the relationship between job satisfaction and professional behaviors among pediatric nurses in Turkey. The results of this study are considered to reveal an important factor that increases job satisfaction among pediatric nurses. This, in turn, will shed light on healthcare institutions, professional organizations, research centers, and all other units related to nursing, providing guidance for planned projects in the field.

Aim

This study was conducted to determine the relationship between job satisfaction and professional behaviors in pediatric nurses.

Research questions

- What are the job satisfaction scores of pediatric nurses according to their professional characteristics?
- What are the professional behavior scores of pediatric nurses according to their professional characteristics?
- Is there a relationship between the job satisfaction and professional behavior scores of pediatric nurses?

Material and methods

Ethical approval

For conducting the study, an ethical approval was obtained from the non-interventional clinical research ethics committee of a training and research hospital (decision no: 18/10, decision date: 16.01.2018), and institutional permissions were obtained from the hospitals where the study was conducted (Number: E-77597247-604.02/E-50687469-799/E-98206329/770). The study was conducted in line with the Declaration of Helsinki, and the nurses were informed about the purpose and content of the study. They were also explained that their data would be used only for scientific purposes, and then their written consent was obtained.

Design and sample

This descriptive correlational study was conducted with pediatric nurses working in three tertiary university hospitals in İzmir and Ankara provinces in Turkey, which provide services in accordance with the Quality Standards in Health. The total sample in the three hospitals consisted of 320 pediatric nurses. Therefore, no sampling method was used. Twenty nurses who were on maternity leave, on sick leave, on annual leave, etc., and 56 nurses who did not agree to participate in the study were not included in the sample. A total of 244 nurses who agreed to participate in the study were included in the study. The participation rate in the study was 76.25%. The power of the study was calculated using the "G. Power-3.1.9.2" program with α=0.05. Based on the analysis conducted on 244 participants, the effect size was found to be 0.342, and the post-hoc power of the study was calculated as 0.99. The minimum power value required for post-hoc analysis is 0.67. Therefore, the achieved power level is acceptable, and the data size is sufficient.[23]

Instruments

The data were obtained through face-to-face interviews using a personal information form, the Job satisfaction scale for nurses (JSSN), and the behavioral inventory form for professionalism in nursing (BIPN).

Personal information form

This form consists of 10 questions regarding the pediatric nurses' demographic and professional character-

istics, including gender, education and marital status, nursing experience, motivation for choosing nursing, working clinic, pediatric nursing experience, type of work shift, participation in scientific activities, and membership in professional organizations.

Job satisfaction scale for nurses (JSSN)
This scale was developed by Muya et al. in Japan. It consists of 27 items and four subscales, including "Positive emotions toward work", "Appropriate support from superiors", "Perceived significance in the workplace", and "Pleasant working environment".[24] Its Turkish validity and reliability study was conducted by Türe Yıldırım and Yılmaz.[25] This is a 5-point Likert type scale, scoring from "1=strongly disagree" to "5=strongly agree." The 6th and 20th items in the scale are reverse scored. There is no cut-off point for the scale. A higher scale score indicates greater job satisfaction. The internal consistency of the scale cronbach's alpha was reported as 0.90. In this study, the cronbach's alpha value for the scale was determined as 0.91.

Behavioral inventory form for professionalism in nursing (BIPN)
This scale was developed by Miller et al. and its Turkish validity and reliability study was conducted by Karadağ et al.[26,27] It consists of a 7-item demographic information form and two sections with 39 items, measuring professional behaviors across nine subscales. The subscales include educational preparation, publication, research, participation in a professional organization, community service, competence and continuing education, code for nurses, theory, autonomy. Each subscale has a total score of 3, and the total possible weighted score on the scale is 27. A higher scale score indicates greater level of professional behaviors. The Cronbach's alpha value of the scale is 0.78-0.87, and the test-retest reliability coefficient is 0.86. In this study, the Cronbach's alpha value for the scale was determined as 0.80.

Data collection
The study was conducted between February 1, 2018, and April 29, 2018, at three university hospitals located in Ankara and Izmir provinces of Turkey. These hospitals were chosen because they provide specialized inpatient treatment services to patients with various health problems in the field of pediatrics, allowing access to a wide population of nurses specializing in pediatrics. During data collection, the unit head nurses were initially informed about the study, followed by the full-time nurses who agreed to participate in the study. It was emphasized that the participation was voluntary. Both surveys and informed consent forms were provided to pediatric nurses in sealed envelopes. The data collection forms did not include the names of the nurses. Filling out the data collection tools lasted around 10 minutes. The participants left the completed surveys at the designated drop-off locations in each unit, and the authors returned to the study site on the same day to collect the completed surveys.

Evaluation of data
The data were analyzed by using the Statistical Package for Social 22.0 program (SPSS, IBM, Armonk, NY, USA) and evaluated using descriptive statistics such as frequency and percentage. The skewness and kurtosis statistics were utilized to test whether the data were normally distributed.[28] The student's t test was used to compare the means between two groups, and ANOVA to compare the means among three or more groups. Post-Hoc analyses were performed where appropriate using Bonferroni correction. Pearson's correlation analysis was used to determine the relationship between job satisfaction and professional behaviors of pediatric nurses. Simple linear regression analysis was conducted to examine the effect of professional behaviors on job satisfaction. The level of significance was accepted as $p<0.05$ in all analyzes.

Results

The characteristics of pediatric nurses
Of the pediatric nurses, 91% were female, 67.6% were married, 75.4% had a bachelor's degree, 59.0% had over 5 years of experience in the nursing profession, 50.4% chose the nursing profession due to job opportunities, 28.7% worked in general pediatric departments, 57.8% had 5 years or more of nursing experience in pediatric clinics, 61.1% worked in rotating day and night shifts, 91.0% did not participate in any scientific nursing activities, and 93.4% were not members of a nursing association (Table 1).

Job satisfaction scale for nurses and behavioral inventory form for professionalism in nursing scores
The pediatric nurses' JSSN and BIPN mean scores were 3.42±0.59 and 5.69±4.05, respectively (Table 2).

Comparison of the pediatric nurses' JSSN and BIPN scores according to their professional characteristics
A statistically significant difference was found between the pediatric nurses' JSSN mean scores according to work experience in pediatric units, willingness to choose nursing profession, working department, and shift type ($p<0.05$). An advanced analysis was conducted to determine which groups the difference originated from. Accordingly, the pediatric nurses with more than 5 years of work experience had significantly higher JSSN mean score than those with 0–2 years and 2–5 years of experience ($p=0.002$). In addition, the pediatric nurses who willingly chose the nursing profession had significantly

Table 1. Demographic and professional characteristics of the pediatric nurses (n=244)

Variables	Group	n (%)
Gender	Female	222 (91)
	Male	22 (9)
Marital Status	Married	165 (67.6)
	Single	79 (32.4)
Educational level	Associate degree	42 (17.2)
	Bachelor degree	184 (75.4)
	Master degree	18 (7.4)
Working experience (year)	0–5	144 (59)
	6–10	60 (24.6)
	11–15	19 (7.8)
	16–20	8 (3.3)
	21 or more	13 (5.3)
Willingness to choose nursing profession	Willingly	75 (30.7)
	At my parent's request	46 (18.9)
	Due to job opportunities	123 (50.4)
Department	General child clinic	70 (28.7)
	Pediatric hematology oncology clinic	24 (9.8)
	Pediatric intensive care unit	36 (14.8)
	Outpatient clinic	6 (2.5)
	Pediatric emergency	27 (11.1)
	Pediatric surgery	22 (9.0)
	Neonatal intensive care unit	59 (24.1)
Working experience in pediatric units (year)	0–2	57 (23.4)
	3–5	46 (18.8)
	5 or more	141 (57.8)
Shift type	Day	88 (36.1)
	Day/Night Rotation	149 (61.1)
	Night	7 (2.8)
Attended the scientific conference	Yes	22 (9)
	No	222 (91)
Member of a professional association	Yes	16 (6.6)
	No	228 (93.4)
Total		244

Table 2. JSSN and BIPN scores*

JSSN and subscales	Mean±SD	min–max
Positive emotions toward work	3.58±0.76	1.5–5
Appropriate support from superiors	3.3±1	1–5
Perceived significance in the workplace	3.91±0.6	1.6–5
Pleasant working environment	2.52±0.83	1–5
Total mean score	3.42±0.59	1.3–4.9
BIPN and subscales		
Educational preparation	0.93±0.69	0–3
Publication	0.18±0.52	0–2
Research	0.47±0.84	0–3
Participation in a professional organization	0.36±0.5	0–2.5
Community service	0.35±0.9	0–3
Competence and continuing education	1.27±0.69	0.5–3
Code for nurses	0.19±0.39	0–1
Theory	1.67±1.09	0–3
Autonomy	0.23±0.48	0–2
Total mean score	5.69±4.05	0.5–19.5

* JSSN – job satisfaction scale for nurses; BIPN – behavioral inventory form for professionalism in nursing; SD – standard deviation

higher JSSN mean score than those who chose the profession due to their family's request or job opportunities ($p<0.001$). Furthermore, the pediatric nurses from pediatric intensive care and pediatric emergency departments had significantly higher JSSN mean scores than those from general pediatric departments ($p<0.001$). The pediatric nurses working pediatric intensive care, pediatric emergency, and neonatal units hid significantly higher JSSN mean scores than those working in pediatric surgical departments ($p<0.001$). Finally, the pediatric nurses who worked in day shifts had significantly higher JSSN mean score than those who worked in rotating or night shifts ($p=0.016$) (Table 3).

A statistically significant difference was also found between the pediatric nurses' BIPN mean scores according to department and shift type ($p<0.05$). The pediatric nurses from the pediatric intensive care, pediatric surgery and neonatal units had significantly higher BIPN mean scores than those from the pediatric emergency unit ($p=0.003$). In addition, the pediatric nurses who worked in day shifts had significantly higher BIPN mean score than those who worked in rotating or night shifts ($p=0.034$) (Table 3).

Table 3. Comparison of the nurses' job satisfaction and professional behaviors by professional characteristics

Variables	Group	JSSN			BIPN		
		Mean	SD	F test	Mean	SD	F test
Working experience in pediatric units (year)	0–2 (1)	3.27	0.72	0.002*	5.03	3.14	0.189
	3–5 (2)	3.27	0.53	3>1, 3>2	5.30	2.85	
	5 or more	3.54	0.53		6.09	4.64	
Willingness to choose nursing profession	Willingly (1)	3.67	0.64	<0.001**	5.55	3.45	0.445
	At my parent's request (2)	3.34	0.52	1>2, 1>3	6.38	4.02	
	Due to job opportunities (3)	3.31	0.54		5.53	4.39	
Department	General child clinic (1)	3.26	0.62	<0.001**	5.64	4.25	0.003*
	Pediatric hematology oncology clinic (2)	3.49	0.56	3>1, 5>1,	4.71	2.51	3>5, 6>5,
	Pediatric intensive care unit (3)	3.70	0.37	3>6, 5>6,	6.76	4.21	7>5
	Outpatient clinic (4)	3.64	0.61	7>6	7.33	2.16	
	Pediatric emergency (5)	3.65	0.53		2.98	1.58	
	Pediatric surgery (6)	3.01	0.66		6.80	3.88	
	Neonatal intensive care unit (7)	3.45	0.55		6.17	4.64	
Type of working shift	Day (1)	3.57	0.58	0.016*	6.57	4.47	0.034*
	Day/Night Rotation (2)	3.34	0.60	1>2	5.16	3.78	1>2
	Night (3)	3.31	0.25		5.93	1.79	

* JSSN – job satisfaction scale for nurses; BIPN – behavioral inventory form for professionalism in nursing; SD – standard deviation; Independ – F: One-Way ANOVA

The relationship between job satisfaction and professional behaviors of pediatric nurses

There was a moderately positive statistically significant relationship between the pediatric nurses' professional behaviors and job satisfaction (r=0.342; p<0.001) (Table 4).

Table 4. The relationship between behavioral inventory form for professionalism in nursing and job satisfaction scale for nurses (n=244)*

		JSSN
Behavioral Inventory Form for Professionalism in Nursing	R	0.342
	p	<0.001

* SD – standard deviation; R – correlation coefficient; p – Sig.2 tailed-values

Examining the effect of professional behaviors on job satisfaction in pediatric nurses by simple linear regression

Table 5 present the results of the regression analysis performed to determine whether professional behaviors of pediatric nurses affect their job satisfaction. Accordingly, the regression model was statistically significant (F=32.107; p<0.001), and revealed that professional behaviors of pediatric nurses positively and significantly affected their job satisfaction (β=0.050, p<0.001), where professional behaviors explained 11.7% of the variance in job satisfaction (R^2=0.117).

Table 5. The results of regression analysis between behavioral inventory form for professionalism in nursing and job satisfaction scale for nurses (n=244)

Independent Variables	B	SE	β	t	p	%95 CI LL	%95 CI UL	VIF
Constant	3.138	0.062		50.876	<0.001	3.017	3.260	
BIPN	0.050	0.009	0.342	5.666	<0.001	0.003	0.067	1.000
F=32.107, p=0.000, R^2=0.117, Durbin Watson: 1.830								

B – unstandardized beta coefficients; SE – standard Error; β – standardized beta coefficients; CI – confidence interval; LL – lower limit; VIF – variance inflation factor; Independent variable: BIPN – behavioral inventory form for professionalism in nursing; Dependent variable: JSSN – job satisfaction scale for nurses

Discussion

This study examined the relationship between job satisfaction and professional behaviors in pediatric nurses.

Job satisfaction scale for nurses and behavioral inventory form for professionalism in nursing scores

This study found that the pediatric nurses had moderate level of job satisfaction. Several studies have also reported moderate or above-average levels of job satisfaction for pediatric nurses scores.[2,19,21,29-32] The result of this study was consistent with those in previous studies. Furthermore, this result is significant as it suggests that job satisfaction of nurses working in pediatric care clinics will reflect in their caregiving practices.

This study determined that the pediatric nurses had low level of professional behaviors. Several studies have also reported low levels of professional behavior for nurses.[33-37] However, a study reported moderate levels of professional behavior for nurses in Poland and Belarus.[38] Higher levels of professionalism are important for nurses to enhance their autonomy, empowerment, recognition, establishment of nursing care standards, and provision of quality services.[9,39] This result of our study may be because pediatric nurses have low participation in scientific activities and are not members of nursing associations.

Comparison of the pediatric nurses' JSSN and BIPN scores according to their professional characteristics

In this study, the pediatric nurses who had worked in pediatric units for more than 5 years had significantly higher job satisfaction scores. It is expected that employees who have worked in a workplace for a long time generally have a better understanding of the workplace, a harmony between their experiences and expectations, the ability to make quick decisions in times of crisis, and improved professional problem-solving skills, leading to higher levels of job satisfaction.[40] Studies of pediatric nurses have also found a positive relationship between the duration of professional experience and job satisfaction.[7,22]

In our study, the pediatric nurses who willingly chose the nursing profession had significantly higher job satisfaction scores. Studies emphasize that choosing nursing as a profession due to family preferences or job security reasons can negatively affect job satisfaction.[22,41] Nurses who do not only consider nursing as an economic necessity but also have a motivation to improve their profession and contribute to society can nurture their personal feelings of achievement. Güdücü Tüfekçi et al. found higher job satisfaction among pediatric nurses who chose nursing willingly.[29] This may be due to the multidimensional care provided by pediatric nurses who care for children with different biological, psychological, and social characteristics from birth to adolescence, which can enhance their intrinsic satisfaction.

In this study, the pediatric nurses who worked on continuous day shifts had significantly higher levels of job satisfaction professional behaviors. Studies indicate that night shifts, which involve long and exhausting working hours, can decrease job satisfaction, making nurses consider leaving the profession.[21,42-44] Two studies found that pediatric nurses working only on day shifts had higher job satisfaction compared to others.[21,22] This may be because working night shifts affects bot physiological and psychological well-being of nurses, disrupts their sleep patterns, reduces their participation in so-

cial life, and has a negative impact on patient safety due to the increased workload during long hours of night shifts.[45] Considering these factors mentioned in the literature, job satisfaction of nurses is negatively affected by working in night shifts.

This study found that the pediatric nurses working in pediatric intensive care, emergency, and neonatal intensive care units had significantly higher job satisfaction. Several studies have also found high job satisfaction among nurses working in units that employ technological applications, require rapid critical thinking in stressful situations, and provide health care services for complex patients.[46,48] This may be because nurses working in these pediatric units provide care to pediatric patients, which increases their autonomy and level of interdisciplinary communication.

This study determined that the pediatric nurses working in pediatric intensive care, pediatric surgery and neonatal units exhibited significantly higher levels of professional behavior. This may be because nurses working in specialized pediatric units in Turkey are generally certified and use guidelines for care practices based on current knowledge and skills as required by institutional policies.

The relationship between job satisfaction and professional behaviors in pediatric nurses

This study examined the relationship between job satisfaction and professional behaviors in pediatric nurses, and found a significant moderate positive relationship between their job satisfaction and professional behaviors. This result is consistent with those in the literature, suggesting that as professional behaviors of nurses increase, their job satisfaction also increase.[35,49,50] This result may be because increased professional behaviors are associated with increased nursing roles, knowledge and practical success, which positively reflects on job satisfaction.

Examining the effect of professional behaviors on job satisfaction in pediatric nurses through simple linear regression

Our study suggests that professional behaviors of pediatric nurses affect their job satisfaction. This result is consistent with those in the literature, suggesting that an increase in professional behaviors increases job satisfaction in pediatric nurse.[35,51,52] In our study, the professional behavior of pediatric nurses accounted for 11.7% of the variation in their job satisfaction.

Study limitations

As one of the limitations of the study, the results of this study cannot be generalized according to gender groups, since the majority of the participants are female.

Conclusion

This study determined that pediatric nurses had a moderate level of job satisfaction and a low level of professional behaviors. The professional characteristics of pediatric nurses influenced their job satisfaction and professional behaviors. As the professional behaviors of pediatric nurses increased, their job satisfaction also increased. Additionally, the professional behaviors of pediatric nurses had an impact on job satisfaction. Based on these results, it is recommended to conduct further studies on the factors influencing professional behaviors of pediatric nurses in order to enhance their job satisfaction, and to develop, implement and evaluate relevant strategies to increase professional behaviors and job satisfaction in pediatric nurses.

Declarations

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Author contributions

Conceptualization, F.D. and D.Y.; Methodology, F.D. and D.Y.; Software, F.D. and D.Y.; Validation, F.D. and D.Y.; Formal Analysis, F.D. and D.Y.; Investigation, F.D. and D.Y.; Resources, F.D. and D.Y.; Data Curation, F.D. and D.Y.; Writing – Original Draft Preparation, F.D. and D.Y.; Writing – Review & Editing, F.D. and D.Y.; Visualization, F.D. and D.Y.; Supervision, F.D. and D.Y.; Project Administration, F.D. and D.Y.; Funding Acquisition, F.D. and D.Y.

Conflicts of interest

The authors declare that there is no conflict of interest.

Data availability

Data available on request from the authors.

Ethics approval

An ethical approval was obtained from the non-interventional clinical research ethics committee of a training and research hospital (decision no: 18/10, decision date: 16.01.2018).

References

1. World Health Organization. State of the world's nursing report. https://www.who.int/publi cations-detail/nursing--report-2020. Accessed April 14, 2023.
2. World Health Organization. Nursing and midwifery. https://www.who.int/news-room/fact-sheets/detail/nursing-and-midwifery. Accessed April 10, 2023.
3. Alves DFS, Guirardello EB. Safety climate, emotional exhaustion and job satisfaction among Brazilian paediatric professional nurses. *Int Nurs Rev.* 2016;63(3):328-335. doi: 10.1111/inr.12276

4. Kaya A, İşler-Dalgıç A. Evaluating the job satisfaction of nurses working in pediatric clinics in terms of eleven dimensions and the influencing factors. *FN Journal of Nursing.* 2020;28(3):312-320. doi: 10.5152/FNJN.2020.19101
5. Zahednezhad H, Hoseini MA, Ebadi A, Farokhnezhad Afshar P, Ghanei Gheshlagh R. Investigating the relationship between organizational justice, job satisfaction, and intention to leave the nursing profession: A cross-sectional study. *J Adv Nurs.* 2021;77(4):1741-1750. doi: 10.1111/jan.14717
6. Adwan JZ. Pediatric nurses'grief experience, burnout and job satisfaction. *J Pediatr Nurs.* 2014;29:329-336. doi: 10.1016/j.pedn.2014.01.011
7. Roberts-Turner R, Hinds PS, Nelson J, et al. Effects of leadership characteristics on pediatric registered nurses' job satisfaction. *Pediatr Nurs.* 2014;40(5):236-241.
8. Klopper HC, Madigan E, Vlasich C, et al. Advancement of global health: Recommendations from the Global Advisory Panel on the Future of Nursing & Midwifery (GAPFON®). *J Adv Nurs*. 2020;76(2):741-748. doi: 10.1111/jan.14254
9. Cao H, Song Y, Wu Y, et al. What is nursing professionalism? A concept analysis. *BMC Nurs.* 2023;22(1):34. doi: 10.1186/s12912-022-01161-0
10. Barać I, Prlić N, Plužarić J, Farčić N, Kovačević S. The mediating role of nurses' professional commitment in the relationship between core self-evaluation and job satisfaction. *Int J Occup Med Environ Health.* 2018;31(5):649-658. doi: 10.13075/ijomeh.1896.01256
11. Tanaka M, Taketomi K, Yonemitsu Y, Kawamoto R. Professional behaviours and factors contributing to nursing professionalism among nurse managers. *J Nurs Manag.* 2016;24(1):12-20. doi: 10.1111/jonm.12264
12. Ichikawa N, Yamamoto-Mitani N, Takai Y, Tanaka M, Takemura Y. Understanding and measuring nurses' professionalism: Development and validation of the Nurses' Professionalism Inventory. *J Nurs Manag.* 2020;28(7):1607-1618. doi: 10.1111/jonm.13116
13. Miller BK. A model for professionalism in nursing. *Todays OR Nurse.* 1988;10(9):18-23.
14. Zengin M, Yayan EH, Yıldırım N, et al. Effect of professional values upon professional attitudes of pediatric nurses. *Arch Health Sci Res.* 2018;5:316-323. doi:10.17681/hsp.324725
15. Ayar D, Bektaş M, Bektaş İ, Bülbül Ö, Cesur A. The impact of professional values and job satisfaction of pediatric nursing on their intercultural sensitivity. *J Educ Res Nurs.* 2021;18(3): 323-328. doi: 10.5152/jern.2021.44522
16. Kaya A, Çelik D, İşler Dalgıç A. Professional values and job satisfaction levels of pediatric nurses and influencing factors: A cross sectional study. *J Pediatr Res.* 2020;7(1):58-63. doi: 10.4274/jpr.galenos.2019.68815
17. Castaneda GA, Scanlan JM. Job satisfaction in nursing: Aconcept analysis. *Nurs Forum*. 2014;49(2):130-138. doi: 10.1111/nuf.12056
18. Lee SE, MacPhee M, Dahinten VS. Factors related to perioperative nurse' job satisfaction and intention to leave. *Jpn J Nurs Sci*. 2020;17(1):e12263. doi: 10.1111/jjns.12263
19. Akman O, Oztürk C, Bektaş M, Ayar D, Armstrong MA. Job satisfaction and burnout among paediatric nurses. *J Nurs Manage.* 2016;24(7):923-933. doi: 10.1111/jonm.12399
20. Meyer RM, Li A, Klaristenfeld J, Gold JI. Pediatric novice nurses: examining compassion fatigue as a mediator between stress exposure and compassion satisfaction, burnout, and job satisfaction. *J Pediatr Nurs*. 2015;30(1):174-183. doi: 10.1016/j.pedn.2013.12.008
21. Torun T, Çavuşoğlu H. Examining burnout and job satisfaction of nurses working in pediatric units. *HEAD.* 2018;15(2):71-80. doi: 10.5222/HEAD.2018.071
22. Kaya A, İşler Dalgiç A. Examination of job satisfaction and burnout status of pediatric nurses: A cross-sectional and correlational study using online survey research in Turkey. *Perspect Psychiatr Care.* 2021;57:800-808. doi: 10.1111/ppc.12617
23. Cohen J. *Statistical Power Analysis for the Behavioral Sciences*. 2nd. United States of America: Lawrence Erlbaum Associates;1988:8-14.
24. Muya M, Katsuyama K, Ozaki F, Aoyama H. Development of a scale measuring the job satisfaction of Japanese hospital nurses. *Jpn J Nurs Sci.* 2014;11(3):160-170. doi: 10.1111/jjns.12017
25. Türe Yılmaz A, Yıldırım A. Validity and reliability of the nurse job satisfaction scale in Turkish. *SHYD.* 2016;3(3):158-168. doi:10.5222/SHYD.2016.158
26. Miller BK, Adams D, Beck LA. Behavioral inventory for professionalism in nursing. *J Prof Nurs*. 1993;9(5):290-295. doi: 10.1016/8755-7223(93)90055-h
27. Karadağ A, Hisar F, Elbaş NÖ. A behavioural inventory for professionalism in nursing: A sample from Turkey. *Hemşirelik Forumu*. 2004;7:14-22.
28. George D, Mallery P. *IBM SPSS Statistics 26 Step by Step: A Simple Guide and Reference*.(16th ed.). Routledge; 2019. doi: 10.4324/9780429056765
29. Güdücü Tüfekçi F, Kurudirek F, Baran G. The levels of job satisfaction and description of pediatric nurses. *Gümüşhane Üniversitesi Sağlık Bilimleri Dergisi.* 2015;4:70-76.
30. Roney LN, Acri MC. The cost of caring: An exploration of compassion fatigue, compassion satisfaction, and job satisfaction in pediatric nurses. *J Pediatr Nurs*. 2018;40:74-80. doi: 10.1016/j.pedn.2018.01.016
31. Xu S, Tao L, Huang H, Little J, Huang L. Pediatric nurses' turnover intention and its association with calling in China's tertiary hospitals. *J Pediatr Nurs.* 2020;52:e51-e56. doi: 10.1016/j.pedn.2020.01.005
32. Khatatbeh H, Al-Dwaikat T, Oláh A, et al. The relationships between paediatric nurses' social support, job satisfaction and patient adverse events. *Nurs Open.* 2021;8(6):3575-3582. doi: 10.1002/nop2.907
33. Hisar F, Karadağ A. Determining the professional behaviour of nurse executives. *Int J Nurs Pract.* 2010;16(4):335-341. doi: 10.1111/j.1440-172X.2010.01849.x
34. Kim-Godwin YS, Baek HC, Wynd CA. Factors influencing professionalism in nursing among Korean American

registered nurses. *J Prof Nurs.* 2010;26(4):242-249. doi: 10.1016/j.profnurs.2009.12.007

35. Çelik S, Hisar F. The influence of the professionalism behaviour of nurses working in health institutions on job satisfaction. *Int J Nurs Pract.* 2012;18(2):180-187. doi: 10.1111/j.1440-172X.2012.02019.x
36. Solomon Y, Beker J, Belachew T. Professionalism and its predictors among nurses working in Jimma Zone Public Hospitals, South West Ethiopia. *J Nurs Care.* 2015;5(4):292. doi: 10.4172/2167-1168.1000292
37. Dikmen Y, Karataş H, Arslan GG, Ak B. The level of professionalism of nurses working in a hospital in Turkey. *J Caring Sci.* 2016;5(2):95-102. doi:10.15171/jcs.2016.010
38. Marcinowicz L, Shpakou A, Piatrou S, et al. Behavioural categories of professionalism of nurses in Poland and Belarus: A comparative survey. *J Clin Nurs.* 2020;29(9-10):1635-1642. doi: 10.1111/jocn.15226
39. Huber TH. Nursing professionalism. *Ky Nurse.* 2015;63(1):15.
40. Kalisch BJ, Lee H, Rochman M. Nursing staff teamwork and job satisfaction. *J Nurs Manag.* 2010;18(8):938-947. doi: 10.1111/j.1365-2834.2010.01153.x
41. Sabancıoğulları S, Doğan S. Relationship between job satisfaction, Professional identy and intention to leave the profession among nurses in Turkey. *J Nurs Manag.* 2015;23(8):1076-1085. doi: 10.1111/jonm.12256
42. Dobrowolska B, Visintini C, Pokorná A, et al. Exploring the meaning of night shift placement in nursing education: a European multicentre qualitative study. *Int J Nurs Stud.* 2020;112:103687. doi: 10.1016/j.ijnurstu.2020.103687
43. Larsen AD, Ropponen A, Hansen J, et al. Working time characteristics and long-term sickness absence among Danish and Finnish nurses: A register-based study. *Int J Nurs Stud.* 2020;112:103639. doi: 10.1016/j.ijnurstu.2020.103639
44. Smith A, McDonald AD, Sasangohar F. Night-shift nurses and drowsy driving: A qualitative study. *Int J Nurs Stud.* 2020;112:103600. doi: 10.1016/j.ijnurstu.2020.103600
45. Tambağ H, Kahraman Y, Şahpolat M, Can R. The effect of nurses' work environment on job satisfaction. *Bakırkoy Medical Journal.* 2015;11(4):143-149. doi:10.5350/BTDMJB201511402
46. McDonald K, Rubarth LB, Miers LJ. Job satisfaction of neonatal intensive care nurses. *Adv Neonatal Care.* 2012;12(4):E1-8. doi: 10.1097/ANC.0b013e3182624eb1
47. Sekol MA, Kim SC. Job satisfaction, burnout, and stress among pediatric nurses in various specialty units at an acute care hospital. *J Nurs Educ and Pract* 2014;4(12):115-124. doi: 10.5430/JNEP.V4N12P115
48. Snapp B, McCutchon EW, Moore TA, Teel D. Neonatal nurse practitioner job satisfaction, workforce environment, and mental well-being. *J Am Assoc Nurse Pract.* 2022;1;34(9):1058-1065. doi: 10.1097/JXX.0000000000000754
49. Hwang JI, Lou F, Han SS, et al. Professionalism: the major factor influencing job satisfaction among Korean and Chinese nurses. *Int Nurs Rev.* 2009;56(3):313-318. doi: 10.1111/j.1466-7657.2009.00710.x
50. Kantek F, Kaya A. Professional values, job satisfaction, and ıntent to leave among nursing managers. *J Nurs Res.* 2017;25(4):319-325. doi: 10.1097/JNR.0000000000000164
51. Zhang W, Meng H, Yang S, Liu D. The influence of professional ıdentity, job satisfaction, and work engagement on turnover intention among township health inspectors in China. *Int J Environ Res Public Health.* 2018;14;15(5):988. doi: 10.3390/ijerph15050988
52. Wang C, Xu J. Zhang TC, Li QM. Effects of professional identity on turnover intention in China's hotel employees: The mediating role of employee engagement and job satisfaction. *Journal of Hospitality and Toursim Management.* 2020;45:10-22. doi: 10.1016/j.jhtm.2020.07.002

Wydawnictwo UR 2023
ISSN 2544-1361 (online)
doi: 10.15584/ejcem.2023.3.23

http://www.ejcem.ur.edu.pl
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2023; 21 (3): 561–566

ORIGINAL PAPER

Predictors of hospitalization in patients presenting to emergency department with an acute exacerbation of COPD – a single-center study in Turkey

Tuğba Sanalp Menekşe [1], Ekrem Taha Sert [2]

[1] Department of Emergency Medicine, Education and Research Hospital, Aksaray University, Aksaray, Turkey
[2] Department of Emergency Medicine, Faculty of Medicine, Aksaray University, Aksaray, Turkey

ABSTRACT

Introduction and aim. In this study, we evaluated parameters that might be associated with hospitalization in patients admitted to the emergency department (ED) with an acute exacerbation of chronic obstructive pulmonary disease (AECOPD).

Material and methods. Patients with COPD who presented to ED due to AECOPD between January 1, 2020 and December 31, 2021 were included in the study. Patient data were obtained from the hospital database. Univariable and multivariable logistic regression methods were used to identify the relationship between hospitalization and clinical parameters.

Results. The study included 237 patients divided into two groups: inpatients (n=124) and outpatients (n=113). We found significant differences between the two groups in terms of temperature, oxygen saturation, respiratory rate, C-reactive protein, white blood cell count, procalcitonin, albumin, arterial blood pH, pCO_2, and non-invasive mechanical ventilation (NIMV) requirement. Multivariable logistic regression analysis showed that body temperature [odds ratio (OR):1.62;95% confidence interval (CI):1.21–4.91; p<0.001], oxygen saturation (OR:0.73, 95% CI:0.39-0.94, p<0.001), respiratory rate (OR:1.96; 95% CI: 1.07–6.14; p<0.001), albumin (OR:0.71; 95% CI:0.41–0.93; p=0.042), procalcitonin (OR:2.93; 95% CI:1.22–4.84; p<0.001), arterial blood pH (OR:0.78; 95% CI:0.29-0.91; p=0.038), pCO_2 (OR:2.45; 95% CI:1.24–4.65; p<0.001), and NIMV requirement (OR:2.31; 95% CI:1.41–5.13; p<0.001) were the independent predictors of hospitalization.

Conclusion. Our findings may help identify patients who will require hospitalization at an early stage.

Keywords. chronic obstructive pulmonary disease, emergency department**,** hospitalization

Introduction

Chronic obstructive pulmonary disease (COPD) is a chronic inflammatory lung disease characterized by progressive, completely irreversible airflow limitation. COPD is an important cause of morbidity and mortality with its high prevalence and increasing incidence.[1] COPD is a preventable and treatable disease that presents with exacerbations.[2] Acute exacerbations negatively affect the quality of life of patients, hospitalization rates, disease progression, and mortality.[3,4]

In the Global Initiative for Chronic Obstructive Lung Disease guidelines, the acute exacerbations of COPD (AECOPD) requiring hospitalization are defined as "severe exacerbations". Mild to moderate exacerbations can usually be treated on an outpatient basis.[5] The demographic and clinical characteristics of patients play an important role in determining severe exacerbations requiring hospitalization. In addition, comorbidities, blood gas parameters, and inflammatory biomarkers are also important factors in hospitalization.[6] Airway in-

Corresponding author: Tuğba Sanalp Menekşe, e-mail: tugba.sanalp@hotmail.com

Received: 7.05.2023 / Revised: 26.06.2023 / Accepted: 30.06.2023 / Published: 30.09.2023

Menekşe TS, Sert ET. *Predictors of hospitalization in patients presenting to emergency department with an acute exacerbation of COPD – a single-center study in Turkey*. Eur J Clin Exp Med. 2023;21(3):561–566. doi: 10.15584/ejcem.2023.3.23.

flammation is frequently increased in AECOPD.[7] Various inflammatory markers such as, C-reactive protein (CRP), white blood cell count (WBC) have been shown to be increased in AECOPD.[8]

Aim

Due to the respiratory and systemic adverse effects of the disease, one of the most important goals of treating patients with COPD is the effective treatment and prevention of exacerbations. In an acute attack, it is extremely important to identify high-risk groups for hospitalization, especially in terms of exacerbation management, detection of conditions that require special treatment, and reducing treatment costs. Therefore, in this study, we aimed to examine the general characteristics of patients hospitalized for AECOPD and investigate parameters that might be associated with hospitalization.

Material and methods

Ethical approval

Approval for the study was obtained from the clinical research ethical committee of Aksaray University Faculty of Medicine (approval number: 2022/01-08).

Study design

This retrospective descriptive study was conducted in the emergency department (ED) of a single tertiary center in Turkey. Patients with a diagnosis of COPD who applied to the emergency department with cough, sputum and shortness of breath between January 1, 2020 and December 31, 2021 and required additional treatment were included in the study. Patients without a diagnosis of COPD, those whose records could not be reached, those who were transferred to another hospital for any reason without treatment, and those with malignancies, bronchial asthma, tuberculosis, and advanced liver or kidney failure were excluded from the study. The flowchart is shown in Figure 1.

The clinical data of the patients were obtained from the hospital's electronic database and by retrospectively screening the patient files. The patients' age, gender, vital signs (temperature, blood pressure, respiratory rate and oxygen saturation), comorbid diseases (coronary artery disease, diabetes mellitus and hypertension), laboratory parameters, NIMV and invasive mechanical ventilation (IMV) requirements, intensive care unit admission, and in-hospital mortality rates were recorded. The clinical diagnosis, treatment, and follow-up of the patients were provided according to international standard guidelines.[5]Acute respiratory failure, sudden worsening of dyspnea at rest, high respiratory rate, severe symptoms such as decreased oxygen saturation, confusion, drowsiness, onset of new physical symptoms (e.g. cyanosis, peripheral edema), failure to respond to medical therapy, presence of serious comorbidities, inadequate home support was determined as the criteria for hospitalization. Discharge criteria were defined as mild to moderate AECOPD that improved with treatment according to the guideline.[5] NIMV (Non-invasive mechanical ventilation) indications included respiratory acidosis ($pCO_2 \geq 45$ mmHg and arterial pH≤7.35), severe dyspnea with use of accessory respiratory muscles, and persistent hypoxemia despite supplemental oxygen therapy. IMV indications included status post-respiratory or cardiac arrest, diminished consciousness, psychomotor agitation inadequately controlled by sedation, massive aspiration or persistent vomiting, persistent inability to remove respiratory secretions, severe hemodynamic instability without response to fluids and vasoactive drugs, severe ventricular or supraventricular arrhythmias, life-threatening hypoxemia in patients unable to tolerate NIMV.[5] The effects of the patients' clinical parameters on hospitalization were evaluated.

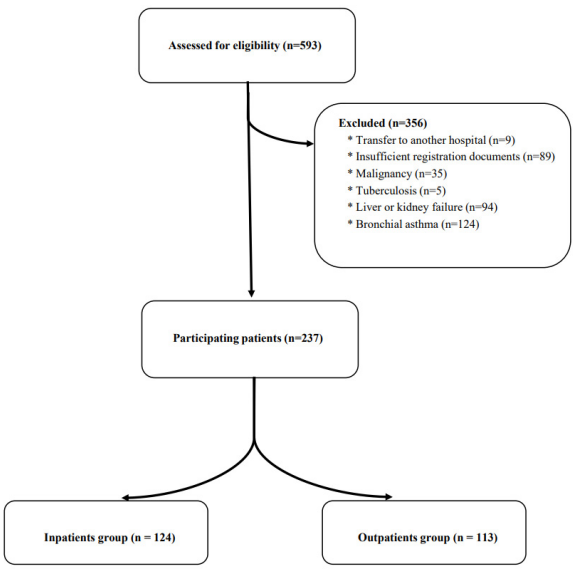

Fig. 1. Recruitment process flowchart

Statistical analysis

SPSS v. 22.0 (SPSS Inc, Chicago, IL, USA) was used for the statistical analysis of the data obtained from the study. Numerical variables were expressed as mean ± standard deviation or median (25–75% quartiles) and qualitative variables as numbers and percentages. The conformity of the results to the normal distribution was checked with the Kolmogorov-Smirnov test. The chi-square test was used to determine whether there was a difference between the groups in terms of qualitative variables. Student's t-test was used to compare normally distributed data between the two groups, and Mann-Whitney U test was used to compare non-normally distributed data. The univariable and multivariable logistic regression analyses were undertaken to determine the relationship between hospitalization and possible clinical variables (Body temperature, oxygen saturation, respiratory rate, white blood cell, albumin, procalcitonin, C-reactive protein, arterial blood pH,

pCO_2, NIMV requirement). The multivariable logistic regression analysis was applied to the variables with a p value of <0.05 in the univariable logistic regression analysis. p<0.05 was considered statistically significant.

Results

During the study, 593 patients were evaluated for eligibility. 356 patients were excluded from the study. A total of 237 patients who met the criteria were included in the study. The patients were divided into two groups: inpatients (n=124) and outpatients (n=113). The mean age of the patients was 70.7±9.2 years, 77.2% (n=183) were men, and 22.8% (n=54) were women. The most common comorbidity was hypertension (77.2%). In ED, 2.1% of the patients were treated with IMV and 11.8% with NIMV. Of all the patients, 6.8% were admitted to the intensive care unit (n=16), and the in-hospital mortality rate was 3.4% (n = 8). Table 1 shows the demographic data and clinical characteristics of the patients who presented to ED due to AECOPD.

Table 1. Demographic data and clinical characteristics of the study population (n=237)*

Variable	
Age, years, mean ± SD	70.7 ± 9.2
Male, n (%)	183 (77.2%)
Vital signs at presentation to ED	
Systolic blood pressure, mmHg, median (25th–75th percentile)	135 (126–150)
Diastolic blood pressure, mmHg, median (25th–75th percentile)	80 (80–90)
Body temperature, (°C), median (25th–75th percentile)	36.7 (36.5–36.9)
Oxygen saturation, (%), median (25th–75th percentile)	85 (75–88)
Respiratory rate, per/minute, median (25th–75th percentile)	29 (26–34)
Hypertension, n (%)	157(66.2%)
Diabetes mellitus, n (%)	38 (16.0%)
Heart disease, n (%)	95 (40.1%)
Hemoglobin, g/dL, mean ± SD	14.2±2.2
White blood cell, x10^9/L, median (25th–75th percentile)	10.2 (7.9–12.4)
Glucose, mg/dL, median (25th–75th percentile)	127 (104–155)
Creatinine, mg/dL, median (25th–75th percentile)	0.80 (0.63–1.01)
Albumin, g/dL, median (25th–75th percentile)	37.3 (34.2–40.0)
Procalcitonin, ng/mL, median (25th–75th percentile)	0.06 (0.03–0.14)
C–reactive protein, mg/dL, median (25th–75th percentile)	18 (7.3–78.4)
Lactate/albumin ratio, median (25th–75th percentile)	0.04 (0.03–0.06)
Arterial blood pH, median (25th–75th percentile)	7.34 (7.30–7.39)
pCO_2, mmHg, median (25th–75th percentile)	54 (47–66)
Arterial blood lactate, mmol/L, median (25th–75th percentile)	1.7(1.2–2.3)
Invasive MV requirement, n (%)	5 (2.1%)
Non–invasive MV requirement, n (%)	28 (11.8%)
ICU admission, n (%)	16 (6.8%)
In–hospital mortality, n (%)	8 (3.4%)

* Data are presented as mean ± standard deviation, median (25%–75% percentiles), or n (%); ED – emergency department; MV – mechanical ventilation; ICU – intensive care unit; pCO_2 – partial pressure of carbon dioxide

When the vital signs were examined, a statistically significant difference was found between the two groups in terms of respiratory rate, oxygen saturation, and body temperature (p<0.05). There were statistically significant differences in the WBC, albumin, procalcitonin, and CRP parameters between the inpatient and outpatient groups (p=0.02; p=0.011, p<0.001, and p<0.001, respectively). In addition, the arterial partial pressure of carbon dioxide (pCO_2) value was significantly higher in the inpatient group (p < 0.001). The laboratory parameters and clinical characteristics of the groups are given in Table 2.

Table 2. Comparison of the demographic and clinical characteristics between the outpatient and inpatient groups*

Variable	Inpatients (n=124)	Outpatients (n=113)	p
Age, years, mean ± SD	71.0±9.3	70.4±9.2	0.725
Male, n (%)	96 (77.4%)	87 (77.0%)	0.937
Vital signs at presentation to ED			
Systolic blood pressure, mmHg, median (25th–75th percentile)	140 (125–157)	130 (125–140)	0.168
Diastolic blood pressure, mmHg, median (25th–75th percentile)	80 (80–90)	80 (75–85)	0.170
Body temperature, °C, median (25th–75th percentile)	36.8 (36.5–37.0)	36.7 (36.5–36–8)	<0.001
Oxygen saturation, %, median (25th–75th percentile)	79 (67–85)	88 (85–90)	<0.001
Respiratory rate, per/minute, median (25th–75th percentile)	32 (28–35)	28 (25–30)	<0.001
Hypertension, n (%)	89 (71.8%)	68 (60.2%)	0.059
Diabetes mellitus, n (%)	21 (16.9%)	17 (15.0%)	0.692
Heart disease, n (%)	54 (43.5%)	41 (36.3%)	0.254
Hemoglobin, g/dL, mean ± SD	14.5 ± 2.4	13.9 ± 2.0	0.051
White blood cell, x10^9/L, median (25th–75th percentile)	10.6 (8.3–13.5)	9.8 (7.7–11.8)	0.020
Glucose, mg/dL, median (25th–75th percentile)	130 (110–165)	119 (100–151)	0.096
Creatinine, mg/dL, median (25th–75th percentile)	0.78 (0.61–0.99)	0.81 (0.66–1.02)	0.268
Albumin, g/dL, median (25th–75th percentile)	36.4 (33.4–38.9)	37.6 (35.7–41.0)	0.011
Procalcitonin, ng/mL, median (25th–75th percentile)	0.08 (0.04–0.17)	0.04 (0.02–0.09)	<0.001
C–reactive protein, mg/dL, median (25th–75th percentile)	35.6 (10.9–116.7)	11.6 (3.6–43.6)	<0.001
Lactate/albumin ratio, median (25th–75th percentile)	0.04 (0.03–0.07)	0.04 (0.03–0.05)	0.237
Arterial blood pH, median (25th–75th percentile)	7.34 (7.29–7.39)	7.36 (7.31–7.40)	0.015
pCO_2, mmHg, median (25th–75th percentile)	60.5 (49–73)	51.0 (45–58)	<0.001
Arterial blood lactate, mmol/L, median (25th–75th percentile)	1.75 (1.2–2.6)	1.70 (1.2–2.3)	0.264
Invasive MV requirement, n (%)	5 (4.0%)	0	0.038
Non–invasive MV requirement, n (%)	27 (21.8%)	1 (0.9%)	<0.001
ICU admission, n (%)	16 (12.9%)	0	<0.001
In–hospital mortality, n (%)	8 (6.5%)	0	<0.001

* Data are presented as mean ± standard deviation, median (25%–75% percentiles), or n (%); ED – emergency department; MV – mechanical ventilation; ICU – intensive care unit; pCO_2 – partial pressure of carbon dioxide

Univariable and multivariable logistic regression analyses were used on the statistically significant parameters. The multivariable logistic regression analysis revealed that body temperature [odds ratio (OR): 1.62; 95% confidence interval (CI): 1.21–4.91; p<0.001], oxygen saturation (OR: 0.73; 95% CI: 0.39–0.94; p<0.001), respiratory rate (OR: 1.96; 95% CI: 1.07–6.14; p<0.001), albumin (OR: 0.71; 95% CI: 0.41–0.93; p=0.042), procalcitonin (OR: 2.93; 95% CI: 1.22–4.84; p<0.001), arterial blood pH (OR: 0.78, 95% CI: 0.29–0.91, p=0.038), pCO2 (OR: 2.45; 95% CI: 1.24–4.65; p<0.001), and NIMV requirement (OR: 2.31; 95% CI: 1.41–5.13; p<0.001) were the independent predictors of hospitalization (Table 3).

Table 3. Predictors of hospitalization as determined by the univariable and multivariable logistic regression analyses*

Variables	Univariable logistic regression		Multivariable logistic regression	
	OR (95% CI)	p	OR (95% CI)	p
Body temperature, °C	1.41 (1.09–3.66)	<0.001	1.13 (1.03-4.77)	<0.001
Oxygen saturation, %	0.61 (0.44–0.92)	<0.001	0.73 (0.39–0.94)	<0.001
Respiratory rate	1.82 (1.13–5.17)	<0.001	1.96 (1.07–6.14)	<0.001
White blood cell, x10^9/L	1.76 (1.55–3.97)	0.023		
Albumin, g/dL	0.64 (0.43–0.89)	0.012	0.71 (0.41–0.93)	0.042
Procalcitonin, ng/mL	2.51 (1.26–3.32)	<0.001	2.93 (1.22–4.84)	<0.001
C-reactive protein	1.33 (1.15–3.25)	<0.001		
Arterial blood pH	0.71 (0.26–0.82)	0.015	0.78 (0.29–0.91)	0.038
pCO_2	2.23 (1.39–3.89)	<0.001	2.45 (1.24–4.65)	<0.001
Non-invasive MV requirement	2.01 (1.38–4.33)	<0.001	2.31 (1.41–5.13)	<0.001

* OR – odds ratio; CI – confidence interval; MV – mechanical ventilation, pCO_2 – partial pressure of carbon dioxide

Discussion

In this study, we investigated parameters that could affect the hospitalization of patients who presented to ED due to AECOPD. On completion of the study, body temperature, oxygen saturation, respiratory rate, arterial blood pH, pCO_2, albumin, procalcitonin, and NIMV requirement were identified as factors determining hospitalization.

The effect of age and gender on hospitalization remains controversial. Previous studies have showed that the rates of AECOPD and hospitalization are higher in women because they are more sensitive to external factors. The increase in women's exposure to tobacco and biomass in the last 15 years has increased the frequency of COPD. In addition, some theories have been put forward to explain the sensitivity of women to these substances. Some of these theories are estrogen effect, impaired gas exchange in the lungs and small bronchi.[9-11] Similarly, Antonela et al. showed that male gender was a higher risk factor for COPD-related morbidity and mortality.[12] In contrast, recent studies have shown no statistically significant difference between genders in terms of hospitalization rates.[13,14] There are also researchers reporting that the length of hospital stay in COPD increases as patient age increases.[15,16] In the current study, no significant difference was found between the inpatient and outpatient groups in relation to age and gender. Therefore, we consider that gender or age alone is not sufficient to determine hospitalization and discrepancies between studies are also related to regional and cultural differences.

Fever shows the severity of the clinical manifestations of the infection causing the exacerbation and supports the decision for hospitalization.[17] Similar to Lieberman et al., we found fever to be an important clinical finding for making the hospitalization decision in patients who presented to ED with AECOPD.[17] Another cardinal symptom of COPD, dyspnea, is the most common reason for referral to ED. Increasing dyspnea is an indicator of hypoxemia and causes hypercapnia.[18,19] In our study, the pCO_2 value was significantly higher in the inpatients, which is consistent with the literature. Hypercapnia is an important indicator of emerging respiratory failure and predicts hospitalization requirement.[18]

Respiratory rate can be easily measured with non-invasive methods and information about the severity of the disease can be obtained. In studies, it has been shown that respiratory rate is 18-20 per minute in patients admitted to ED with AECOPD and treated as an outpatient, while it is >24 in inpatients.[20] In another study, it was stated that respiratory rate <24 breaths per minute is mild AECOPD and outpatient treatment is sufficient.[21] In our study, Tseng et al. similar to his study, it was shown that the respiratory rate was statistically significantly higher in hospitalized patients.[22]

With increasing age, the number of COPD exacerbations and mortality rate increase as a result of the deterioration of respiratory function and the increasing incidence of comorbid diseases. Similar to our study, Raherison et al. found the most common comorbid disease to be hypertension in their cohort.[23] Infectious factors, especially increased airway inflammation are the most critical factors triggering a COPD attack. As a result, changes are observed in infectious parameters in the blood.[24] In a recent study, procalcitonin levels were shown to predict the hospitalization and prognosis of patients.[8] In another study, the authors reported a significant relationship between procalcitonin levels and disease severity and NIMV requirement in patients with AECOPD followed up in the intensive care unit.[25] CRP is a highly sensitive indicator of inflammation but cannot always be used to distinguish between bacterial and non-bacterial inflammation. In a meta-analysis that included 12 studies comparing procalcitonin and CRP levels in the diagnosis of bacterial infection in hospitalized patients, the former was found to be more useful than the latter in differentiating bacterial infection from other non-infectious causes

of systemic inflammation.[26] In addition, high procalcitonin and CRP values in COPD exacerbations have been suggested to be significant predictors of short-term (one-month) and long-term (two-year) mortality.[27] Albumin is a negative acute phase protein that decreases in inflammatory conditions, and hypoalbuminemia is associated with chronic diseases. It has been showed that albumin plays an important role in the prognosis of COPD cases accompanied by comorbidities.[28] In the present study, we found a statistically significant difference in the albumin and procalcitonin values between the inpatients and outpatients with AECOPD.

Arterial blood gas parameters are important to evaluate patients with AECOPD and determine their mechanical ventilation and hospitalization requirements. In the literature, it has been reported that the pH value in blood gas has a critical prognostic importance.[29] Respiratory acidosis in arterial blood gas and an increased pCO_2 level in AECOPD indicate the need for intensive care, NIMV, or IMV.[30] It has been observed that at the time of their presentation to the hospital, patients with COPD require mechanical ventilator therapy during exacerbation periods when respiratory acidosis becomes more evident.[31]

Study limitations

This study has certain limitations. First, due to the retrospective and single-center design of the study, the data were limited to the records that we were able to access from the hospital database. In addition, the single-center study design inherent carries risks of bias. Second, some patients had recurrent presentations during the study period, but only the data from their first visit were taken into consideration. Lastly, the measured blood parameters were obtained only at the time of the patients' presentation to ED, and changes in these biomarkers during follow-up were not examined. Despite these limitations, we consider that the results of this study are important in terms of guiding future research on this subject.

Conclusion

We determined that body temperature, oxygen saturation, respiratory rate, arterial blood pH, pCO_2, albumin, procalcitonin, and NIMV requirement were associated with hospitalization in patients with AECOPD. Our findings suggest that the hospitalization requirement is associated with parameters indicating the severity of the disease. It should be kept in mind that COPD attacks will be more severe in these patients, and therefore they may require hospitalization at any time.

Declarations

Funding

The authors declared that this study has received no financial support.

Author contributions

Conceptualization, T.S.M. and E.T.S.; Methodology, T.S.M.; Software, T.S.M.; Validation, T.S.M. and E.T.S.; Formal Analysis, E.T.S.; Investigation, T.S.M.; Resources, T.S.M..; Data Curation, T.S.M.; Writing – Original Draft Preparation, T.S.M. and E.T.S.; Writing – Review & Editing, T.S.M.; Visualization, T.S.M. and E.T.S..; Supervision, T.S.M.; Project Administration, T.S.M.; Funding Acquisition, T.S.M.

Conflicts of interest

No conflict of interest was declared by the authors.

Data availability

The datasets used and/or analyzed during the current study are open from the corresponding author on reasonable request.

Ethics approval

All subjects gave their informed consent for inclusion before they participated in the study. This study protocol was approved by Clinical Research Ethical Committee of Aksaray University Faculty of Medicine with a protocol number of 2022/01-08 and conducted in accordance with the Declaration of Helsinki and Good Clinical Practices.

References

1. Hurst JR, Han MK, Singh B, et al. Prognostic risk factors for moderate-to-severe exacerbations in patients with chronic obstructive pulmonary disease: a systematic literature review. *Respir Res.* 2022;23(1):213. doi: 10.1186/s12931-022-02123-5
2. Halpin D, Celli B, Criner G, et al. The GOLD Summit on chronic obstructive pulmonary disease in low--and middle-income countries. *Int J Tuberc Lung Dis.* 2019;23(11):1131-1141. doi: 10.5588/ijtld.19.0397
3. Erhabor G, Adeniyi B, Arawomo A, et al. Acute Exacerbation of COPD: Clinical Perspectives and Literature Review. *West Afr J Med.* 2021;38(11):1129-1142.
4. Çolak Y, Afzal S, Marott JL, et al. Prognosis of COPD depends on severity of exacerbation history: a population-based analysis. *Respir Med.* 2019;155:141-147.doi: 10.1016/j.rmed.2019.07.021
5. Global Initiative for Chronic Obstructive Lung Disease. 2022 GOLD Report. Global strategy for the diagnosis, management and prevention of COPD. 2022. https://goldcopd.org/2022-gold-reports-2/. Accessed February 2, 2022.
6. Westerik JA, Metting EI, van Boven JF, Tiersma W, Kocks JW, Schermer TR. Associations between chronic comorbidity and exacerbation risk in primary care patients with COPD. *Respir Res.* 2017;18(1):31. doi: 10.1186/s12931-017-0512-2
7. Younus M, Farooq F, Anwar S, Nasir Y, Sciences H. Frequency of Hypomagnesaemia in Acute Exacerbation of

Chronic Obstructive Pulmonary Disease. *Pakistan J Medical Health Sci.* 2022;16(07):441.
8. Gong C, Yang Y, Chen M, Xie Z. Effect of procalcitonin on the prognosis of patients with COPD. *Biomed Rep.* 2020;12(6):313-318. doi: 10.3892/br.2020.1298
9. Goto T, Yoshida K, Faridi MK, Camargo CA, Hasegawa K. Contribution of social factors to readmissions within 30 days after hospitalization for COPD exacerbation. *BMC Pulm Med.* 2020;20(1): 107. doi:10.1186/s12890-020-1136-8
10. Zysman M, Raherison-Semjen C. Women's COPD. *Front Med.* 2021;8:600107. doi:10.3389/fmed.2021.600107
11. DeMeo DL, Ramagopalan S, Kavati A, et al. Women manifest more severe COPD symptoms across the life course. *Int J Chron Obstruct Pulmon Dis.* 2018;13:3021-3029. doi: 10.2147/COPD.S160270
12. Antonela SA, Carone M. Hospitalizations for chronic obstructive pulmonary disease exacerbations and their impact on disease and subsequent morbidity and mortality. *Expert Rev Pharmacoecon Outcomes Res.* 2013;13(2):187-189. doi: 10.1586/erp.13.9
13. Kırhan İ, Üzer F. Bir üniversite hastanesine KOAH alevlenme ile yatan hastaların genel özelliklerinin incelenmesi. *J Harran Univ Med Fac.* 2018;15(3):230-205.
14. Montagnani A, Mathieu G, Pomero F, et al. Hospitalization and mortality for acute exacerbation of chronic obstructive pulmonary disease (COPD): an Italian population-based study. *Eur Rev Med Pharmacol Sci.* 2020;24(12):6899-6907. doi: 10.26355/eurrev_202006_21681
15. Kırkıl G, Deveci F, Turgut T, Muz MH, Türkoğlu S. Factors Related with Duration of Hospital Stay in Patients with COPD. *Firat Med J.* 2013;18(3):171-175.
16. Agboado G, Peters J, Donkin L. Factors influencing the length of hospital stay among patients resident in Blackpool admitted with COPD: a cross-sectional study. *BMJ Open.* 2012;2(5):e000869. doi: 10.1136/bmjopen-2012-000869
17. Lieberman D, Shmarkov O, Gelfer Y, Varshavsky R, Lieberman DV. Prevalence and clinical significance of fever in acute exacerbations of chronic obstructive pulmonary disease. *Eur J Clin Microbiol Infect Dis.* 2003;22(2):75-78. doi: 10.1007/s10096-002-0872-4
18. MacLeod M, Papi A, Contoli M, et al. Chronic obstructive pulmonary disease exacerbation fundamentals: Diagnosis, treatment, prevention and disease impact. *Respirology.* 2021;26(6):532-551. doi: 10.1111/resp.14041
19. Sheng F, Chen L, Lin H, Wu H. Systematic review and meta-analysis: value of venous blood gas in the diagnosis of acute exacerbation of chronic obstructive pulmonary disease in Emergency Department. *Ann Palliat Med.* 2022;11(4):1473-1481. doi: 10.21037/apm-22-336
20. Elvekjaer M, Aasvang EK, Olsen RM, et al. WARD-Project Group. Physiological abnormalities in patients admitted with acute exacerbation of COPD: an observational study with continuous monitoring. *J Clin Monit Comput.* 2020;34:1051-1060. doi: 10.1007/s10877-019-00415-8
21. Celli BR, Fabbri LM, Aaron SD, et al. An updated definition and severity classification of chronic obstructive pulmonary disease exacerbations: the Rome proposal. *Am J Respir Crit Care Med.* 2021;204(11):1251-1258. doi: 10.1164/rccm.202108-1819PP
22. Tseng CY, Chang JCY, Chen YC, et al. Changes of heart rate variability predicting patients with acute exacerbation of chronic obstructive pulmonary disease requiring hospitalization after emergency department treatment. *J Chin Med Assoc.* 2018;81:47-52. doi: 10.1016/j.jcma.2017.10.003
23. Raherison C, Ouaalaya E-H, Bernady A, et al. Comorbidities and COPD severity in a clinic-based cohort. *BMC Pulm Med.* 2018;18(1):117. doi: 10.1186/s12890-018-0684-7
24. Hakamy A, McKeever TM, Steiner MC, Roberts CM, Singh SJ, Bolton CE. The use of the practice walk test in pulmonary rehabilitation program: National COPD Audit Pulmonary Rehabilitation Workstream. *Int J Chron Obstruct Pulmon Dis.* 2017;12:2681-2686. doi: 10.2147/COPD.S141620
25. Daubin C, Parienti JJ, Vabret A, et al. Procalcitonin levels in acute exacerbation of COPD admitted in ICU: A prospective cohort study. *BMC Infect Dis.* 2008;8:145. doi: 10.1186/1471-2334-8-145
26. Simon L, Gauvin F, Amre D, Saint-Louis P, Lacroix J. Serum procalcitonin and C-reactive protein levels as markers of bacterial infection: a systematic review and meta-analysis. *Clin Infect Dis.* 2004;39(2):206-217. doi: 10.1086/421997
27. Lacoma A, Prat C, Andreo F, et al. Value of procalcitonin, C-reactive protein, and neopterin in exacerbations of chronic obstructive pulmonary disease. *Int J Chron Obstruct Pulmon Dis.* 2011;6:157-169. doi: 10.2147/COPD.S16070
28. Zinellu E, Fois AG, Sotgiu E, et al. Serum albumin concentrations in stable chronic obstructive pulmonary disease: a systematic review and meta-analysis. *J Clin Med.* 2021;10(2):269. doi: 10.3390/jcm10020269
29. Steriade AT, Johari S, Sargarovschi N, et al. Predictors of outcome of noninvasive ventilation in severe COPD exacerbation. *BMC Pulm Med.* 2019;19(1):131. doi: 10.1186/s12890-019-0892-9
30. Crisafulli E, Barbeta E, Ielpo A, Torres A. Management of severe acute exacerbations of COPD: an updated narrative review. *Multidiscip Respir Med.* 2018;13:36. doi: 10.1186/s40248-018-0149-0
31. Ahmed SM, Athar M. Mechanical ventilation in patients with chronic obstructive pulmonary disease and bronchial asthma. *Indian J Anaesth.* 2015;59(9):589-598. doi: 10.4103/0019-5049.165856

Wydawnictwo UR 2023
ISSN 2544-1361 (online)
doi: 10.15584/ejcem.2023.3.24

http://www.ejcem.ur.edu.pl
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2023; 21 (3): 567–575

ORIGINAL PAPER

Role of sulfide anion in the development of chronic alcoholic hepatitis under the conditions of modulation of adenosine monophosphate kinase – a correlational study

Andrii Mykytenko [1], Oleh Akimov [2], Oleksandr Shevchenko [3], Karine Neporada [1]

[1] Department of Biological and Bioorganic Chemistry, Poltava State Medical University, Poltava, Ukraine
[2] Department of Pathophysiology, Poltava State Medical University, Poltava, Ukraine
[3] Department of General and Clinical Pathological Physiology, Kharkiv National Medical University, Kharkiv, Ukraine

ABSTRACT

Introduction and aim. Hydrogen sulfide (H_2S) has attracted the attention of researchers as a novel signaling molecule that affects vascular metabolism, immune function, stress and inflammation. It plays an important role in pathophysiological disorders under the conditions of the development of obesity, diabetes, non-alcoholic fatty liver disease and cardiovascular diseases. The purpose of this work is to establish correlation ratios of H_2S concentration with markers of oxidative-nitrosative stress and extracellular matrix metabolism of the liver during chronic alcoholic hepatitis modeling and AMPK modulation by phenformin and doxorubicin.

Material and methods. The experiments were performed on 36 white, sexually mature male Wistar rats, weighing 180-220 g. Alcoholic hepatitis was modelled by alcohol administration, on the background of alcoholic hepatitis animals received phenformin orally at a dose of 10 mg/kg or doxorubicin at a dose of 1.25 mg/kg intraperitoneally. Statistical processing of the results of biochemical studies was carried out using the non-parametric method of Spearman to determine correlations.

Results. H_2S during alcoholic hepatitis inversely proportionally strongly correlates with the concentration of nitrites, oxyproline and arginase activity. Phenformin administration during alcoholic hepatitis leads to formation of inversely proportionally strongly correlation of H_2S with the production of superoxide anion radical, the concentration of malondialdehyde, activities of constitutive NO-synthases, nitrite reductases, nitrate reductases, and arginase. Doxorubicin administration during alcoholic hepatitis leads to formation of directly proportional strongly correlation of H_2S with the activity of constitutive NO-synthases, nitrite reductases, nitrate reductases.

Conclusion. Administration of phenformin or doxorubicin expands correlations between H_2S and indicators of oxidative-nitrosative stress.

Keywords. AMPK, chronic alcohol hepatitis, doxorubicin, liver, phenformin, sulfide anion

Introduction

In recent years, hydrogen sulfide (H_2S) has attracted the attention of researchers as a novel signaling molecule that affects vascular metabolism, has influence on immune function, changes stress and inflammation progression. It plays an important role in pathophysiological disorders under the conditions of the development of obesity, diabetes, non-alcoholic fatty liver disease and cardiovascular diseases.[1] H_2S exerts physiological functions by targeting proteins, enzymes, and transcription factors through a post-translational mod-

Corresponding author: Andrii Mykytenko, e-mail: mykytenkoandrej18@gmail.com

Received: 2.05.2023 / Revised: 17.06.2023 / Accepted: 3.07.2023 / Published: 30.09.2023

Mykytenko A, Akimov O, Shevchenko O, Neporada K. *Role of sulfide anion in the development of chronic alcoholic hepatitis under the conditions of modulation of adenosine monophosphate kinase – a correlational study.* Eur J Clin Exp Med. 2023;21(3):567–575. doi: 10.15584/ejcem.2023.3.24.

ification known as persulfidation.[2] The main effects of H_2S are neuromodulation, regulation of vascular tone, cytoprotection, anti-inflammatory action, sensing (reception) of oxygen, angiogenesis and energy generation.[3-4]

Enzymatic formation of H_2S is catalyzed by cystathionine γ-lyase (EC 4.4.1.1, CSE), cystathionine β-synthase (EC 4.2.1.22, CBS) and 3-mercaptopyruvate sulfurtransferase (EC 2.8.1.2, MST).[5] All these three enzymes are present in the liver and through the synthesis of H_2S regulate its functions. A small part of endogenous H_2S is formed by the non-enzymatic reduction of sulfur contained in certain metabolites (persulphides, thiosulphates and polysulphides).[2] Hepatic H_2S metabolism affects glucose metabolism, insulin sensitivity, lipoprotein synthesis, mitochondrial biogenetics, and biogenesis. H_2S can be involved in many liver diseases such as fibrosis, cirrhosis and liver cancer.[5-6]

Several pathways are involved in the pathogenesis of ethanol-induced liver disease. One of the central pathways involves the induction of cytochrome P450 2E1 by ethanol, which leads to the induction of lipid peroxidation in hepatocytes. The second pathway involves ethanol regulation of transcription factors associated with lipid metabolism. Ethanol also affects the activity of enzymes involved in energy metabolism, including AMP-activated protein kinase (AMPK) and sirtuin-1 (SIRT1) [7]. Ethanol-mediated dysregulation of hepatic AMPK, a master regulator of lipid metabolism, is one of the main mechanisms in the pathogenesis of alcoholic fatty liver disease, because impaired AMPK signaling accelerates lipid accumulation and inhibits lipid catabolism, ultimately leading to the development of alcoholic fatty liver disease in animals.[8]

Among the chemicals and pharmacological preparations that can enhance the activity of AMPK we should note the effect of biguanides (phenformin, buformin, metformin, etc.).[9-10] Among the biguanides, it should be noted that phenformin has a higher ability to phosphorylate AMPK (50 times more active than metformin) and thereby activate AMPK-dependent transcription cascades.[11-12] Doxorubicin has a powerful inhibitory effect on AMPK activity. The use of doxorubicin at a dose of 2.5 mg/kg has a persistent inhibitory effect on AMPK activity in the heart.[13] A single intraperitoneal injection of doxorubicin at a dose of 20 mg/kg also causes a persistent decrease in AMPK expression and leads to development of oxidative stress due to a decrease in the expression of antioxidant enzymes (superoxide dismutase and catalase).[14] Thus, most of the negative effects of doxorubicin (cytotoxicity, damage to mitochondria, development of oxidative stress) are associated with its ability to inhibit AMPK activity.[15-17]

It has been reported, that diallyl disulfide (DADS) has hepatoprotective effects against alcoholic liver disease (ALD), while the underlying mechanisms of action of H_2S remain largely unknown. Research by Shi-Xuan Liu et al. (2022) reported that DADS ameliorated ethanol-induced downregulation of peroxisome proliferator-activated receptor α (PPARα), of carnitine palmitoyltransferase 1 (CPT1) and phosphorylated AMP-activated protein kinase in mouse liver and AML12 cells. These results demonstrate that DADS can prevent ethanol-induced hepatic steatosis and early inflammation by regulating the gut-liver axis and supporting fatty acid catabolism.[18]

The search for ways to reduce oxidative-nitrosative damage to the liver under the conditions of the development of chronic alcoholic hepatitis led us to believe that the modulation of the AMPK cascade plays an important role in the pathogenesis of this disease. Considering the antioxidant and regulatory potential of hydrogen sulfide, which undoubtedly changes its metabolism under conditions of chronic alcoholic hepatitis, the question arises as to what is the role of hydrogen sulfide in changing metabolism of the hepatocyte. Establishing correlations between indicators of oxidative-nitrosative stress and indicators of metabolism of the extracellular matrix of the liver will bring us closer to establishing the role of hydrogen sulfide in the pathogenesis of chronic alcoholic hepatitis.

Aim

We aimed to establish correlation ratios of H_2S concentration with markers of oxidative-nitrosative stress (total NO-synthase activity, activity of constitutive and inducible isoforms of NO-synthase, concentration of nitrosothiols and nitrites, concentration of peroxynitrites of alkali and alkaline earth metals, the activity of nitrite- and nitrate reductases, arginases, superoxide dismutase and catalase, concentration of malondialdehyde, oxidation-modified proteins and production of superoxide anion) and parameters of extracellular matrix metabolism of the liver (total concentration of glycosaminoglycans, concentrations of heparin-heparan, keratan-dermatan and chondroitin fractions, concentration of free oxyproline and sialic acids) during AMPK modulation by phenformin and doxorubicin under conditions of chronic alcoholic hepatitis modeling.

Materials and methods

Ethical approval

Research was conducted in accordance with the standards of the Council of Europe Convention on Bioethics "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (1997), general ethical principles of experiments on animals approved by the First National Congress on Bioethics of Ukraine (September 2001) and other international agreements and national leg-

islation in this area. The rats were kept in a vivarium accredited in accordance with the "Standard rules of order, equipment and maintenance of experimental biological clinics (vivarium)". All experimental procedures were approved by Bioethical Committee of Poltava State Medical University (Record № 197 from 23.09.2021).

Sample and experimental groups

The experiments were performed on 36 white, sexually mature male Wistar rats, weighing 180-220 g. The animals were divided into 6 groups:

I – control (n=6). The control group included animals that were subjected to similar manipulations throughout the study period, but were injected with a physiological solution.

II – phenformin group (n=6), received phenformin hydrochloride according to phenformin injection protocol;

III – alcoholic hepatitis group (n=6), received alcohol according to chronic alcoholic hepatitis protocol.

IV – alcoholic hepatitis + phenformin group (n=6), was subjected to chronic alcoholic hepatitis and phenformin injection protocols.

V – doxorubicin group (n=6) which received doxorubicin hydrochloride according to doxorubicin injection protocol.

VI – alcoholic hepatitis + doxorubicin group (n=6), was subjected to chronic alcoholic hepatitis and doxorubicin injection protocols.

The conditions for keeping animals in the vivarium were standard. Animals were removed from the experiment on the 63rd day by blood sampling from the right ventricle of the heart under thiopental anesthesia. Devices used for research have passed metrological control.

Phenformin injection protocol

Phenformin hydrochloride (phenformin, Sigma-Aldrich), as activator of AMP-activated protein kinase was introduced orally at a dose 10 mg/kg daily for 63 days.[19]

Chronic alcoholic hepatitis modelling protocol

Chronic alcoholic hepatitis in rats was modeled by the method of forced intermittent alcoholization for 5 days, with a repeat after two days by intraperitoneal injection of 16.5% ethanol solution in 5% glucose solution, at the rate of 4 ml/kg of body weight. After that, they were transferred to 10% ethanol as the only source of drinking.[20] Modelling lasted for 63 days.

Doxorubicin injection protocol

Doxorubicin hydrochloride (doxorubicin, S.C. Sindan-Pharma S.R.L.), as inhibitor of AMP-activated protein kinase, was injected intraperitoneally at a dose 1,25 mg/kg four times a week for 63 days.[21]

Biochemical analysis

For biochemical analysis we used 10% liver tissue homogenate and blood serum. Liver tissue homogenate was obtained after homogenization of 1 g of rat liver with 9 ml of 0.2 M Tris-buffer solution (Trisaminomethane-hydrochloric acid buffer, pH=7.4). Then it was centrifugated at 3000 g for 10 minutes. Upper layer (supernantant) was used for further biochemical analysis. Blood plasma was obtained after addition of 0,109 M sodium citrate at ratio 9:1 and subsequent centrifugation at 3000 g for 10 minutes.

In the blood plasma of rats, the activity of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined using diagnostic kits, produced by NPP "Filisit-Diahnostyka". We also calculated the de Ritis coefficient (AST/ALT).

Concentration of sulfide anion (calculated as H_2S concentration) specifically reacts with N-N-dimethyl-para-phenylenediamine in the presence of Fe^{3+} ions and excess of hydrochloric acid to form a red-pink chromogen with a maximum light absorption at a wavelength of 667 nm.[22]

Total NO-synthase activity (gNOS) was evaluated by the increase of nitrites after incubation of 10% tissue homogenate (0.2 ml) for 30 min in the incubation solution (2.5 ml of 0.1 M trisbuffer, 0.3 ml of 320 mM aqueous solution of L-arginine and 0.1 ml of 1 mM NADPH$^+$H$^+$ solution). To determine the activity of cNOS 1% solution of aminoguanidine hydrochloride was used and the incubation time was extended to 60 min.[23-24] The activity of iNOS was calculated by the formula: iNOS= gNOS-cNOS.

The method for the determination of nitrosothiols was based on the determination of the difference in the concentration of nitrites (NO_2^-) using Griess reagent (modified by Ilosvay) before and after oxidation of nitrosothiol complexes (S-NO) to nitrites with a solution of mercuric chloride ($HgCl_2$).[25]

The concentration of nitrite and peroxynitrite of alkali and alkaline earth metals, the activity of nitrite- and nitrate reductases, arginases, superoxide dismutase (SOD) and catalase, concentration of malondialdehyde (MDA), oxidation-modified proteins and production of superoxide anion, GAG fractions (heparin-heparan, keratan- dermatan and chondroitin), the concentration of free oxyproline and sialic acids were studied in rat liver 10% homogenate.[23,26-33]

Statistical analysis

Statistical processing of the results of biochemical studies was carried out using the non-parametric method of Spearman to determine correlations (with the exception of groups where the studied parameters corresponded to a normal distribution with very small values of the standard deviation, where the Pearson method was

used). All statistical calculations were performed in the Microsoft Office Excel program and its extension Real Statistics 2019. Correlation was considered statistically significant at $p<0.05$.

Results

The role of the AMPK cascade in the development of chronic hepatitis remains unclear. But it is known that modulation of AMPK activity leads to changes in the pathogenesis of chronic alcoholic hepatitis. Establishing correlational and pathogenetic relationships between the concentration of hydrogen sulfide and biochemical indicators of oxidative-nitrosative stress and markers of the metabolism of the extracellular matrix of the liver under the conditions of chronic alcoholic hepatitis will allow the use of donors and scavengers of hydrogen sulfide in the pathogenetic therapy of alcoholic liver disease.

Blood biochemical markers of chronic alcoholic hepatitis (AST, ALT activity and de Ritis coefficient) under the conditions of modulation of AMPK cascade are shown in Fig. 1. Activity of AST, ALT and de Ritis coefficient proved a presence of cytolytic process in rat liver in chronic alcohol hepatitis group.

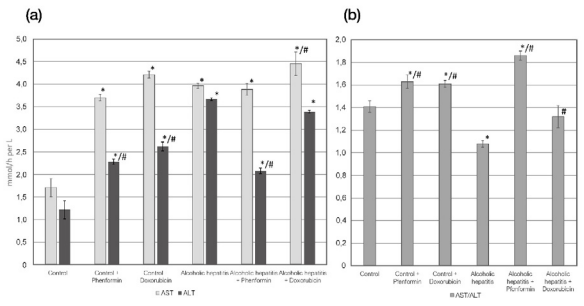

Fig. 1. Biochemical indicators of blood plasma of rats under the conditions of modeling chronic alcoholic hepatitis and modulation of AMPK cascade: (a) activity of aspartate aminotransferase (AST) and alanine aminotransferase (ALT); (b) the de Ritis coefficient (AST/ALT); * – indicates that difference is statistically significant compared to control group; # – indicates that difference is statistically significant compared to alcoholic hepatitis group

In the control group of animals, no statistically significant correlations were found between the concentration of sulfide anion and other biochemical parameters. In the group of animals injected with phenformin, it was found that the concentration of sulfide anion is inversely proportionally strongly correlated with the activity of superoxide dismutase and inversely proportionally strongly correlated with the concentration of the keratan-dermatan fraction of glycosaminoglycans (Table 1).

Table 1. Correlation analysis of biochemical indicators of the liver of rats under the conditions of modeling chronic alcoholic hepatitis and stimulation of AMPK cascade activation[a]

Correlation relationships of biochemical parameters	Group							
	Control		Control+ Phenformin		Alcoholic hepatitis		Alcoholic hepatitis + Phenformin	
	rho	p	rho	p	rho	p	rho	p
Sulfide anion (μmol/g) / Catalase (μkat/g)	0.739	0.09	-0.029	0.96	-0.243	0.64	-0.319	0.54
Sulfide anion (μmol/g) / Superoxide dismutase (c.u.)	-0.739	0.09	-0.828	0.04	-0.176	0.74	-0.478	0.34
Sulfide anion (μmol/g) / Superoxide anion radical (nmol/s per g)	0	1	0.478	0.34	-0.179	0.73	-0.956	0.003
Sulfide anion (μmol/g) / MDA (μmol/g)	-0.134	0.8	-0.429	0.4	-0.7	0.12	-0.886	0.02
Sulfide anion (μmol/g) / Oxidation-modified proteins (c.u.)	0.618	0.19	0.696	0.12	-0.582	0.23	0.6	0.21
Sulfide anion (μmol/g) / Inducible NO synthase (μmol/min per g of protein)	0.088	0.87	0.486	0.33	-0.203	0.7	-0.543	0.27
Sulfide anion (μmol/g) / Constitutive NO synthases (μmol/min per g of protein)	0.045	0.93	0.486	0.33	0.029	0.96	-0.947*	0.004
Sulfide anion (μmol/g) / Nitrite reductase activity (μmol/min per g of protein)	-0.265	0.61	0.6	0.21	0.348	0.5	-0.996*	p<0.001
Sulfide anion (μmol/g) / Nitrate reductase activity (μmol/min per g of protein)	-0.618	0.19	0.486	0.33	0.406	0.42	-0.947*	0.004
Sulfide anion (μmol/g) / $ONOO^-$ (μmol/g)	0.091	0.86	-0.714	0.11	-0.696	0.12	0.943	0.005
Sulfide anion (μmol/g) / S-NO (μmol/g)	-0.088	0.87	-0.116	0.83	0.667	0.15	0.754	0.08
Sulfide anion (μmol/g) / NO_2 concentration (nmol/g)	0.091	0.86	0	1	-0.882	0.02	-0.478	0.34
Sulfide anion (μmol/g) / Arginase activity (μmol/min per g of protein)	0,177	0.74	0.486	0.33	-0.812	0.0499	-0.83	0.04
Sulfide anion (μmol/g) / Concentration of heparin-heparan fraction (μmol/L)	-0.739	0.09	0.66	0.16	-0.294	0.57	0.543	0.27
Sulfide anion (μmol/g) / Concentration of keratin-dermatan fraction (μmol/L)	-0.739	0.09	-0.986	0.0003	-0.176	0.74	0.94	0.005
Sulfide anion (μmol/g) / Concentration of chondroitin fraction (μmol / l)	-0.739	0.09	0.66	0.16	-0.35	0.49	-0.94	0.004
Sulfide anion (μmol/g) / Concentration of free oxyproline (μmol / g)	0.739	0.09	0.657	0.16	-0.912	0.01	-0.486	0.33
Sulfide anion (μmol/g) / Concentration of sialic acids (mg / g)	-0.582	0.23	0.086	0.87	-0.09	0.87	-0.412	0.42

[a] * – the correlation coefficient was calculated by Pearson's method (r^2)

In a group of rats with chronic alcoholic hepatitis, it was found that sulfide anion is inversely proportionally strongly correlated with the concentration of nitrites, oxyproline and arginase activity.

Table 2. Correlation analysis of biochemical indicators of the liver of rats under the conditions of modeling chronic alcoholic hepatitis and blockade of AMPK-cascade activation

Correlation relationships of biochemical parameters	Group							
	Control		Control+ doxorubicin		Alcoholic hepatitis		Alcoholic hepatitis + doxorubicin	
	rho	p	rho	p	rho	p	rho	p
Sulfide anion (μmol/g) / Catalase (μkat/g)	0.739	0.09	0.377	0.46	-0.243	0.64	0.543	0.27
Sulfide anion (μmol/g) / Superoxide dismutase (c.u.)	-0.739	0.09	-0.478	0.34	-0.176	0.74	-0.478	0.34
Sulfide anion (μmol/g) / Superoxide anion radical (nmol/s per g)	0	1	0.478	0.34	-0.179	0.73	0.478	0.34
Sulfide anion (μmol/g) / MDA (μmol/g)	-0.134	0.8	0.377	0.46	-0.7	0.12	-0.714	0.11
Sulfide anion (μmol/g) / Oxidation-modified proteins (c.u.)	0.618	0.19	-0.429	0.4	-0.582	0.23	0.406	0.42
Sulfide anion (μmol/g) / Inducible NO synthase (μmol/min per g of protein)	0.088	0.87	-0.486	0.33	-0.203	0.7	0.586	0.33
Sulfide anion (μmol/g) / Constitutive NO synthases (μmol/min per g of protein)	0.045	0.93	-0.486	0.33	0.029	0.96	0.943	0.004
Sulfide anion (μmol/g) / Nitrite reductase activity (μmol/min per g of protein)	-0.265	0.61	0.429	0.4	0.348	0.5	0.943	0.005
Sulfide anion (μmol/g) / Nitrate reductase activity (μmol/min per g of protein)	-0.618	0.19	0.429	0.4	0.406	0.42	0.943	0.005
Sulfide anion (μmol/g) / $ONOO^-$ (μmol/g)	0.091	0.86	0.429	0.4	-0.696	0.12	-0.2	0.7
Sulfide anion (μmol/g) / S-NO (μmol/g)	-0.088	0.87	0.371	0.47	0.667	0.15	-0.986	0.0003
Sulfide anion (μmol/g) / NO_2 concentration (nmol/g)	0.091	0.86	0.828	0.04	-0.882	0.02	-0.478	0.34
Sulfide anion (μmol/g) / Arginase activity (μmol/min per g of protein)	0,177	0.74	-0.429	0.4	-0.812	0.0499	0.486	0.33
Sulfide anion (μmol/g) / Concentration of heparin-heparan fraction (μmol/L)	-0.739	0.09	-0.429	0.4	-0.294	0.57	-0.886	0.02
Sulfide anion (μmol/g) / Concentration of keratin-dermatan fraction (μmol/L)	-0.739	0.09	-0.319	0.54	-0.176	0.74	0.89	0.02
Sulfide anion (μmol/g) / Concentration of chondroitin fraction (μmol/L)	-0.739	0.09	-0.43	0.4	-0.35	0.49	-0.54	0.27
Sulfide anion (μmol/g) / Concentration of free oxyproline (μmol/g)	0.739	0.09	0.429	0.4	-0.912	0.01	-0.943	0.005
Sulfide anion (μmol/g) / Concentration of sialic acids (mg/g)	-0.582	0.23	-0.088	0.87	-0.09	0.87	0.429	0.4

During phenformin correction of chronic alcoholic hepatitis in rats, it was found that sulfide anion is inversely proportionally strongly correlated with the production of superoxide anion radical, the concentration of malondialdehyde and the chondroitin fraction of glycosaminoglycans, as well as with activities of constitutive NO-synthases, nitrite reductases, nitrate reductases, and arginase. It was also found that the sulfide anion is directly proportional strongly correlated to the concentration of peroxynitrite and the keratan-dermatan fraction of glycosaminoglycans (Table 1).

Doxorubicin injection to rats on background of chronic alcoholic hepatitis led to following changes: concentration of sulfide anion directly proportional strongly correlated to the concentration of nitrites, the activity of constitutive NO-synthases, nitrite reductases, nitrate reductases and the concentration of the keratan-dermatan fraction of glycosaminoglycans. It was also found that sulfide anion is inversely proportionally strongly correlated with the concentration of nitrosothiols, free oxyproline and the heparin-heparan fraction of glycosaminoglycans (Table 2).

Discussion

The summary of correlation ratios between H_2S and biochemical parameters of rat liver are presented in Fig. 2. In brief, during excessive alcohol intake sulfide anion receives strong negative correlation bonds with nitrite content, concentration of free L-oxyproline and arginase activity (ARG), which were absent under normal conditions. Stimulation of AMPK cascade by phenformin leaves correlation of sulfide anion with arginase intact, but removes its correlation with nitrites and L-oxyproline, while creating new correlations with nitrite reductases (NiR), nitrate reductases (NaR), MDA, cNOS, peroxynitrite ($ONOO^-$) and superoxide.

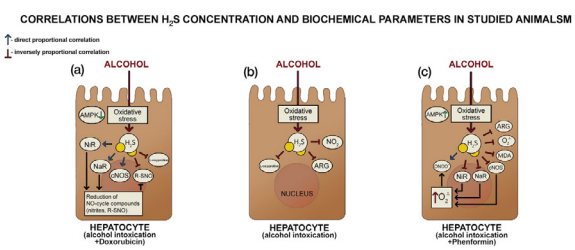

Fig. 2. Correlation of H_2S concentration with markers of oxidative-nitrosative stress and extracellular matrix metabolism of the liver during chronic alcoholic hepatitis modeling and AMPK modulation: (a) influence of alcohol intoxication and doxorubicin on H_2S correlation with biochemical parameters, (b) influence of alcohol intoxication on H_2S correlation with biochemical parameters, (c) influence of alcohol intoxication and phenformin on H_2S correlation with biochemical parameters

Despite its inversed correlation with superoxide production, sulfide anion can potentially cause increased production of superoxide from NiR and NaR (as parts of xanthine oxidoreductase complex), thus leading to in-

crease of peroxynitrite formation, which may explain appearance of direct correlation between sulfide anion and peroxynitrite during combined influence of phenformin and alcohol. Inhibition of AMPK cascade by doxorubicin leaves correlation of sulfide anion with L-oxyproline intact, but removes its correlation with nitrites and arginase, while creating new correlations with NiR, NaR, S-NO, and cNOS. It is worth mentioning, that correlations between sulfide anion and NiR, NaR, and cNOS in doxorubicin+alcoholic hepatitis group have different vector compared to phenformin+alcoholic hepatitis group. In doxorubicin+ alcoholic hepatitis group sulfide anion can potentially create conditions under which main source of NO production will shift towards predominance of L-arginine-independent pathway.

The absence of statistically significant correlations in the control group of animals may indicate the non-linearity of the relationship between the sulfide anion content and the investigated biochemical parameters. At the same time, modulation of AMPK activity leads to the appearance of statistically significant correlations. The appearance of an inversely proportional strong relationship between SOD activity and H_2S concentration under the conditions of stimulation of AMPK activation by phenformin may be associated with a decrease in the production of reactive oxygen species (ROS) by mitochondria under the influence of AMPK.[34] The appearance of a correlation relationship similar in direction and strength between H_2S and the concentration of the keratan-dermatan fraction of GAG is related to the ability of AMPK to directly affect the concentration of different fractions of sulfated GAG.[35] Reduction of the degree of AMPK activation by doxorubicin leads to the appearance of a direct strong relationship between H_2S and nitrite concentration, which may indicate the ability of H_2S to enhance gene expression of constitutive and inducible NOS isoforms.[36]

Under the conditions of chronic alcoholic hepatitis, the appearance of an inversely proportional strong relationship between H_2S and nitrite concentration is noted, which may be related to the ability of ethanol to induce the expression of genes of the inducible isoform of NOS, regardless of H_2S concentration.[37] The appearance of the relationship with similar direction and strength between H_2S and arginase activity in the group of chronic alcoholic hepatitis is also related to the biological effects of alcohol, namely its ability to decrease arginase activity, while H_2S can increase its activity.[38-39] The inversely proportional strong relationship between the concentrations of H_2S and L-oxyproline under conditions of chronic alcoholic hepatitis can be explained by the origin of free L-oxyproline, which under conditions of excessive accumulation of alcohol is released from collagen fibers during oxidative stress, while H_2S is a powerful antioxidant.[40]

Stimulation of the activation of the AMPK cascade in the background of simulation of alcohol intoxication significantly enhances the effect of H_2S on the nitric oxide system. The appearance of inversely proportional strong relationships with the activities of constitutive NOS isoforms, nitrate reductases and nitrite reductases can be explained by the joint inhibitory effect of both the AMPK cascade and alcohol on the activity of the xanthine oxidoreductase complex, especially on its reductase domain.[41-43] Considering the fact that inhibition of AMPK cascade activation by doxorubicin completely reverses the relationship between H_2S and the enzymes described above to a directly proportional strong one, it can be assumed that AMPK can affect the biological function of H_2S in relation to the xanthine oxidoreductase complex.

H_2S has the ability to stimulate the conversion of the xanthine oxidoreductase complex into nitrite reductase and promote the formation of nitric oxide from this source.[44] The blockade of the transition of the xanthine oxidoreductase complex, due to the activation of AMPK, to nitrite reductase can contribute to the excessive formation of ROS from the oxidase domain, which explains the directly proportional strong relationship between H_2S and $ONOO^-$ in the group of combined exposure to phenformin and chronic alcoholic hepatitis. Conversely, blockade of AMPK activation and H_2S-dependent stimulation of conversion of the xanthine oxidoreductase complex to nitrite reductase may explain the directly proportional strong relationship between H_2S and nitrosothiols in a group of animals under combined exposure to doxorubicin and chronic alcoholic hepatitis.

The disappearance of the relationship between H_2S and arginase activity in a group of animals with combined exposure to doxorubicin and chronic alcoholic hepatitis may be associated with a redistribution of the effect of H_2S on the nitrate-nitrite reductase pathway of nitric oxide formation towards the predominance of the effect on nitrosothiols, which can modulate the activity of arginase by releasing nitrous oxide.[45]

Modulation of AMPK activity does not change the direction and strength of the relationship between H_2S and the concentration of free L-oxyproline under conditions of chronic alcoholic hepatitis. Changes in the relationships between H_2S and the concentration of different GAG fractions depend to a greater extent on the influence of AMPK on the concentration of the latter and require further investigation.[35]

The limitation of this study is that we did not access the expression of AMPK in studied groups.

Perspectives of further research

Perspective of further research lies in establishing of causation between sulfide anion concentration and

changes in biochemical parameters with which it has shown strong correlations. On the studied models of modulation of AMPK cascade during alcohol intoxication we can establish dependence of abovementioned biochemical parameters, especially those, that showed significant correlations, from changes in concentration of sulfide anion in liver, which can be achieved by addition of sulfide donors and/or scavengers. Estimation of pathogenetic role of sulfide anion in development of alcoholic hepatitis and its interplay with AMPK cascade may open a path for usage of sulfide anion as a pathogenetically sound treatment of alcoholic hepatitis, free from negative impacts of direct influence on AMPK by specific modulators.

Conclusion

Modeling of chronic alcoholic hepatitis leads to the appearance of correlations between the concentration of endogenous H_2S and the activity of arginases, the concentration of nitrites and free L-oxyproline in the liver of rats.

Administration of phenformin under the conditions of chronic alcoholic hepatitis modeling expands correlations between endogenous H_2S and indicators of oxidative-nitrosative stress in the liver of rats, due to new correlations with superoxide anion-radical, peroxynitrite, nitrate-nitrite reductases, constitutive NO-synthases and malondialdehyde. However, the administration of phenformin leads to the loss of the correlation between endogenous H_2S and the concentrations of free L-oxyproline and nitrite.

Administration of doxorubicin under the conditions of chronic alcoholic hepatitis modeling expands correlations between endogenous H_2S and indicators of oxidative-nitrosative stress in the liver of rats, due to new correlations with peroxynitrite, nitrate-nitrite reductases, constitutive NO-synthases and nitrosothiols. However, administration of doxorubicin leads to a loss of correlation between endogenous H_2S and arginase activity and nitrite concentration.

Declarations

Funding

The authors declare no financial support.

Author contributions

Conceptualization, A.M. and K.N.; Methodology, A.M.; Software, O.A.; Validation, A.M., O.A., O.S. and K.N.; Formal Analysis, A.M.; Investigation, A.M. and O.A.; Resources, A.M.; Data Curation, A.M. and O.A.; Writing – Original Draft Preparation, A.M.; Writing – Review & Editing, O.S. and K.N.; Visualization, A.M.; Supervision, O.S. and K.N.; Project Administration, A.M.; Funding Acquisition, A.M.

Conflicts of interest

The authors declare that no conflicts exist.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Ethics approval

Research was conducted in accordance with the standards of the Council of Europe Convention on Bioethics "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (1997), general ethical principles of experiments on animals approved by the First National Congress on Bioethics of Ukraine (September 2001) and other international agreements and national legislation in this area. Research was approved by Ethical Committee of Poltava State Medical University.

References

1. Mateus I, Prip-Buus C. Hydrogen sulphide in liver glucose/lipid metabolism and non-alcoholic fatty liver disease. *Eur J Clin Invest.* 2022;52(3):e13680. doi: 10.1111/eci.13680
2. Comas F, Moreno-Navarrete JM. The Impact of H_2S on Obesity-Associated Metabolic Disturbances. *Antioxidants (Basel).* 2021;10(5):633. doi: 10.3390/antiox10050633
3. Sukmansky OI. Sulfur-containing gaseous signaling molecules. *Fiziol Zh.* 2017;63(6):106-117. doi: 10.15407/fz63.06.106
4. Zhang XN, Zhao N, Guo FF, Wang YR, Liu SX, Zeng T. Diallyl disulfide suppresses the lipopolysaccharide-driven inflammatory response of macrophages by activating the Nrf2 pathway. *Food Chem Toxicol.* 2022;159:112760. doi: 10.1016/j.fct.2021.112760
5. Lee JH, Im SS. Function of gaseous hydrogen sulfide in liver fibrosis. *BMB Rep*. 2022;55(10):481-487. doi:10.5483/BMBRep.2022.55.10.124
6. Read E, Milford J, Zhu J, Wu L, Bilodeau M, Yang G. The interaction of disulfiram and H_2S metabolism in inhibition of aldehyde dehydrogenase activity and liver cancer cell growth. *Toxicol Appl Pharmacol.* 2021;426:115642. doi: 10.1016/j.taap.2021.115642
7. Ma Y, Ding Q, Qian Q, et al. AMPK-Regulated Autophagy Contributes to Ursolic Acid Supplementation-Alleviated Hepatic Steatosis and Liver Injury in Chronic Alcohol-Fed Mice. *ACS Omega.* 2022;8(1):907-914. doi: 10.1021/acsomega.2c06252
8. Na AY, Yang EJ, Jeon JM, Ki SH, Song KS, Lee S. Protective Effect of Isoliquiritigenin against Ethanol-Induced Hepatic Steatosis by Regulating the SIRT1-AMPK Pathway. *Toxicol Res.* 2018;34(1):23-29. doi: 10.5487/TR.2018.34.1.023
9. Jiménez-Vacas JM, Herrero-Aguayo V, Montero-Hidalgo AJ, et al. Clinical, Cellular, and Molecular Evidence of the Additive Antitumor Effects of Biguanides

and Statins in Prostate Cancer. *J Clin Endocrinol Metab.* 2021;106(2):e696-e710. doi: 10.1210/clinem/dgaa877
10. Zhao H, Swanson KD, Zheng B. Therapeutic Repurposing of Biguanides in Cancer. *Trends Cancer.* 2021;7(8):714-730. doi: 10.1016/j.trecan.2021.03.001
11. Wu T, Zhou S, Qin M, et al. Phenformin and ataxia-telangiectasia mutated inhibitors synergistically co-suppress liver cancer cell growth by damaging mitochondria. *FEBS Open Bio.* 2021;11(5):1440-1451. doi: 10.1002/2211-5463.13152
12. Jaidee R, Kongpetch S, Senggunprai L, Prawan A, Kukongviriyapan U, Kukongviriyapan V. Phenformin inhibits proliferation, invasion, and angiogenesis of cholangiocarcinoma cells via AMPK-mTOR and HIF-1A pathways. *Naunyn Schmiedebergs Arch Pharmacol.* 2020;393(9):1681-1690. doi: 10.1007/s00210-020-01885-3
13. Wang T, Yuan C, Liu J, et al. Targeting Energy Protection as a Novel Strategy to Disclose Di'ao Xinxuekang against the Cardiotoxicity Caused by Doxorubicin. *Int J Mol Sci.* 2023;24(2):897. doi: 10.3390/ijms24020897
14. Xu X, Liu Q, Li J, et al. Co-Treatment With Resveratrol and FGF1 Protects Against Acute Liver Toxicity After Doxorubicin Treatment via the AMPK/NRF2 Pathway. *Front Pharmacol.* 2022;13:940406. doi: 10.3389/fphar.2022.940406
15. Kawano I, Adamcova M. MicroRNAs in doxorubicin-induced cardiotoxicity: The DNA damage response. *Front Pharmacol.* 2022;13:1055911. doi: 10.3389/fphar.2022.1055911
16. Luo F, Zhao J, Liu S, et al. Ursolic acid augments the chemosensitivity of drug-resistant breast cancer cells to doxorubicin by AMPK-mediated mitochondrial dysfunction. *Biochem Pharmacol.* 2022;205:115278. doi: 10.1016/j.bcp.2022.115278
17. Ma L, Gong Q, Chen Y, Luo P, Chen J, Shi C. Targeting positive cofactor 4 induces autophagic cell death in MYC-expressing diffuse large B-cell lymphoma. *Exp Hematol.* 2023;119-120:42-57.e4. doi:10.1016/j.exphem.2023.01.001
18. Liu SX, Liu H, Wang S, Zhang CL, Guo FF, Zeng T. Diallyl disulfide ameliorates ethanol-induced liver steatosis and inflammation by maintaining the fatty acid catabolism and regulating the gut-liver axis. *Food Chem Toxicol.* 2022;164:113108. doi: 10.1016/j.fct.2022.113108
19. Dilman VM, Berstein LM, Zabezhinski MA, Alexandrov VA, Bobrov JF, Pliss GB. Inhibition of DMBA-induced carcinogenesis by phenformin in the mammary gland of rats. *Arch Geschwulstforsch.* 1978;48(1):1-8.
20. Mykytenko AO, Akimov OY, Neporada KS. Influence of lipopolysaccharide on the development of oxidative-nitrosative stress in the liver of rats under conditions of chronic alcohol intoxication. *Fiziol Zh*. 2022;68(2):29–35. doi: 10.15407/fz68.02.029
21. Yarmohmmadi F, Rahimi N, Faghir-Ghanesefat H, et al. Protective effects of agmatine on doxorubicin-induced chronic cardiotoxicity in rat. *Eur J Pharmacol*. 2017;796:39-44. doi: 10.1016/j.ejphar.2016.12.022
22. Sugahara S, Suzuki M, Kamiya H, et al. Colorimetric Determination of Sulfide in Microsamples. *Anal Sci*. 2016;32(10):1129-1131. doi: 10.2116/analsci.32.1129
23. Akimov O Ye, Kostenko VO. Functioning of nitric oxide cycle in gastric mucosa of rats under excessive combined intake of sodium nitrate and fluoride. *Ukr. Biochem. J*. 2016;88(6):70-75. doi: 10.15407/ubj88.06.070
24. Yelins'ka AM, Akimov OYe, Kostenko VO. Role of AP-1 transcriptional factor in development of oxidative and nitrosative stress in periodontal tissues during systemic inflammatory response. *Ukr Biochem J*. 2019;91(1):80-85. doi: 10.15407/ubj91.01.080
25. Gaston B, Reilly J, Drazen JM, et al. Endogenous nitrogen oxides and bronchodilator S-nitrosothiols in human airways. *Proc Natl Acad Sci USA*. 1993;90(23):10957-10961. doi: 10.1073/pnas.90.23.10957
26. Mykytenko AO, Akimov OYe, Yeroshenko GA, Neporada KS. Influence of NF-κB on the development of oxidative-nitrosative stress in the liver of rats under conditions of chronic alcohol intoxication. *Ukr Biochem J*. 2022;94(6):57-66. doi: 10.15407/ubj94.06.057
27. Korolyuk MA, Ivanova LI, Mayorova IG. Method for determination of catalase activity. *Laboratory science*. 1988;1:16-19.
28. Matsytska YK, Akimov OY, Mykytenko AO. Influence of corvitin and metformin on biochemical changes in lacrimal glands of rats during water avoidance stress modeling. *Oftalmologicheskii Zhurnal*. 2022;97(3):39–44. doi: 10.31288/oftalmolzh202233944
29. Mykytenko AO, Matsytska YK, Akimov OY. Influence of lipopolysaccharide and the general adaptation syndrome on the development of oxidative-nitrosative stress in the lacrimal glands of rats. *Fiziol Zh*. 2023;69(2):71-77. doi: 10.15407/fz69.02.071
30. Kostenko VO, Tsebrzhins'kii OI. Production of superoxide anion radical and nitric oxide in renal tissues sutured with different surgical suture material. *Fiziol Zh*. 2000;46(5):56-62.
31. Volpi N. Purification of heparin, dermatan sulfate and chondroitin sulfate from mixtures by sequential precipitation with various organic solvents. *J Chromatogr B Biomed Appl*. 1996;685(1):27-34. doi: 10.1016/0378-4347(96)00154-5
32. Tatyanets SS. Method for determination of free oxyproline in blood serum. *Laboratory work*. 1985;1:61-62.
33. Menshikova VV. Methodical guidelines for the application of unified clinical laboratory methods of research. 1973:96-97.
34. Wu S, Zou MH. AMPK, Mitochondrial Function, and Cardiovascular Disease. *Int J Mol Sci*. 2020;21(14):4987. doi: 10.3390/ijms21144987
35. Shrikanth CB, Jagannath S, Chilkunda ND. AMPK differentially alters sulphated glycosaminoglycans under normal and high glucose milieu in proximal tubular cells. *J Biochem*. 2021;169(1):75-86. doi: 10.1093/jb/mvaa094

36. Yilmaz-Oral D, Kaya-Sezginer E, Asker H, Gur S. Co-administration of sodium hydrosulfide and tadalafil modulates hypoxia and oxidative stress on bladder dysfunction in a rat model of bladder outlet obstruction. *Int Braz J Urol*. 2022;48(6):971-980. doi: 10.1590/S1677-5538.IBJU.2022.0207
37. Sudhakaran G, Prathap P, Guru A, et al. Reverse pharmacology of Nimbin-N2 attenuates alcoholic liver injury and promotes the hepatoprotective dual role of improving lipid metabolism and downregulating the levels of inflammatory cytokines in zebrafish larval model. *Mol Cell Biochem*. 2022;477(10):2387-2401. doi: 10.1007/s11010-022-04448-7
38. Trapeznikova SS, Gurtovenko VM, Navasardiants DG. Arginase activity in various tissues of rats in alcohol intoxication. *Vopr Med Khim*. 1983;29(4):95-8.
39. Liu Z, Zhu Z, He Y, et al. A Novel Hydrogen Sulfide Donor Reduces Pilocarpine-Induced Status Epilepticus and Regulates Microglial Inflammatory Profile. *Front Cell Neurosci*. 2021;15:780447. doi: 10.3389/fncel.2021.780447
40. Koneru M, Sahu BD, Gudem S, et al. Polydatin alleviates alcohol-induced acute liver injury in mice: Relevance of matrix metalloproteinases (MMPs) and hepatic antioxidants. *Phytomedicine*. 2017;27:23-32. doi: 10.1016/j.phymed.2017.01.013
41. Yang KJ, Kim JH, Chang YK, Park CW, Kim SY, Hong YA. Inhibition of xanthine oxidoreductase protects against contrast-induced renal tubular injury by activating adenosine monophosphate-activated protein kinase. *Free Radic Biol Med*. 2019;145:209-220. doi: 10.1016/j.freeradbiomed.2019.09.027
42. Castro GD, Delgado de Layño AM, Costantini MH, Castro JA. Cytosolic xanthine oxidoreductase mediated bioactivation of ethanol to acetaldehyde and free radicals in rat breast tissue. Its potential role in alcohol-promoted mammary cancer. *Toxicology*. 2001;160(1-3):11-18. doi: 10.1016/s0300-483x(00)00433-9
43. Yamamoto T, Moriwaki Y, Takahashi S, Suda M, Higashino K. Ethanol as a xanthine dehydrogenase inhibitor. *Metabolism*. 1995;44(6):779-785. doi: 10.1016/0026-0495(95)90192-2
44. Pardue S, Kolluru GK, Shen X, et al. Hydrogen sulfide stimulates xanthine oxidoreductase conversion to nitrite reductase and formation of NO. *Redox Biol*. 2020;34:101447. doi: 10.1016/j.redox.2020.101447
45. Ckless K, van der Vliet A, Janssen-Heininger Y. Oxidative-nitrosative stress and post-translational protein modifications: implications to lung structure-function relations. Arginase modulates NF-kappaB activity via a nitric oxide-dependent mechanism. *Am J Respir Cell Mol Biol*. 2007;36(6):645-653. doi: 10.1165/rcmb.2006-0329SM

Wydawnictwo UR 2023
ISSN 2544-1361 (online)
doi: 10.15584/ejcem.2023.3.25

http://www.ejcem.ur.edu.pl
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2023; 21 (3): 576–581

ORIGINAL PAPER

The effect of different blood groups on visual evoked potentials

Mehmet Tahir Eski [1], Ahmet Yabalak [2], Halime Şahan [2], Alper Aziz Hüdai Ayaslı [3], Taha Sezer [4]

[1] Department of Ophthalmology, Neon Hospital, Erzincan, Turkey
[2] Department of Neurology, Düzce University Faculty of Medicine, Düzce, Turkey
[3] Department of Neurology, Düzce Atatürk State Hospital, Düzce, Turkey
[4] Department of Ophthalmology, Düzce University Faculty of Medicine, Düzce, Turkey

ABSTRACT

Introduction and aim. Purpose of the study is to determine whether it is required to use different standards when evaluating visual evoked potential (VEP) measurements of healthy individuals with different blood groups.

Material and methods. The study consisted of healthy individuals with different blood groups who have applied to the ophthalmology and neurology outpatient clinic of Düzce University Medical Faculty from January to December 2022. The patients went through detailed ophthalmologic examination and VEP test and only the ones with normal results were included to the study.

Results. The study consisted of 119 individuals, with a blood group distribution of 30 A, 29 B, 30 AB and 30 O. VEP latency and amplitude changes were compared and no significant difference was observed within 4 groups in terms of P100 and N70 latency and amplitudes. There was N70 latency prolongation in Rh- group and this difference was found to be statistically significant (p=0.009). Rh+ group was found to be high in terms of P100 amplitudes and this was considered statistically significant (both p=0.023).

Conclusion. There was no statistically significant difference in the VEP parameters of the individuals with the ABO blood groups hence same VEP normal values can be used for ABO blood groups.

Keywords. blood groups, visual evoked potential, Rh factor

Introduction

ABO system which is known as Blood Group System is considered to be the most important system in our body, serving as the code of our body. Recognition of this importance is followed by amplified awareness and constantly increasing number of related studies.[1] Due to immunological barriers, ABO blood group system compatibility is required for all processes from blood transfusions to organ transplantation. Besides, blood group distribution is not similar in all societies. There are even studies suggesting that some conditions like tumors and infections and cardiovascular and gastroduodenal diseases are more prevalent in certain blood groups.[1–5] ABO blood group antigen exists in various tissues like erythrocyte, thrombocyte and endothelium. The effect of different blood groups on retinal nerve thickness was reported in a previous ophthalmological study.[6]

Pattern visual evoked potential (VEP) test is an important electrophysiological test commonly employed in neurology and ophthalmology clinics for the purpose of reinforcing the results of unexplained vision loss, neurological disorders or optic nerve damages. VEP test also provides quantitative data about optic tracts from reti-

Corresponding author: Ahmet Yabalak, e-mail: yabalakahmet@gmail.com

Received: 27.04.2023 / Revised: 7.07.2023 / Accepted: 12.07.2023 / Published: 30.09.2023

Eski MT, Yabalak A, Şahan H, Ayaslı AAH, Sezer T. *The effect of different blood groups on visual evoked potentials.* Eur J Clin Exp Med. 2023;21(3):576–581. doi: 10.15584/ejcem.2023.3.25.

na to brain through optic disc.[7] When literature is reviewed, various studies can be found on the relations between optic disorder and blood groups.[8–12] However, no previous study was found related to the effects of blood groups on VEP test.

Aim

Based on all being said and found, we established the hypothesis of a possible difference the blood groups might have on Pattern Visual Evoked Potential (pVEP) test also considering the intense antigen cells in the optic and brain tissues. Our purpose was to evaluate whether it is required to use different standards in the evaluation process of VEP measurements of healthy individuals with different blood groups.

Material and methods

The ophthalmology and neurology outpatient clinic of our hospital conducted this study in compliance with the Declaration of Helsinki principles and the approval of the institutional ethics committee (182/2022 – Clinical Trial Protocol). Our study was carried out in the VEP room of Düzce University, Faculty of Medicine, Neurology. First of all, the patients underwent eye examination. Considering the inclusion and exclusion criteria, VEP test examinations of the patients who were suitable for eye and neurologic examination were performed. The study covers the evaluation of healthy individuals within the age group of 18-50. Each patient has not only been informed on the protocols of the procedures as well as the purpose of the study itself but they were asked to submit their fully signed consent forms as well.

The participating patients were eliminated according to the following criteria: patients with addiction, drug use, alcohol and smoking history; patients with eye surgery history or high intraocular pressure, glaucoma or any kind of systemic disease like high blood pressure or diabetes; patients with neurological conditions or some kind of disorder that might reflect on optic disc, like papilledema, optic neuritis, sclerosis or similar; patients with eye conditions such as astigmatism of over 1D, anisocoria, pupil size of under 3 mm, amblyopia, diplopia, cataract, myopia or hyperopia of over 3D.

Several tests such as direct and indirect light reflexes, slit lamp biomicroscopy, best corrected visual acuity, Goldman Applanation Tonometry intraocular pressure measurement, eye movement test and fundus examination were performed for each patient. Considering any possible changes in the results after the dilation, VEP test was completed before the dilated fundus examination. The possibility of any diurnal difference was avoided by recording all measurements between 9:00–11:00 hours.

In our study, 4 different blood groups according to A and B antigens, namely A, B, AB and O as well as groups for Rh+ and Rh- according to RH factor were analyzed.

Keypoint (Dantec, Denmark) device and a 16-inch screen were used for VEP measurements. Pattern VEP (pVEP) test recording was performed by placing the three electrodes (active, reference, ground) such that active electrode was 2 cm above the protuberant occipitalis externa of occipital bone, reference electrode was on vertex and ground electrode was on the forehead, right at the hairline border. The patient was than seated in a dark room, 1 meter away from a screen with a moving board of chess designs and asked to stare at a fixed point on the board while his/her electrical potentials emerging in the bilateral occipital cortex were recorded. The recording was done for each eye separately, using the same procedure while keeping the unmeasured eye closed. The specifications of the screen were set to; 12*16 pieces of 2-inch equal-sized squares, 99% contrast based on Michelson constant, sweep rate of 30 ms/D and 5uV/D, sensitivity of 30 uV/D and filter of 1 Hz-200 Hz. Averaging was done by giving 250 stimuli and average measurements were calculated automatically. An experienced electrophysiology technician was accompanying the patient throughout the measurements in order to monitor his/her staring at the fixation point. Patients wearing glasses were measured with their glasses on. ISCEV publication on standardization criteria and detailed information of visual stimuli respond recording were taken as the base of measurements and all recordings of our study were done in compliance with the stated criteria.[8]

SPSS 21.0 (IBM Corp., Armonk, NY, USA) software was used to assess the findings. Numbers and percentages were used for categorical variables and average SD ± was used for countable variables. In order to evaluate the difference between countable variable groups, one-way analysis of variance (ANOVA) was employed for variables of normal distribution and Kruskal Wallis method was employed for variables of non-normal distribution. In cases of variables where a significant difference is determined between groups, Post Hoc analysis was employed for variables of normal distribution and Mann-Whitney U test was employed for non-normal distribution in order to determine which groups displayed that difference. $P<0.05$ was defined to be significant.

Results

The demographic specifications and the VEP latency and amplitude differences according to the ABO grouping were compared and the obtained results were analyzed in a table (Table 1).

Table 1. Comparison of demographic data and VEP results between groups[a]

Blood groups	A (30)	B (29)	AB (30)	0 (30)	p
Age	38.47±7.07	33.86±9.25	34.5±12.21	37.43±9.19	0.193*
Gender M/F	12/18	11/18	18/12	15/15	0.297[2]
L N70 (ms)	78.7±6.09	76.81±6.75	74.23±6.4	76.69±7.26	0.083*
L P100 (ms)	108.17±4.87	107.23±6.36	108.02±5.53	107.0±6.02	0.824*
R N70 (ms)	78.68±5.89	76.31±6.81	74.79±7.08	75.59±8.01	0.166*
R P100 (ms)	107.84±5.52	105.94±5.98	107.38±5.2	105.8±6.62	0.440*
LN70 amp. (μV)	2.23±2.59	1.91±3.13	1.6±1.68	1.11±1.72	0.453[1]
LP100 amp.(μV)	-6.18±2.61	-7.08±5.48	-7.33±3.4	-7.6±4.16	0.463[1]
RN70 amp. (μV)	2.62±2.79	2.33±3.41	1.57±1.55	1.01±1.75	0.058*
RP100 amp.(μV)	-6.96±2.17	-8.07±3.75	-7.99±3.43	-7.95±3.71	0.528*

[a] *One-way analysis of variance (Anova) test; [1] – Kruskal-Wallis test; [2] – Chi square test; M – male; F – female; L – left; R – right; amp – amplitude; ms –millisecond; μV – microvolt

119 individuals were included to the study out of which 30 were blood group A, 29 were blood group B, 30 were blood group AB and 30 were blood group O. Furthermore, the age average of the participating patients was similar among the study groups such as 38.47±7.07 for blood group A, 33.86±9.25 for blood group B, 34.5±12.21 for blood group AB and 37.43±9,19 for blood group O, hence the difference was found to be statistically not significant (p=0.193). Likewise, the study groups were similar in terms of gender distribution (M/F), such as 12/18 for blood group A, 11/18 for blood group B, 18/12 for blood group AB and 15/15 for blood group O, hence the difference was found to be statistically not significant (p=0.297).

Table 2. Comparison of data according to Rh blood groups[a]

Rh blood groups	Rh + (101)	Rh- (18)	p
Age	35.51±39.28	39.28±10.70	0.232[2]
Gender M/F	44/57	12/6	0.070[1]
L N70 (ms)	74.41±6.37	77.62±8.72	0.231[2]
L P100 (ms)	107.59±5.89	107.72±4.34	0.997[2]
R N70 (ms)	75.53±6.99	80.88±5.68	0.009[2]
R P100 (ms)	106.63±6.07	107.38±4.52	0.829[2]
LN70 amp. (μV)	1.65±2.41	2.08±2.08	0.417[2]
LP100 amp. (μV)	-7.33±4.21	-5.43±2.17	0.023[2]
RN70 amp. (μV)	1.80±2.55	2.34±2.46	0.436[2]
RP100 amp.(μV)	-8.04±3.28	-6.03±3.02	0.023[2]

[a] [1] – Chi square test; [2] – Mann-Whitney U test; M – male; F – female; L – left; R – right; amp – amplitude; ms – millisecond; μV – microvolt

According to our findings there was no statistically significant difference between the P100 and N70 latencies of both right and left eye of the participants in all four blood groups (p=0.440, p=0.166 right eye respectively and p=0.824, p=0.083 left eye respectively). Likewise, there was no statistically significant difference between the P100 and N70 amplitudes of all four blood groups (p=0.528, p=0.058 right eye respectively and p=0.463, p=0453 left eye respectively).

The demographic specifications and the VEP latency and amplitude differences according to the Rh classification were compared and the obtained results were analyzed in a table (Table 2).

The age average of the participating patients was similar among the study groups such as 35.51±39.28 for Rh+ blood group and 39.28±10.70 for Rh- blood group, hence the difference was found to be statistically not significant (p=0.232). Likewise, the study groups were similar in terms of gender distribution (M/F), such as 44/57 for Rh+ blood group and 12/6 for Rh- blood group, hence the difference was found to be statistically not significant (p=0.070).

According to our findings there was no statistically significant difference between the P100 latencies of both right and left eye of the participants in both groups (p=0.829, p=0.997 respectively). A prolongation of N70 latency was observed in the left eyes of Rh- group and the difference was found to be statistically significant (p=0.009). Furthermore, P100 amplitudes of both right eye and left eye of Rh+ group were found statistically high (p=0.023 both eyes). There was no statistically significant difference between the other parameters.

Discussion

In our study, we found that ABO/Rh blood groups had a statistically significant effect on some of the values related to VEP.

We know that blood groups have various effects on the body. There are studies showing that cancer cases are seen more frequently in certain blood groups.[13,14] There are also studies showing some effects of blood groups on vascular systems.[15–17] Furthermore, there are studies showing the effects of blood groups on brain and nerve systems.[15,18,19] Studies related to nerve system have also been detailed in terms of effects of blood groups on sense of pain, sense of taste and hearing (Cochlear nerve).[20–22] It is a known fact that there are studies related to the impact of blood groups on eye conditions.[6,8,9,23] In other words, there are studies covering the effect of blood groups on tissues starting from cornea and going onto brain. But there are no studies related to their effect on VEP. Comparison of data was not achieved since our study is the first study on this topic in literature.

When literature on the effects of ABO blood groups on eye conditions is reviewed, it can be seen that there is no consensus. Naderan et al. found no statistically significant correlation in their study of 626 individuals on blood groups and refraction errors relationship.[9] Furthermore, Leske et al. did not find any correlation between the ABO blood groups and primary open-angle glaucoma (POAG).[23] Yet in another study, it was con-

cluded that both zonular cataract and corneal dystrophy were seen more commonly among the people with blood group A whereas blood group B was found to have a correlation with zonular cataract and blood group O was found to have a correlation with myopia and nuclear cataract.[24] As a result of their study with POAG patients, Khan et al. found a correlation between the blood groups B and Rh-.[11] According to the study conducted by Garg and Pahwa, primary open angle glaucoma (POAG) and primary close angle glaucoma (PCAG) were more commonly seen in blood groups A and B compared to blood groups AB and O which were observed much less.[25] Additionally, Lavinsky et al. conducted a study with 16 patients who went through retrobulbar/peribulbar anesthesia due to cataract surgery where they performed VEP examinations 1 month prior and 1 month after the anesthesia and found no statistically significant difference.[26] In their blepharospasm study, Eski MT et al. showed that the decrease of the pressure on optic disc caused some changes on the VEP.[27] Teberik et al., in their blood group study including retina, choroid and optic nerve, found retina thickness were no statistically significant.[6] In similar to our study, there was no statistically significant difference in P100, P70 latency and amplitudes with respect to ABO phenotyping but there was prolongation in the N70 latencies of Rh- group and an increase in P100 amplitude in Rh+ group in terms of Rh phenotyping. Neuroophthalmological abnormalities like loss of visual field are where differential glycosylation patterns are most commonly seen. Earlier, the different features of these abnormalities have been proven to be the suppressed synthesis of some oligosaccharides portions of glycoproteins which were originating from the defects in genes that encode glycosyltransferases. Furthermore, glycosyltransferases enzymes have been shown to reflect the expression of carbohydrate markers of ABO/Rh blood groups. Therefore, the expressed glycosyltransferase in individuals with A/B or H antigen may indicate an eye disease.

On the other hand, we have not found any studies on blood groups and VEP in our literature review. It would be valuable to assess blood subgroups with regards to neuroophthalmological conditions. Further analyses ae highly recommended to get a better understanding the reasons underlying this relation.

Smith et al., conducted a study on taste bud in terms of the effects of blood groups on nerve systems and found that some senses are more dominant in certain blood groups.[22] Blood antigens which are developed either by adding N-acetyl galactosamine residue or galactose residue to H antigens of A- or B- positive individuals respectively, were only found in cell membranes of the granular cell layer. However, the relation between the antigen expression and epithelial cell differentiation is quite different with rats. That is, in case of rats, N-acetyllactosamine is in the upper spinous and granular cell layers whereas, B antigen is in the basal and parabasal layers and H antigen is in the spinous cell layer. Even though the sequence of the relation is opposite of what is observed in humans, the expression of molecules cross-reacting with ABO / H antigens of rats is related to nervous cellular differentiation. The H antigen was also observed on the surface of not only the nerve cells in the tongue epithelium but also on the majority of the nerve cells. This observation in the study suggests that carbohydrate structure can be considered as the feature of nerve cells at intermediate stage of differention.[22] We believe that there might be an effect on VEP through a similar mechanism of nerves.

Study limitations

There are some limitations to this preliminary study. First of all, it consists of relatively small sample quantity. Secondly, the lack of previous studies on VEP prevented any possible data comparison studies. Lastly, the study has been conducted with healthy patients, therefore, we do not know what the results would have been with cardiovascular patients. On the other hand, the strength of our study is the fact that it is the first and only study in literature. Hence, we do believe that this study will serve its purpose of providing a basic information of blood groups in healthy individuals, in the literature.

Conclusion

VEP test, with an ever-increasing importance, is a non-invasive measurement method which is being frequently used in neuro-ophthalmologic evaluations. On the other hand, various effects of blood groups on the body are observed with an elevating importance. Furthermore, it is highly important to know the normal values and the factors that might affect these normal values during the evaluation of VEP results. The elevating importance of the VEP test, when combined with the lack of sufficient blood group studies despite the current improvements driven us to the idea of pursuing this issue. In our study, we found statistically significant difference in VEP values related to Rh antigen. Therefore, it suggests that different VEP normal values can be used for patients according to the Rh group. Nevertheless, we do suggest to conduct further studies on a multi-centered level with a higher quantity of participants.

Declarations

Funding

There is no person or organization to support this work financially. The authors do not have a relationship with the sponsor or a commercial company regarding the study.

Author contributions

Conceptualization, M.T.E. and A.Y.; Methodology, M.T.E. and A.Y.; Software, A.Y.; Validation, M.T.E, A.Y., A.A.H.A, H.Ş. and T.S.; Formal Analysis, M.T.E, A.Y., A.A.H.A, H.Ş. and T.S.; Investigation, M.T.E, A.Y., A.A.H.A, H.Ş. and T.S.; Resources, M.T.E, A.Y., A.A.H.A, H.Ş. and T.S.; Data Curation, A.Y., A.A.H.A, H.Ş. and T.S.; Writing – Original Draft Preparation, M.T.E, A.Y., A.A.H.A, H.Ş. and T.S.; Writing – Review & Editing, M.T.E, A.Y., A.A.H.A, H.Ş. and T.S.; Visualization, M.T.E and A.Y.; Supervision, M.T.E. and A.Y.; Funding Acquisition, A.Y.

Conflicts of interest

The authors declare that they have no conflict of interest.

Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval

Ethics committee of Düzce University approved the study design (no. 2022-182) on 07/11/2022.

References

1. Pisk SV, Vuk T, Ivezić E, Jukić I, Bingulac-Popović J, Filipčić I. ABO blood groups and psychiatric disorders: a Croatian study. *Blood Transfus*. 2019;17(1):66. doi: 10.2450/2018.0266-17
2. Wang Y, Zhou BY, Zhu CG, et al. Distribution of ABO blood groups and coronary artery calcium. *Heart Lung Circ.* 2017;26(6):593-598. doi: 10.1016/j.hlc.2016.10.014
3. Shim HJ, Lee R, Shin MH, et al. Association between ABO genotype and risk of hepatocellular carcinoma in Koreans. *Asian Pac J Cancer Prev.* 2015;16(7):2771-2775. doi: 10.7314/apjcp.2015.16.7.2771
4. Pourafkari L, Ghaffari S, Ahmadi M, Tajlil A, Nader ND. Association of ABO blood types with the risk of in-stent restenosis. *Perfusion*. 2015;30(6):507-513. doi: 10.1177/0267659115573098
5. Franchini M, Favaloro EJ, Targher G, Lippi G. ABO blood group, hypercoagulability, and cardiovascular and cancer risk. *Crit Rev Clin Lab Sci.* 2012;49(4):137-149. doi: 10.3109/10408363.2012.708647
6. Teberik K, Eski MT. Association of ABO blood groups and Rh factor with retinal and choroidal thickness. *Eur J Ophthalmol.* 2019;29(2):234-238. doi: 10.1177/1120672118783629
7. Tamcelik N. *Ocular Electrophysıology.* 2011th ed. Istanbul: Turkish Ophthalmological Association Education Publications; 2011.
8. Chan JH, Dua HS, Powell-Richards A, Jones DRE, Harris IM. Effect of ABO blood group mismatching on corneal epithelial cells: an in vitro study. *Br J Ophthalmol.* 2001;85(9):1104-1109. doi: 10.1136/bjo.85.9.1104
9. Naderan M, Rajabi MT, Shoar S, et al. Distribution of ABO and Rh Blood Groups in Patients With Keratoconus: A Case–Control Study. *Eye & Contact Lens*. 2015;41(4):214-217. doi: 10.1097/ICL.0000000000000098
10. Ardjomand N, Komericki P, Klein A, Mattes D, El-Shabrawi Y, Radner H. AB0 blood group expression in corneal allograft failures. *Ophthalmologe* 2005;102:981-986. doi: 10.1007/s00347-005-1199-1
11. Khan MI, Micheal S, Akhtar F, Naveed A, Ahmed A, Qamar R. Association of ABO blood groups with glaucoma in the Pakistani population. *Can J Ophthalmol.* 2009;44(5):582-586. doi: 10.3129/i09-104
12. Dua HS, Chan J, Gomes JAP, Azuara-Blanco A. Adverse effect of blood group ABO mismatching on corneal epithelial cells. *Lancet*. 1998;352(9141):1677-1677. doi: 10.1016/s0140-6736(05)61448-1
13. Englisch C, Moik F, Nopp S, Raderer M, Pabinger I, Ay C. ABO blood group type and risk of venous thromboembolism in patients with cancer. *Blood Adv.* 2022;6(24):6274-6281. doi: 10.1182/bloodadvances.2021006283
14. Cote DJ, Downer MK, Zoltick ES, Smith TR, Stampfer MJ. ABO blood group and risk of glioma. *Neuro Oncol.* 2017;19(6):871-873. doi: 10.1093/neuonc/nox013
15. Lotz RC, Welter C da S, Ramos SA, Ferreira LE, Cabral NL, FRANÇA PHC de. ABO blood group system and occurrence of ischemic stroke. *Arq Neuropsiquiatr.* 2021;79:1070-1075. doi: 10.1590/0004-282X-ANP-2020-0219
16. Dentali F, Sironi AP, Ageno W, Crestani S, Franchini M. ABO blood group and vascular disease: an update. In: *Semin Thromb Hemost.* Thieme Medical Publishers; 2013:049-059. doi: 10.1055/s-0033-1363460
17. Huang X, Zou Y, Li L, Chen S, Hou J, Yu B. Relation of ABO blood groups to the plaque characteristic of coronary atherosclerosis. *Biomed Res Int.* 2017;2017:2674726. doi: 10.1155/2017/2674726
18. Richards O, Cromie KJ, Akhunbay-Fudge C, et al. ABO blood group in aneurysmal subarachnoid haemorrhage—a pilot study. *Acta Neurochir (Wien).* 2022;164(2):507-515. doi: 10.1007/s00701-021-05079-5
19. Kumarguru BN, Pallavi P, Manjunath GV, Vasan TS, Rajalakshmi BR. Histopathological study of central nervous system lesions: emphasizing association of neoplasms with ABO blood groups. *J Clin Diagn Res. JCDR.* 2017;11(4):EC15. doi: 10.7860/JCDR/2017/23863.9644
20. Simoni AH, Jerwiarz A, Randers A, Gazerani P. Association between ABO blood types and pain perception. *Somatosens Mot Res.* 2017;34(4):258-264. doi: 10.1080/08990220.2018.1425675
21. Yang Y, Hood LJ, McPherson B. Association between ABO blood group status and cochlear/neural function: auditory brainstem response findings. *Acta Otolaryngol.* 2021;141(3):273-278. doi: 10.1080/00016489.2020.1858236
22. Smith DV, Klevitsky R, Akeson RA, Shipley MT. Taste bud expression of human blood group antigens. *J Comp Neurol.* 1994;343(1):130-142. doi: 10.1002/cne.903430110

23. Leske MC, Nemesure BB, He Q, Mendell N, Polednak A. Open-angle glaucoma and blood groups: the Barbados Eye Study. *Arch Ophthalmol.* 1996;114(2):205-210. doi: 10.1001/archopht.1996.01100130199017
24. Padma T, Murty JS. Association of genetic markers with some eye diseases. *Acta Anthropogenet*. 1983;7(1):1-12.
25. Garg MP, Pahwa JM. Primary glaucoma and blood groups. *Indian J Ophthalmol*. 1965;13(4):127.
26. Lavinsky J, Gus PI, Ehlers JA, Roehe D, Nora DB. Visual-evoked potentials: assessment of retrobulbar and peribulbar anesthesia. *J Cataract Refract Surg*. 2000;26(10):1529-1532. doi: 10.1016/s0886-3350(00)00447-8
27. Eski MT, Teberik K, Sezer T, Ayasli AH, Ozturk A. Visual evoked potential changes following Botox administration in patients with blepharospasm. *Int Ophthalmol.* 2023:43(6):2101-2107. doi: 10.1007/s10792-023-02639-9

Wydawnictwo UR 2023
ISSN 2544-1361 (online)
doi: 10.15584/ejcem.2023.3.26

http://www.ejcem.ur.edu.pl
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2023; 21 (3): 582–589

ORIGINAL PAPER

Nursing care for symptoms seen in patients undergoing palliative surgery – a retrospective study

Özge Uçar , Sevim Çelik , Elif Karahan , Sibel Altıntaş

Department of Nursing, Faculty of Health Sciences, Bartin University, Bartin, Turkey

ABSTRACT

Introduction and aim. Palliative care is an approach to improve the quality of life of patients and their families facing problems associated with life-threatening illnesses or old age, to manage their pain, distress, and other symptoms, improving their quality of life, and providing psychological support. This study was designed to identify the care practices planned by nurses for the health issues of palliative surgical patients.

Material and methods. This is a retrospective, cross-sectional, descriptive study. The data was collected from digital records of the two state hospitals in the Western Black Sea Region of Turkey between January 2019 and January 2020. The research was completed with a total of 391 data of patients undergoing palliative surgery. Frequency and percentage distributions were used in descriptive statistics.

Results. The frequency of symptoms observed in patients were determined as cough and sputum (81.8%), dehydration (73.9%), dyspnea (71.9%), fatigue (49.9%), loss of appetite-weight (49.9%), insomnia (44%), pain (37.6%), nausea (29.2%), and constipation (23.8%). The planned nursing diagnoses for these symptoms were identified as risk for falls, imbalanced nutrition, risk for impaired skin integrity, risk for infection, risk for aspiration, deficient knowledge, risk for impaired respiratory function, constipation, ineffective airway clearance, pain, risk for deficient fluid volume, sleep deprivation, impaired gas exchange respectively.

Conclusion. This study highlights the insufficient planning of nursing care for the symptoms of palliative surgical patients receiving palliative care in our country. Therefore, it is recommended that palliative care nurses be supported with in-service training on appropriate care planning topics.

Keywords. nursing, nursing care, palliative care, palliative surgery, symptom management

Introduction

The advancement of medical care with technology and the development of new treatments for chronic illnesses have increased the fight against diseases and extended survival times. However, these advancements also lead to longer experiences of chronic illness and more pain and suffering caused by these illnesses.[1,2] In this sense, centers where patients and healthy individuals can easily access healthcare services, receive continuous, comprehensive, and integrated care, and particularly receive effective support in palliative care are crucial for family and community health.[3,4]

Palliative care is an approach to improve the quality of life of patients and their families facing problems associated with life-threatening illnesses or old age, to manage their pain, distress, and other symptoms, improving their quality of life, and providing psychological support (WHO, 2020). Palliative care is provided to individuals with life-threatening illnesses such as motor neuron diseases like Alzheimer's and dementia, cancer,

Corresponding author: Özge Uçar, e-mail: ozgeenginucar@gmail.com

Received: 12.04.2023 / Revised: 25.06.2023 / Accepted: 16.07.2023 / Published: 30.09.2023

Uçar Ö, Çelik S, Karahan E, Altıntaş S. *Nursing care for symptoms seen in patients undergoing palliative surgery – a retrospective study*. Eur J Clin Exp Med. 2023;21(3):582–589. doi: 10.15584/ejcem.2023.3.26.

heart disease, cirrhosis, chronic obstructive pulmonary disease, diabetes, HIV/AIDS, kidney failure, multiple sclerosis, rheumatoid arthritis, Parkinson's disease, and tuberculosis.[3,5] The aim is to prevent the symptoms associated with all of these illnesses such as pain, dyspnea, fatigue, and others through the use of pharmacological and non-pharmacological methods, and to provide the patient with a comfortable life until the end of life.[6-8]

Palliative surgery is a surgical intervention aimed at relieving a patient's symptoms and thereby improving their quality of life, typically in those with a poor prognosis or life-threatening illness. The treatment and care techniques used in palliative surgery run parallel to those used in curative surgery.[9] Nowadays, "palliative surgery" is a term used for all surgical procedures aimed at alleviating symptoms caused by advanced disease and improving quality of life. Overlooking the necessity of palliative care in surgical patients can result in greater discomfort for patients in the later stages of the disease or even in the end-of-life period.[6] Clinicians and nurses need to have appropriate experience and knowledge to provide good palliative care.[2,6] Palliative surgery is a series of planned surgical procedures aimed at supporting patients to live as well as possible for as long as possible and determining appropriate treatment. Patient and family participation in care should be ensured to help them cope with the situation in their remaining life.[10]

During this period, nurses have significant responsibilities. It is undeniable that timely and effective nursing interventions facilitate symptom management for patients requiring palliative treatment and care, and lead to positive patient outcomes such as improving patient and family quality of life.[11]

Palliative care nursing aims to approach the patient and family holistically, in collaboration with all disciplines, in order to improve the patient's quality of life.[12] In pursuit of this goal, nurses play a prominent role not only as evidence-based care providers, but also as educators and leaders.[13] Given all of this, it is essential that nurses possess the necessary knowledge and skills in palliative care.[6] Particularly in the palliative care phase where patients may be struggling with symptoms, nurses should have a strong understanding of symptom management and develop and implement comprehensive care plans that encompass physical, psychosocial, and spiritual dimensions.[6] Studies have shown that proper symptom management in palliative care reduces treatment time and costs, and shortens hospital stays.[1,13] In the literature, many studies are showing that palliative surgery reduces symptoms. On the other hand, there are findings related to symptom management and nursing care in all palliative patients. However, no data investigating the effect of nursing care on symptoms in palliative surgery patients were found. However, it is thought that the success of palliative surgery may also be affected by the quality of nursing care provided during and after the perioperative period.

Aim

This study was designed to identify nursing diagnoses addressed by nurses for the health problems and symptoms of palliative surgical patients hospitalized in a palliative care unit.

Material and methods

Study design

This study is a retrospective cross-sectional descriptive research.

Study questions

- What are the common symptoms in palliative surgery patients?
- What are the common treatment methods used in palliative surgery patients?
- What are the commonly used nursing diagnoses in palliative surgery patients?

Sample

This retrospective study was conducted on palliative surgical patients who were treated at the Palliative Care Centers of two state hospitals located in the Western Black Sea region between January 2019 and January 2020. Palliative surgical patients who were over 18 years of age, had undergone at least one surgical procedure, had been hospitalized at least once, and had been discharged with complete data were included in the study. Patients who had not undergone palliative surgery as their hospitalization purpose but had undergone it once during their hospitalization were also included in the study. For patients with multiple hospitalizations, only the first hospitalization was taken into account. A total of 391 patient records were included in the study.

Data collection

In this study, data was collected over a period of 1 year by the researcher in the palliative care centers of two state hospitals located in the Western Black Sea region. The palliative care centers were 16-bed and 18-bed units accepting adult palliative patients. There were 12 nurses and 14 nurses working in the unit, all with at least a bachelor's degree. The nurses' years of experience ranged from 2 to 22 years. Data were obtained retrospectively by examining the hospital's digital archives of 527 patients who were followed up and treated in the palliative care center. Only the first admission of patients with multiple admissions was evaluated. 136 patients who did not meet the inclusion criteria, stayed less than 24 hours or had insufficient medical records were excluded from the study. A total of 391 patients were in-

cluded in the study, and their demographic characteristics, admission indications, comorbidities, presence and type of malignancy, length of stay, Glasgow coma scale, pain scale, symptoms, nursing diagnoses, and discharge status were recorded using a data collection form prepared by the literature, based on the evaluation of their first admission.

Table 1. Characteristics of patients (n=391)[a]

Characteristics		n	%
Gender	Female	201	41.5
	Male	190	39.3
Education status	Illiterate	98	25.1
	Literate	220	56.3
	Primary school	18	4.6
	High school	43	11.3
	University	11	2.8
Smoking status	Smoking	120	30.7
	Not smoking	271	69.3
Nutrition	Oral	61	15.6
	NG	25	6.4
	PEG	305	78.0
Dependent Mobilization	Dependent	299	76.5
	Independent	92	23.5
		Mean ±SD	**Min.-Max.**
Age		74.63±11.28	44–96
BMI		25.31±4.03	15.43–34.13
Length of stay		26.12±12.31	3–92
Assessment scales	Glasgow Coma Scale	9.73±1.48	8–13
	Pain Scale*	3.69±2.44	0–6
	Braden Scale	11.49±1.61	0–12

[a] SD – standard deviation; BMI – body mass index; * – the VAS (Visual Analog Scale) pain scale has been used

Statistical analysis

The collected data was analyzed usiwereIBM SPSS Statistics (V25.0, IBM Corporation). Descriptive statistics such as numbers, percentages, means, and standard deviations were used to evaluate the obtained data. Cross table was used for Symptoms, palliative surgery operations and nursing diagnosis. Skewness and kurtosis coefficients of the scores were examined and it was observed that these coefficients were within the range of ±2 (George and Mallery, 2010). No missing data was detected in the dataset.

Ethics approval

This study was conductedbyh the Helsinki Declaration. The study was approved by the institutional ethics committee of Bartin University (No. 2020-BRT-08). Institutional permission was obtained from the Ministry of Health of the Republic of Turkey, the Provincial Health Directorate to which the hospital is affiliated, and the hospital (2020-05-20T-14). Oral consent was obtained from the responsible physician and nurses of the palliative care unit of the state hospital where the data was collected. Since this was a retrospective study and personal data ofthe patients were not included, informed consent from patients was not necessary and the data were obtained from the hospitals' digital databases.

Results

The mean age of the patients was 74.63±11.28. The average length of stay in the palliative care center was 26.12±12.31 days. Of the patients, 41.5% were female and 39.3% were male. 76.5% of the patients had impaired motor activity, and 30.7% were smokers. The mean body mass index of the patients was found to be 25.31±4.03. 15.6% of the patients were receiving oral nutrition, 6.4% were receiving nasogastric tube feeding, and 78% were receiving percutaneous endoscopic gastrostomy tube feeding. The average Glasgow Coma Scale score of the patients was 9.73, the average Braden Scale score for pressure ulcer risk assessment was 11.49, and the average pain score measured with VAS was 3.69 (Table 1).

Nursing diagnoses planned by nurses for the symptoms were determined as follows: risk for falls (98.2%) was the most common nursing diagnosis, followed by Imbalanced nutrition (87.7%), Risk for impaired skin integrity (72.6%), Risk for infection (63.4%), Risk for aspiration (60.1%), Deficient knowledge (50.9%), Risk for impaired respiratory function (48.6%), Constipation (17.4%), Ineffective airway clearence (15.6%), Pain (10.7%), Risk for deficient fluid volume (10.7%), Impaired gas exchange (10.2%), and Sleep deprivation (8.2%) (Table 2).

Table 2. Nursing diagnoses applied to palliative care patients (n=391)[a]

Nursing Diagnoses	n	%
Risk for aspiration	235	60.1
Risk for impaired respiratory function	190	48.6
Risk for infection	248	63.4
Risk for impaired skin integrity	284	72.6
Risk for falls	384	98.2
Imbalanced nutrition	343	87.7
Pain	42	10.7
Deficient knowledge	199	50.9
Risk for deficient fluid volume	42	10.7
Ineffective airway clearance	61	15.6
Anxiety	33	8.4
Constipation	68	17.4
Sleep deprivation	32	8.2
Impaired gas exchange	40	10.2
Total	391	100

[a] Multiple responses

The frequency of symptoms observed in patients was determined as wounds (93.9%), cough (81.8%), dehydration (73.9.2%), dyspnea (71.9%), fatigue (49.9%),

loss of appetite-weight (49.9%), insomnia (44%), pain (37.6%), nausea and vomiting (29.2%), and constipation (23.8 %) (Table 3).

According to Table 3, it was determined that nurses most frequently provided care to patients with a diagnosis of fall risk for all symptomsWhen evaluating nursing diagnoses related to experienced symptoms, it was found that there were relatively low frequencies of diagnoses for patients experiencing specific symptoms. Among patients with pain symptoms, the diagnosis of pain was given in 3.3% of cases. For those experiencing insomnia, disruptions in sleep patterns were diagnosed in 4.3% of cases. Constipation diagnoses were assigned for patients with constipation issues at a rate of 4.1%. In cases where patients developed wounds, the diagnoses of infection risk (59.3%) and impaired skin integrity risk (67.5%) were more prevalent. For patients with dehydration, the diagnosis of fluid volume imbalance risk was identified in 8.7% of cases. Similarly, among patients complaining of cough and sputum, nursing diagnoses such as ineffective breathing pattern (40.2%), ineffective airway clearance (13.6%), and impaired gas exchange (5.4%) were identified by nurses with less frequency compared to the diagnosis of fall risk. Nursing diagnoses of nutritional imbalance (45.5%) and fluid volume imbalance (5.4%) were also assigned less frequently as nursing diagnoses compared to the diagnosis of fall risk in patients experiencing appetite and weight lossPatients experiencing dyspnea were diagnosed with a risk of impaired breathing pattern at a rate of 32.7%, ineffective airway clearance at a rate of 11.3%, and impaired gas exchange at a rate of 7.9%. In patients suffering from weakness and fatigue, the most common diagnosis was a risk of falls, accounting for 48.6% (Table 3).

According to Table 4, the most common palliative surgical treatments were as follows: percutaneous endoscopic gastrostomy (PEG) at a rate of 71.6%, wound surgery at a rate of 71.1%, tracheostomy at a rate of 60.9%, colostomy at a rate of 31.4%, cystostomy procedure at a rate of 30.6%, and biopsy at a rate of 27%.

Table 4 includes a cross-tabulated table comparing nursing diagnoses according to palliative surgical treatments. According to this, the most commonly assigned nursing diagnosis in patients, regardless of the type of palliative surgical procedure, was the risk of falls. In patients with PEG, the nursing diagnosis of risk of falls was followed by diagnoses of nutritional imbalance (60.3%), risk of aspiration (44.4%), and fluid volume imbalance

Table 3. Distribution of nursing diagnoses according to patients' symptoms in palliative care centers (n=391)[a]

Nursing Diagnosis*		Risk for aspiration	Risk for impaired respiratory function	Risk for infection	Risk for impared skin integrity	Risk for falls	Imbalanced Nutrition	Pain	Deficient Knowledge	Risk for deficient fluid volume	Ineffective airway clearance,	Anxiety	Constipation	Sleep deprivation	Gas Exchange, impaired	Total
		Symptoms*														
Pain	n	94	80	94	103	146	131	13	77	12	25	11	26	14	14	147
	%	24.0	20.5	24.0	26.3	37.3	33.5	3.3	19.7	3.1	6.4	2.8	6.6	3.6	3.6	37.6
Nausea and vomiting	n	67	54	77	85	110	104	9	55	12	20	8	25	12	11	114
	%	17.1	13.8	19.7	21.7	28.1	26.6	2.3	14.1	3.1	5.1	2.0	6.4	3.1	2.8	29.2
Insomnia	n	96	85	99	126	169	147	15	96	15	22	10	27	17	12	172
	%	24.6	21.7	25.3	32.2	43.2	37.6	3.8	24.6	3.8	5.6	2.6	6.9	4.3	3.1	44.0
Constipation	n	55	43	56	69	92	84	13	48	9	16	11	16	6	12	93
	%	14.1	11.0	14.3	17.6	23.5	21.5	3.3	12.3	2.3	4.1	2.8	4.1	1.5	3.1	23.8
Wounds**	n	223	178	232	264	362	321	42	186	42	55	33	63	29	39	367
	%	57.0	45.5	59.3	67.5	92.6	82.1	10.7	47.6	10.7	14.1	8.4	16.1	7.4	10.0	93.9
Dehidratation	n	176	138	190	202	284	252	37	139	34	44	28	48	25	33	289
	%	45.0	35.3	48.6	51.7	72.6	64.5	9.5	35.5	8.7	11.3	7.2	12.3	6.4	8.4	73.9
Fatique- weakness	n	123	73	119	145	190	166	23	109	27	18	18	21	12	24	195
	%	31.5	18.7	30.4	37.1	48.6	42.5	5.9	27.9	6.9	4.6	4.6	5.4	3.1	6.1	49.9
Coughing-sputum	n	184	157	203	235	313	280	33	161	32	53	27	61	24	34	320
	%	47.1	40.2	51.9	60.1	80.1	71.6	8.4	41.2	8.2	13.6	6.9	15.6	6.1	8.7	81.8
Loss of appetite-weight	n	121	105	130	131	191	178	28	86	18	34	21	33	22	21	195
	%	30.9	26.9	33.2	33.5	48.8	45.5	7.2	22.0	4.6	8.7	5.4	8.4	5.6	5.4	49.9
Dyspnea	n	164	128	183	206	276	247	31	142	31	44	25	53	22	31	281
	%	41.9	32.7	46.8	52.7	70.6	63.2	7.9	36.3	7.9	11.3	6.4	13.6	5.6	7.9	71.9
Total	n	235	190	248	284	384	343	42	199	42	61	33	68	32	40	391
	%	60.1	48.6	63.4	72.6	98.2	87.7	10.7	50.9	10.7	15.6	8.4	17.4	8.2	10.2	100

[a] * – multiple responses; ** – diabetic ulcers, pressure ulcers, arterial ulcers, venous leg ulcers

Table 4. Distribution of nursing diagnoses according to palliative surgical treatments in palliative care centers (n=391)[a]

Nursing Diagnosis*		Risk for aspiration	Risk for impaired respiratory function	Risk for infection	Risk for impared skin integrity	Risk for falls	Imbalanced Nutrition	Pain	Deficient Knowledge	Risk for deficient fluid volume	Ineffective airway clearance,	Anxiety	Constipation	Sleep deprivation	Gas Exchange, impaired	Total
		Surgical treatments for palliative purposes*														
Drains	n	76	55	76	92	117	106	15	59	13	19	13	18	11	17	121
	%	20.9	15.2	20.9	25.3	32.2	29.2	4.1	16.3	3.6	5.2	3.6	5.0	3.0	4.7	33.3
PEG	n	161	124	161	187	254	219	29	136	31	34	21	40	21	28	260
	%	44.4	34.2	44.4	51.5	70.0	60.3	8.0	37.5	8.5	9.4	5.8	11.0	5.8	7.7	71.6
Tracheostomy	n	125	111	145	160	215	193	23	104	25	35	18	43	20	22	221
	%	34.4	30.6	39.9	44.1	59.2	53.2	6.3	28.7	6.9	9.6	5.0	11.8	5.5	6.1	60.9
Colostomy	n	69	52	73	87	109	101	13	55	12	19	11	19	9	14	114
	%	19.0	14.3	20.1	24.0	30.0	27.8	3.6	15.2	3.3	5.2	3.0	5.2	2.5	3.9	31.4
Cystostomy	n	69	53	73	84	107	97	14	52	13	16	13	19	10	15	111
	%	19.0	14.6	20.1	23.1	29.5	26.7	3.9	14.3	3.6	4.4	3.6	5.2	2.8	4.1	30.6
Wound surgery**	n	159	129	162	184	252	217	28	135	28	35	20	42	23	26	258
	%	43.8	35.5	44.6	50.7	69.4	59.8	7.7	37.2	7.7	9.6	5.5	11.6	6.3	7.2	71.1
Biopsy	n	58	45	60	76	94	87	11	50	9	16	10	15	7	13	98
	%	16.0	12.4	16.5	20.9	25.9	24.0	3.0	13.8	2.5	4.4	2.8	4.1	1.9	3.6	27
Total	n	217	180	230	264	356	315	39	183	42	56	30	64	29	37	363
	%	59.8	49.6	63.4	72.7	98.1	86.8	10.7	50.4	11.6	15.4	8.3	17.6	8.0	10.2	100

[a] * – multiple responses; ** – greft, flept, debritman, VAC

(8.5%). In patients who underwent tracheostomy, nursing diagnoses included a risk of aspiration at a rate of 34.4%, risk of impaired breathing pattern at a rate of 30.6%, ineffective airway clearance at a rate of 9.6%, and impaired gas exchange at a rate of 6.1%. In patients who underwent wound surgery procedures such as grafting, flaps, debridement, and vacuum-assisted closure (VAC), the most common nursing diagnosis after the risk of falls was impaired skin integrity risk, which was identified at a rate of 50.7%. Among patients who underwent biopsy procedures, the highest percentage of diagnoses was the risk of falls at 25.9%, followed by impaired skin integrity risk at 20.9%, knowledge deficit at 13.8%, and pain at 3% (Table 4).

Discussion

Palliative care has become an important field that the whole world has been giving importance to in recent years due to the increasing aging population and related chronic diseases. In the terminal phase of patients, it is evident that palliative care is necessary for maximizing their quality of life as much as possible in their final moments, and for the participation of both the patient and their family. This retrospective cross-sectional study was conducted with 391 patients to determine the palliative care profile in Turkey and the interventions of nurses.

In our current study, the most common symptoms were coughing, dehydration, wounds, dyspnea, fatigue, insomnia, pain, nausea, and constipation. In a study on care in the terminal phase, 72% of the patients had cancer.[14] Thrane et al. found that 60.5% of patients were admitted to palliative care due to pain, 53.9% due to neurological diseases, and 51.2% due to congenital disorders.[15] In a systematic review of palliative care in Australia, the main reasons for admission were cancer (42%), non-cancer diseases (8%), and terminal conditions (12%), such as heart failure and renal failure.[16] The same study reported that pain was more common in male patients, and the most common symptoms for all patients were difficulty breathing, nausea/vomiting, and dementia.[16] In a study conducted in Turkey, the most common reasons for admission were inadequate nutrition, care education, pain, and pressure ulcers. The frequency of reasons for admission to palliative care can vary from region to region, and the low number of patients admitted for cancer in our study may be due to the lack of oncology specialists in our region. Another study conducted in Turkey showed similar reasons for admission to palliative care.[17] Therefore, while our study is parallel to Miniksar and Aydın's study, it differs from other studies in terms of reasons for admission. However, differences in reasons for admission were observed in all other studies.

In our study, the most common symptoms observed were cough, dehydration, dyspnea, weight loss, fatigue, insomnia, pain, nausea, and constipation in order of frequency. According to an analysis conducted by Pang et al., fatigue was identified as the most frequently encountered symptom, with a rate of 96%. This was followed by sleep problems at 94.8% and pain at 92.5%.[18] In a study conducted by Cantero et al. on symptom management in palliative care patients, the most common symptoms

were ranked as pain, fatigue, nausea, depression, anxiety, drowsiness, shortness of breath, anorexia, sleep disorders, and malaise.[19] In a study conducted in 2022, the most common problems in palliative patients were reported as nausea, pain, fatigue, and sleep problems.[20] In a study conducted on 145 patients in Denmark, the most common symptoms were recorded as pain and deterioration in physical condition.[21] In another study, the most common symptoms in palliative patients were reported as nausea, pain, drowsiness, shortness of breath, and loss of appetite, respectively.[22] Considering all these results, similar symptoms were observed in palliative care patients at different frequencies. This may be due to cross-cultural differences in the populations of patients admitted to palliative care clinics and shortcomings in healthcare. When examining the nursing diagnoses used according to patients' symptoms, it can be observed that nurses most frequently assigned the diagnosis of fall risk for all symptoms. It has been determined that nursing diagnoses consistent with the symptoms were assigned; however, the rates were found to be quite low. This situation suggests that nurses may not be actively and accurately utilizing nursing diagnoses according to patients' symptoms. Furthermore, the diagnosis of anxiety was assigned to a total of 8.4% of all patients. No nursing diagnoses related to the spiritual and religious care of palliative patients were found. In previous studies, it has been noted that the most common nursing diagnoses in palliative patients are anxiety and spiritual care diagnoses.[23,24]

According to our research, the most commonly performed palliative surgical procedures for patients in palliative care clinics include PEG placement, wound surgery, tracheostomy, colostomy, cystostomy, and biopsy procedures. It has been observed that the most commonly used nursing diagnosis in patients, regardless of the type of surgical procedure, is the risk for fall. The utilization rate of diagnosis specific to surgical procedures is indeed quite low. This situation indicates that nurses are not utilizing specific diagnoses for palliative surgical patients. On the other hand, it is also considered that this situation may stem from the nurses' inadequacy in documentation, despite providing comprehensive care in all areas.

Patients receiving palliative care require planned and comprehensive nursing care during their end-of-life period. When evaluating the data obtained, it was found that the most common diagnosis given to patients was malnutrition. However, it is noteworthy that diagnoses such as risk for fall, risk for infection, risk for impaired skin integrity, and risk for impaired respiratory function followed closely. According to a study published by Carpenter et al., nursing interventions focused on three main strategies: (1) advanced care planning, (2) personnel education (e.g.. teaching general concepts in a classroom or seminar) or patient/family education (e.g. guiding practical palliative care skills), and (3) integration of the palliative care team.[25] In their study on palliative patients with lung cancer, Naito et al. conducted a feasibility study that included early multimodal interventions for nutrition, exercise and mobility, and risk for fall, and found that early interventions were beneficial for patients.[26] A study conducted by Zongo et al. in oncology patients who underwent palliative surgery in Africa, it was reported that patients experienced an 80% reduction in symptoms after palliative surgery.[27] However, no extensive nursing diagnoses or interventions specifically targeting symptoms seen in palliative care patients have been found in the literature. Nevertheless, our study identified similar nursing diagnoses such as the risk for fall and imbalanced nutrition based on limited data. However, our research revealed that the nursing diagnoses were not specific to patient symptoms, and there was a statistical increase in the same diagnoses. On the other hand, it is notable that risk diagnoses were used more frequently than actual diagnoses. Therefore, according to the research results, patients should be primarily diagnosed with actual diagnoses such as impaired respiratory function, imbalanced fluid volume, fatigue, sleep deprivation, pain, nausea, and constipation. Considering the characteristics of the nurses working in the palliative care centers where the data was collected, it is worth considering that palliative care nurses may be inadequate in determining the current situation of patients and planning appropriate nursing care and diagnoses.

Study limitations

This research was obtained from the records of a palliative care clinics in the two state hospitals located in the Western Black Sea region of Turkey. Due to the lack of an oncologist and the responsible physician being a general surgeon in the hospital where the study was conducted. patients admitted to the clinic are mostly followed up for palliative surgical purposes. Therefore, this study cannot be generalized to all palliative patients and palliative care services.

Conclusion

This study highlights the wide range of symptoms are frequently seen in patients receiving palliative care surgery in Turkey. It is seen that the duration and variety of medical treatments given with these symptoms may increase. In addition, it was found that the nursing diagnoses planned for symptoms were not parallel and related to the symptoms and that similar diagnoses were often made for all patients. Considering the effects of nursing care on symptom management in palliative surgery patients, it emphasizes that the nursing care planning applied for symptoms in patients is inadequate. Therefore, it is recommended that palliative care nurs-

es be supported with in-service training on appropriate care planning. On the other hand, it is recommended that future studies be conducted on repeated hospitalizations in palliative patients in order to determine the frequency of recurrence of certain symptoms and needs analysis for this purpose.

Declarations

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Author contributions

Conceptualization, Ö.U. and S.Ç.; Methodology, Ö.U.; Software, Ö.U.; Validation, Ö.U., S.Ç. and E.K.; Formal Analysis, Ö.U. and S.A.; Investigation, Ö.U.; Resources, E.K. and S.A.; Data Curation, Ö.U. and S.Ç.; Writing – Original Draft Preparation, Ö.U., S.Ç., E.K. and S.A.; Writing – Review & Editing, Ö.U. and S.Ç.; Visualization, Ö.U.; Supervision, S.Ç.

Conflict of interest

No conflict of interest has been declared by the authors.

Data availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethics approval

This study was approved by the local ethics committee (Ethics Committee of Bartin University-Bartin date: 15.08.2020 decision number: 2020-08).

References

1. Graney BA, Au DH, Barón AE, et al. Advancing symptom alleviation with palliative treatment (ADAPT) trial to improve quality of life: A study protocol for a randomized clinical trial. *Trials*. 2019;20(1):355. doi: 10.1186/s13063-019-3417-1
2. Harden K, Price D, Duffy E, Galunas L, Rodgers C. Palliative care: improving nursing knowledge, attitudes, and behaviors. *Clin J Oncol Nurs.* 2017;21(5):232-238. doi: 10.1188/17.CJON.E232-E238
3. Schlick CJR, Bentrem DJ. Timing of palliative care: When to call for a palliative care consult. *J Surg Oncol.* 2019;20(1):30-34. doi: 10.1002/JSO.25499
4. Sekse RJT, Hunskår I, Ellingsen S. The nurse's role in palliative care: A qualitative meta-synthesis. *J Clin Nurs.* 2018;27(1-2):e21-e38. doi: 10.1111/JOCN.13912
5. Sawatzky R, Porterfield P, Roberts D, et al. Embedding a palliative approach in nursing care delivery: an integrated knowledge synthesis. *ANS Adv Nurs Sci.* 2017;40(3):263-279. doi: 10.1097/ANS.0000000000000163
6. Fahy BN. Introduction: Role of palliative care for the surgical patient. *J Surg Oncol*. 2019;120(1):5-9. doi: 10.1002/JSO.25449
7. Temel JS, Greer JA, El-Jawahri A, et al. Effects of early integrated palliative care in patients with lung and gı cancer: A randomized clinical trial. *J Clin Oncol.* 2017;35(8):834-841. doi: 10.1200/JCO.2016.70.5046
8. Uzelli Yilmaz D, Akın Korhan E, Khorshid L. Evaluation of nursing care quality in a palliative care clinic. *Journal of Human Sciences*. 2017;14(3):2968. doi: 10.14687/jhs.v14i3.4828
9. Bergdahl E, Ternestedt BM, Berterö C, Andershed B. The theory of a co-creative process in advanced palliative home care nursing encounters: A qualitative deductive approach over time. *Nursing Open*. 2018;6(1):175-188. doi: 10.1002/NOP2.203
10. Mounsey L, Ferres M, Eastman P. Palliative care for the patient without cancer. *Australian Journal of General Practice*. 2018;47(11):765-769. doi: 10.31128/AJGP-07-18-4625
11. Hagmann C, Cramer A, Kestenbaum A, et al. Evidence-based palliative care approaches to non-pain physical symptom management in cancer patients. *Semin Oncol Nurs.* 2018;34(3): 227-240. doi: 10.1016/j.soncn.2018.06.004
12. Bostwick D, Wolf S, Samsa G, et al. Comparing the palliative care needs of those with cancer to those with common non-cancer serious illness. *J Pain Symptom Manage.* 2017;53(6):1079-1084. doi: 10.1016/j.jpainsymman.2017.02.014
13. Chow K, Dahlin C. Integration of palliative care and oncology nursing. *Semin Oncol Nurs.* 2018;34(3):192-201. doi: 10.1016/J.SONCN.2018.06.001
14. Lokker ME, van der Heide A, Oldenmenger WH, van der Rijt CCD, van Zuylen L. Hydration and symptoms in the last days of life. *BMJ Support Palliat Care.* 2021;11(3):335-343. doi: 10.1136/bmjspcare-2018-001729
15. Thrane SE, Maurer SH, Cohen SM, May C, Sereika SM. Pediatric palliative care: A five-year retrospective chart review study. *J Palliat Med.* 2017;20(10):1104-1111. doi: 10.1089/jpm.2017.0038
16. Khalil H, Downie A, Ristevski E. Mapping palliative and end of care research in Australia (2000–2018). *Palliat Support Care.* 2020;18(6):713-721. doi: 10.1017/S1478951519001111
17. Miniksar ÖH, Aydın A. Retrospective analysis of hospitalized patients in our palliative care unit. *Journal of Contemporary Medicine*. 2020;10(3):429-433. doi: 10.16899/jcm.624878
18. Pang L, de la Cruz M, Wu J, et al. Symptom frequency and change of oldest old cancer patients. *Support Care Cancer.* 2019;27(11):4165-4170. doi: 10.1007/s00520-019-04702-7
19. Valero-Cantero I, Martínez-Valero FJ, Espinar-Toledo M, et al. Complementary music therapy for cancer patients in at-home palliative care and their caregivers: protocol for a multicentre randomised controlled trial. *BMC Palliat Care.* 2020;19(1):61. doi: 10.1186/S12904-020-00570-9

20. Chung V, Sun V, Ruel N, Smith TJ, Ferrell BR. Improving palliative care and quality of life in pancreatic cancer patients. *J Palliat Med.* 2022;25(5):720. doi: 10.1089/JPM.2021.0187
21. Skjoedt N, Johnsen AT, Sjøgren P, et al. Early specialised palliative care: interventions, symptoms, problems. *BMJ Support Palliat Care.* 2021;11(4):444-453. doi: 10.1136/BMJSPCARE-2019-002043
22. de Nooijer K, Van Den Noortgate N, Pype P, Van den Block L, Pivodic L. Palliative care symptoms, concerns and well-being of older people with frailty and complex care needs upon hospital discharge: a cross-sectional study. *BMC Palliat Care.* 2022;21(1):173. doi: 10.1186/S12904-022-01065-5
23. Abreu-Figueiredo RM, Sá LO, Lourenço TM, Almeida SS. Death anxiety in palliative care: validation of the nursing diagnosis. *Acta Paul Enferm*. 2019;32(2):178-185.
24. Caldeira S, Timmins F, de Carvalho EC, Vieira M. Clinical validation of the nursing diagnosis spiritual distress in cancer patients undergoing chemotherapy. *Int J Nurs Knowl*. 2017;28(1):44-52. doi:10.1111/2047-3095.12105
25. Carpenter JG, Lam K, Ritter AZ, Ersek M. A systematic review of nursing home palliative care interventions: Characteristics and outcomes. *J Am Med Dir Assoc.* 2020;21(5):583. doi: 10.1016/J.JAMDA.2019.11.015
26. Naito T, Mitsunaga S, Miura S, et al. Feasibility of early multimodal interventions for elderly patients with advanced pancreatic and non-small-cell lung cancer. *J Cachexia Sarcopenia Muscle.* 2019;10(1):73. doi: 10.1002/JCSM.12351
27. Zongo N, Yameogo PB, Savadogo JT, et al. The outcomes of palliative surgical treatment in a context of rising incidence of gastrointestinal cancers in Burkina Faso, Africa. *Eur J Surg Oncol.* 2022;48(10):2174-2180. doi: 10.1016/j.ejso.2022.07.002

Wydawnictwo UR 2023
ISSN 2544-1361 (online)
doi: 10.15584/ejcem.2023.3.27

http://www.ejcem.ur.edu.pl
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2023; 21 (3): 590–594

ORIGINAL PAPER

Morbidity profile and outcome of new-born admitted in sick newborn care units of Uttar Pradesh, India

Satya Prakash Singh [1], Harish Chandra Paliwal [2], Saket Shekhar [1], Poonam Kushwaha [1]

[1] Community Medicine, Rama Medical College Hospital & Research Center, Kanpur, India
[2] Dr. B S Kushwaha Institute of Medical Sciences, Kanpur, India

ABSTRACT

Introduction and aim. Reduction in child morbidity and mortality is result of upgradation of infrastructure and quality of public health care services. India alone accounts for 30% of the global neonatal deaths occur in India that occurs due to preventable cause. So, the aim of this study was to assess the pattern and status of neonatalmortality observed in SNCUs of Uttar Pradesh, India.

Material and methods. Descriptive study was conducted based on secondary data obtained from sick new born care units (SNCU) online database from 89 Government-supported SNCUs of Uttar Pradesh, from April 2014 to March 2016. Data obtained included age, weight, sex, diagnosis, and outcome.

Results. 22933 neonates admitted in SNCU were included in study with 14269 (62.2%) were males and 8664 (37.8%) females. Majority of the subject (20070; 87%) were in 0-5 days old age group. Most (72.5%) of admitted new-born improved and discharged. Low birth weight was significantly ($\chi^2 = 1334.2$, $p<0.001$) related with outcome. Birth asphyxia contributed to maximum (36.11%) number of deaths, followed by respiratory distress syndrome (25.21%), sepsis (15.38%), prematurity and extremely low birth weight (5.8%).

Conclusion. Improved antenatal care, improved access to health facility, timely referral of high-risk cases, capacity building, intensive interventional management can reduce neonatal mortality and its complications. Study also warrants, in-depth community-based qualitative study to identify gender-specific, equity issues.

Keywords. low birth weight, morbidity profile, mortality profile, respiratory distress

Introduction

An estimated 130 million babies are born worldwide each year from which about 4 million die in the neonatal period. About 30% of the global neonatal deaths occur in India and little progress has been made in reducing it in the last decade. Universal outreach and family-community care intervention like essential new-born care, resuscitation of the new-born, emergency new-born care, family care of the new-born, and care for low-birth-weight babies at 90% coverage has been estimated to avert Neo Natal mortality from 18 to 37%.[1-5]

Facility based newborn care (FBNC) has a significant potential for improving newborn survival. Provision of newborn care facilities at various levels of health facilities will not only increase the confidence in the health care delivery system but also increase the coverage of services at the time of greatest risk i.e., birth and the first few days of life and thus address the challenge of bringing down neonatal mortality in the country. Newborn care corner (NBCC), newborn stabilization unit (NBSU) and sick newborn care unit (SNCU) are newborn care facilities at MCH level I (PHC/SC), MCH lev-

Corresponding author: Saket Shekhar, e-mail: drsaketshekhar@gmail.com

Received: 1.06.2023 / Revised: 14.07.2023 / Accepted: 17.07.2023 / Published: 30.09.2023

Singh SP, Paliwal HC, Shekhar S, Kushwaha P. *Morbidity profile and outcome of newborn admitted in sick new born care units of Uttar Pradesh, India.* Eur J Clin Exp Med. 2023;21(3):590–594. doi: 10.15584/ejcem.2023.3.27.

el II (CHC/FRU) and MCH level III (district hospital/ medical college/tertiary care hospital) respectively.[5-11] Traditional practices like applying something on the umbilical stump, oil instillation into the nose, etc. also contribute to the newborn risk of morbidity and mortality.[2] FBNC at various levels is critical to strengthen the care of sick, premature, and low birth weight newborns.

Aim

The current study aimed to assess the pattern of neonate mortality observed in SNCUs of Uttar Pradesh and medical college-attached hospitals.

Material and methods

Study design and settings

A cross-sectional descriptive study was conducted based on secondary data collected from SNCUs of Uttar Pradesh. The study includes all Government-supported 89 designated SNCUs during the year April 2014–March 2016. SNCU is a neonatal unit nearby labour room which provides special care (all care except assisted ventilation and major surgery) for sick newborns. As per government guidelines, any facility with more than 3000 deliveries per year should have an SNCU. Most district and few sub-district hospitals fulfilled this criterion. In our study, we have included SNCU data from 75 district hospitals and 14 Subdistrict Hospitals. Approval from the Institutional Ethics Committee (IEC) was taken prior to starting the study (IEC/Rama medical college/2023/5678).

Study population

Neonates admitted in SNCUs were considered as study participants. These were categorized into two sections: 1) Inborn – who have delivered in same facility, and 2) Out born – who have referred to the facility from peripheral health facilities.

Study period

January 2022 to March 2022 was the study period and aggregated yearly data were taken for the study.

Data collection

The source of information was SNCU monthly reports generated from admitted neonates (Indoor cases only). In SNCUs, neonates are referred from MCH level facilities 1-2, private health facilities and from community by direct contact admission or by frontline health workers: Accredited Social Health Activist, Auxiliary Nurse Midwife, or Multi-Purpose Worker. The primary data have been recorded in predefined registers and case sheets of SNCUs filled up by paediatricians and staff nurses.

Inclusion criteria

The study included all neonates, who had ≤28 days of life admitted in SNCUs.

The SNCU monthly report is predefined format from Ministry of Health and Family Welfare, Government of India, which includes data on admission information, reasons of admission, course of admission, and mortality reasons (if any) with treatment outcomes. It also includes information on gender, birth weights, gestation age, and duration of stay. Ethical permission was sought from the concerned authority of State Health and Family Welfare Department, Government of Gujarat. The aggregated data of SNCU reports were analyzed and due efforts were made to conceal identity of hospitals and patients.

Data analysis

Statistical analysis was done analyzing the percentages, proportions, and Chi-square using Statistical Package for the Social Sciences (SPSS) version 17 (SPSS-Inc., IBM, USA). After analysis, the efforts were made to share the analysis with concerned SNCU in charge to take corrective actions in consultation with State officials.

Limitation of analysis

Detailed information of each neonate had not been collected. The aggregated data of indicators was taken into the study. Only Government-supported SNCUs were studied. Data pertaining to gestational age of neonates was not available. The follow-up on discharged, leaving against medical advice (LAMA), and referred neonates were not done during the study.

Results

Total 22933 neonates admitted in SNCU were included in study. We can depict age and sex distribution of study subjects from Table 1. 14269 (62.2%) were males and 8664 (37.8%) were females. Majority of the subject (20070; 87%) were in 0–5 days old age group, among these 12469 were (62.1%) male and 7601 (37.9%) females.

Table 1. Age and gender wise distribution of neonates admitted in SNCU (n=22933)

Age Group (days) (0.0%)	Sex	
	Male	Female
0–5 (20070; 87.5%)	12469 (62.1%)	7601 (37.9%)
6–10 (1356; 5.9%)	832 (61.4%)	524 (38.6%)
11–20 (929; 4.1%)	593 (63.8%)	336 (36.2%)
>20 (578; 2.5%)	375 (64.9%)	203 (35.1%)
Total	14269 (62.2%)	8664 (37.8%)

Outcome of neonates admitted in SNCU among different birth weight groups is described in Table 2 and Figure 1. Most of them 16628 (72.5%) improved and discharged in satisfactory condition, 2790 (12.2%) referred to higher centres, 1983 (8.6%) expired and 1503 (6.6%) left against medical advice. This relationship between birth weight group of newborn and outcome was found statistically significant (χ^2=1334.2, df =15,

p<0.001). Figure 2 describes causes of neonatal death, maximum death 36.1% occurred due to HIE/moderate-severe birth asphyxia.

Table 2. Outcome of neonates admitted in SNCU among different birth weight groups (n=22904)

Weight (kg)	Outcome				Total
	Discharged	Expired	LAMA	Referred	
0–1.5	1246 (50%)	654 (26.3%)	244 (9.8%)	340 (13.7%)	2484 (100%)
1.6–2	2696 (72.3%)	359 (9.6%)	243 (6.5%)	426 (11.4%)	3724 (100%)
2.1–2.5	4332 (76.4%)	389 (6.9%)	315 (5.6%)	631 (11.1%)	5667 (100%)
≥ 2.5	8354 (75.6%)	581 (5.3%)	701 (6.3%)	1393 (12.6%)	11029 (100%)
Total	16628 (72.5%)	1983 (8.6%)	1503 (6.6%)	2790 (12.2%)	22904 (100%)

$\chi^2 = 1334.2$, df =15, p<0.001

Causes of neonatal death admitted in SNCU among different birth weight groups. Out of total 1983 (8.6%) neonatal deaths, most of the neonatal deaths 716 were occurred due to HIE/moderate-severe birth asphyxia, of which maximum 323 (45.1%) occurred among birth weight group >2.5 kg followed by 201 (28.1%) among birth weight group 2.1–2.5 kg, 104 (14.5%) among birth1.6–2 kg weight group and rest 88 (12.3%) were amongst birth weight group 0-1.5 kg. Total 500 deaths due to respiratory distress syndrome maximum 186 (37.2%) were among birth weight group 0–1.5 kg, 125 (25.0%) among birth weight group 1.6–2 kg, 106 (21.2%) among >2.5 kg and rest 83 (16.6%) among 2.1–2.5 kg weight group. Out of total 305 deaths were due to sepsis; most of these 108 (35.4%) were among birth weight group 0–1.5 kg, 72 (23.6%) among 1.6–2 kg, 66 (21.6%). Total 151 deaths occurred due to prematurity (<28 weeks of gestation) 118 (78.1%) were among birth weight group 0-1.5, 19 (12.6%) among birth weight group 1.6–2 kg rest 14 (9.3%) among >2 kg birth weight. Total 118 deaths due to extremely low birth weight (ELBW) (weight less than 1000 g) 105 (91.3%) were among birth weight 0–1.5 kg (Table 3).

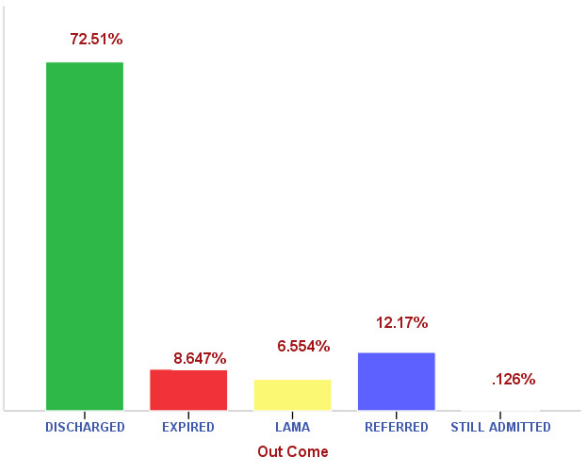

Fig. 1. Outcome of newborns admitted in SNCU

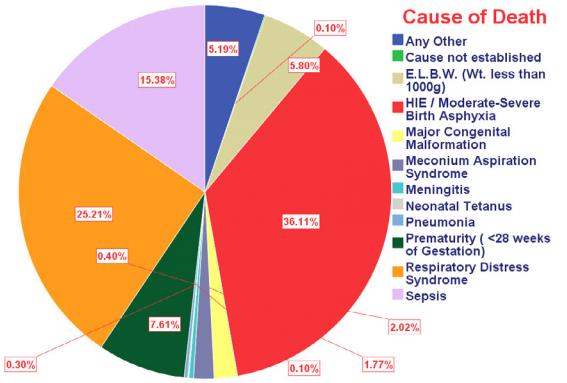

Fig. 2. Causes of death among newborns in SNCU

Table 3. Causes of neonatal death admitted in SNCU among different birth weight groups

Cause of Death	Weight Group (kg)				Total
	0–1.5	1.6–2	2.1–2.5	>2.5	
Cause not established	1 (50%)	0 (0%)	0 (0%)	1 (50%)	2 (100%)
(ELBW, weight<1000g)	105 (91.3%)	10 (8.7%)	0 (0%)	0 (0%)	115 (100%)
HIE/moderate-severe birth asphyxia	88 (12.3%)	104 (14.5%)	201 (28.1%)	323 (45.1%)	716 (100%)
Major congenital malformation	3 (7.5%)	7 (17.5%)	12 (30%)	18 (45.0%)	40 (100%)
Meconium aspiration syndrome	5 (14.3%)	6 (17.1%)	9 (25.7%)	15 (42.9%)	35 (100%)
Meningitis	4 (50.0%)	1 (12.5%)	2 (25.0%)	1 (12.5%)	8 (100%)
Neonatal tetanus	1 (50.0%)	0 (0.0%)	0 (0.0%)	1 (50.0%)	2 (100%)
Pneumonia	0 (0.0%)	1 (16.7%)	2 (33.3%)	3 (50.0%)	6 (100%)
Prematurity (<28 weeks of gestation)	118 (78.1%)	19 (12.6%)	1 (0.7%)	13 (8.6%)	151 (100%)
Respiratory distress Syndrome (RDS)	186 (37.2%)	125 (25.0%)	83 (16.6%)	106 (21.2%)	500 (100%)
Sepsis	108 (35.4%)	66 (21.6%)	59 (19.3%)	72 (23.6%)	305 (100%)
Any other	35 (34%)	20 (19.4%)	20 (19.4%)	28 (27.2%)	103 (100%)
Total	654 (33%)	359 (18.1%)	389 (19.6%)	581 (29.3%)	1983 (100%)

Discussion

This study aimed at understanding morbidity pattern, causes of mortality among newborns admitted across 89 government supported SNCUs across Uttar Pradesh, a northern state in India. Improvement of new-born health outcome aimed at comprehensive continuum of maternal and child health care services with context-specific investment. Maximum reduction in child morbidity and mortality can be met if, high-coverage programme of universal outreach and family-community care becomes an integrated intervention.[2,3] Reduction in child morbidity and mortality is result of upgradation of infrastructure in public health care facilities, with increased number of SNCUs, which are advanced newborn care centres in tertiary level health care centres.[12-15]

Present study reflects that male admission outnumbered female admission (62.2% vs 32.8%) simulating findings in other studies.[5,12,16-18] This may be due to gen-

der bias prevalent in India, where male children are given more importance. This societal gender bias is also evident in Sex Ratio at birth in India. National family Health Survey conducted in 2109-20 also reported sex ratio at birth to be 929 females per 1000 males. Biological vulnerability of the male gender could also be the other reason behind male ponderance in SNCU admissions. It warrants in-depth community-based observations to identify gender-specific, equity issues.[12,16]

SNCU admissions were maximum in early neonate period (0-5 days). Over 90 % admissions were within first 10 days of birth in our study. This is consistent with many previous studies.[4,5,10] This finding emphasizes that the most vulnerable period is the first week of life. This period must be prioritized to further decrease neonatal mortality and thereby infant mortality. Infant mortality rate have long been used as overall health status indicator of countries.

Most of the admitted new-born (72.5%) improved and discharged in satisfactory condition, 12.2% referred and 8.6% expired. These findings are better than outcome status reported by other studies.[12,19] This could be due to regional differences and upgradation of infrastructure with time in various facilities. Indian government launched a big initiative with name of India Newborn Action Plan (INAP) in 2014. INAP targets towards the goals of Single Digit Neonatal Mortality Rate and Single digit Stillbirth Rate by 2030. INAP has been implemented under within the existing RMNCH+A framework. It has been guided under principles of integration, equity, gender, quality of care, convergence, accountability and partnerships with six pillars of intervention packages. It focusses on preconception and antenatal care; care during labour and childbirth; immediate newborn care, care of healthy newborn, care of small and sick newborn and care beyond newborn survival.[20-23]

Low birth weight was significantly related with outcome simulating findings of Mahajan et al.[13] This findings emphasizes on the fact that to curb neonatal mortality we will have to ensure good preconception and antenatal care prioritizing maternal nutrition. Food related myths are quite prevalent in the Indian society. Raising awareness about them can be big step towards improving maternal nutrition. This can be achieved by periodic training of frontline workers like ASHA, Anganwadi Workers etc.

HIE/moderate-severe birth asphyxia contributed to maximum (36.11%) number of deaths, followed by respiratory distress syndrome (25.21%), sepsis (15.38%), prematurity and ELBW (5.8%) predominant causes of death in our study. Our study present better finding than other previous studies which reported, neonatal jaundice, prematurity, low birth weight, perinatal asphyxia and sepsis as major causes for SNCU admission and also for morbidity and mortality.[5,13,15-17,19,24,25] This may be due to improved health care system and infrastructure. Moderate-severe birth asphyxia being the top cause of neonatal mortality in SNCUs suggests importance of upscaling of resuscitation at SNCUs and Human resource employment and periodic training.

Conclusion

Neonatal mortality is one of the major contributors to the Infant Mortality. To address the issues of higher neonatal and early neonatal mortality, FBNC services at health facilities have been emphasized by setting up of facilities for care of sick newborn such as SNCU at different levels is a thrust area.

The present study showed that greater number of male infants admitted to SNCU. Male preponderance is still prevalent in spite of various efforts of government to create awareness about female health. Further qualitative studies are required to explore reasons of the male predominance.

The commonest cause for death was birth asphyxia followed by respiratory distress syndrome, sepsis, prematurity and E.L.B.W. All health care personnel involved in new-born care should undergo skill development training on simple immediate newborn care and resuscitation in equipped peripheral institutions. Prevalence of sepsis among new-born born is a matter of grave concern. Proper aseptic procedure, sanitation and hygiene should be maintained to prevent it.

Declarations

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Author contributions

Conceptualization, S.P.S. and H.C.P.; Methodology, P.K.; Software, S.P.S.; Validation, S.S., P.K. and S.P.S.; Formal Analysis, H.C.P.; Investigation, S.P.S.; Resources, P.K.; Data Curation, H.C.P.; Writing – Original Draft Preparation, S.P.S.; Writing – Review & Editing, P.K.; Visualization, S.P.S.; Supervision, S.S.

Conflicts of interest

No conflict of interest has been declared by the authors.

Data availability

Data will be provided on demand from the corresponding author.

Ethics approval

This study was approved by the local ethics committee (IEC/Rama medical college/2023/5678).

References

1. Narayanan I, Litch JA, Srinivas GL, Onwona-Agyeman K, Abdul-Mumin A, Ramasethu J. At-Risk Newborns: Overlooked in Expansion From Essential Newborn Care to Small and Sick Newborn Care in Low- and Middle-Income Countries. *Glob Health Sci Pract*. 2023;11(1):e2200099. doi: 10.9745/GHSP-D-22-00099
2. Darmstadt GL, Bhutta ZA, Cousens S, Adam T, Walker N, De Bernis L. Evidence-based, cost-effective interventions: how many newborn babies can we save? *Lancet*. 2005;365(9463):977-988. doi: 10.1016/S0140-6736(05)71088-6
3. Lassi ZS, Kedzior SG, Bhutta ZA. Community-based maternal and newborn educational care packages for improving neonatal health and survival in low- and middle-income countries. Cochrane *Database Syst Rev.* 2019;2019(11):CD007647. doi: 10.1002/14651858.CD007647.pub2
4. Saini N, Chhabra S, Chhabra S, Garg L, Garg N. Pattern of Neonatal Morbidity and Mortality: A Prospective Study in a District Hospital in Urban India. *J Clin Neonatol*. 2016;5(3):183. doi: 10.4103/2249-4847.191258
5. Uppal K, Ashwani N, Jeelani K, Prabhakar K, Yadaiah D. Profile of neonatal mortality in SNCU district hospital. *Galore Int J Health Sci Res*. 2019;4(1):6–8.
6. Child Health Programmes :: National Health Mission. https://nhm.gov.in/index1.php?lang=1&level=3&sublinkid=1179&lid=363. Accessed April 11, 2023.
7. Shiras T, Bradley SEK, Johns B, Cogswell H. Sources for and quality of neonatal care in 45 low- and middle-income countries. *PLoS One*. 2022;17(7):e0271490. doi: 10.1371/journal.pone.0271490
8. Neogi SB, Khanna R, Chauhan M, et al. Inpatient care of small and sick newborns in healthcare facilities. *J Perinatol*. 2016;36(3):18-23. doi: 10.1038/jp.2016.186
9. Gathwala G. Neonatal care in India. *Indian Journal of Community and Family Medicine*. 2015;1(1):17-22. doi 10.4103/2395-2113.251600
10. Kumaravel KS, Ganesh J, Balaji J, Pugalendhiraja KV, Ramesh BB. A study on impact of NRHM on neonatal care and clinical profile of neonates admitted in a SNCU of a Rural Medical College. *J Evol Med Dent Sci*. 2015;4(82):14335-14348. doi: 10.14260/JEMDS/2015/2039
11. Venugopal S, Patil RB, Thukral A, et al. Feasibility, Sustainability, and Effectiveness of the Implementation of „Facility-Team-Driven" Approach for Improving the Quality of Newborn Care in South India. *Indian J Pediatr.* 2023;10.1007/s12098-023-04518-8. doi: 10.1007/s12098-023-04518-8
12. Shah HD, Shah B, Dave PV, Katariya JB, Vats KP. A step toward healthy newborn: An assessment of 2 years' admission pattern and treatment outcomes of neonates admitted in special newborn care units of Gujarat. *Indian J Community Med Off Publ Indian Assoc Prev Soc Med*. 2018;43(1):14.
13. Mahajan S, Bajaj M, Vaid A, Thakur A. A one year review of clinical profile and treatment outcomes of neonates admitted to Sick Newborn Care Unit (SNCU) at Regional hospital in Himachal Pradesh draining a rural area. *Journal of Medical Sciences and Clinical Research*. 2019;7(8):235-241. doi: 10.18535/jmscr/v7i8.40
14. Kardum D, Serdarušić I, Biljan B, Šantić K, Živković V. Readmission of late preterm and term neonates in the neonatal period. *Clinics (Sao Paulo)*. 2022;77:100005. doi: 10.1016/j.clinsp.2022.100005
15. Budhiarta KDL, Kardana IM. Neonatal Profile and Outcome in Neonatal Intensive Care Unit Sanglah Hospital. *Am J Pediatr*. 2020;6(3):289-294.
16. Rakholia R, Rawat V, Bano M, Singh G. Neonatal morbidity and mortality of sick newborns admitted in a teaching hospital of Uttarakhand. *CHRISMED J Health Res*. 2014;1(4):228.
17. Jena D, Tripathy R, Pradhan S, Sethi G. Assessment of socio-clinical profile of neonates admitted in sick neonatal care unit of tertiary care hospital: Odisha. *Int J Res Med Sci*. 2017;5(9):4077-4081. doi: 10.18203/2320-6012.ijrms20173986
18. Rohit M, Bhavesh M, PatelJaiminkumar, Punitha KM. Study of the morbidity and the mortality pattern in the neonatal intensive care unit at a tertiary care teaching hospital in Gandhinagar District, Gujarat, India. *J Res Med Dent Sci*. 2015;3(3):208-212.
19. Panda PK, Panda PK. Clinical profile and outcome of newborns admitted to a secondary-level neonatal intensive care unit in tribal region of Odisha. *J Clin Neonatol*. 2019;8(3):155.
20. Khurmi M, Karpe V, Kaur P. India launches India newborn action plan. *Indian J Child Health*. 2015;2(1):43-44. doi: 10.32677/IJCH.2015.v02.i01.015
21. Mohanasundari SK, Padmaja A. National Health Mission (NHM) and India Newborn Action Plan (INAP) Services in Newborn Health -An Overview. *Int J Adv Nurs Manag*. 2019;7(4):366-370. doi: 10.5958/2454-2652.2019.00086.6
22. Datta V, Ghosh S, Aquino LD. Progressing towards SDG 2030 goals with system changes: the India Newborn Action Plan. *BMJ Open Qual*. 2022;11:e001971. doi: 10.1136/bmjoq-2022-001971
23. Kumar J, Saini SS, Kumar P. Care During Labour, Childbirth, and Immediate Newborn Care in India: A Review. *Indian J Pediatr.* 2023;10.1007/s12098-023-04721-7. doi: 10.1007/s12098-023-04721-7
24. Mandal S. A Study on Clinical Profile and Outcome of Sick Neonates in a District Level SNCU. *J Evid Based Med Healthc*. 2020;7(1):29-33. doi: 10.18410/jebmh/2020/7
25. Iyer DCR, Gornale DVK, Pj DH, Katwe DN, V SP, Y K. Morbidity and Mortality pattern of neonatal intensive care unit in a Medical College Hospital from South India. *Pediatr Rev Int J Pediatr Res*. 2015;2(4):77-82. doi: 10.17511/ijpr.2015.i04.08

Wydawnictwo UR 2023
ISSN 2544-1361 (online)
doi: 10.15584/ejcem.2023.3.30

http://www.ejcem.ur.edu.pl
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2023; 21 (3): 595–604

ORIGINAL PAPER

The difficulties experienced in patient communication by nursing students taking the clinical practice course for the first time – a qualitative study

Pinar Harmanci

Faculty of Health Sciences, Kahramanmaraş İstiklal University, Kahramanmaraş, Turkey

ABSTRACT

Introduction and aim. Nursing fields begin to face a wide variety of challenges when they first enter clinical practice. The aim of this study is to determine the difficulties experienced in patient communication by nursing students taking the clinical practice course for the first time

Material and methods. Focus group interview design was used for this study. Focus group design is used to give information about the opinions and experiences of the sample group on any subject. It is reported as a convenient design to identify different perspectives on a subject within the scope of the sample. Focus group interviews are a widely used method as data collection technique.

Results. The findings of the study continue on 4 main themes at level 3, by identifying the similarities and differences in the codes determined by the three experts (researchers consulted and the researcher conducting/reporting the study). The findings of the study were evaluated in comparison with the findings of the studies reached in the literature and conducted in regions such as Israel, Iran, Sri-Lanka, Kenya, and Africa.

Conclusion. There are many problems in student-patient communication in many different dimensions. The difficulties arising due to these problems generally focus on unknown clinical environment, lack of knowledge, differences between theory and practice, and mentor interaction.

Keywords. difficulties first practice, nursing students, patient communication

Introduction

Nursing education consists of an important process covering theoretical, laboratory, and clinical areas and practical training. Clinical teaching covers an important part of nursing education (usually half) and it appears as the most important education process.[1,2] Clinical practice is also known as the most important educational field that can be given to nursing students to improve their professional aspects. At the same time, clinical practice courses teach students "how to communicate" with the patient or healthy individual they will treat.[3,4] Nurses are considered to be leading health professionals who need to develop effective communication with the individual they care for in order to provide effective nursing care and to get the correct nursing diagnosis.[5]

Learning motivations of nursing students mostly vary depending on the environmental conditions in the clinic (the attitude of the professionals in the clinic, the physical environment of the clinic…). When these conditions are not adequately met, the nursing student sees herself as inadequate and unsuccessful. This situation is also reflected in patient communication.[6,7]

Corresponding author: Pinar Harmanci, e-mail: pinar.harmanci@istiklal.edu.tr

Received: 22.06.2023 / Accepted: 21.07.2023 / Published: 30.09.2023

Harmanci P. *The difficulties experienced in patient communication by nursing students taking the clinical practice course for the first time – a qualitative study.* Eur J Clin Exp Med. 2023;21(3):595–604. doi: 10.15584/ejcem.2023.3.30.

A systematic review revealing how nursing students' communication with patients is improved, proposes that students will benefit from trainings such as role play, or live simulation performed spontaneously using cases.[8] The review reports that realistic, patient-centered, and feedback-included communication approaches described in theoretical courses are important issues that would minimize communication problems in practice areas.[9,10]

Nursing students need to learn communication difficulties during theoretical training in order to adopt innovative communication education approaches and gain the ability to continue learning and developing in the field of practice.[8,11,12]

In a systematic review, it is stated that the best teaching intervention in which nursing students can recognize and solve the difficulties in their communication with the patient is the use of live simulation.[8]

Social skills as well as academic achievements of nursing students contribute to coping with difficulties in communication. For example, when a group project is given and each student takes responsibility for herself, the effective evaluation of the project results also contributes to the development of students' social skills at the basic level. Students learn to intervene in crisis to solve any problem and to develop interpersonal relations and high-level thinking skills with small peer groups. This situation provides a nursing student, who will take the clinical practice course for the first time, with social skills as well as academic knowledge in order to solve patient-centered communication difficulties that she will encounter in the field of practice.[13,14]

The results of a study conducted in Taiwan in 2016 suggest that nursing students should develop their communication skills and communication-related social skills according to their clinical needs (situation-specific). The study reported that students have traumatic-negative communication experiences with individuals with cancer.[15] Moreover, cultural differences related to issues such as illness/health/loss may be a reason for communication problems that nursing students experience in clinical fields.[15,16] A study conducted in Turkey in 2022 reports that nursing students, who encounter a death/terminal period patient for the first time, have inadequacies in communication and cannot receive adequate/comprehensive mentor training in clinic.[17] It is also seen in the results of the study that it is an important issue for nursing students to receive education including realistic and therapeutic techniques (practice-oriented) in theoretical training.[13-17] It is known that nursing students who will enter the field of practice for the first time may encounter many different patient groups.[16,17]

Aim

The aim of this study is to determine the difficulties experienced in patient communication by nursing students taking the clinical practice course for the first time. It is thought that the study will guide future quantitative and experimental studies and contribute to the identification of difficulties.

Material and methods

Study design

Focus group interview design was used to determine "The Difficulties Experienced in Patient Communication by Nursing Students Taking the Clinical Practice Course for the First Time." Focus group design is used to give information about the opinions and experiences of the sample group on any subject. It is reported as a convenient design to identify different perspectives on a subject within the scope of the sample. Focus group interviews are a widely used method as a data collection technique. It is expressed as a common and useful way of collecting qualitative data within the sample (in a predetermined group).[18,19] Focus group interviews, which are qualitative data collection techniques for group interviews, are expressed as a method in which the meanings of words are examined (classified) and real emotions are observed.[19,20] They are used to evaluate and determine processes such as attitudes, thoughts, and knowledge about a subject in the sample group and in the group represented by the sample, and to create a background about the subject.[19] It is seen that focus group interviews are used to determine what the sample thinks about an event/situation right after the process or months later, what the sample experiences, what the sample learns, and the emotion that this process creates in the individual.[20-22]

Study universe and sample

For the study, 1st year nursing students at a university in the north-east of Turkey were included in the study. All the students in the 1st year constituted both the universe and the sample of the study. Within the scope of the research, there were 60 (total number of students in the 1st grade and the universe) nursing students. However, 32 students (10 students did not participate in the study, 18 students could not participate in the interviews/dropped out) who accepted to cooperate and could participate in the study process were included in the study (Fig. 1). The participants were asked 7 basic open-ended interview questions in the form in which the study data would be collected. These questions were determined as follows:

1. Which patient problem in the clinic affects you most during practice?
2. In which situations do you feel most at a dead end in communication with patients during practice?
3. How do you feel when there is a problem in your communication with a patient?
4. What do you think about patient-nurse interactions?

5. What do you do when you have problems communicating with a patient?
6. What are the difficulties you experience in communicating with a patient?
7. Do you think you can cope with the difficulties you experience in communicating patients?

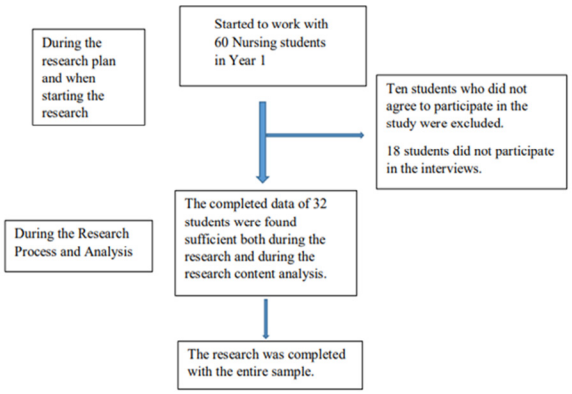

Fig. 1. Flowchart of study

Data collection

While making the study plan, 2 separate forms were created in accordance with the sample and the nature of the study. One of these forms is the socio-demographic data form where we could collect the data of the socio-demographic characteristics (characteristics such as age, sex, marital status, place of residence) of the participants (nursing students), and the second form is the data form that would be collected for the purpose of the qualitative study, which was created by taking expert opinions (3 experts). The second form, which consists of 7 questions, consists of open-ended questions to collect data belonging to the qualitative study. For the 2nd form, group interviews were conducted with all the participants (n: 32 and 6 different groups consisting of 5 students were determined by lottery method). These interviews were recorded in all groups, and verbal and written consent was obtained from the students to record the focus group interviews. In the study, an expert observer was included in the group (like an outside student) without being declared an expert and an observer. This observer observed mimics, behaviors, and gestures in order to evaluate especially nonverbal records. The data of the study were collected and recorded (with a voice recorder) in accordance with the focus group interview design. The interviews were conducted in a calm, quiet, well-ventilated environment where the interviewer could answer the questions well. The interviews were conducted in a suitable classroom of a faculty with a nursing department and lasted approximately 20-40 minutes.

Data analysis

Three steps were followed in the focus group interview analysis:

1. After each interview, an evaluation was made, and the main themes of the interview were dwelled on. Some notes were taken on the importance of the data obtained from the participants and the interview process was reviewed.
2. The obtained records were listened to repeatedly (three expert listeners), themes were defined, and sub-themes were formed.
3. Moreover, while listening to the answers to the questions, the responses given by nonverbal communication were also noted by comparing the records and the notes. Features such as mimics and tone of voice were evaluated in a mutual interaction. Nonverbal responses generating cues appropriate to the content were also evaluated.

The evaluation steps making up this part are examined in two ways:

a. Classification and arrangement of words/Classifications according to the theme/Creation of themes and sub-themes
b. Reviewing nonverbal communication sources/ Behaviors revealed at the time of gestures and responses

Table 1. Demonstration of 3 levels of coding/themes/sub-themes

Level 1 codes (general classification)	Level 2 codes (themes)	Level 3 codes (sub-themes)
Fear of communication failure	Lack of self-confidence	Unknown communication process/ Clinical Anxiety
Fear of giving wrong information		Lack of sufficient knowledge about the patient/disease process
Fear of inability to communicate effectively	Feeling inadequate in communication	Differences between ideal theoretical knowledge and area of practice
		Inadequate clinical supervision given to overcome difficulties in communication
Inability to identify difficulties in communication	Difficulty in transforming theoretical knowledge into behavior	
Inability to cope with difficulties in communication	Difficulty in compliance with hospital procedures	
Lack of knowledge	Difficulty in recognizing difficult patients and their behaviors	
Fear of the "unknown" that is likely to be encountered in communication	Anxiety in communicating with an unknown patient/ disease process	

According to the qualitative content analysis approaches developed by Graneheim and Lundman, 6 different themes were determined at level 2.[23] In the interviews developed by Stewart and Shamdasani, in which examined paragraph by paragraph, subject by subject were examined, important situations were coded according to the identified topics.[24] Three experts

(researchers consulted and the researcher conducting/reporting the study) determined the similarities and differences in the codes they determined and finally divided them into 4 main themes at level 3.

In the study, 3 basic level coding was performed.

Level 1 codes consisted of codes that covered the general and reflected the main themes, which were formed after the important and specific answers to the questions asked to the participants.

Level 2 codes were usually prepared comparatively. When the 1st Level codes were created, it was requested to gather them under a more general title in a way covering the determined 1st Level codes.[23,25]

Level 3 codes were the coding level made in the form of main title(s) describing the psycho-social process created by the other coded levels.

The codes were also created/evaluated by 2 different experts (3 experts in total) other than the researcher. Table 1 shows the level-by-level coding results/themes. Since all data cannot be presented due to the nature of qualitative studies, some basic expressions and guidelines for the formation of themes are included in the findings.[21] The researcher who conducted the study and the experts whose opinions were consulted have a significant and long education/experience in the field. The purpose and importance of the study were explained to the students in advance so that they could reveal their real feelings, thoughts, and behaviors. It was tried to provide an environment where the participant students could express themselves freely. Since this is a qualitative study, it was tried to be strengthened in terms of reliability with expert opinion, observation, and basic coding approaches/content analysis.

Results

The socio-demographic characteristics of the sample group included in the study are given in Table 2.

Unknown communication process/Clinical anxiety

When the level 1 and level 2 codes in Table 1 are examined, it is seen that nursing students have problems such as communication failure and fear of giving wrong information, inability to communicate effectively and to identify difficulties in communication. These codes negatively affect students' self-confidence. Some important examples of the development of clinical anxiety of students (including the unknown communication process) are given below.

"*Sometimes I know the answers to the questions patients ask me. These are ordinary theoretical knowledge. However, I still refrain from answering. I also get worried about what if he misunderstands me. And even though I'm sure, I stay very quiet when I think maybe there are things I don't know. Because of this situation, patients sometimes do not make me do simple practices, even if I am accompanied by a mentor. I guess I should be more go-ahead about the things I know.*"

(Male S7)

The anxiety experienced in informing patients about clinical routines and nursing student's inability to answer questions create deficits in "self-confidence".

"*In one case, I informed the patient that his blood pressure was a little high during vital signs monitoring. The patient's blood pressure value, which I measured after 20 minutes, was even higher. I thought it was because I told him his blood pressure was high. I was very worried that day. In my subsequent hospital practices, I avoided informing patients of their vital signs. When patients asked about their vital signs, I usually said that my mentor nurse would give information and left. Sometimes it was really hard for me to go to practice. Saying something wrong without knowing might take me to court.*"

(Female S9)

Nursing students generally refrain from giving information to patients during clinical practice. They get worried that they can cause emotional and spiritual harm to patients. This situation sometimes makes students more anxious when they come to clinical practice. Some students (Male [M] S11, Female [F] S14, M S21, F S9…) think that they can experience legal proceedings because of a statement they say (How much information they can give to patients can be made into a procedure). It is seen that this situation causes nursing students not to be able to adequately answer to patients' questions in the clinic and to experience anxiety while coming to/during clinical practice. Students also say that they "lost their self-confidence" in giving information or wrong practices (M S13, F S17, M S28, M S24, F S22…..).

Lack of sufficient knowledge about the patient/disease process

When the codes in Table 1 are examined, it is seen that the nursing students have significant communication difficulties such as fear of inadequacy in effective communication, fear of inadequacy in identifying difficulties in communication, and fear of lack of information. These processes can have multiple causes. However, the nursing students with whom the study was conducted often stated the lack of recognition of comorbid diseases or the primary disease creating the care process, and the fact that individuals did not recognize their unique symptoms/reactions as difficulties in communication.

"*A patient's relative called me to the sick room when the serum was finished. When I entered into the room, the patient was rocking back and forth on the bed. I asked the patient's relative why he did this. I checked his vital signs. I tried to communicate with the patient. I called him. However, he did not look at me. The patient contin-*

ued to rock back and forth in the bed and started shouting. Again, I asked the patient's relative if he could not hear me. At that moment my mentor nurse came. She immediately removed the empty IV set from his arm. "How long has he been in this situation?" he asked. The patient's relative said "it is about 10 minutes". Then the mentor nurse came in and gave a medicine from the patient's order. After observing together, we left the room. I could never enter into that room again. Even if I entered, what would I talk to the patient or his relative? For example, while listening to a topic about communication, I listened to the importance of eye communication with the patient at school. I did not know how to establish eye communication with this patient."

(Female S18)

The fact that students do not know some diseases and symptoms, do not know patients' medical histories, and do not have an idea about their comorbid diseases also affects their communication processes and the speed in the communication network (Students do not recognize the symptoms and communication difficulties to share with their mentors). The nursing students stated that they had more difficulties in the communication process they established with patients with additional diseases that they did not know.

"When I entered into the patient's room, I introduced myself, but then the patient repeatedly asked me who I was. I introduced myself a few more times. When I went to his room for a blood pressure measurement that was in his clinical routine, he did not give me permission to take the measurement. He asked me to call the nurse. When I called the mentor nurse, she always said that she did not know me and that I had not introduced myself to her before. Apparently, the patient had forgotten all the moments when I introduced myself to him. But I don't know why he forgot. At that moment, I did not know what to think about the patient's condition, diagnosis/behaviors. Since this caused me a lot of anxiety, I tried to avoid any possible interaction with the patient."

(Female S23)

The nursing students have difficulty in assessing whether a patient's symptoms develop after a medication, due to an additional disease, or as a result of the primary disease being cared for.

Differences between ideal theoretical knowledge and area of application

Depending on many processes, in the clinical environment, different and incompatible times/environments/events can be experienced from the theoretical teachings. The nursing students stated that they experienced almost all the problems causing theoretical knowledge not to be applied to the clinic.

"Actually, I wanted to catch a quiet time when we could hear each other, in order to communicate with the patient in an effective process and to create an environment of trust. However, unfortunately, we could not find the time to initiate/maintain secure communication with my mentor nurse in the patient rooms. My mentor nurse was constantly taking on intensive care duties, and I participated in these care processes by watching her and helping her from time to time. Although I learned a lot about care, I did not have much time for effective communication. Because I had difficulty in providing time and environment for many communication techniques and processes that I learned at school."

(Female S16)

The nursing students say that they have more problems in initiating and maintaining communication, especially with individuals with neurological, neuropsychiatric, and psychiatric problems (M S11, F S18, M S13, F S19, F S23, F S32, M S31, F S17…).

"When I entered into room 20, the patient had taken off the intravenous catheter we had just put and was dressed. There was blood everywhere. As far as I remembered from a lecture we were taught about communication, I needed to create a safe environment for the patient. When I told him to lie down on the bed and I was going to apply pressure to his arm to stop the bleeding, he suddenly threw the blood contaminated IV set at me. The upper part of my body, including my eyes, was smeared with blood. I remember the patient had been well a few hours ago. After the routine maintenance of the clinic was over, I thought of going to his room to get the data I needed for my course. However, this situation, which developed in just a few hours, took me by surprise. In that situation, it became impossible for me to communicate."*

(Female S32)

It is understood that the students do not know the acute situations that they may experience during the communication process in the internal and surgical units and the symptoms of psychiatric comorbid diagnoses. The student encountered a case of delirium above, and the theoretical (theoretical communication subjects received includes a healthy and routine communication training) approach was insufficient.

It is seen that students coming to clinical practice for the first time may experience deficiencies in various aspects in such cases. As the best way to manage these processes, it is necessary to show what can be done in the face of pathological communication environments and processes under the supervision of a mentor. As a matter of fact, as seen in Table 1, "insufficient clinical supervision given to overcome difficulties in communication", which is one of the level 3 themes, emerges as a final difficulty.

Inadequate clinical supervision to overcome difficulties in communication

The nursing students could not get effective and evidence-based answers for some possible situations they asked their mentor nurses.

Female S26: "How should we respond when a six-year-old child asks us what happened to his mother in the accident?"

Nurse (N) 1: "Of course you will tell the truth!" (Yes, "correct" but "incomplete", this should include a communication/notification process. The general health, emotional, and mental state of the six-year-old child patient should be considered.)

N2: "Explaining this to an individual being treated makes him worse."

(Taken from a few examples FS 26 gave in answering the research questions.)

"We entered into a patient's room for a routine observation. When a patient whose treatment was over said, "I wish I wasn't discharged from the hospital right away, because I still have respiratory distress", the mentor nurse said, "You can't stay here forever, your treatment will continue at home." About an hour later, when I entered into the room, I saw the patient crying. I asked if I could do something for him, and he shook his head no. When I conveyed this to the mentor nurse, she said that some patients may behave like this. In fact, the part that did not sit well with me was this: maybe the patient cried not because of respiratory distress, but because he was afraid of being alone at home or dying. So were we going to just leave the patient alone?"

A student who has not yet received a psychiatry education (a student who came to the clinic for the first time to practice) also noticed the situations in which the mentor's communication deficit is obvious. The student responses reveal different characteristics in the attitudes of mentor nurses regarding communication.

While some students (M S11, F S23, M S28, F S14, M S27…) explained in their answers that the mentor nurses neglected the communication issue due to the intensity of their other work (care/treatment and other non-nurse-related jobs in the service…);

"Last week, when I said to my mentor nurse, 'You said you would go back to the patient's room and listen to her,' she replied, 'I am busy with transfusion right now and unfortunately she is my patient, too and my priorities are different'. I think the nurses are a little busy. However, I could not observe because my mentor nurse could not go to the interview, she was going to have in the patient room." (Female S23)

Student observations that the mentor nurse was busy were frequently expressed. However, many simplified the situation of one of the patients (which can be very important to the patient) when stating priorities, similar to this statement. This is one of the most common communication mistakes.)

Table 2. Descriptive characteristics of the participants

Participants	Age	Marital status	Living place	Working status	Family type	Family income
M S1	18	Single	City	Non-working	Wide	Average
F S2	19	Single	Big city	Non-working	Core	Average
F S3	20	Single	Village	Non-working	Wide	Low
F S4	19	Single	City	Non-working	Core	Average
F S5	19	Single	City	Non-working	Core	Average
M S6	19	Single	Village	Non-working	Wide	Average
M S7	19	Single	City	Non-working	Core	Average
F S8	20	Single	Village	Non-working	Wide	Average
F S9	19	Single	City	Non-working	Core	Average
F S10	19	Single	City	Non-working	Core	Average
M S11	20	Single	City	Non-working	Core	Average
F S12	19	Single	Big City	Non-working	Core	Average
M S13	19	Single	City	Non-working	Core	Average
F S14	19	Single	Big City	Non-working	Core	Average
F S15	19	Single	City	Non-working	Wide	Average
F S16	19	Single	Village	Non-working	Wide	Average
F S17	20	Single	Village	Non-working	Core	Average
F S18	20	Single	City	Non-working	Core	Average
F S19	20	Single	City	Non-working	Core	Average
M S20	22	Single	City	Non-working	Core	Average
F S21	20	Single	City	Non-working	Core	Average
F S22	20	Single	Big City	Non-working	Core	Average
F S23	19	Single	Village	Non-working	Core	Average
M S24	21	Single	Big City	Non-working	Wide	Low
M S25	19	Single	City	Non-working	Core	Average
F S26	19	Single	City	Non-working	Core	Low
M S27	19	Single	City	Non-working	Core	Average
M S28	19	Single	Village	Non-working	Core	Average
F S29	20	Single	City	Non-working	Core	Average
F S30	21	Single	Big City	Non-working	Core	Average
M S31	19	Single	Big City	Non-working	Core	Average
F S32	19	Single	City	Non-working	Core	Average

Other student answers (M S31, F S17, M S13, F S20, M S6…) state that the mentor nurses have a lack of knowledge in patient communication.

("A patient who had to walk in the corridor from time to time, said that he was tired and asked if he was walking enough when my mentor nurse and I were passing by. The mentor nurse told him that she did not see the time he was walking, that she was not sure that he -the patient- could tell the time and distance correctly, so he could not answer. When the patient said that his pain increased as he walked, she said, 'then sit down." – Male S31)

We understand that even when there is not a busy work schedule (even if there is), the nurse treats the patient as a competitor rather than as her caregiver and simplifies the patient's pain and fatigue. This situation reveals that they do not use communication with its methods, they do not make an effort to do this, and they have incomplete information about the patient-nurse communication processes.

Discussion

In this section, 4 themes, which were determined by experts and whose findings were exemplified as a result of the analysis were discussed in the light of the literature. The *"Unknown communication process/Clinical Anxiety"* in the study are revealed in the literature with various dimensions. In a study examining the stress experienced by the nursing students who came to clinical practice for the first time in Israel in 2014 and the ways of coping with this stress, it was reported that the nursing students experienced intense stress due to their lack of clinical communication skills, their relationship with complex patients, and the complexity of the clinical environment.[26] It was reported that the nursing students who came to clinical practice for the first time in Taiwan and Iran could not establish a 'therapeutic relationship' with the patients and the mentors, and therefore experienced intense anxiety during clinical practice.[27,28] In two different studies conducted in Iran in 2015, it was stated that the students defined the unknown clinical environment as "stressful".[29,30] Similarly, the study stated that the nursing students had problems in communicating and identifying their deficiencies in communication, and this often created an environment for making mistakes. When the answers of the nursing students were examined, we saw that they had some difficulties in communicating with the patients and that they had problems of orientation to the clinical environment. Similarly, in a study conducted in Taiwan, it was reported that the students' lack of communication skills caused deeper communication failures in the unknown clinical environment.[31] From a different viewpoint, the results of a study in Ireland reported that the nursing students who came to clinical practice for the first time were very young and were weak against the communicative, emotional, and social problems they would experience in the clinical environment.[32] The fact that the students in the study had no previous clinical experience supports the fact that these students experience communication-related fears in the face of the complexity of the clinical environment. Another study in Iran showed that the fear and communication problems experienced by nursing students in the clinical environment also negatively affected their self-confidence.[33] When the study findings are examined, fear of communication and self-confidence problems created by the unknown clinical environment emerge as frequently detected issues.

When the literature is examined, students' lack of knowledge about care and treatment, low communication skills, insufficient knowledge, and an unfamiliar clinical environment are always shown as sources of stress and anxiety for students.[26-34] In particular, the lack of theoretical knowledge (knowledge of communication, knowledge of clinical environment, knowledge of diseases and illnesses) causes students to be unable to integrate their theoretical knowledge into the clinic.[35] Nursing students who theoretically take inadequate communication training and come to the clinic with insufficient communication skills can also give inadequate answers (or wrong/ineffective) in their interactions with the patient.[36] When they have to cope with a difficult patient or a comorbid diagnosis they do not know, they lose all their motivation. This situation creates an important anxiety environment for them.[37] In a study conducted in Northern Tanzania, 17.6% of nursing students' communication difficulties were due to lack of knowledge.[38] The students' responses and emerging themes in the study show that our findings are in line with the literature. Since nursing students have clinical experience for the first time, they do not know comorbid diagnoses or whether changes in communication with the patient while treatment is ongoing are an acute biochemical/neurological change. This causes fear and withdrawal from communication. It was observed that first-year nursing students, who have very limited theoretical communication knowledge, have problems integrating their existing theoretical knowledge. The reason for this is the "*Differences between ideal theoretical knowledge and area of practice*", which is another theme of the study.

The data of two studies conducted in Malawi and South Africa show us that the clinical practice environment can be very different, the discussion environment in the clinical environment is idealized in theory, and this ideal environment knowledge that students learn in theory and the clinical environment are very different.[39,40] In a study conducted in Israel, it was reported that nursing students were strengthened theoretically, but theoretical knowledge could not be reflected in the clinical field, which has a more complex structure. The study draws attention to gaps in theoretical knowledge and practice.[41] In cases where the clinical environment and theoretical knowledge are incompatible, nursing students generally feel the lack of communication more. This situation may bring to mind the question of "where am I doing wrong" for them. For example, it is clearly seen in our findings that the students learned that they should establish "eye communication" during communication, but the complex clinical environment brought an acute neurological/neuropsychiatric patient to them. At this point, a mentor-student relationship is required, showing how idealized theoretical knowledge can be used in complex situations.

In the mentor-student relationship, the issues related to mentors, such as using their own value judgments, reflecting their own attitudes and efforts to integrate their norms into communication/clinic· ignoring student nurses, and mentors' inadequacy in the clinical environment etc. come to the fore.[42-45] The data of a study in Kenya concluded that 54% of the student nurses stated that

mentor nurses had insufficient communication-interaction.[45] In a study conducted in Africa, it was reported that mentor-registered nurses spent very little time with the nursing students and could not afford enough knowledge/dedication to transform theoretical knowledge into practice.[46,47] In the study, it is clearly seen that the students' answers were that the mentor nurses generally gave insufficient supervision and the reason for this was mostly lack of knowledge (although various reasons were reported) and lack of communication.

According to a study conducted in Sri-Lanka, there are basically two parameters affecting the student-mentor relationship. One of them is reported as workload and the other as socio-cultural differences.[48] Mentor nurses cannot provide supervision that will add value to nursing education in an overcrowded and complex environment.[49] Moreover, the fact that nurses are separated as academic and clinical staff creates a mentor confusion. The fact that they have little or no connection with each other also reveals mentors' knowledge and empathy problems in the clinical environment.[50-51]

Considering that the same problems are present in the study, the fact that mentor nurses are separated from the academic staff and that they do not have enough knowledge/empathy/mentoring training may reveal these problems.

Study limitations

Since the student sample with which the study was conducted was 1st grade, a group that had not received any communication training was selected. When the nursing course contents are examined, it is seen that some schools offer communication-based courses, but some do not. Therefore, the data has a limitation in explaining the communication difficulties of all 1st year nursing students. However, this situation makes the study important in terms of making comparisons with other studies (2nd, 3rd, 4th grade nursing students) in the discussion.

Conclusion

The results of the study show that the clinical environment contains an unknown and students begin by experiencing communication-related fears in this unknown environment. Although orientation programs are useful in this regard, the main issue is how students integrate theoretical knowledge into the clinic. Therefore, 1st year students need to learn this basic theoretical knowledge in the clinic by taking a course such as "health communication". In this case, mentor nurses are responsible for learning that "things are not always as written in the books" in the clinic. However, all literature shows that mentor nurses cannot provide sufficient clinical supervision. In our study, it is thought that there are important problems related to mentor nurses such as ignoring students, heavy workload, and lack of knowledge/ignoring patient communication, as far as it is especially understood from students' answers. The fact that clinical orientations should be a long process that does not include only a few hours, that mentor nurses receive a mentoring training from staff in this context, and that students undergo a basic communication training can be some of the things to be done in this regard.

Declarations

Funding

This research is not funded by any institution or organization.

Author contributions

Conceptualization, P.H. and P.H.; Methodology, P.H.; Software, P.H.; Validation, P.H., P.H. and P.H.; Formal Analysis, P.H.; Investigation, P.H.; Resources, P.H.; Data Curation, P.H.; Writing – Original Draft Preparation, P.H.; Writing – Review & Editing, P.H.; Visualization, P.H.; Supervision, P.H.; Project Administration, P.H.; Funding Acquisition, P.H

Conflicts of interest

There is no conflict of interest in this study.

Data availability

The data set of this study is not shared openly in any medium.

Ethics approval

The ethical permission of the study, numbered 07-22 and dated 30.03.2022, was obtained from Tokat Gaziosmanpaşa University Scientific Research and Publication Ethics. Before this permission, written study permission was obtained from the school of the nursing students where the study would be conducted. The participants were informed verbally and the Helsinki declaration was read to each of them. Informed consent was signed by the participants.

Reference

1. Dinmohammadi M, Jalali A, Peyrovi H. Clinical learning experiences of Iranian student nurses: A qualitative study. *Nursing Practice Today.* 2016;3(1):31-39.
2. Kelly C. Student's perceptions of effective clinical teaching revisited. *Nurse Educ Today.* 2007;27(8):885-892. doi: 10.1016/j.nedt.2006.12.005
3. Başar G, Semiha A, Durna Z. Evaluation of problem solving and communication skills in nurses and nursing students. *Gümüşhane Üniversitesi Sağlık Bilimleri Dergisi.* 2015;4(1):125-147.
4. Kourkouta L, Papathanasiou IV. *Communication in nursing practice. Mater Sociomed*. 2014;26(1):65-67.
5. Shorey S, Kowitlawakul Y, Devi MK, Chen HC, Soong SKA, Ang E. Blended learning pedagogy designed for

communication module among undergraduate nursing students: A quasi-experimental study. *Nurse Educ Today*. 2018;61:120-126. doi: 10.1016/j.nedt.2017.11.011

6. Aktaş YY, Karabulut N. A Survey on Turkish nursing students' perception of clinical learning environment and its association with academic motivation and clinical decision making. *Nurse Educ Today.* 2016;36:124-128. doi: 10.1016/j.nedt.2015.08.015
7. Fadana FP, Vember HF. Experiences of undergraduate nursing students during clinical practice at health facilities in Western Cape, South Africa. *Curationis.* 2021;44(1):e1-e10. doi: 10.4102/curationis.v44i1.2127
8. Gutiérrez-Puertas L, Márquez-Hernández VV, Gutiérrez--Puertas V, Granados-Gámez G, Aguilera-Manrique G. Educational interventions for nursing students to develop communication skills with patients: a systematic review. *Int J Environ Res Public Health*. 2020;17(7):2241. doi: 10.3390/ijerph17072241
9. Fioravanti MA, Hagle H, Puskar K, et al. Creative learning through the use of simulation to teach nursing students screening, brief intervention, and referral to treatment for alcohol and other drug use in a culturally competent manner. *J Transcult Nurs.* 2018;29(4):387-394. doi: 10.1177/1043659617727832
10. Kaplonyi J, Bowles KA, Nestel D, Kiegaldie D, Maloney S, Haines T, Williams C. Understanding the impact of simulated patients on health care learners' communication skills: A systematic review. *Med Educ*. 2017;51:1209-1219.
11. Henderson A, Rowe J, Watson K, Hitchen-Holmes D. Graduating nurses' self-efficacy in palliative care practice: An exploratory study. *Nurse Educ Today.* 2016;39:141-146. doi: 10.1016/j.nedt.2016.01.005
12. Henderson A, Harrison P, Rowe J, Edwards S, Barnes M, Henderson S. Students take the lead for learning in practice: A process for building self-efficacy into undergraduate nursing education. *Nurse Educ Pract.* 2018;31:14-19. doi: 10.1016/j.nepr.2018.04.003
13. Earl Grace L. Using cooperative learning for a drug information assignment. *Am J Pharm Educ*. 2009; 73(7):132. doi: 10.5688/aj7307132
14. Van Der Laan S, Joyce Spindle Roxanne M. The impact of group formation in a cooperative learning environment. *Journal of Accounting Education.* 2007;25(4): 153-167.
15. Lin MF, Hsu WS, Huang MC, Su YH, Crawford P, Tang CC. "I couldn't even talk to the patient": Barriers to communicating with cancer patients as perceived by nursing students. *Eur J Cancer Care (Engl).* 2017;26(4):12648. doi: 10.1111/ecc.12648
16. Shahzad S, Ali N, Younas A, Tayaben JL. Challenges and approaches to transcultural care: An integrative review of nurses' and nursing students' experiences. *J Prof Nurs.* 2021;37(6):1119-1131. doi: 10.1016/j.profnurs.2021.10.001
17. Gül Ş, Demir Karabulut S, Eren H, et al. Nursing students' experiences with death and terminal patients during clinical education. *OMEGA-Journal of Death and Dying.* 2022;85(3):628-649.
18. Krueger RA. *Focus Groups: A Practical Guide for Applied Research*. Sage Publications: California; 1988.
19. Denzin NK. *The Research Act. 3rd edition. Prentice Hall: Englewood Cliffs*. New Jersey;1989.
20. Stewart DW, Shamdasani PN. *Analysing focus group data. In Focus Groups: Theory and Practice*. Shamdasani PN (ed.) Sage Publications: Newbury Park; 1990.
21. Barbour RS, Kitzinger J. *Developing focus group research: politics, theory and practice.* Sage Publications: Newbury Park; 1999.
22. Patton MQ. *Qualitative Evaluation and Research Methods. 2nd edition*. Sage Publications: Newbury Park; 1990.
23. Graneheim UH, Lundman B. Qualitative content analysis in nursing research: concepts, procedures and measures to achieve trustworthiness. *Nurse Educ Today*. 2004;24:105-112. doi: 10.1016/j.nedt.2003.10.001
24. Stewart DW, Shamdasani PN. *Analysing focus group data. In Focus Groups: Theory and Practice.* Shamdasani PN (ed). Sage Publications: Newbury Park;1990
25. Polit DF, Hungler BP. *Nursing research: Principles and Methods*. Philadelphia, New York; 1999.
26. Jan Liora K, Popescu L. Israel's Nursing Students' Stress Sources and Coping Strategies During Their First Clinical Experience in Hospital Wards-A Qualitative Research. *Social Work Review/Revista de Asistenta Sociala*. 2014;13(4):78560642.
27. Fard MY, Pouladi S, Kamali F, et al. The stressing factors in clinical education: the viewpoints of students. *Iranian Journal of Medical Education*. 2008;8(2).
28. Sun FK, Long A, Tseng YS, Huang HM, You JH, Chiang CY. Undergraduate student nurses' lived experiences of anxiety during their first clinical practicum: A phenomenological study. *Nurse Educ Today.* 2016;37:21-26. doi: 10.1016/j.nedt.2015.11.001
29. Joolaee S, Jafarian Amiri SR, Farahani MA, Varaei S. Iranian nursing students' preparedness for clinical training: A qualitative study. *Nurse Educ Today.* 2015; 35(10):e13-e17. doi: 10.1016/j.nedt.2015.07.026
30. Damodaran DK, Kandasamy M. Clinical Environment: Experiences of Undergraduate Student Nurses. *International Journal of Advances in Nursing Management*. 2015;1(1):25-30.
31. Sheu S, Lin HS, Hwang SL. Perceived stress and physio-psycho-social status of nursing students during their initial period of clinical practice: the effect of coping behaviors. *International Journal of Nursing Studies.* 2002;39(2):165-175.
32. O'brien F, Keogh B, Neena K. Mature students' experiences of undergraduate nurse education programmes: The Irish experience. *Nurse Educ Today.* 2009;29(6):635-640. doi: 10.1016/j.nedt.2009.01.008
33. Rafati F, Rafati S, Khoshnood Z. Perceived Stress Among Iranian Nursing Students in a Clinical Learning Envi-

ronment: A Cross-Sectional Study. *Adv Med Educ Pract.* 2020;11:485-491. doi: 10.2147/AMEP.S259557

34. Jamshidi N, Molazem Z, Sharif F, Torabizadeh C, Najafi Kalyani M. The challenges of nursing students in the clinical learning environment: A qualitative study. *Scientific World Journal.* 2016;2016:1846178.
35. Günay U, Kilinç G. The transfer of theoretical knowledge to clinical practice by nursing students and the difficulties they experience: A qualitative study. *Nurse Educ Today.* 2018;65:81-86. doi: 10.1016/j.nedt.2018.02.031
36. Luhanga F, Yonge O, Myrick F. Precepting an unsafe student: the role of the faculty. *Nurse Educ Today.* 2008;28(2):227-231. doi: 10.1016/j.nedt.2007.04.001
37. Polit DF, Beck CT. *Nursing research Principles and methods.* Lippincott Williams&Wilkins; 2004.
38. Gemuhay HM, Kalolo A, Mirisho R, Chipwaza B, Nyangena E. Factors affecting performance in clinical practice among preservice diploma nursing students in Northern Tanzania. *Nurs Res Pract.* 2019; 2019:3453085. doi: 10.1155/2019/3453085
39. Mabuda BT, Potgieter E, Alberts UU. Student nurses' experiences during clinical practice in the Limpopo Province. *Curationis*. 2008;31(1):19-27. doi: 10.4102/curationis.v31i1.901
40. Msiska G, Smith P, Fawcett T. The "lifeworld" of Malawian undergraduate student nurses: The challenge of learning in resource poor clinical settings. *International Journal of Africa Nursing Sciences.* 2014;1:35-42.
41. Akram AS, Mohamad A, Akram S. The role of clinical instructor in bridging the gap between theory and practice in nursing education. *Int J Caring Sci.* 2018;11(2):876-882.
42. Levett-Jones T, Lathlean J, Higgins I, McMillan M. Staff–student relationships and their impact on nursing students' belongingness and learning. *J Adv Nurs.* 2009;65(2):316-324. doi:10.1111/j.1365-2648.2008.04865.x
43. Baluyot CMA, Blomberg K. Challenges in Assessment Situations in Clinical Studies: How do Nurse Mentors in Home-Based Health Care Services Experience Assessment Situations in Supervision of 2nd Year Bachelor Nursing Students. *Nur Primary Care.* 2019;3(1):1-8.
44. Amoo SA, Menlah A, Garti I, Appiah EO. Bullying in the clinical setting: Lived experiences of nursing students in the Central Region of Ghana. *PLoS One.* 2021;16(9):e0257620. doi: 10.1371/journal.pone.0257620
45. Tuitoek D. Mutinda A, Kubende P. Nursıng Students'perceptıon On Clınıcal Teachıng And Learnıng At Kenya Methodıst Unıversıty, Kenya. *Journal of Health, Medicine and Nursing.* 2022;8(2):48-68.
46. Kerthu HS, Nuuyoma V. Theory-Practice Gap: Challenges Experienced by Nursing Students at the Satellite Campus of a Higher Education Institution in Namibia. *International Journal of Higher Education.* 2019;8(5):21-28.
47. Rashawn Mohamed Abd-Elhady T, Hamdy Nasr Abdelhalim E, Abd El Reheem Abd El Reheem H, Mosaad Mohamed Elghabbour G. Nursing Students' Experience and Satisfaction with the Clinical Learning Environment. *International Egyptian Journal of Nursing Sciences and Research.* 2022;3(1):437-454.
48. Aluwihare-Samaranayake D, Ogilvie L, Cummings GG, Gellatly IR. The nursing profession in Sri Lanka: Time for policy changes. *International Nursing Review.* 2017;64(3):363-370.
49. Da Silva KA, Rodrigues MS, Ramos Moreira FB, et al. Soil survey using geostatistics in an agricultural experimental area in Petrolina, Pernambuco state, Brazil. *Comunicata Scientiae.* 2017;8(1):175-180.
50. Rathnayake S, Ekanayaka J. Depression, anxiety, and stress among undergraduate nursing students in a public university in Sri Lanka. *Int J Caring Sci.* 2016;9(3):1020-1032.
51. Ross JG, Dunker KS. New clinical nurse faculty orientation: A review of the literature. *Nurs Educ Perspect.* 2019;40(4):210-215. doi: 10.1097/01.NEP.0000000000000470

Wydawnictwo UR 2023
ISSN 2544-1361 (online)
doi: 10.15584/ejcem.2023.3.6

http://www.ejcem.ur.edu.pl
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2023; 21 (3): 605–616

REVIEW PAPER

Pathogenesis of selected multiple primary neoplasms

Marcin Kazimierz Witek, Sabina Skrzynecka, Mateusz Bartoszek, Julia Michalik, Jakub Pudźwa

Students' Scientific Association at the Medical College, Rzeszow University, Rzeszow, Poland

ABSTRACT

Introduction and aim. Multiple primary tumors are defined as having more than one primary tumor in a different organ location in the same person. Therefore, it is important to know pathogenesis of multiple primary neoplasms to discover new forms of primary prevention and secondary prevention, especially connected with genetic tests which are important for the future of medicine as a part of personalized medicine. The aim of the study is to present selected aspects of the pathogenesis of multiple primary neoplasms.

Material and methods. PubMed databases and Google Scholar were searched.

Analysis of the literature. The rising risk of developing multiple primary cancers is a consequence of the progressive growth and ageing of the population and development of cancer in patients previously treated for cancer. The formation of secondary neoplasms may be multifactorial – to a large extent it is associated with genetic factors that may facilitate neoplastic transformation, for example as a result of radiation therapy, chemotherapy, inherited syndromes, environmental factors such as tobacco or alcohol, sometimes random somatic mutations.

Conclusion. Knowledge of the pathogenesis of multiple primary tumors can contribute to a better understanding of the problem, as well as help in the prevention or early diagnosis of multiple primary tumors (primary and secondary prevention).

Keywords. pathogenesis, multiple primary neoplasms, multiple primary tumors, oncogenetics, personalized medicine, tumor biology

Introduction

Multiple tumors are understood to mean two or more primary tumors in an individual that come from a primary place or tissue and are not extension, recurrence or metastatic. In cancer patients, the risk of developing new primary cancer is 20% higher than in the general population. Of those who have had cancer over the age of 60, about a one third are diagnosed with more than one other neoplasm.[1] Multiple primary neoplasms (MPN) are divided into three groups (Fig. 1): (1) neoplasms related to previous treatment, (2) neoplasms associated with disease syndromes, accompanied by an increased risk of cancer, (3) neoplasms in which typical factors play a role in the development of environmental or genetic predispositions. Moreover, multiple tumors can also develop by chance.[2]

Aim

The aim of the study is to present selected mechanisms of the pathogenesis of MPN.

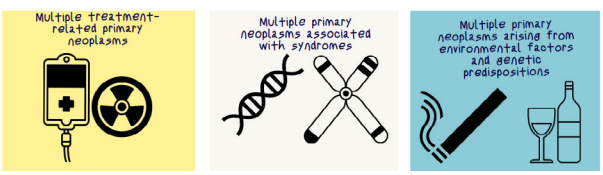

Fig. 1. Etiology of MPN pathogenesis

Corresponding author: Marcin Kazimierz Witek, e-mail: martinusvitek@gmail.com

Received: 23.02.2023 / Revised: 2.05.2023 / Accepted: 9.05.2023 / Published: 30.09.2023

Witek MK, Skrzynecka S, Bartoszek M, Michalik J, Pudźwa J. *Pathogenesis of selected multiple primary neoplasms.* Eur J Clin Exp Med. 2023;21(3):605–616. doi: 10.15584/ejcem.2023.3.6.

Material and methods

There were searched PubMed databases and Google Scholar.

Analysis of the literature

Multiple treatment-related primary neoplasms

Secondary gliomas developing after radiation therapy

Gliomas are common secondary neoplasms associated with previous radiotherapy to the area of the brain or the cerebrospinal axis. Most often they belong to high-grade astrocytomas, which have a poor prognosis. López et al. conducted genetic profiling of 12 gliomas associated with prior radiotherapy to determine their molecular pathogenesis. The primary tumors were medulloblastoma, intracranial germinoma, leukaemia, Hodgkin's lymphoma, craniopharyngioma, and pineocytoma. The interval between radiotherapy and secondary diagnosis of glioblastoma was between 4 to 41 years (mean 16 years). Ten of the secondary gliomas were high-grade infiltrating astrocytomas – they showed clearly aneuploid genomes, with significantly increased numbers of intrachromosomal breakpoints and focal amplification/homozygous deletions. The changes found were probably the result of DNA double-strand breaks caused by ionizing radiation. In secondary gliomas, a high frequency of TP53 mutations, CDK4 amplification or homozygous CDKN2A deletion, and amplification or rearrangement of receptor tyrosine kinase and Ras-Raf-MAP kinase pathway genes (PDGFRA, MET, BRAF, and RRAS2) were noted. It is worth mention that changes in the CDKN2A and CDK4 genes as well as a similar activation of the receptor tyrosine kinase and Ras-Raf-MAP pathway genes typically occur in high-grade spontaneous gliomas in both children and adults. López et al. speculated that the TP53 mutation is selected early in the development of radiation-induced gliomas, which allows the neoplasm initiating cell to survive and expand with numerous chromosomal rearrangements induced by ionizing radiation. It is worth mention that TP53 mutations have also been identified with high frequency in radiation-induced sarcomas. Of note, in secondary neoplasms, no changes were found in IDH1, IDH2, H3F3A, HIST1H3B, HIST1H3C, TERT (containing the promoter region) and PTEN, which are typical of the major subtypes of diffuse gliomas in children and adults. Unlike wild-type IDH gliomas in the adult cerebral hemispheres, high-grade radiation-associated gliomas only rarely contain an EGFR amplification or mutation, and are instead more likely to contain PDGFRA or MET amplification or mutations. Compared to both high-grade gliomas in children and wild-type IDH glioblastomas in adults, high-grade gliomas associated with radiation only rarely contain NF1 inactivation, and instead more frequently show BRAF rearrangement or high-grade focal RRAS2 amplification.

The other two types of gliomas observed in the study were anaplastic astrocytoma and glioblastoma multiform. It is worth mentioning that in both of these low-grade gliomas, there was no increase in DNA intrachromosomal copy number breakpoints, and focal amplification/homozygous deletions that are typically seen in radiation-associated high-grade gliomas. It is therefore uncertain whether these two tumors were actually caused by radiation. However, pathogenic changes identified in both tumors (KIAA1549-BRAF fusion and homozygous SMARCB1 deletion) were due to chromosome breaks rather than single nucleotide variants, could have been induced by ionizing radiation during treatment of the primary malignant neoplasm.[3]

Secondary leukemias associated with previous chemotherapy

The definition of MPN corresponds to tumors. However, the secondary primary malignancies can also be hematological malignancies such as leukaemias.[4] Secondary acute myeloid leukemia refers to acute myeloid leukemia, which arises based on for example received chemotherapy or radiotherapy due to treating breast cancer as the first primary cancer. Breast cancer, non-Hodgkin's lymphomas, and Hodgkin's lymphomas are the three most frequently primary cancers that develop secondary AML after treatment.[5] The incidence of secondary AML associated with prior oncological treatment ranges from 0.8 to 6.3% after 20 years of conventional therapy, with a significant reduction in risk after 10 years.[6] Secondary acute myeloid leukemia has a worse prognosis than its *de novo* counterparts, with a 5-year overall survival <30% despite advanced insight into pathogenesis and new treatment methods available. Poor prognosis in these cancers depends both on patient-related factors and those related to AML.[5]

Genetic aberrations in hematopoietic stem cells and progenitor cells exposed to cytotoxic therapy may provide a survival advantage that leads to clonal proliferation and eventually manifests as secondary AML.[6] The bone marrow microenvironment, including pluripotent mesenchymal cells and their descendants, endothelium of bone marrow sinusoids, fibroblasts, reticular cells, adipocytes and catecholaminergic nerve fibers, regulates almost all functions of hematopoietic stem cells - altered functions of these cells, caused by the therapy, may also contribute to the pathogenesis of secondary AML. Cytotoxic drugs cause the release of many pro-inflammatory cytokines (TNF alpha, IL-6, TGF beta) and the generation of free radicals that damage both enzyme cells and niche autonomic nerve fibers. Their altered functions in several mice models have been shown to be sufficient for the occurrence of AML.

The use of drugs belonging to alkylating compounds and topoisomerase II inhibitors (TOPII inhibitors) is as-

sociated with an increased risk of developing secondary AML. The mechanism behind this is due to the formation of double-strand breaks, which result in cell growth and apoptosis arrest. If double-strand breaks are not repaired, can generate chromosomal changes and genomic instability characteristic of these drugs.[5]

Alkylating agents

Alkylating compounds form cross-link DNA, leading to single-strand and double-strand breaks during the repair process. The result of these lesions are deletions in chromosomes 5 or 7 and haploinsufficiency of the EGR1 neoplasm suppressor gene on chromosome 5q, which are events initiating the pathogenesis of AML.[6] The development of AML seems to be gradual, is often preceded by MDS and occurs after a period of about 5-7 years, caused by the deactivation of many suppressor genes.[5]

TOPII inhibitors

The mechanism of topoisomerase II inhibitors responsible for the development of secondary AML is based on their interference with DNA re-ligation and chromosome breakage.[6] This results in chromosomal translocations that most often include the KMT2A genes on chromosome 11q23, RUNX1 on 21q22, and PML/RARA. Leukemia takes 1-3 years to develop and is almost never preceded by MDS. This short time is due to translocations that result in a dominant oncogene such as KMT2A fusing at 11q23, so fewer subsequent mutations are needed to transform to a leukemic phenotype.[5,6]

Radiotherapy-related breast cancer

Ionizing radiation directly and indirectly causes DNA damage and increases the production of reactive oxygen and nitrogen species (RONS). RONS lead to DNA damage and epigenetic changes that result in mutations and genomic instability. The proliferation of RONS increases the effects of DNA damage and mutations. This causes inflammation which additionally contributes to further carcinogenesis.[7]

We know that treatment neoplasms in childhood is connect with more recently occurrence of secondary primary tumors in the future. Brown et al. indicate in his study that people who have been treated at neoplasm in their childhood – have secondary primary neoplasms including breast cancers with poorer overall survivor due to poor prognosis risk factors. Although less than 3% of childhood neoplasm survivors develop second primary neoplasm within 15 years of their initial diagnosis, the cumulative incidence approaches 10% by 30 years after diagnosis in high-risk populations.[8] Hodgkin lymphoma survivors had greater risk of secondary breast cancer influenced by age at treatment (higher among women exposed early to irradiation with the highest relative risk at 15 years or younger, and to be not significant among women diagnosed after the age of 40 or 50 years) and importantly time since treatment and dose (>40 Gy yielding greatest risk).[9] Moskowitz et al. claim that highest risk of developing breast cancer have patients who received lower doses of radiotherapy (14 Gy) to a large area of the chest (whole lung field), which meant that a larger area of breast tissue was covered. Receive high-dose radiotherapy (30-40 Gy) to a smaller area of the chest (mantle field) have a similar or lower risk of developing breast cancer (mediastinal field), but an increased risk compared to women who have not been previously irradiated.[7] Thurkaa Shanmugalingam et al. suggest a IGF-1 role in increasing breast cancer risk which is the most common second primary cancer that develops in young women treated for Hodgkin's lymphoma with supradiaphragmatic irradiation. Pubertal growth of the mammary gland is mediated predominantly by the actions of IGF-1 and GH via estrogen. Among younger women more prone to developing a secondary breast cancer due to the increased levels of both IGF-1 and estrogen during puberty, and the promoting effects of IGF-1. In breast cancer, IGF-1 is mainly expressed in the stromal cells (especially fibroblasts) and also rarely in the breast epithelium. Consider also an endocrine role of IGF-1 effects on the malignant transformation of breast tissue. IGF-1 protect breast cancer cells from apoptosis and induces survival. Also suggest that IGF-1 produced by the stromal cells is increased in breast cancer. This may then promote growth of a second primary breast cancer by endocrine way.[10] Radiotherapy breaks DNA, especially microsatellite alterations, which is more common in secondary than not-related with radiotherapy.[11] Studies suggest increase survival of mammary epithelial cells with accumulating DNA damage and stepwise carcinogenesis due to premenopausal women with high IGF-1 levels were at risk of higher IGF1R activation in these cells.[10]

MPN associated with syndromes

Pathogenesis of MPN on the example of germ cell tumors – Wilms tumor and hepatoma in Beckwith-Wiedemann syndrome

Nephroblastoma

Wilms' tumor – also known as nephroblastoma, is a malignant neoplasm, the second most frequent in the pediatric population. This solid tumor occurs with a frequency of about 1 in 10,000 cases, while a bilateral Wilms tumor occurs in about 6 out of 100 children. This tumor is more common in girls than in boys, and the vast majority of cases, about 90%, are detected in children under 7 years of age.[12] The family form occurs only in about 1% of patients.

The pathogenesis of this neoplasm is well described in the literature. A significant relationship has been shown between the presence of nephroma and the pres-

ence of mutations in the WT1 and WT2 genes, which are located on chromosome 11 (11p13, 11p15) and CTNNB1, WTX, TP53, MYNC.[13]

The presence of mutations in the TP53 gene and the loss of heterozygosity in chromosomes1p, 1q, 11p15, 16q are negative prognostic factors.

Nephrotic nephroblastoma is believed to develop from persistent metanephric tissue or residual nephrogenic tissue that should normally disappear during childhood.

Nephroblastoma is more common in children with other syndromes, such as Perlman syndrome, Denys-Drash syndrome, Beckwith-Wiedemann syndrome or WAGR syndrome (Wilms tumour-aniridia syndrome). The risk of Wilms tumour in paediatric patients with Denys-Drash syndrome is estimated at 90%.[14]

Hepatoblastoma

Hepatoblastoma is a malignant tumor of the liver that occurs most frequently in children under 2 years of age.[15] This tumor originates from immature liver precursor cells, and in terms of histology, two main types are distinguished: epithelial and mesenchymal.

The epithelial type includes the following subtypes: fetal, pleomorphic, embryonal, macrotrabecular, small cell undifferentiated, cholangioblastic and mixed epithelial variants. The mesenchymal type, on the other hand, is divided into a subtype with and without teratoid features. Increased serum levels of alpha-fetoprotein (AFP) are found in 90% of patients.

In about 30% of patients with hepatoblastoma, coexistence of Beckwith-Wiedemann syndrome, FAP, Edwards syndrome, Li-Fraumeni syndrome, nephroblastoma and trisomy of 21 pair of chromosomes is found. However, most cases of hepatoblastoma occur sporadically.[16]

Literature data indicate the involvement of a defect in the WNT signaling pathway in the pathomechanism. This abnormality is responsible for the accumulation of beta-catenin. Moreover, in the case of aggressive hepatoblastoma, activation of TERT and MYC has also been demonstrated.[17]

In Beckwith-Wiedemann syndrome, a significant role of factors predisposing to the formation of hepatoblastoma, such as cell cycle regulators, MAPK kinase and (PI3K)/AKT, has been demonstrated.

Factors specific to hepatoblastoma oncogenesis – Beckwith-Wiedemann syndrome oncogenesis network – have been identified. Changes in 11p15 participate in hepatoblastoma oncogenesis by initially disrupting the balance of cell cycle regulators and chromatin organizers, including histone deacetylase 1 (HDAC1), ATP-dependent helicase X, and F-Box and WD repeat domain. In addition, oncogenic factors such as dickkopf WNT 1 and 4 signaling pathway inhibitor, WNT16, forkhead box O3 (FOXO3) and MAPK10 are differentially expressed in 11p15 altered HB both against BWS and non-syndromic background.[18,19]

When comparing nephroblastoma and hepatoblastoma in terms of pathomechanism, an association consisting in the presence of a defect on chromosome 11 in both of these neoplasms can be noticed.

Pathogenesis of MPN on the example of neoplasms of epithelial origin – breast cancer and ovarian cancer in HBOC syndrome

Hereditary breast and ovarian cancer (HBOC) associated with BRCA1 and BRCA2 variants is characterized by an increased risk of breast cancer in women and men, ovarian cancer (including fallopian tube cancer and primary peritoneal cancer) and, to a lesser extent, other cancers such as prostate cancer, pancreatic cancer and melanoma, mainly in people with the pathogenic BRCA2 variant. The risk of concomitant cancer varies depending on whether HBOC is caused by the BRCA1 or BRCA2 pathogenic variant.[20]

HBOC is a genetic cancer syndrome most commonly caused by germline mutations in the BRCA1 and BRCA2 genes. These are tumor suppressor genes located on chromosomes 17q21 and 13q12.3 respectively. These genes encode proteins involved in the repair of DNA double-strand breaks through homologous recombination, which is one of the key mechanisms for maintaining DNA integrity. To perform this function, BRCA proteins interact with many other molecules that together form a protein complex; without a functional BRCA complex, the cell relies on alternative DNA repair mechanisms, some of which are prone to errors and may further contribute to the development of genetic aberrations. Because of this phenomenon, HBOC patients with germline BRCA1 and BRCA2 mutations have an increased risk of developing a number of neoplasms, particularly those arising in the breast, as well as the ovaries and fallopian tubes.[21]

BRCA1 and BRCA2 encode proteins, subject to expression in the cell nucleus in different tissues during S and G2 phases. BRCA genes are essential for maintaining chromosome structure. Therefore, due to their neoplasm suppressor function, they have been called "caretakers" of the genome because correct structure and function ensures genome stability. In contrast, any abnormality of the "caretakers" results in genome instability, which is an important factor in the pathomechanism of neoplasm.

The starting point for elucidating the functions of BRCA1 and BRCA2 relevant to cancer predisposition is the observation that mouse cells with deficient of the BRCA2 homologue show spontaneous aberrations in chromosome structure that accumulate during division in culture. Microscopically, abnormalities include not only

broken chromosomes and chromatids, but also triple and quadruple-stranded structures, markers of defective mitotic recombination typical of human diseases, Bloom syndrome and Fanconi anemia, also associated with increased susceptibility to neoplasm (including breast cancer).[22]

Individuals with germline mutations in any of the BRCA1/2 genes are at higher risk of developing certain types of neoplasms compared to the general population. Most BRCA mutation-induced cancers develop in the breast or ovary. Some studies indicate a role for oxidative stress during the menstrual cycle in the development of ovarian neoplasms. In addition, hormone regulation, especially estrogen, increases the frequency of double-strand brakes, indicating tissue specificity.[23]

Both BRCA genes are involved in DNA repair. Expression levels of BRCA1, BRCA2 and RAD51 increase in cells when they enter S phase, which indicate that they function during or after DNA replication. This means that BRCA1 and BRCA2 function in a common pathway that is responsible for genome integrity and maintaining chromosome stability. They form complexes that activate double-strand break repair and initiate homologous recombination. RAD51, on the other hand, is a key component of this mechanism. However, it appears that the roles played by BRCA1 and BRCA2 in this process are different.

Ubiquitination is the process by which proteins are tagged for degradation by the proteasome. BRCA1 acts with BARD1 in this ubiquitination process. Small ubiquitin-like ligases are required to localize BRCA1 in locations of DNA damage, and BRCA1 itself, post-translationally modified by sumoylation, acts with BARD1 as an E3 ligase and further ubiquitinates local proteins.

BRCA1 is part of the BRCA1-associated genome surveillance complex (BASC). This complex includes MSH2, MSH6, MLH1, ATM, BLM, the RAD50-MRE11-NBS1 complex and DNA replication factor C. All members of this complex play roles in the recognition of abnormal or damaged DNA, suggesting that BASC may serve as a sensor of DNA damage and as a regulator of the post-replication repair process.

BRCA1 also acts as a checkpoint, playing a crucial role in cell survival by preventing the propagation of DNA damage through cell cycle progression before DNA repair occurs.

BRCA1 is an integral part of the DNA damage signaling cascade, suggesting the existence of a positive feedback loop amplifying the DNA damage response. In addition, BRCA1 regulates the expression of G2M cell cycle checkpoint proteins, preventing an unplanned transition of the cell into mitosis at multiple levels of regulation. BRCA1 also plays a role in both transcription-coupled repair and global genome repair.[21]

BRCA2 plays a role through its close interaction with RAD51 via BRC repeats. In addition, RAD51 also interacts with the C-terminal region of BRCA2, TR2. This part of BRCA2 play a regulatory role in recombination repair. Phosphorylation of this part of BRCA2 may have a dual function, causing inhibition or activation during homologous recombination. BRCA2 also plays a role in homologous recombination at meiosis by interacting with RAD51 and DMC1. This suggests that BRCA2 not only plays a role in carcinogenesis, but additionally contributes to fertility problems in affected carriers. In summary, both BRCA genes are involved in DNA repair and both act on a common pathway that is responsible for genome integrity and maintaining chromosome stability.[21]

BRCA1 breast cancers

Most BRCA1 mutation-associated neoplasms are invasive ductal adenocarcinomas, accounting for 74%.[21] Morphologically, they are highly malignant carcinomas with no special type and show minimal, if any, tubular or glandular formation, markedly pleomorphic nuclei (significant variation in size and shape), vesicular chromatin, prominent nucleoli and high mitotic activity.[24] However, compared to sporadic breast cancer, a much higher incidence of BRCA1-associated neoplasms are classified as medullary carcinomas, 2% vs 13%, respectively. The other histological types of breast cancer are found more or less equally in tumors associated with BRCA1 mutations as in sporadic breast cancer. With regard to other histopathological features, it is observed that BRCA1 mutations more often accompanied by a low degree of differentiation (G3), high mitotic activity, frequent areas of necrosis and reduced tubular formation, with a higher degree of pleomorphism. All these features indicate a more aggressive phenotype. In addition, the tumors are often well delimited and show a significant degree of lymphoplasmocyte infiltration and a high incidence of lymphatic vessel invasion.

HER-2/neu amplification is rarely found in tumors with BRCA1 mutations. One explanation may be that in the BRCA1 germline mutation background, HER-2/neu is lost during loss of heterozygosity at the BRCA1 locus because HER-2/neu is located close to BRCA1 on chromosome 17. Gene expression profile analysis has provided a tools to distinguish between different breast cancer subtypes. Based on this data, BRCA1-related breast cancers are classified as basal like type. The gene expression profile of BRCA1-associated neoplasms includes genes that have been found to have functions in proliferation, angiogenesis, cell motility, cell adhesion, transcription and DNA repair. As mentioned above, breast tumors with BRCA1 mutations show expression of basal markers such as CK 5/6, CK14, EGFR, P-cadherin and caveolin 1, vimentin and laminin, confirming the basal subtype specified by immunohistochemistry. These data additional highlight that carcinogenesis in BRCA1 ger-

mline mutation carriers very often occurs as part of the „basal" pathway of progression.

Promoter hypermethylation of tumor suppressor genes has been shown to be slightly less abundant in breast cancers associated with a germline BRCA1 mutation, although it is still markedly higher than in normal tissue.

Copy number alterations commonly found in BRCA1-associated breast cancers are gain of 3q, 7p, 8q, 10p, 12p, 16p and 17q and loss of 2q, 3p, 4p, 4q, 5q, 12q, 16p and 18q. This only partly coincides with the copy number changes found in sporadic breast cancers associated with germline BRCA2 mutations.[21]

BRCA2 breast cancers

Similar to BRCA1-related breast cancers, the most common histological type of BRCA2 tumors is invasive ductal carcinoma (76%). Reports have been published of a higher incidence of neoplasms belonging to the invasive (pleomorphic) lobular, tubular and situs carcinomas in BRCA2-associated breast cancers compared with sporadic breast cancer. BRCA2 tumors are more often moderately or poorly differentiated carcinomas (grades 2 and 3) due to less tubular formation, greater nuclear pleomorphism and higher mitotic rates compared to sporadic neoplasms.

In a study using gene expression analysis to distinguish BRCA2-related neoplasms, the differentiating genes were those related to transcription, signal transduction, cell proliferation, cell adhesion and extracellular matrix remodelling. In this study, relatively high expression of FGF1 and FGFR2 was observed, which was confirmed by immunohistochemistry.[21] Using the gene expression profile mentioned earlier, the majority of BRCA2-associated breast cancers were classified as luminal. Looking more specifically at the molecular genetics, BRCA2-associated breast cancers show patterns of chromosome copy number gain and loss that do not occur in sporadic breast cancers. Copy number alterations more common in BRCA2-associated breast cancers are gain of 8q, 17q22-q24 and 20q13 and loss of 8p, 6q, 11q and 13q.[21]

BRCA1/2 ovarian cancers

BRCA1/2 germline mutations are found in approximately 15% of women with epithelial ovarian cancers, of which tubo-ovarian tumor is the most common subtype. The characteristic histopathological diagnosis of HBOC-associated fallopian tube-ovarian cancer with a BRCA mutation is high-grade serous carcinoma, and the prevalence of germline BRCA1 and BRCA2 mutations rises to approximately 25% in patients diagnosed with these neoplasms.

Morphologically, classic high-grade serous carcinoma shows expansive and infiltrative glandular and papillary growth with slit-like spaces. Cell nuclei are generally enlarged and irregular, with prominent nucleoli and active mitoses, including atypical forms. Immunohistochemically, serous carcinomas with a high degree of malignancy show p53 expression in an abnormal pattern (usually nuclear overexpression or complete absence of expression, and less often expression according to a cytoplasmic pattern), in addition to CK7, PAX8 and WT-1. P16 expression is usually diffuse, strong and block-like.[24]

In the context of BRCA-associated high-grade serous carcinoma, a variety of morphological features have been described. It was shown that tubo-ovarian carcinomas of BRCA1 germline mutation carriers tended to show high-grade and serous/undifferentiated histology, prominence of tumor-infiltrating lymphocytes, prominent nuclear atypia with giant/weird forms and abundant mitotic figures; these features had a negative predictive value of >94% and a positive predictive value of 21% for BRCA1 germline mutation status. Tumors with BRCA2 mutations also had pseudo-endometrioid and transitional features, but tended towards relative tumor-infiltrating lymphocytes deficiency and necrosis.[24]

MPN arising from environmental factors

Head and neck cancers are mainly squamous cell carcinoma and consists oral cavity, pharynx (nasopharynx, oropharynx, hypopharynx), larynx, paranasal sinuses and nasal cavity, salivary glands (but salivary glands, sinuses, or muscles or nerves of head and neck cancers are not squamous cell carcinoma and much less common). Head and neck cancers share the same risk factors and it seems due to the anatomical proximity of each part and histopathology of these cancers.[25,26]

Contribution of tobacco consumption to the carcinogenesis of oral squamous cell carcinomas

Oral squamous cell carcinoma accounts for more than 90% of histopathological cases of oral cancer.[27] Cianfriglia et al. described 200 cases of patients who had the first primary neoplasm as oral squamous cell carcinoma. In median follow-up of 3.2 years, the incidence rate of second primary neoplasm was 14%: 39% arose in the oral cavity, 18% in the oropharynx, 10% in the lung and 7% both in the lip and larynx. The second primary neoplasm in 96% of cases was also squamous cell carcinoma. One risk factor was the consumption of tobacco products.[28] Eberl et al. indicated especially high larynx, pharynx and oral cavity for males and oesophagus, oral cavity and urinary tract for females secondary primary cancers smoking-related after treated lung cancer.[29]

Jiang et al. point to 4 possible pathways (Fig. 2) for tobacco smoke-induced carcinogenesis of squamous cell carcinoma: epigenetic changes of oral epithelial cells, inhibition of multiple systemic immune functions,

changes in oxidative stress and a possible "tobacco-virus collaboration."

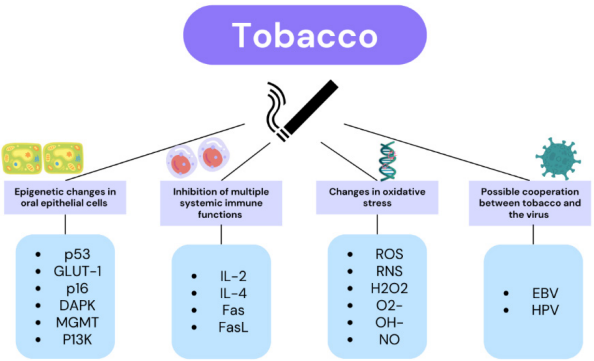

Fig. 2. Four possible pathways for tobacco smoke-induced carcinogenesis of squamous cell carcinoma and factors participate in pathogenesis

Epigenetic changes in epithelial cells

Several studies have shown that tobacco can cause abnormal expression of p53, GLUT-1, p16 (MTS, multiple tumor suppressor 1), DAPK (death-associated protein kinase), MGMT (O6-methylguanine-DNA methyltransferase), PI3K (the phosphatidylinositol 3-kinase) and other genes in the oral epithelium, which is associated with oral squamous cell carcinoma.

It has been observed that p53 aberrations are an initial alteration in the development of oral cancer. A high percentage of p53 protein overexpression has been found in pre-neoplastic lesions and oral neoplasms in epithelial cells among patients with heavy tobacco use.

Increased expression of GLUT-1, a glucose transporter required for glucose uptake by the cell, is observed in neoplasm cells. This indicates increased proliferative activity, energy requirements, aggressive develop and a poor response to ionizing radiation. GLUT-1 expression correlates significantly with the histological malignancy and stage (pTNM) of oral squamous cell carcinoma. Furthermore, GLUT-1 has been shown to increase the incidence of oral cancer in tobacco smokers.

Epigenetic alterations of the p16, DAPK, MGMT, PI3K, c-myc genes are frequently found in people with oral cancer.

Moreover, generally in squamous cell carcinoma of head and neck region, expression of alpha-7 nicotinic acetylcholine receptors (nAChRs) promotes proliferation and migration through phosphorylation of epidermal growth factor receptor (EGFR), protein kinase B (Akt), mammalian target of rapamycin (mTOR) and stimulation of beta-adrenergic receptors. Nicotine upregulates the expression of mesenchymal proteins (fibronectin and vimentin) and downregulates epithelial proteins (beta-catenin and E-cadherin), thereby supporting cell motility and invasion through induction of epithelial-mesenchymal transition (EMT).[27] Nicotine can interfere with drug efficacy via cytochrome P450 (CYP)-mediated metabolism, glucuronidation and/or protein binding, which may affect the efficacy of anticancer drugs. Tobacco use also promotes a pro-inflammatory tumor microenvironment, which further supports tumor growth.[30]

Inhibition of multiple systemic immune functions

Dysfunction of the immune system plays an important role in the escape of neoplasm cells from effector immune functions, leading to neoplasm development. The incidence of malignant neoplasms in immunocompromised patients is 100 times higher than in healthy individuals.

Tobacco-associated oral cancer is likely to be associated with multiple systemic immune abnormalities, particularly impaired CD4+ and CD3+ T lymphocytes and different regulation of IL-4 and IL-2 in subsets of CD8+ and CD4+ T lymphocytes in the peripheral blood. The findings suggest that tobacco may reduce IL-4 gene transcription.

The Fas receptor and FasL (Fas Ligand) system are associated with neoplasm immune escape. In tobacco-associated oral squamous cell carcinoma, up-regulation of FasL and down-regulation of the Fas receptor are observed.[27]

Changes in oxidative stress

Tobacco, which is a foreign substance, has been proved to stimulate the body to produce more free radicals, which with increasing concentrations, can damage cellular components, ultimately leading to denaturation or mutation, parasitic infections, inflammatory diseases and neoplasms. The development of oral cancer has been proven to be linked to oxidative stress. Free radicals (i.e. reactive oxygen species – ROS, reactive nitrogen species – RNS and reactive oxygen metabolites such as hydrogen peroxide – H_2O_2, superoxide anions – O_2^-, hydroxyl radicals – OH^-, nitric oxide – NO and malondialdehyde) can cause DNA damage. These include strand breaks, DNA-protein cross-links, base modifications, inhibition of DNA repair. In addition, lipid peroxides and reaction products with cell membrane fatty acids are formed. There is also reduced superoxide dismutase activity. In addition, one study found that smokers had a significantly higher risk of developing oral cancer than non-smokers based on erythrocyte glutathione reductase, superoxide dismutase, catalase and plasma thiol.[27]

Possible cooperation between tobacco and the virus

Epstein-Barr virus (EBV), or human herpes virus 4 (HHV-4), resides in a latency period in healthy carriers and, under the influence of changing external factors, the virus can be periodically reactivated. EBV is often associated with various malignancies, such as Burkitt's

lymphoma, Hodgkin's disease, gastric cancer and nasopharyngeal carcinoma.

Several EBV proteins are expressed in oral squamous cell carcinoma tissues. This is associated with specific tumor phenotypes, indicating a correlation between EBV infection and neoplasm development. The induction of oral and nasopharyngeal squamous cell carcinoma is explained by tobacco-induced activation of EBV and HPV16. Further epidemiological and experimental studies are needed to confirm the interaction between tobacco use and EBV activation in cancer development and to reveal potential mechanisms.[27]

Contribution of alcohol consumption and metabolism genes of this xenobiotic to the carcinogenesis of upper gastrointestinal cancers including cancers of the upper aerodigestive tract

Lv et al. in an epidemiological study of 161 patients with multiple primary cancers indicated that the main carcinogens of gastrointestinal and respiratory cancers were tobacco and alcohol use.[31]

A link between chronic alcohol consumption and the incidence of cancer in humans has been proved.[32] Smoking cessation and abstinence or moderation of alcohol consumption are key factors in the prevention of oral and pharyngeal and esophageal cancers.[33] The mechanisms through which alcohol drinking confers an increased risk of first or second primary cancers are likely to be quite similar among cancers of the upper aerodigestive tract (oral cavity, pharynx, larynx, and esophagus).[34] The mechanism of carcinogenesis in various alcohol-induced cancers is not fully understood, although likely events include genotoxic effect of acetaldehyde, generation of reactive oxygen species via cytochrome P450 2E1 (CYP2E1), abnormal folic acid and retinoid metabolism, increased estrogen levels and genetic polymorphisms.[32] The results of the study indicate that in upper gastrointestinal cancer survivors, continued alcohol abuse were associated with a more than 2-fold increased risk of subsequent upper gastrointestinal cancers.[34] Alcohol absorption begins in the mucosa of the upper gastrointestinal tract and takes place mainly in the stomach and small intestine. Acetaldehyde production occurs mainly in the liver, but acetaldehyde formation begins in the mouth and progresses along the gastrointestinal tract.[35] Ethanol is oxidized in the cytosol to acetaldehyde by alcohol dehydrogenase, and acetaldehyde is then oxidized in the mitochondrion to acetate by aldehyde dehydrogenase.[32] Exposure to acetaldehyde leads to molecular changes and mutagenesis, including the formation of DNA adducts, cross-linking of DNA-protein complexes, DNA strand breaks and chromosome aberrations. Locally, these changes can lead to dysplasia and further to oral and pharyngeal cancer.[33] Various studies indicate that the disproportion between alcohol dehydrogenase and aldehyde dehydrogenase activity plays a key role in alcohol-induced cancers. Significantly higher activities of different isoforms of these enzymes are observed in esophageal cancer, liver cancer and cervical cancer, making it possible to attribute polymorphisms of related genes to these isoforms.[32]

Tamura et al. in a study conducted on rat epithelial cells showed that ethanol is not only an inducer of gastric epithelial cell necrosis, but is also an inducer of oxidative stress and causes increased lipid peroxidation.[36] It is likely that the mechanism is related to the accumulation of lipid peroxidation products such as malondialdehyde and 4-hydroxynonenal, which in turn form exocyclic DNA adducts. Reactive oxygen species can act as messengers in intracellular signaling pathways leading to the transformation of a normal cell into a cancer cell. These pathways affect alter cell cycle changes by activating NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) and AP-1 (activator protein 1) (c-jun and c-fos) expression and promoting cell metastasis by regulating MAPK (mitogen-activated protein kinases). Accumulation of reactive oxygen species leads to up-regulation of vascular endothelial growth factor (VEGF) and monocyte chemotactic protein (MCP-1), which are key mediators of angiogenesis and tumor dissemination. Increased expression of the reactive oxygen species-mediated metalloproteinases MMP2 and MMP9 leads to extracellular matrix damage, increases cell motility and promotes distant metastasis.

Metabolism gene variants

Significant interactions between heavy drinking and the MTHFR (methylenetetrahydrofolate reductase gene) TT genotype (homozygous variant with mutation) have been described for head and neck area cancer, esophageal cancer and colorectal cancer. Compared to people with the CC (homozygous normal) genotype, those with the TT or CT (heterozygous) genotype have MTHFR activities of approximately 30% and 65%, respectively.

An increased risk of esophageal squamous cell carcinoma in heavy drinkers is associated with the CYP2E1c1/c1 or CYP2E1c1/c2 genotype. Observations indicate that the c2 variant allele shows 10-fold higher transcriptional activity, elevated protein levels and increased enzymatic activity compared to the c1 allele.

The ADH1B gene shows several polymorphisms and is associated with the risk of developing various cancers. Two studies in Asian populations showed a significantly higher risk of upper gastrointestinal, oral cavity and hypopharynx cancer in moderate or heavy drinkers with the ADH1B*1/*1 genotype than in those with ADH1B*1/*2 or ADH1B*2/*2. The enzyme encoded by ADH1B*1/*1 has only 1% and 0.5% oxidative capacity against those encoded by ADH1B*1/*2 and ADH1B*2/*2, respectively.[32]

The presence of the ALDH2 Lys allele has been shown to have a significantly increased direct effect on the risk of developing head and neck, esophageal and gastric cancers, even in people who have never smoked. In contrast, acetaldehyde-related carcinogenesis occurs locally in organs exposed to alcohol. It is assumed that alcohol abusers with the ALDH2 Lys allele are exposed to increased local acetaldehyde concentrations two to three times (via saliva) and five to six times (via gastric juice) higher than those without this allele. Increased acetaldehyde concentrations lead, among other things, to more DNA adducts and DNA damage.[35]

Bacterial flora and acetaldehyde metabolism

Acetaldehyde plays a major role in the carcinogenesis of alcohol-related upper gastrointestinal cancers, which is locally (oral mucosa and salivary glands) produced by microorganisms and also supplied with drinks and food products. Ethanol fermentation is a specific feature of microorganisms. Under hypoxia, food sugars such as glucose and fructose are converted to ethanol and acetaldehyde is formed as a by-product.

The microbial formation of acetaldehyde at mutagenic concentrations begins in the saliva and gastric juice in HCl-deficient stomachs immediately after alcohol consumption and continues as long as ethanol is present in the human body. The key role of the oral microbiome, particularly bacteria, is already well understood in this process.[33]

HPV-related secondary primary tonsil cancer

HPV infection is connected with head and neck squamous cell carcinomas. The molecular mechanisms of HPV-associated carcinogenesis involve the insertion of HPV genomic DNA into basal epithelial cells, leading to the expression of the viral oncoproteins E6 and E7. As a result, key cellular signaling pathways responsible for cell cycle control are altered by degradation of the tumor suppressor protein p53 via E6 and the retinoblastoma protein (pRb) via E7, leading to malignant transformation and immortalization of cells. In addition, HPV E6 protein interacts with c-myc to form the c-myc/E6 complex, which activates transcription of the human telomerase catalytic subunit (hTERT), contributing to tumor cell immortalization. Head and neck squamous cell carcinomas mainly are connected with tonsil cancer and likely same with HPV-related second primaries in this area apart tongue base and others. W. Strober et al. suggest that spreading HPV-related secondary tumour links with a single viral infection resulting in infection of cells at more than one anatomic location via intra-host spread (lymphatic, hematogenous, or salivary), infection of different tissue locations with the same virus at the same time, or on subsequent exposure or intralymphatic intraoropharyngeal metastases of tumor cells. Moreover, they claim that patient-specific (genetic and immunologic) and virus specific (such as particularly aggressive sublineages) could play a role. Multiple versus single primaries (younger patients, have small T1 tumor, no adenopathy N0) suggests different biological pathogenesis process but it needs more investigation.[30,37]

Diet and microbiome bacteria in second primary squamous cell carcinoma of head and neck

Certain bacteria are involved in the metabolic activation of carcinogenic chemicals such as acetaldehyde, which can promote carcinogenesis through genomic mutations. Chronic inflammation induced by persistent bacterial infection also supports several hallmark capabilities. Bacterial products, such as endotoxins, enzymes and metabolic waste, can cause DNA damage and consequently alter cell cycle control and signaling pathways, leading to further genomic instability and mutation. Certain immune cell responses to gut commensal bacteria are also associated with immunotherapy response. Red meat and processed meat also contain carcinogens (cooking at high temperatures produces carcinogenic substances such as PAHs, N-nitroso compounds (NOCs) and heterocyclic aromatic amines (HAA) that can cause genomic instability and mutations. All this factors can help in synchronous or metachronous carcinogenesis.[30]

Summary

Risk factors for the development of MPN include a previous treatment for neoplasm and should be considered an adverse effect of chemotherapy and radiotherapy. Therefore, an important element in the prophylaxis of secondary neoplasms is increased surveillance of people who have undergone neoplasm treatment, have a hereditary predisposition to neoplasms and have a specific lifestyle predisposing them to neoplasm (smoking, excessive alcohol consumption) or are exposed to environmental factors.[38]

Secondary neoplasms are considered the most serious complication of anticancer therapy and involve both chemotherapy and radiotherapy. The most common secondary neoplasm after chemotherapy is acute myeloid leukemia, which is usually preceded by myelodysplastic syndrome.[39] Secondary leukemias are a consequence of the use of alkylating drugs (cisplatin, carboplatin, cyclophosphamide, ifosfamide) and topoisomerase II inhibitors (etoposide, irinotecan).[40,41] Topoisomerase II inhibitors, including etoposide and teniposide, often cause rearrangements involving the mixed-lineage leukemia gene (MLL otherwise KMT2A) on chromosome 11q23. The prognosis is very poor for leukemias associated with rearrangements in the MLL gene, including secondary leukemias associated with etoposide. Typically, treatment-induced AML occurred after multiple

doses given in short intravenous infusions. The cumulative risk of complications over 4 to 5 years ranged from 0% to 18.4% in patients treated with cumulative doses ranging from 5200 mg/m^2 to 19200 mg/m^2.[42]

Radiotherapy can also have a significant impact on the development of secondary neoplasms. One example is radiotherapy for Hodgkin lymphoma. Although it significantly reduces the risk of regional recurrence and increases survival time as a part of treatment, but it is associated with an increased risk of secondary neoplasm in the irradiated area. Less than 3% of childhood neoplasm survivors develop second primary neoplasm within 15 years of their initial diagnosis, the cumulative incidence approaches 10% by 30 years after diagnosis in high-risk populations.[8]

According to a 2017 study, the development of a second primary neoplasm in children, adolescents and young adults has a significantly worse prognosis than in older patients. The differences in survival rate after secondary and first neoplasm are most significant in those under 40 years of age.[43] Therefore, it is important to know about the syndromes that run in the patient's family, which are associated with the occurrence of MPN and often manifest themselves at a young age, in order to implement appropriate prophylaxis.

Carcinogens such as tobacco smoke and alcohol are responsible for the development of numerous changes in tissues that are exposed to these substances. Smoking is a well-known factor responsible for the development of cancer in the respiratory tract, which is mainly related to direct exposure of the respiratory tract to the carcinogens contained in tobacco smoke. Long-term smoking can contribute to the simultaneous development of several neoplasms. The number of older people, as well as the number of people who have undergone treatment for neoplasm, is steadily increasing, which may result in an increased incidence of MPN in the future.[1,38] Therefore, the timing of exposure to carcinogens and the age of the patient may be important factors in the occurrence of MPN.

Conclusion

MPN are the subject lots of research and scientific analysis. However, looking at the increasing incidence of both primary and secondary primary tumors, it is also a public health concern. Increasing knowledge of secondary neoplasm formation, analysis of epidemiological data and understanding of risk factors allows effective primary prevention using modern methods such as genetic screening to detect predisposing mutations, as well as secondary prevention with screening of those at increased risk of secondary neoplasms. The problem of the occurrence of MPN may become one of the elements of personalized medicine in the future.

Declarations

Funding

This research received no external funding.

Author contributions

Conceptualization, M.K.W.; Methodology, M.K.W.; Software, M.K.W.; Validation, M.K.W.; Formal Analysis, M.K.W., S.S., M.B., J.M. and J.P.; Investigation, M.K.W., S.S., M.B., J.M. and J.P.; Resources, M.K.W., S.S., M.B., J.M. and J.P.; Data Curation, M.K.W., S.S., M.B., J.M. and J.P.; Writing – Original Draft Preparation, M.K.W., S.S., M.B., J.M. and J.P.; Writing – Review & Editing, M.K.W. and J.M.; Visualization, M.K.W.; Supervision, M.K.W.; Project Administration, M.K.W.; Funding Acquisition, M.K.W.

Conflict of interest

The authors declare no conflict of interest.

Data availability

Data supporting the results of this study shall, upon appropriate request, be available from the corresponding author.

References

1. Soerjomataram I, Coebergh JW. Epidemiology of multiple primary cancers. *Methods Mol Biol.* 2009;471:85-105. doi: 10.1007/978-1-59745-416-2_5
2. Sakellakis M, Peroukides S, Iconomou G, Boumpoucheropoulos S, Kalofonos H. Multiple primary malignancies: a report of two cases. *Chin J Cancer Res.* 2014;26(2):215-218.
3. López GY, Van Ziffle J, Onodera C, et al. The genetic landscape of gliomas arising after therapeutic radiation. *Acta Neuropathol.* 2019;137(1):139-150. doi: 10.1007/s00401-018-1906-z
4. Petru E, Schmähl D. Cytotoxic chemotherapy-induced second primary neoplasms: clinical aspects. *Neoplasma.* 1991;38(2):147-155.
5. Capelli D, Menotti D, Fiorentini A, Saraceni F, Olivieri A. Secondary Acute Myeloid Leukemia: Pathogenesis and Treatment. In: Li W. editor. *Leukemia.* Brisbane (AU): Exon Publications, 2022. doi: 10.36255/exon-publications-leukemia-secondary-acute-myeloid-leukemia
6. Cheung E, Perissinotti AJ, Bixby DL, et al. The leukemia strikes back: a review of pathogenesis and treatment of secondary AML. *Ann Hematol.* 2019;98(3):541-559. doi: 10.1007/s00277-019-03606-0
7. Smolarz B, Nowak AZ, Romanowicz H. Breast Cancer—Epidemiology, Classification, Pathogenesis and Treatment (Review of Literature). *Cancers.* 2022;14(10):2569. doi: 10.3390/cancers14102569
8. Brown AL, Arroyo VM, Agrusa J, et al. Survival Disparities for Second Primary Malignancies Diagnosed among Childhood Cancer Survivors: A Population-based Assessment. *Cancer.* 2019;125(20):3623-3630. doi: 10.1002/cncr.32356

9. Bakkach J, Mansouri M, Loudiyi A, et al. Secondary breast cancer after Hodgkin lymphoma: a case report and literature review. *Ecancermedicalscience* 2018;12:810. doi: 10.3332/ecancer.2018.810
10. Shanmugalingam T, Bosco C, Ridley AJ, Van Hemelrijck M. Is there a role for IGF-1 in the development of second primary cancers? *Cancer Med*. 2016;5(11):3353-3367. doi: 10.1002/cam4.871
11. Behrens C, Travis LB, Wistuba II, et al. Molecular changes in second primary lung and breast cancers after therapy for Hodgkin's disease. *Cancer Epidemiol Biomarkers Prev.* 2000;9(10):1027-1035.
12. Caldwell BT, Wilcox DT, Cost NG. Current Management for Pediatric Urologic Oncology. *Adv Pediatr*. 2017;64(1):191-223. doi: 10.1016/j.yapd.2017.04.001
13. Leslie SW, Sajjad H, Murphy PB. *Wilms Tumor*. In: StatPearls. Treasure Island (FL): StatPearls Publishing; 2022.
14. MacFarland SP, Duffy KA, Bhatti TR, et al. Diagnosis of Beckwith-Wiedemann syndrome in children presenting with Wilms tumor. *Pediatr Blood Cancer*. 2018;65(10):e27296. doi: 10.1002/pbc.27296
15. Sharma D, Subbarao G, Saxena R. Hepatoblastoma. *Semin Diagn Pathol*. 2017;34(2):192-200. doi: doi: 10.1053/j.semdp.2016.12.015
16. Finegold MJ, Lopez-Terrada DH, Bowen J, Washington MK, Qualman SJ, College of American Pathologists. Protocol for the examination of specimens from pediatric patients with hepatoblastoma. *Arch Pathol Lab Med*. 2007;131(4):520-529. doi: 10.5858/2007-131-520-PFTEOS
17. Czauderna P, Lopez-Terrada D, Hiyama E, Häberle B, Malogolowkin MH, Meyers RL. Hepatoblastoma state of the art: pathology, genetics, risk stratification, and chemotherapy. *Curr Opin Pediatr* 2014;26(1):19-28. doi: 10.1097/MOP.0000000000000046
18. Schnater JM, Kohler SE, Lamers WH, von Schweinitz D, Aronson DC. Where do we stand with hepatoblastoma? A review. *Cancer*. 2003;98:668-678. doi: 10.1002/cncr.11585
19. Koch A, Denkhaus D, Albrecht S, Leuschner I, von Schweinitz D, Pietsch T. Childhood hepatoblastomas frequently carry a mutated degradation targeting box of the beta-catenin gene. *Cancer Res*. 1999;59:269-273.
20. Petrucelli N, Daly MB, Pal T. BRCA1-and BRCA2-associated hereditary breast and ovarian cancer. GeneReviews®, 2016. Accessed 3 January, 2023.
21. Van Der Groep P, Van Der Wall E, Van Diest PJ. Pathology of hereditary breast cancer. *Cell Oncol.* 2011;34(2):71-88. doi: 10.1007/s13402-011-0010-3
22. Venkitaraman AR. Cancer susceptibility and the functions of BRCA1 and BRCA2. *Cell*. 2002;108(2):171-182. doi: 10.1016/S0092-8674(02)00615-3
23. Neff RT, Senter L, Salani R. BRCA mutation in ovarian cancer: testing, implications and treatment considerations. *Ther Adv Med Oncol.* 2017;9(8):519-531. doi: 10.1177/1758834017714993
24. Hodgson A, Turashvili G. Pathology of hereditary breast and ovarian cancer. *Front Oncol.* 2020;10:531790. doi: 10.3389/fonc.2020.531790
25. Chow, Laura QM. Head and neck cancer. *N Engl J Med*. 2020;382(1):60-72. doi: 10.1056/NEJMra1715715
26. Son E, Panwar A, Mosher CH, Lydiatt D. Cancers of the major salivary gland. *J Oncol Pract.* 2018;14(2):99-108. doi: 10.1200/JOP.2017.026856
27. Jiang X, Wu J, Wang J, Huang R. Tobacco and oral squamous cell carcinoma: A review of carcinogenic pathways. *Tob Induc Dis*. 2019;17:29. doi: 10.18332/tid/105844
28. Cianfriglia F, Di Gregorio DA, Manieri A. Multiple primary tumours in patients with oral squamous cell carcinoma. *Oral Oncol*. 1999;35(2):157-163. doi: 10.1016/S1368-8375(98)00105-5
29. Eberl M, Tanaka LF, Kraywinkel K, Klug SJ. Incidence of Smoking-Related Second Primary Cancers After Lung Cancer in Germany: An Analysis of Nationwide Cancer Registry Data. *J Thorac Oncol.* 2022;17(3):388-398. doi: 10.1016/j.jtho.2021.11.016
30. Miranda-Galvis M, Loveless R, Kowalski LP, Teng Y. Impacts of Environmental Factors on Head and Neck Cancer Pathogenesis and Progression. *Cells.* 2021;10:389. doi: 10.3390/cells10020389
31. Lv M, Zhang X, Shen Y, et al. Clinical analysis and prognosis of synchronous and metachronous multiple primary malignant tumors. *Medicine (Baltimore)*. 2017;96(17):e6799. doi: 10.1097/MD.0000000000006799
32. Ratna A, Mandrekar P. Alcohol and Cancer: Mechanisms and Therapies. *Biomolecules*. 2017;7(3):61. doi: 10.3390/biom7030061
33. Nieminen MT, Salaspuro M. Local Acetaldehyde-An Essential Role in Alcohol-Related Upper Gastrointestinal Tract Carcinogenesis. *Cancers (Basel)*. 2018;10(1):11. doi:10.3390/cancers10010011
34. Druesne-Pecollo N, Keita Y, Touvier M, et al. Alcohol drinking and second primary cancer risk in patients with upper aerodigestive tract cancers: a systematic review and meta-analysis of observational studies. *Cancer Epidemiol Biomarkers Prev.* 2014;23(2):324-331. doi:10.1158/1055-9965.EPI-13-0779
35. Koyanagi YN, Suzuki E, Imoto I, et al. Across-Site Differences in the Mechanism of Alcohol-Induced Digestive Tract Carcinogenesis: An Evaluation by Mediation Analysis. *Cancer Res*. 2020;80(7):1601-1610. doi: 10.1158/0008-5472.CAN-19-2685
36. Tamura M, Matsui H, Kaneko T, Hyodo I. Alcohol is an oxidative stressor for gastric epithelial cells: detection of superoxide in living cells. *J Clin Biochem Nutr*. 2013;53(2):75-80. doi: 10.3164/jcbn.13-32
37. Strober W, Shishido S, Wood B, Lewis JS, Kuhs K, Ferris RL, Faden DL. Two for the price of one: Prevalence, demographics and treatment implications of multiple HPV mediated Head and Neck Cancers. *Oral Oncol.* 2020;100:104475. doi: 10.1016/j.oraloncology.2019.104475

38. Copur MS, Manapuram S. Multiple Primary Tumors Over a Lifetime. *Oncol.* 2019;33(7):629384.
39. Schaapveld M, Aleman BM, van Eggermond AM, et al. Second Cancer Risk Up to 40 Years after Treatment for Hodgkin's Lymphoma. *N Engl J Med.* 2015;373(26):2499-2511. doi: 10.1056/NEJMoa1505949
40. Pobudejska A, Sońta-Jakimczyk D, Pawełczak-Szastok M. Ocena stanu zdrowia i jakości życia osób leczonych w dzieciństwie z powodu ostrej białaczki limfoblastycznej. *Pediatr Pol.* 2008;83.6:656-661. doi: 10.1016/S0031-3939(08)70190-2
41. Kawashiri T, Kobayashi D, Uchida M, et al. Analysis of Secondary Leukemia and Myelodysplastic Syndrome After Chemotherapy for Solid Organ Tumors Using the Food and Drug Administration Adverse Event Reporting System (FAERS). *J Pharm Pharm Sci.* 2021;24:499-508. doi: 10.18433/jpps31862
42. Ezoe S. Secondary leukemia associated with the anti-cancer agent, etoposide, a topoisomerase II inhibitor. *Int J Environ Res Public Health.* 2012;9(7):2444-2453. doi: 10.3390/ijerph9072444
43. Printz C. Second primary cancers deadlier for younger patients. *Cancer*. 2017;123(17):3210-3211. doi: 10.1002/cncr.30929

Wydawnictwo UR 2023
ISSN 2544-1361 (online)
doi: 10.15584/ejcem.2023.3.15

http://www.ejcem.ur.edu.pl
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2023; 21 (3): 617–626

REVIEW PAPER

Drug-induced thrombocytopenia – etiology and alternative therapeutic approaches

Vani Rajashekaraiah, Anusha Berikai Ananthakrishna

Department of Biotechnology, School of Sciences, JAIN (Deemed-to-be University), Bengaluru, Karnataka, India

ABSTRACT

Introduction and aim. The cumulative incidence of drug-induced thrombocytopenia (DIT) is 10 cases per one million people per year with a prevalence of approximately 25% in critically ill patients. This review provides a comprehensive view of drug-induced thrombocytopenia, diagnosis, underlying mechanisms, common strategies in therapeutics, and potential alternatives.

Material and methods. Databases such as "Google Scholar", "PubMed", "Medline" and "MDPI" was used for literature review with the keywords, "platelets", "platelet disorders", "thrombocytopenia", "drug-induced", "oxidative stress" "plant extracts", "phytochemicals", "antioxidants", for the articles published between 2013-2023 and written in the English language.

Analysis of the literature. Several antimicrobials, anti-cancer drugs, and antivirals are often reported to cause adverse effects during treatment, such as thrombocytopenia. A thorough understanding of the underlying pathophysiology is important for appropriate treatment. Even though an improvement in platelet count is observed after the discontinuation of the causative drug, there is a dire need for treatment in some cases due to associated complications. There are various pitfalls with conventional treatments which include clinical complications and lack of effectiveness.

Conclusion. Interventions in therapeutics through antioxidants can aid in faster recovery. Various plant extracts and phytochemicals have been employed as therapeutics in platelet disorders due to their exceptional antioxidant activity. It is imperative to explore the bioactive components of natural products and their influence on platelet efficacy. Also, it highlights how antioxidants can be used as a safe, yet effective option as therapeutics for treating a complicated disorder such as DIT or be used as supplements to prevent adverse effects of existing treatments involving antibiotics and chemotherapeutics.

Keywords. alternative therapeutics, drug-induced thrombocytopenia, phytochemicals, platelets

Introduction

Platelets or thrombocytes are blood cells that are continuously produced from megakaryocytes in a process called Thrombopoiesis, mainly in the bone marrow. These anucleate cells range from ~150,000–450,000/µL in healthy individuals.[1] They are found circulating in the blood for 8-10 days and are eliminated in the spleen and liver.[2] The main function of the platelet is to react to vascular damage and form a blood clot at the site to stop bleeding.[3] Nonetheless, platelets are also involved in several other functions such as affecting tumor progression by stimulating angiogenesis, separating the lymphatic circulation during embryogenesis, and helping to close ductus arteriosus at the time of birth. They are a significant part of primary immunity and contribute inflammatory mediators.[4]

Platelet disorders are primarily due to following reasons: (i) an increase in platelet number, (ii) a decrease in platelet numbers, or (iii) platelet dysfunction.[5] These can lead to either defective hemostatic plug formation leading to bleeding or spontaneous clot formation leading to thrombosis.[6]

Corresponding author: Vani Rajashekaraiah, e-mail: vani.rs@jainuniversity.ac.in

Received: 27.04.2023 / Revised: 25.05.2023 / Accepted: 26.05.2023 / Published: 30.09.2023

Rajashekaraiah V, Ananthakrishna AB. *Drug-induced thrombocytopenia – etiology and alternative therapeutic approaches.* Eur J Clin Exp Med. 2023;21(3):617–626. doi: 10.15584/ejcem.2023.3.15.

Platelet disorders are classified into:

i. Platelet function disorders: Inherited or acquired, due to loss or gain of platelet function, change in size and morphology of the platelets, and associated with clinically important bleeding defects.[7]
ii. High platelet count conditions or Thrombocytosis: Abnormal accumulation of platelets in the blood is further classified into primary or essential and secondary or reactive thrombocytosis.[8,9]
iii. Low platelet count conditions or Thrombocytopenia: Atypically low platelet levels (under 20,000 per mm^3) due to an underlying cause.[10]

Aim

This review provides a comprehensive view of drug-induced thrombocytopenia, its diagnosis, underlying mechanisms, common strategies in therapeutics, and potential alternatives such as phytochemicals.

Material and methods

Databases such as "Google Scholar", "PubMed", "Medline" and "MDPI" was used for literature review with the keywords, "platelets", "platelet disorders", "thrombocytopenia", "drug-induced", "oxidative stress" "plant extracts", "phytochemicals", "antioxidants", for the articles published between 2013-2023 and written in English language.

Analysis of the literature

Thrombocytopenia

Thrombocytopenia is characterized by a massive reduction in platelets in the peripheral blood, caused by a myriad of both congenital and acquired causes. Acute and severe decrease in platelet count exposes the individuals to an increased risk of developing spontaneous hemorrhage or thrombosis. Hemostasis entirely depends upon the functioning and the number of platelets in circulating blood and any deviation in the number can cause many complications.[11]

The major causes of thrombocytopenia can be summarized as follows:[12,13]

a. Decrease in differentiation of hematopoietic stem cells in bone marrow
b. Impairment of megakaryocyte maturation
c. Disruption of endothelial adhesion of megakaryocytes
d. Increase in peripheral platelet destruction due to immune and non-immune mediated pathways
e. Increase in splenic sequestration
f. Accelerated clearance from the circulation
g. Oxidative Stress

Drug-induced thrombocytopenia (DIT)

The main threat in any treatment procedure is the side effects caused by therapeutic drugs. Drugs administered for the treatment of a clinical symptom are capable of causing a different disorder altogether, based on their mode of action at the molecular level. DIT is one such disorder with a cumulative incidence of about 10 cases per million population per year and a prevalence as high as 25% in critically ill patients.[14] DIT is often underdiagnosed and underreported and puts the patients at an increased risk of hemorrhage.

Studies have been conducted during the past two decades based on the types of drugs that specifically induce thrombocytopenia. Around 300 drugs have been clinically implicated in causing DIT as an adverse effect during the treatment procedure. Commonly reported drugs can be categorized into antibiotics, chemotherapeutic drugs, steroids and cardiovascular drugs. Chemotherapy regimens involving dexamethasone + cytarabine + cisplatin, isophosphamide + carboplatin + etoposide, gemcitabine + dexamethasone + cisplatin and gemcitabine + and oxaliplatin are most commonly reported to cause DIT in cancer patients.[15] Some of the glycoprotein IIb/IIIa (GPIIb/IIa) inhibitors have also been reported in DIT cases.[14,16] Drug-related disorders are common in small molecules as well as biotherapeutics. Although they were believed to be safe due to their specific action, many biotherapeutic drugs have been reported to lead to adverse reactions leading to loss in blood cell count and functions.[16] Some of the clinically important cases of DIT are listed in Table 1.

A detailed history of the patient and timely diagnosis is necessary to understand the underlying etiology. However, DIT often manifests in two ways:

a. One to two weeks after beginning a new drug or suddenly, after a single dose when the drug was previously taken intermittently.[22]
b. Immediately after the first administration of antithrombotic agents that block fibrinogen binding to platelet GP IIb-IIIa.[14,37]

DIT varies extensively from case to case and is often misdiagnosed as immune thrombocytopenic purpura and patients receive inappropriate treatment, leading to several complications. There have been cases where patients may not consider that self-regulated medications, beverages, foods, or herbal remedies are relevant to their bleeding symptoms and may not report them.[38]

Thorough understanding of the underlying pathophysiology plays a crucial role in devising a treatment of DIT. Identification of the trigger for the sudden fall in platelet count is the first and foremost step in the diagnosis of DIT. Secondly, excluding pseudothrombocytopenia is critical which occurs due to *in vitro* factors such as anticoagulants used during blood collection. There is no gold standard for the diagnosis of DIT, however, following a certain protocol in diagnosis and treatment as outlined in Fig. 1 can be favourable.[39-41]

Table 1. Cases of drug-induced thrombocytopenia and treatments

Drug class	Drugs	Medical condition	Time of diagnosis	Plausible mechanisms	Treatments
Antibiotics	Acyclovir[17]	Systemic lupus erythematosus (SLE)	Day 5	Drug-dependent immune mechanism	Discontinued Acyclovir; started IVIG at 0.4 g per kg/day for 4 days
	Benznidazole[18]	Chagas disease	Day 15	Toxicity due to reactive metabolites (free radicals) formed during the bioactivation	Discontinued Benznidazole; Prednisone at 1.5 mg/kg for 4 days with subsequent tapering of dose
	Azithromycin[19]	Nonpruritic maculopapular rash	Day 5	Drug-dependent and drug-independent antibodies	Discontinued Azithromycin; administered dexamethasone 40 mg IV + intravenous immunoglobulin (IVIG) for 10 days
	Piperacillin-Tazobactam[20]	Sepsis	Day 1	Platelet destruction due to hapten-dependent antibodies	Replaced the drugs with imipenem on day 9
	Vancomycin; Ciprofloxacin[21]	Left total knee arthroplasty	Day 18	Drug-dependent platelet antibodies	Replaced vancomycin and rifampin with IV daptomycin
	Indinavir[22]	HIV infection	Month 15	Platelet destruction due to antibody-mediated lysis and decreased production in the bone marrow due to underlying infection	Switched to Stavudine + Didanosine + efavirenz.
	Nitrofurantoin[23]	Urinary tract infection	Week 3	Immune-mediated adverse reactions	Intravenous corticosteroids + intravenous immunoglobulin (IVIG) + 4-units of platelet transfusion
Antineoplastic drugs	Cisplatin[24]	Bladder tumor	Day 28	Myelosuppression	Prophylactic platelet transfusion
	Etoposide[25]	Metastatic rectal neuroendocrine carcinoma	Month 4	Immune-mediated adverse reactions	Platelet transfusion + Rituximab
	Carboplatin[26]	Glioblastoma multiforme	Week 5	Platelet destruction	Drug discontinuation + Platelet transfusions + Papaya leaf extract liquid supplementation
	Oxaliplatin[27]	Small Bowel Adenocarcinoma	24 h	Bone marrow suppression	Drug discontinuation + Platelet transfusions
Biotherapeutics	Trastuzumab[28]	Breast Cancer	Day 2	Platelet-reactive IgG and IgM targeting glycoprotein IIb/IIIa and Ib/V/IX complexes	Drug discontinuation + Oral prednisolone
	Durvalumab[29]	Non-small cell lung cancer	Week 6	Anti-human platelet antigen auto-antibodies	Drug discontinuation
	Atezolizumab[30]	Small cell lung cancer	Month 7	immune checkpoint inhibitor-induced	Methylprednisolone + platelet transfusion + thrombopoietin
Cardiovascular drugs	Abciximab[31]	Percutaneous coronary intervention of left anterior descending artery	Day 8	Formation of antibodies against its chimeric structural peptide sequence	Oral prednisone + intravenous immunoglobulin (IVIG) for 7 days
	Atorvastatin[32]	Hyperlipidemia	Week 10	Platelet apoptosis in vivo	Discontinued atorvastatin + IVIG for 2 days + Prednisone 80 mg for 5 days + Rituximab
	Tirofiban[33]	Percutaneous transluminal coronary angioplasty	<24 h	Drug-dependent antibody-mediated platelet destruction	Drug discontinuation
	Eptifibatide[34]	Acute coronary syndrome	Day 2	Platelet destruction	Drug discontinuation
	Carvedilol[35]	Hemodialysis	Week 2	Drug-dependent antibody-mediated destruction of platelets	Drug discontinuation
	Heparin[36]	COVID-19	Day 11	Anti-PF4/ heparin antibody	Drug discontinuation
	Quinine[37]	Restless leg syndrome	48 h	Immune reactivation of drug specific antibodies	Platelet transfusion

Mechanisms

Majorly, the mechanisms can be classified into (i) platelet destruction and (ii) platelet production.

i. Platelet destruction: Platelet clearance due to increase in platelet desialylation by NanA neuraminidase and the Ashwell-Morell receptor (AMR)-dependent pathway can be the major causes of DIT during infection.[42] Cross-Reaction of the drugs with endogenous thrombopoietin can lead to the neutralization of platelets in the blood stream.[43] Off-target platelet binding and activation due to the overloading of the therapeutic drug in blood can result in platelet activation and eventually destruction.[44] The immune system can also play a major role in platelet destruction by inducing antiplatelet antibodies against the murine sequences of the chimeric Fab molecule.[31]

ii. Platelet production: Low platelet levels may be due to the inhibition of thrombopoietin signaling in early megakaryokinesis leading to decreased platelet production.[45] Myelosuppression/toxicity by targeting the epidermal growth factor receptor pathway, vascular endothelial growth factor (VEGF) pathway, and BCR-ABL pathway can contribute to low platelet count as there is an abnormality at

the production level or induction of platelet apoptosis.[37,46,47] Bone marrow toxicity caused by some drugs can inhibit megakaryocyte differentiation and maturation which can impact platelet numbers.[48] Platelet consumption can also occur due to endothelial dysfunction from lack of VEGF or suppression of hematopoiesis.[49] Table 2 outlines various mechanisms that reduce platelet numbers based on different classes of drugs and their target pathways.

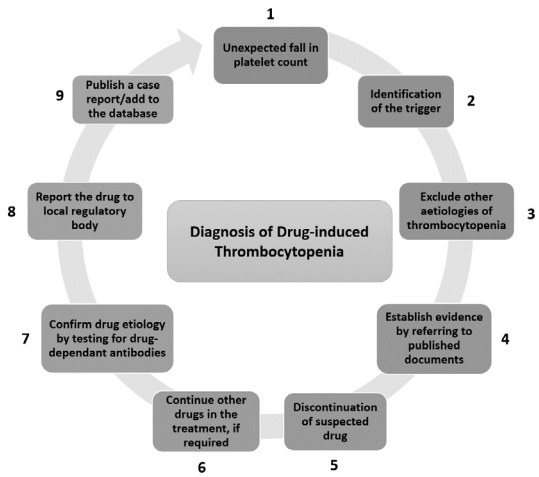

Fig. 1. Diagnosis of Drug-induced thrombocytopenia

Table 2. Mechanisms of the drugs inducing thrombocytopenia

Drugs	Mechanisms
Antimicrobials[42]	Increased platelet desialylation
Selinexor[45]	Inhibition of thrombopoietin signaling in early megakaryokinesis
Tyrosine and serine/threonine kinase inhibitors (KIs)- gefitinib and erlotinib[46]	Myelosuppression/toxicity by targeting epidermal growth factor receptor pathway
Tyrosine and serine/threonine kinase inhibitors (KIs)- sorafenib and sunitinib[47]	Myelosuppression/toxicity by targeting vascular endothelial growth factor pathway
Tyrosine and serine/threonine kinase inhibitors (KIs)- imatinib and dasatinib[37]	Myelosuppression/toxicity by targeting BCR-ABL pathway
Antibody-drug conjugates (ADCs)- brentuximab vedotin, trastuzumab emtansin; Inotuzumab ozogamicin[48]	Bone marrow toxicity and inhibits megakaryocyte differentiation and maturation
Bevacizumab[49]	Platelet consumption due to endothelial dysfunction from lack of VEGF
Anti-CD40L mAbs[50]	Inhibits disaggregation of ADP-aggregated platelets
Abciximab[51]	Induction of antiplatelet antibodies directed against the murine sequences of chimeric Fab molecule
TNF-α inhibitors[52,53]	Induces autoantibodies including antiplatelet antibodies or suppression of hematopoiesis
AMG X and LY2541546[54,55]	Platelet activation by direct binding to platelets and/or megakaryocytes causes the release of serotonin
Thrombopoietic agents- rHu-TPO or PEG-rHuMGDF[43]	Cross-Reacts with endogenous thrombopoietin and neutralizes
CH12 humAb[44]	Off-target platelet binding and activation resulting in platelet destruction

Treatments

Treatment strategies for DIT focus primarily on the recovery of lost platelets. In most cases, physicians identify the drug responsible for the condition and take the first step by switching the suspected drug to an alternate drug. Cessation of administering a suspected drug generally reverses the condition of DIT in most cases.[14] However, treatment is required in cases where the therapeutic drug is crucial for the patient or when patients experience life-threatening bleeding. Conventional treatment is advised based on the following strategies outlined below.

Common approaches

The outline of commonly used strategies in the treatment of DIT is summarized in Fig. 2 and can be explained as follows:

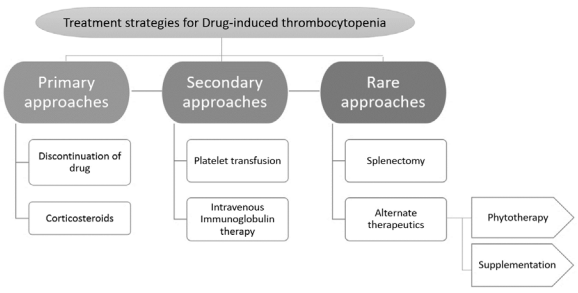

Fig. 2. Therapeutic strategies for Drug-induced thrombocytopenia

i. Discontinuation of the suspected drug: The first approach of the treatment is by discontinuing the suspected causative agent. This can be done by establishing which of the patient's medications is reported for causing DIT by referring to the available databases.[56]
ii. Corticosteroids: Generally, corticosteroids like prednisone, dexamethasone, and methylprednisolone are used in the treatment of DIT. The mechanism of corticosteroids is primarily based on impairing the clearance of opsonized platelets in the bone marrow and peripheral organs. It also acts by reducing the autoantibody levels and preventing the immune-related platelet destruction, thereby increasing the platelet levels more rapidly than any other treatment.[57,58]
iii. Intravenous (IV) immunoglobulin G (IgG) therapy: Another common approach is the use of IV IgG therapy to impair the clearance of opsonized platelets. IV IgG causes increased clearance of the antiplatelet antibodies by saturating the neonatal Fc salvage receptor for IgG. Even though it is more expensive than other treatments, it is beneficial due to rapid response ranging between 24-48 hours.[59,60]
iv. Platelet transfusion: Transfusion can be attempted when the suspected drug is necessary for an extended duration. Transfusions can become ineffective in some cases, due to other related complications,

making an individualized approach a necessity. Procurement of matched platelets by platelet apheresis are extremely difficult in emergency situations. Nonetheless, utilizing platelets stored in blood banks having limited shelf-life of up to 5 days can be ineffective due to the effect of storage lesions.[61,62]

v. Surgical: Splenectomy is a surgical procedure that partially or completely removes the spleen to increase the platelet count. This procedure aids in the release of splenic sequestered platelets into circulation, thereby instantly elevating the platelets. However, the major drawback of the procedure is that it may adversely affect portal hypertension. Therefore, portal decompression is more preferred to improve thrombocytopenia. Nonetheless, surgical procedures are the least recommended for DIT as it is mostly unnecessary and may cause further damage during the procedure.[63]

vi. Other drugs: Thrombopoietin receptor (TPO-R) agonists are potential therapeutic drugs for DIT which stimulates the platelet production in the bone marrow. These act as replacement for thrombopoietin and elevate the platelet count. Spleen tyrosine kinase (SYK)-inhibitors are yet another approved class of drugs for DIT which can reduce antibody-mediated platelet destruction by immunosuppression mechanism.[64,65] Some of the examples for drugs include romiplostim, eltrombopag, imiglucerase, avatrombopag and fostamatinib.[58]

Alternative approaches

Oxidative stress in cells and tissues due to reactive oxygen species (ROS) is attributed as a primary causative agent in several disease conditions. Free radicals are highly unstable molecules and are highly reactive, thereby attacking the molecules in a biological system, causing metabolic malfunction, and cellular protein destruction, and can cause cell death.[66,67]

Antioxidants scavenge ROS and repair oxidative damage. Primary antioxidants scavenge ROS by hydrogen atom transfer (HAT) mechanism, by donating an H-atom, or act by single electron transfer (SET) mechanism. The secondary antioxidants neutralize the ROS using prooxidant catalysts by quenching free radicals and are thus exhausted.[68] Plant extracts and phytochemicals have excellent antioxidant capacity and are explored to reduce the possibility of the occurrence of disorders. There are reports that antioxidants alleviate the condition when used as an alternative therapeutic strategy or taken as a supplement or in numerous disease conditions.[69,70]

Studies have proven that various plant extracts can be used as therapeutic agents in the treatment of thrombocytopenic conditions. These plant extracts have been routinely tested in animal models for their effectiveness in stimulating the production of platelets in thrombocytopenic condition. This ability of plant extracts is attributed to their antioxidant capacity. There are many studies dedicated to the identification of the bioactive compound in such extracts. Most bioactive components are phenolic acids, polyphenols, flavonoids or alkaloids in nature. The major challenge lies in identifying the specific bioactive compound in these extracts. Also, their influence on platelet functions is extremely under-reported. Nevertheless, specific antioxidants have been proven to be effective in improving platelet functions such as aggregation, granular secretion, activation and apoptosis and increasing the ability of endogenous antioxidant defense.[71,72] Our preliminary studies have indicated that antioxidants such as L-carnitine and vanillic acid are beneficial for platelet survival.[73,74] Table 3 and Table 4 provide details of plant extracts and antioxidants, respectively used under different thrombocytopenic conditions to improve platelet count. These studies are critical to categorize the antioxidants into either platelet agonists or anti-platelet agents as they are applied under rather entirely contrasting pathophysiological circumstances.

Table 3. Plant extracts as potential therapeutics

Plant	Condition	Effects
Carica papaya[75]	Cyclophosphamide induced-thrombocytopenia	Increase in platelet count
Medicago sativa[76]		Increase in platelet count
Amaranthus spinosus[77]	Ethanol-induced thrombocytopenia	Increase in platelet count and decrease in bleeding and clotting time
Malus domestica[78]	$KBrO_3^-$ induced thrombocytopenia	Increase in platelet count from 14.72 to 33.07 and from 11.08 to 32.90 (normal vs. thrombocytopenic) with apple fruit extract-enriched diet (AFE) and Apple seed extract-enriched diet (ASE), respectively
Nigella sativa[79]	Quinine-induced thrombocytopenia	Increase in platelet count by 1.59-fold
Ji-Xue-Teng (JXT, *Spatholobus suberectus*)[80]	Radiation-induced hematopoietic alteration	Attenuates platelet decline on day 21
Syzygium cumini[81]	Hydroxyurea-induced thrombocytopenia	Increase in platelet counts
Achyranthes aspera[82]	Healthy	Increase in platelet count
Annona muricata; Fagara zanthoxyloide[83]	Zidovudine-induced thrombocytopenia	Increase in platelet count by 49.56% (AM) and 51.32% (FZ), pronounced decrease in bleeding time
Bauhinia monandra[84]	Heparin-induced thrombocytopenia	Increased platelet counts and decreased bleeding and clotting time
Psidium guajava[85]	Busulfan induced-thrombocytopenia	Increase in platelet count and reversal of toxic effects
Equisetum hyemale and *Euphorbia hirta*[86]	Aspirin-induced thrombocytopenia	Both plant extracts possess platelet-increasing property in combination or alone

However, there are a few studies on using antioxidants and herbal products as supplements in human subjects with clinically significant thrombocytopenic conditions. These natural products have proven to improvise patient's hematologic parameters and bleeding

score under different pathological conditions.[92-94] Effect of some antioxidants on platelet functions can be dose-dependent. They act as platelet agonists at higher concentration and can become anti-platelet at lower concentrations.[95] Hence, it is essential to evaluate such antioxidants and optimize the dose for use as supplements in alternate therapeutics.

Table 4. Antioxidants as potential therapeutics for thrombocytopenia

Antioxidant	Condition	Effects
Melatonin[87]	Luzindol-induced thrombocytopenia	Increased platelet count and plasma levels of platelet function markers, β-thromboglobulin and PF 4
Mitoquinone[88]	Radiation-induced thrombocytopenia	Restored platelet count to normal levels, restored mitochondrial membrane potential and lowers superoxide production
γ-Tocotrienol[89]		Decreased severity of thrombocytopenia
Carpaine[90]	Busulfan induced-thrombocytopenia	Platelet stimulating activity
Vanillic acid[74]	Hydroxyurea-induced thrombocytopenia	Improved platelet count
Quercetin-3-O-β-D-glucuronide[91]	Cyclosporine A-induced bone marrow failure	Increased the number of platelets, inhibited the mitochondrial pathway-mediated platelet apoptosis and PI3K/AKT pathway

Conclusion

Alternate therapies using phytochemicals can aid in faster recovery and reduce mortality due to their remarkable antioxidant properties. Plant extracts have shown promising results in increasing the number of platelets in patients with thrombocytopenia. Although these natural remedies are not a substitute for conventional therapies, they can be used a supplementary therapy to improve the effectiveness of treatments. Based on the studies conducted to this point, they can also be used as supplements to prevent adverse effects of existing treatments involving antibiotics and chemotherapeutics. Antioxidant therapies are currently followed for various clinical conditions and are a promising arena for further exploration. However, these therapies are still in its infancy, and have gained attention in the recent past due to the awareness and benefits of traditional approaches. Several scientific reports encourage using antioxidants as alternate therapeutic substances. Furthermore, antioxidants are investigated only in terms of enhancement of platelet count for DIT, but it is imperative to study their effect on platelet functions. Nonetheless, these antioxidants should be extensively studied for their mechanistic properties and physiological interactions to be regarded as one of the standard therapeutic strategies.

Acknowledgments

The authors acknowledge Dr. Leela Iyengar, Dr. Manasa Mithun, M Pallavi, Magdaline Christina R, and JAIN (Deemed-to-be University) for their support.

Declarations

Funding

No financial assistance was obtained to conduct this study.

Author contribution

Conceptualization, V.R.; Methodology, V.R. and A.B.A.; Validation, V.R.; Formal Analysis, V.R. and A.B.A.; Investigation, A.B.A.; Data Curation, A.B.A.; Writing – Original Draft Preparation, A.B.A.; Writing – Review & Editing, V.R.; Visualization, V.R.; Supervision, V.R.

Conflicts of interests

The authors declare no competing interests.

Data availability

All data generated or analyzed during this study are included in this published article.

References

1. van der Meijden PE, Heemskerk JW. Platelet biology and functions: new concepts and clinical perspectives. *Nat Rev Cardiol*. 2019;16:166-179. doi: 10.1038/s41569-018-0110-0
2. Harker LA, Roskos LK, Marzec UM, et al. Effects of megakaryocyte growth and development factor on platelet production, platelet life span, and platelet function in healthy human volunteers. *Blood*. 2000;95:2514-2522. doi: 10.1182/blood.V95.8.2514
3. Laki K. Our ancient heritage in blood clotting and some of its consequences. *Ann N Y Acad Sci*. 1972;202:297-307. doi: 10.1111/j.1749-6632.1972.tb16342.x
4. Leslie M. Beyond Clotting: The Powers of Platelets. *Science*. 2010;328:562-564. doi: 10.1126/science.328.5978.562
5. Michelson, D A. *Platelets*. 3rd ed. USA:Academic Press; 2013.
6. Krishnegowda M, Rajashekaraiah V. Platelet disorders: An overview. *Blood Coagul Fibrin*. 2015;26:479-491 . doi: 10.1097/01.mbc.0000469521.23628.2d
7. Hayward CPM, Rao AK, Cattaneo M. Congenital platelet disorders: overview of their mechanisms, diagnostic evaluation and treatment. *Haemophilia*. 2006;12:128-136. doi: 10.1111/j.1365-2516.2006.01270.x
8. Schafer AI. Thrombocytosis. *New Eng J Med*. 2004;350: 1211-1219. doi: 10.1056/NEJMra035363
9. Allen J, Stokol T. Thrombocytosis and Essential Thrombocythemia. In: Schalm's Veterinary Hematology. Wiley; 2022. doi: 10.1002/9781119500537.ch82
10. Stasi R. How to approach thrombocytopenia. Hematology 2010, *American Society of Hematology Education Program Book*. 2012;1:191-197. doi: 10.1182/asheducation.V2012.1.191.3798260
11. Horrell CJ, Rothman J. The etiology of thrombocytopenia. *Dimens Crit Care Nurs*. 2001;20:10-16. doi: 10.1097/00003465-200107000-00004

12. Danese E, Montagnana M, Favaloro EJ, Lippi G. Drug-Induced Thrombocytopenia: Mechanisms and Laboratory Diagnostics. *Semin Thromb Hemost*. 2020;46:264-274. doi: 10.1055/s-0039-1697930
13. Wang TL, Guo DH, Bai Y, Wen K, Han WY, Wang R. Thrombocytopenia in Patients Receiving Prolonged Linezolid May be Caused by Oxidative Stress. *Clin Drug Investig*. 2016;36:67-75. doi: 10.1007/s40261-015-0352-0
14. Kenney B, Stack G. Drug-induced thrombocytopenia. *Arch Pathol Lab Med*. 2009;133:309–14. doi: 10.5858/133.2.309
15. Lu R, Lin Q, Chen S, Ye X. Chemotherapy-induced thrombocytopenia and platelet transfusion in patients with diffuse large B-cell lymphoma. *Transl Cancer Res*. 2020;9:1640-1651. doi: 10.21037/tcr.2020.01.64
16. Everds NE, Tarrant JM. Unexpected hematologic effects of biotherapeutics in nonclinical species and in humans. *Toxicol Pathol*. 2013;41:280-302. doi: 10.1177/0192623312467400
17. Tsappa I, Missouris C, Psarellis S. Acyclovir-induced thrombocytopenia in a patient with SLE. *BMJ Case Rep*. 2018;2018:bcr2018225118. doi: 10.1136/bcr-2018-225118
18. Crespillo-Andújar C, Calbacho Robles M, Norman FF, Pérez-Molina JA. Severe immune thrombocytopaenia in a patient taking benznidazole for chronic Chagas disease. *BMJ Case Rep*. 2018;2018:bcr2017223788. doi: 10.1136/bcr-2017-223788
19. Butt MU, Jabri A, Elayi SC. Azithromycin-Induced Thrombocytopenia: A Rare Etiology of Drug-Induced Immune Thrombocytopenia. *Case Rep Med*. 2019;2019:6109831. doi: 10.1155/2019/6109831
20. Beaulieu C, Kurczewski L, Yajnik V. Cefepime challenge after piperacillin/tazobactam-induced thrombocytopenia. *J Thromb Thrombolysis*. 2019;48:167-170. doi: 10.1007/s11239-019-01848-3
21. Savage-Elliott I, Wu VJ, Sanchez FL. Drug-Induced Thrombocytopenia Secondary to Commonly Used Antibiotics in Total Joint Arthroplasty. *Arthroplast Today*. 2020;6:137-140. doi: 10.1016/j.artd.2020.03.006
22. Alzahrani M, Alrumaih I, Alhamad F, Abdel Warith A. Rapid onset severe thrombocytopenia following reexposure to piperacillin-tazobactam: report of two cases and review of the literature. *Platelets*. 2018;29:628-631. doi: 10.1080/09537104.2018.1468025
23. Ramey C, LePera A. A Possible Case of Nitrofurantoin-Associated Immune Thrombocytopenia in a Healthy 45-Year-Old Caucasian Female. *Cureus*. 2023;15:e34654. doi: 10.7759/cureus.34654
24. Watanabe A, Momo K, Tanaka K, et al. Clinical course involving thrombocytosis and thrombocytopenia in a patient with bladder cancer treated with gemcitabine and cisplatin. *Clin Case Rep*. 2021;9:e05103. doi: 10.1002/ccr3.5103
25. Bancheno WM, Adidam SR, Melaku MA. Metastatic rectal neuroendocrine carcinoma presenting with treatment-refractory immune thrombocytopenia: A case report and literature review. *Medicine*. 2022;101:e29616. doi: 10.1097/MD.0000000000029616
26. Koehler A, Rao R, Rothman Y, et al. A Case Study Using Papaya Leaf Extract to Reverse Chemotherapy-Induced Thrombocytopenia in a GBM Patient. *Integr Cancer Ther*. 2022;21:153473542110684 . doi: 10.1177/15347354211068417
27. Ghazanfar H, Nawaz I, Ali N. Oxaliplatin-Induced Thrombocytopenia: A Case Report and Review of Pathophysiology of Various Speculative Mechanisms. *Cureus*. 2020;12:e9929. doi: 10.7759/cureus.9929
28. Xiaoqi Z, Shanshan J, Yuanyang P, Minfeng L, Changsheng Y, Jianyu D. Trastuzumab-Induced Severe Thrombocytopenia:A Case Report and Literature Review. *Chin Med J*. 2020;35:377. doi: 10.24920/003799
29. Dougherty SC, Lynch AC, Hall RD. Drug-induced immune-mediated thrombocytopenia secondary to durvalumab use. *Clin Case Rep*. 2021;9:e04227. doi: 10.1093/ajcn/53.5.1222
30. Qiu G, Li S, Li B, et al. Immune thrombocytopenia in a small cell lung cancer patient treated with atezolizumab: a case report. *Transl Lung Cancer Res*. 2022;11:2346-2355. doi: 10.21037/tlcr-22-745
31. Jbara M, Bhogal S, Bajaj K, Chhabra L. Abciximab-induced delayed profound thrombocytopaenia. *BMJ Case Rep*. 2017;2017:bcr2017219379. doi: 10.1136/bcr-2017-219379
32. Ghuman J, Manasewitsch NT, Ghuman J, et al. Atorvastatin-Induced Refractory Thrombocytopenia. *Cureus*. 2021;13:e12502. doi: 10.7759/cureus.12502
33. Meghrajani V, Sabharwal N, Namana V, Elsheshtawy M, Topi B. A Case of Hyperacute Severe Thrombocytopenia Occurring Less than 24 Hours after Intravenous Tirofiban Infusion. *Case Rep Hematol*. 2018;2018:1-6. doi: 10.1155/2018/4357981
34. Alamin MA, Al-Mashdali A, Al Kindi DI, Elshaikh EA, Othman F. Eptifibatide-induced acute profound thrombocytopenia: A case report. *Medicine*. 2022;101:e28243. doi: 10.1097/MD.0000000000028243
35. Raimonde AR, Dennis AK. Carvedilol-Induced Thrombocytopenia: A Case Report. *Hosp Pharm*. 2022;57:786-789 . doi: 10.1177/00185787221108719
36. Lingamaneni P, Gonakoti S, Moturi K, Vohra I, Zia M. Heparin-Induced Thrombocytopenia in COVID-19. *J Investig Med High Impact Case Rep*. 2020;8:2324709620944091. doi: 10.1177/2324709620944091
37. Quintás-Cardama A, Santos FPDS, Kantarjian H, et al. Dynamics and management of cytopenias associated with dasatinib therapy in patients with chronic myeloid leukemia in chronic phase after imatinib failure. *Cancer*. 2009;115:3935-3943. doi: 10.1002/cncr.24432
38. Majhail NS, Lichtin AE. What is the best way to determine if thrombocytopenia in a patient on multiple medications is drug-induced? *Cleve Clin J Med*. 2002;69:259-262. doi: 10.3949/ccjm.69.3.259

39. George JN, Aster RH. Drug-induced thrombocytopenia: pathogenesis, evaluation, and management. *Hematology Am Soc Hematol Educ Program*. 2009;1:153-158. doi: 10.7326/0003-4819-129-11_Part_1-199812010-00009
40. Vayne C, Guéry EA, Rollin J, Baglo T, Petermann R, Gruel Y. Pathophysiology and diagnosis of drug-induced immune thrombocytopenia. *J Clin Med*. 2020;9(7):2212. doi: 10.3390/jcm9072212
41. Manasa K, Soumya R, Vani R. Phytochemicals as potential therapeutics for thrombocytopenia. *J Thromb Thrombolysis*. 2016;41:436-440. doi: 10.1007/s11239-015-1257-8
42. Li MF, Li XL, Fan KL, et al. Platelet desialylation is a novel mechanism and a therapeutic target in thrombocytopenia during sepsis: An open-label, multicenter, randomized controlled trial. *J Hematol Oncol*. 2017;10:104. doi: 10.1186/s13045-017-0476-1
43. Cuker A. Toxicities of the Thrombopoietic Growth Factors. *Semin Hematol*. 2010;47:289-298. doi: 10.1053/j.seminhematol.2010.03.002
44. Zhang Y, Sun J, Tan M, et al. Species-Specific Involvement of Integrin aIIbß3 in a Monoclonal Antibody CH12 Triggers Off-Target Thrombocytopenia in Cynomolgus Monkeys. *Mol Ther*. 2018;26:1457-1470. doi: 10.1016/j.ymthe.2018.04.005
45. Machlus KR, Wu SK, Vijey P, et al. Selinexor-induced thrombocytopenia results from inhibition of thrombopoietin signaling in early megakaryopoiesis. *Blood*. 2017;130:1132-1143. doi: 10.1182/blood-2016-11-752840
46. Ranson M, Wardell S. Gefitinib, a novel, orally administered agent for the treatment of cancer. *J Clin Pharm Ther*. 2004;29:95-103. doi: 10.1111/j.1365-2710.2004.00543.x
47. Narayanan PK, Henry S, Li N. Drug-induced thrombocytopenia: mechanisms and relevance in preclinical safety assessment. *Curr Opin Toxicol*. 2019;17:23-30. doi: 10.1016/J.COTOX.2019.10.002
48. de Goeij BECG, Lambert JM. New developments for antibody-drug conjugate-based therapeutic approaches. *Curr Opin Immunol*. 2016;40:14-23. doi: 10.1016/J.COI.2016.02.008
49. Kilickap S, Abali H, Celik I. Bevacizumab, bleeding, thrombosis, and warfarin. *J Clin Oncol*. 2003;21:3542. doi: 10.1200/JCO.2003.99.046
50. Mirabet M, Barrabés JA, Quiroga A, Garcia-Dorado D. Platelet pro-aggregatory effects of CD40L monoclonal antibody. *Mol Immunol*. 2008;45:937-944. doi: 10.1016/j.molimm.2007.08.006
51. Curtis BR, Swyers J, Divgi A, McFarland JG, Aster RH. Thrombocytopenia after second exposure to abciximab is caused by antibodies that recognize abciximab-coated platelets. *Blood*. 2002:99:2054-2059. doi: 10.1182/blood-2017-10-812461
52. Brunasso AMG, Massone C. Thrombocytopenia associated with the use of anti-tumor necrosis factor-a agents for psoriasis. *J Am Acad Dermatol*. 2009;60:781-785. doi: 10.1016/j.jaad.2008.12.001
53. Keystone EC. Tumor necrosis factor-alpha blockade in the treatment of rheumatoid arthritis. *Rheum Dis Clin North Am*. 2001;27(2):427-443. doi: 10.1016/s0889--857x(05)70211-8
54. Santostefano MJ, Kirchner J, Vissinga C, et al. Off-Target Platelet Activation in Macaques Unique to a Therapeutic Monoclonal Antibody. *Toxicol Pathol*. 2012;40:899-917. doi: 10.1177/0192623312444029
55. Rudmann DG, Page TJ, Vahle JL, et al. Rat-specific decreases in platelet count caused by a humanized monoclonal antibody against sclerostin. *Toxicol Sci*. 2012;125:586-594. doi: 10.1093/toxsci/kfr318
56. George JN, Medina PJ, Zondor SD. Treatment of drug--induced thrombocytopenia. *Expert Opin Drug Saf*. 2002;1:173-180. doi: 10.1517/14740338.1.2.173
57. Bakchoul T, Marini I. Drug-associated thrombocytopenia. Hematology 2014. *Hematology Am Soc Hematol Educ Program*. 2018;2018:576-583. doi: 10.1182/asheducation-2018.1.576
58. Sandler SG, Tutuncuoglu SO. Immune thrombocytopenic purpura - Current management practices. *Expert Opin Pharmacother*. 2004;5:2515-2527. doi: 10.1517/14656566.5.12.2515
59. Warkentin TE. High-dose intravenous immunoglobulin for the treatment and prevention of heparin-induced thrombocytopenia: a review. *Exp RevHematol*. 2019;12:685-698. doi: 10.1080/17474086.2019.1636645
60. Samuelsson A, Towers TerriL, Ravetch J. Anti-inflammatory activity of IVIG mediated through the inhibitory Fc receptor. *Science*. 2001;291:485-487. doi: 10.1126/science.291.5503.484
61. Nagrebetsky A, Al-Samkari H, Davis NM, Kuter DJ, Wiener-Kronish JP. Perioperative thrombocytopenia: evidence, evaluation, and emerging therapies. *Br J Anaesth*. 2019;122(1):19-31 . doi: 10.1016/j.bja.2018.09.010
62. Manasa K, Vani R. Influence of Oxidative Stress on Stored Platelets. *Adv Hematol*. 2016;2016: 4091461. doi: 10.1155/2016/4091461
63. Greenberg EM. Thrombocytopenia: a destruction of platelets. *J Infus Nurs*. 2017;40:41-50. doi: 10.1097/NAN.0000000000000204
64. Erickson-Miller CL, DeLorme E, Tian SS, et al. Discovery and characterization of a selective, nonpeptidyl thrombopoietin receptor agonist. *Exp Hematol*. 2005;33:85-93. doi: 10.1016/j.exphem.2004.09.006
65. Singh R, Masuda ES, Payan DG. Discovery and Development of Spleen Tyrosine Kinase (SYK) Inhibitors. *J Med Chem*. 2012;55:3614-3643. doi: 10.1021/jm201271b
66. Gulcin I. Antioxidants and antioxidant methods: an updated overview. *Arch Toxicol*. 2020;94:651-715. doi: 10.1007/s00204-020-02689-3
67. Forman HJ, Zhang H. Targeting oxidative stress in disease: Promise and limitations of antioxidant therapy. Nat Rev Drug Discov. 2021;20:689-709. doi: 10.1038/s41573-021-00233-1

68. Halliwell B and Gutteridge JMC. Free Radicals in Biology and Medicine. 5th Edition. New York: Oxford University Press; 2015. doi: 10.1093/acprof:oso/9780198717478.001.0001
69. Neha K, Haider MR, Pathak A, Yar MS. Medicinal prospects of antioxidants: A review. *Eur J Med Chem*. 2019;178:687-704. doi: 10.1016/J.EJMECH.2019.06.010
70. Bouayed J, Bohn T. Exogenous Antioxidants—Double-Edged Swords in Cellular Redox State: Health Beneficial Effects at Physiologic Doses versus Deleterious Effects at High Doses. *Oxid Med Cell Longev*. 2010;3:228-237. doi: 10.4161/oxim.3.4.12858
71. Bouaziz A, Romera-Castillo C, Salido S, et al. Cinnamtannin B-1 from bay wood exhibits antiapoptotic effects in human platelets. *Apoptosis*. 2007;12:489-498. doi: 10.1007/s10495-006-0014-z
72. Olas B, Saluk-Juszczak J, Wachowicz B. D-glucaro 1,4-lactone and resveratrol as antioxidants in blood platelets. *Cell Biol Toxicol*. 2008;24:189-199. doi: 10.1007/s10565-007-9028-8
73. Mithun M, Rajashekaraiah V. L-Carnitine as an additive in Tyrode's buffer during platelet storage. *Blood Coagul Fibrinolys.* 2018;29:613-621.
74. Rajashekaraiah V, Mithun M. A formulation of vanillic acid to enhance the platelet count during drug-induced thrombocytopenia. IPO Patent Application No. 201941020419; 2019.
75. Anjum V, Arora P, Ansari SH, Najmi AK, Ahmad S. Antithrombocytopenic and immunomodulatory potential of metabolically characterized aqueous extract of Carica papaya leaves. *Pharm Biol*. 2017;55:2043-2056. doi: 10.1080/13880209.2017.1346690
76. Gholamnezhad Z, Rouki V, Rezaee R, Boskabady MH. Medicago sativa ameliorated cyclophosphamide-induced thrombocytopenia and oxidative stress in rats. *Toxin Rev*. 2023;1:10. doi: 10.1007/s10565-007-9028-8
77. Leih Panopio ZB, Dalisay CG, Landicho JR, et al. Anti-thrombocytopenic activity of Amaranthus spinosus in ethanol-induced rat models exhibiting low platelet count. *The Steth*. 2015;9:1-19.
78. Abbas M, Saeed F, Arshad MU, et al. Exploring the therapeutic potential of apple extracts against thrombocytopenia in rat experimental models. *Nutr Food Sci*. 2018;48:397-405. doi: 10.1108/NFS-09-2017-0201
79. Saadia M, Rehman S, Robin S, et al. Potential of Nigella sativa seed aqueous extract in ameliorating quinine-induced thrombocytopenia in rats. *Pak J Pharm Sci*. 2017:30:1679-1690.
80. Dong XZ, Wang YN, Tan X, Liu P, Guo DH, Yan C. Protective effect of JXT ethanol extract on radiation-induced hematopoietic alteration and oxidative stress in the liver. *Oxid Med Cell Longev*. 2018;2018:9017835. doi: 10.1155/2018/9017835
81. Bandiola TMB, Corpuz MJT. Platelet and Leukocyte Increasing Effects of Syzygium cumini (L.) Skeels (Myrtaceae) Leaves in a Murine Model. *Pharm Anal Acta*. 2018;09:2018. doi: 10.4172/2153-2435.1000586
82. Asuquo Etim E, Abdulhakeem Adebayo Y, Emmanuel Ifeanyi O. Effect of Achyranthes Aspera Leaf Extract on Hematological Parameters of Swiss Albino Mice. *World Journal of Public Health*. 2019;4:96. doi: 10.11648/j.wjph.20190404.14
83. Uchechukwu O, Evelyn U. Body weight and platelet count changes in zidovudine administered Wistar albino rats 3 treated with ethanolic extracts of Annona muricata and Fagara zanthoxyloide. *Int Blood Res Rev*. 2019:9:1-18. doi: 10.9734/ibrr/2019/v9i330099
84. Nworie KM, Okorie AN, Ebere DN, Obike OC. Evaluation of antidote potential of methanol leaf extract of Bauhinia monandra on heparin-induced thrombocytopenia in mice. *Biotechnologia*. 2019;100:57-67. doi: 10.5114/bta.2019.83212
85. Ahmad A, Ibrahim M, Chester K, Khan W, Ahmad S, Ansari S. Antithrombocytopenic potential of bioactivity guided fractions of traditionally used Psidium guajava Linn. leaves in busulfan induced-thrombocytopenic rats. *Pharmacogn Mag*. 2019;15:440. doi: 10.4103/pm.pm_608_18
86. Bangayan JMB, Palattao JRM, Chua JMT, Capili JT, Ramirez Jr RB. Evaluation of Equisetum hyemale and Euphorbia hirta leaf extracts in increasing platelet count of sprague dawley rats. *Int J Biosci*. 2020;17(2):58-65. doi: 10.12692/ijb/17.2.58-65
87. Nyagolov Y, Pashalieva I, Stancheva E, Decheva L, Negrev N. Experimental data about melatonin effects on platelet count and functional activity. *C R Acad Bulg Sci*. 2012;65:855-860.
88. Ramsey H, Zhang Q, Wu MX. Mitoquinone restores platelet production in irradiation-induced thrombocytopenia. *Platelets*. 2015;26:459-466. doi: 10.3109/09537104.2014.935315
89. Singh VK, Kulkarni S, Fatanmi OO, et al. Radioprotective Efficacy of Gamma-Tocotrienol in Nonhuman Primates. *Radiat Res*. 2016;185:285-298. doi: 10.1667/RR14127.1
90. Zunjar V, Dash RP, Jivrajani M, Trivedi B, Nivsarkar M. Antithrombocytopenic activity of carpaine and alkaloidal extract of Carica papaya Linn. leaves in busulfan induced thrombocytopenic Wistar rats. *J Ethnopharmacol*. 2016;181:20-25. doi: 10.1016/j.jep.2016.01.035
91. Xia LM, Zhang AP, Zheng Q, et al. Quercetin-3-O-ß-D-glucuronide inhibits mitochondria pathway-mediated platelet apoptosis via the phosphatidylinositol-3-kinase/AKT pathway in immunological bone marrow failure. *World J Tradit Chin Med*. 2022;8:115-122. doi: 10.4103/wjtcm.wjtcm_44_21
92. Subenthiran S, Choon TC, Cheong KC, et al. Carica papaya leaves juice significantly accelerates the rate of increase in platelet count among patients with dengue fever and dengue haemorrhagic fever. *eCAM*. 2013;2013:616737. doi: 10.1155/2013/616737

93. Esmaeili A, Toosi NN, Taher M, Bayani M, Namazi J. Melatonin effect on platelet count in patients with liver disease. *Gastroenterol Hepatol Bed Bench*. 2021;14:356-361.
94. Elalfy MS, Elhenawy YI, Deifalla S, Hegazy M, Sabra A, Abdelaziz Y. Oxidant/antioxidant status in children and adolescents with immune thrombocytopenia (ITP) and the role of an adjuvant antioxidant therapy. *Pediatr Blood Cancer*. 2015;62:830-837. doi: 10.1002/pbc.25434
95. Rukoyatkina N, Shpakova V, Sudnitsyna J, et al. Curcumin at Low Doses Potentiates and at High Doses Inhibits ABT-737-Induced Platelet Apoptosis. *Int J Mol Sci*. 2021;22:5405. doi: 10.3390/ijms22105405

Wydawnictwo UR 2023
ISSN 2544-1361 (online)
doi: 10.15584/ejcem.2023.3.16

http://www.ejcem.ur.edu.pl
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2023; 21 (3): 627–638

REVIEW PAPER

Autoimmune diseases and their various manifestations in the oral cavity – a systematic review

Shin-Yi Lin , Yu-Wei Huang , Katarzyna Błochowiak

Department of Oral Surgery, Periodontal Diseases, and Oral Mucosal Diseases,
Poznan University of Medical Sciences, Poznan, Poland

ABSTRACT

Introduction and aim. Oral manifestation of the disorder is the leading cause of common initial features of most autoimmune diseases. Therefore, this study aimed to present different oral manifestations of selected autoimmune diseases.

Material and methods. We systematically reviewed the etiology, signs and symptoms, oral manifestations, epidemiology, diagnosis, treatment plan, and prognosis. We searched the articles on PubMed, Google Scholar and Web of Science for the following search term: Behcet's disease, lichen planus, mucous membrane pemphigoid and bullous pemphigoid, pemphigus vulgarisms and paraneoplastic pemphigus, rheumatoid arthritis, Sjögren's syndrome, IgG4-related disease, systemic lupus erythematous, and granulomatosis with polyangitis.

Analysis of the literature. We conducted that the disorder's oral manifestation causes most autoimmune illnesses' earliest symptoms.

Conclusion. Clinical-pathological is a piece of requisite knowledge for the dentist to recognize and diagnose in the early phase of the symptoms.

Keywords. autoimmune diseases, Behcet's disease, lichen planus, lupus erythematous, rheumatoid arthritis, Sjögren's syndrome

Introduction

Autoimmune diseases are a complex group with various, often multifactorial, etiologies. The immune system loses the ability to recognize its antigens from foreign ones and produces antibodies against its own cells. They are chronic inflammatory diseases with subsequent active stages and remission involving multiple organs, especially the skin, joints, connective tissue, and oral cavity. The oral manifestations could be the initial character in the early stage of the disease, and the dentist could be the first who discovers the lesion intraorally and extra orally.[1,2] However, some disorders have similar oral lesions, and a differential diagnosis is needed to distinguish the difference.[3] Also, it is indispensable to understand the oral manifestation of autoimmune disease and its characteristics due to the diseases may also lead to fatal in some cases. Acquire detailed knowledge of the oral manifestation of autoimmune diseases and their features would help the dentist recognize and make an early phase of symptoms diagnosis to build an integral future treatment plan for the patients.[1,4]

Aim

To address these needs, we comprehensively present and discuss selected autoimmune diseases that involve oral and maxillofacial areas. In addition, the study aims to present detailed characteristics of the oral manifestation of autoimmune diseases.

Corresponding author: Katarzyna Błochowiak, e-mail: kblochowiak@ump.edu.pl

Received: 26.04.2023 / Revised: 7.06.2023 / Accepted: 11.06.2023 / Published: 30.09.2023

Lin S-Y, Huang Y-W, Błochowiak K. *Autoimmune diseases and their various manifestations in the oral cavity – a systematic review*. Eur J Clin Exp Med. 2023;21(3):627–638. doi: 10.15584/ejcem.2023.3.16.

Material and methods

We conducted a systematic review to identify all case-control studies correlating with autoimmune disease. First, we searched the articles on PubMed, Web of Science, Google Scholar with the data from 2010 to 2021 with the following search term: "autoimmune diseases," "oral manifestation," "oral lesions," "immunosuppressive drug," "Behçet's disease," lichen planus," "pemphigoid," pemphigus," "rheumatoid arthritis," Sjögren's syndrome," IgG4-related diseases, systemic lupus erythematosus," granulomatosis with polyangitis.", by distributing the diseases into eight sections. For each section, we mention the autoimmune disease as the following: etiology, signs and symptoms, oral manifestation, epidemiology, diagnosis, treatment, and prognosis.

Analysis of the literature

Behçet's disease

Behçet's disease (BD) is a chronic inflammatory autoimmune vasculitis and involves multiple systems. Its etiology is still unknown. The lesions include skin, oral mucosa, genital, gastrointestinal, and neurological systems. BD is characterized by at least two of the three key typical factors: oral ulcers, genital ulcers, and eye inflammation. The critical characteristics of BD are ocular lesions with inflammation known as uveitis and conjunctivitis and oral and genital ulcerations. Ocular lesions are present in 30–70% of the cases.[1] Oral lesions could be the initial sign of the disease.[1,2] Chen et al mentioned that the human leukocyte antigen HLA-B51 is related to BD. It also presents greater chances of having ocular lesions and a lesser chance of genital involvement.[4] Environmental factors such as smoking, diet, stress, and infection can also trigger BD.[3] BD patients with vasculitis have found a more significant tumor necrosis factor (TNF)-α and local neutrophil infiltration and mononuclear endothelial cells.[3,5] Recurrent aphthosis is the most common manifestation in the oral cavity for BD individuals.[6] Oral aphthosis is present on the lips, mucosa, tongue, and soft and hard palate.[1] The oral lesions are ulcers of the oral mucosa indistinguishable from the conventional aphthae of the oral mucosa. They are painful and characterized by the cyclic presentation.[1] The oral lesions are usually the initial manifestation. Their diagnosis is a key factor and permits a more favorable prognosis. Many patients present their onset during childhood.[1] Conversely, their differential diagnosis is challenging, considering oral aphthous lesions are common in the general population. Some aphthous lesions could be linked to HIV, Crohn's disease, sarcoidosis, and lupus, given that the dual-site-specific ulcerations seem to be the unique sign to differentiate the BD from different pathologies associated with aphthous lesions.[1] There are three types of oral ulcers of BD, minor aphthae presenting approximately 3 to 10 mm in diameter with erythematous halo, major aphthae developing into 1-3 cm in diameter and healing with scar tissue, and herpetiform ulcers clustering ulcers similar to HSV infection with numerous lesions.[7,8]

Some articles related to BD epidemiology mentioned that its higher incidence is detected in Middle East and Asian populations.[2,3] The population is mainly from the countries along with Silk Road.[9] The highest prevalence is in Turkey (20-420 per 100.000).[6] BD impacts more males than females; the ratio is 1.5-5:10.[9] Greco et al. mentioned that BD is a common disease in males from Middle Eastern countries; females from Japan and Korea demonstrate a higher frequency of BD.[1] BD shows up in third to the fourth decade of life, and the first onset is around the second to third decades.[9]

The BD diagnostic tests are approaches used in clinical practice—three characteristics of ulcers of uveitis of ocular involvement, oral mucosa, and genital ulcerations.[1,5] The pathergy test shows positive when intradermal puncture on the skin forms an erythematous pustule or papule in forty-eight hours.[5] The oral ulceration of the mucosa could be related to HIV, HSV, Crohn's disease, pemphigus vulgaris, and lichen planus.[6] Therefore, dentists should perform a differential diagnosis like biopsy on patients because oral aphthosis is similar to other conditions.[5]

BD treatment is based on administering immunosuppressive drugs, corticosteroids, and anti-inflammatory drugs. The immunosuppressive drug mainly treats BD, and systemic and topical steroids are for anti-inflammation and pain control.[1,3] Colchicine and glucocorticoids can be the first management line for mucocutaneous lesions. Azathioprine, Apremilast, TNFα inhibitors, and interferon-α are used for the second line.[4] Özdede et al. mention that oral lesion treatment with Apremilast demonstrated around seventy-three percent of oral bioavailability. Both in 12 weeks and 24 weeks, applied with the Apremilast group, showed reduced pain compared to the placebo group.[5] Biological drugs tocilizumab, daclizumab, and adalimumab manage B-cell-mediated inflammatory factors[8] BD patients are suggested to use a chlorhexidine mouth rinse to maintain oral hygiene and avoid food irritating the oral mucosa.[9]

Patients with BD harm their quality of life, especially in oral lesion involvement. The BD shows a life-threatening relapse, which can result in death. Early Diagnosis of BD from the oral manifestations can prevent multiple organ involvement.[1] Management with immunosuppressing drugs can improve the prognosis of BD.[5]

Lichen planus

Another autoimmune disease involving the oral cavity is lichen planus (LP). LP is a chronic disease with oral mucosa membranes and cutaneous inflammation.[10] It was

first mentioned by Erasmus Wilson in 1869. LP is mediated by the lymphocyte T cell with an unclear etiology with a higher count of CD4+ and CD8+ in patients.[10,11,12] Forty-five percent of the individuals with oral-involved lichen planus have presented dry mouth (xerostomia) and lower salivary gland function, and eighty-seven percent of them experienced insufficient salivary flow. Oral lichen planus (OLP) is sometimes accompanied by other autoimmune diseases.[10] The OLP involving the gingiva shows desquamative gingivitis.[12,13] Sometimes, accompanied mouth-burning sensation negatively affects patients' quality of life.[13] OLP can also be irritated by spicy or acidic and salty foods.[8,14] Some factors could also induce OLP, such as medications, dental materials, and viruses (HIV, hepatitis B virus [HBV], and hepatitis C virus [HCV]).[12,15] Some authors described that Aquaporins (AQP) is a channel cell protein that works as an intercellular water permeability. In OLP patients, a significant increase in the AQP3 gene prevents xerostomia.[10] Management of pregnant women with OLP should consider the risk to the mother and fetus. The United States Food and Drug Administration has a risk factor classification for the fetus at three trimesters.[11]

The OLP is commonly presented in the tongue, gingiva, buccal, and labial mucosa.[11,13,16] There are six types of OLP: reticular form, plaque form, atrophic form, bullous form, erosive form, and papular form.[11,13,14] In OLP patient, the manifestation exhibit desquamative gingivitis, saw-tooth rete pegs, and buccal mucosa with Wickham's striae for the reticular formation. Sallow ulceration and deep erosion lesions with erythema can also be observed.[12,13]

The prevalence of LP is around 0.22-5% of the world population, and 60% show up with OLP. The range of age is from 30 to 80 years old.[13] It has a higher frequency in females between the 5th and 6th decades of their lives. One of the articles mentions that one of the reasons is that females experience menopause around the 5th decade, and the estrogen level changes making the mucosa change.[15,17] The ratio between men and women is 1:2 or 1:3.[12,14]

Direct immunofluorescence can be found deposition of IgG, IgM, C3, or fibrinogen at the basement membrane zone (BMZ) and the deposition of IgM in the colloid bodies. The indirect immunofluorescence will be negative.[13,18] Oral lichenoid lesion (OLL) is an immune-related disease on the oral mucosa induced by systemic drugs or dental materials (dental amalgam or resin).[12,15,18] Oral manifestations of OLL have a similar clinical feature to OLP. The differential diagnosis should include clinical and histological, and pathological criteria. OLP is familiar with bilateral and symmetric oral manifestation; OLL is involved with unilateral and localized and doesn't involve cutaneous lesions.[18] Under the microscope, OLP exhibits band-like T lymphocyte cell infiltration in the sub-epithelial layer and degenerated basal cells in the Civatte bodies.[13,18]

Treatment of OLP is quite challenging due to the potential side effects of commonly used drugs. If the patient is pregnant, it is important to ensure the health and well-being of both the mother and the developing fetus undergo drug management.[11] For managing OLP, topical corticosteroids such as clobetasol propionate, fluocinolone, and triamcinolone are used to control the local pain, but it shows that they show a potentially harmful effect on the fetus. Systemic steroids can be considered as well.[8,11] Immunosuppressant drugs like tacrolimus and cyclosporine are effective treatments for an erosive type of OLP. But it also shows an adverse effect on the fetus.[11] Chu et al. mention that oral isotretinoin in the dose of 0.25–1 mg/kg per day can effectively manage the OLP.[19] In turn, White et al. mention no significant benefits of curcumin therapy compared with placebo.[13]

OLP is a chronic immune-mediated disease that can last more than twenty years. There is no requirement for treating the patient with an asymptomatic condition. Patients with OLP have fewer than 5% developing oral squamous cell carcinoma.[12,18] Therefore, OLP patients are suggested to have regular check-ups and reassessment for the oral lesion conditions.[18]

Mucous membrane pemphigoid and bullous pemphigoid

Pemphigoid is an autoimmune disease that causes blistering of the skin and mucous membranes.[1,13] There are two main types of pemphigoid: bullous pemphigoid (BP) and mucous membrane pemphigoid (MMP). BP primarily affects the skin, with blistering typically occurring on the arms, legs, and torso. In contrast, MMP predominantly affects the mucous membranes, such as the mouth, eyes, nose, throat, and genitals.[14,20] In MMP), the most commonly targeted protein is collagen XVII, a basement membrane zone (BMZ) component in the skin and mucous membranes. However, antibodies in MMP can also target other proteins in the BMZ, including laminin 5, 6, and 3, as well as BP antigens 180 and 230 kDa.[8,14] In contrast, bullous pemphigoid (BP) is often triggered by certain medications, radiotherapy, and ultraviolet radiation.[17,21] It is characterized by large, tense blisters on the skin that can be itchy and painful. The antibodies in BP typically target BP antigens 180 and 230 kDa, also components of the BMZ.[14,20] Both MMP and BP are autoimmune diseases characterized by the production of autoantibodies against proteins in the BMZ, leading to blistering and erosions. However, they differ in their clinical presentation and the specific proteins targeted by the autoantibodies.

Most cases with mucous membrane pemphigoid exhibit the oral mucosa as the initial lesions. The pain accompanied by an erosive blister, is easily ruptured on

the affected side and left with a scar after healing the wound.[12] Bagan et all. mention that the commonly affected sites include gingiva, buccal mucosa, and hard and soft palate. About 51.6% of patients present the size of a lesion smaller than 3 cm.[21] Most of BP cases present erythematous cutaneous lesions with pruritus and blisters.[12,20] It affects people from the 6th to 8th decades. The female exhibits a higher chance of being diagnosed with the disease.[13,18] The ratio between women and men is 2:1.[8,13] Schifter et al. described that pemphigoid frequency is three times higher than pemphigus. And it's rare for the kids.[12] The prevalence is from 5 to 7.5 for every 10.000 population.[12]

For mucous membrane pemphigoid, the direct immunofluorescence shows that IgG and C3 are deposited in the layer of the basement membrane. It also can be observed that lymphocytes and granulocytes infiltrate the subepithelial layer.[12] We can discover the epithelial separate from connective tissue as well.[1]

BP demonstrates extensive infiltration with the vesicle and degeneration of epithelial cells in the basement membrane zone.[20] Direct immunofluorescence exhibits IgG and C3 deposition; indirect immunofluorescence can discover circulating antibodies.[12] Desquamative gingivitis is characteristic of pemphigus vulgaris, lichen planus, and pemphigoid.[21] Compared to pemphigus, there is no histopathological characteristic of acantholysis.[14] It is noticed that it's less frequency of involvement of gingiva, and the blister is more fragile in pemphigus individuals.[1,22]

Topical drugs corticosteroid is one of the managements of the pemphigoid. We can apply systemic steroids in some severe cases as the treatment plan.[1] Previous studies noticed that 88% of patients were treated with topical corticosteroids for mucous membrane pemphigoid, and 12% were managed with systemic therapy.[22] It is postulated that depleting the B-lymphocytes is one strategy to manage the disorder. Rituximab is directed against the specific antigen CD20 of B cells and rapidly uses up circulating B cells. Another benefit is cutting down the usage of steroids and reducing side-effect.[23] BP can be fatal for patients.[12] MMP shows the potential to cause ocular lesions and damage the eyes' anatomical structure, leading to blindness. Therefore, the patient must have a regular check-up due to the chance of relapse.[1,14,18] Therapy with mild conditions shows a better prognosis treated with topical corticosteroid medicine. Severe cases must be treated with systemic treatment.[12,18]

Pemphigus vulgaris and paraneoplastic pemphigus

Pemphigus is a chronic inflammation of autoimmune diseases involving the supra epithelial layer with the desmosome in a vesiculobullous shape. It exhibits a specific phenomenon called acantholysis.[1,18] Also known as Tzanck cells.[8]

Pemphigus is a group of autoimmune diseases that affect the skin and mucous membranes, and there are six different types. Pemphigus vulgaris (PV) and paraneoplastic pemphigus (PNP) are two of the most common types and are often the focus of research and clinical practice.[8,18,24]

PV is autoantibody that target desmosomal proteins, particularly desmoglein 1 and 3, and 130, 160-kDa cadherin, which plays a critical role in maintaining the adhesion between keratinocytes in the epidermis and mucous membranes.[14,18] When the antibodies attack these proteins, the adhesion between cells is disrupted, forming intraepithelial blisters, which can cause erosions and ulcers. Schifter et al. noticed that desmogleins dominate the oral cavity, and PV affects the oral cavity and is the first location then extended to the cutaneous.[14]

PNP is a rare autoimmune disease often associated with an underlying neoplasm, particularly hematologic malignancies such as non-Hodgkin's lymphoma.[25] Autoantibodies in PNP target various skin and mucous membrane proteins, including desmoplakin I and II, essential components of desmosomes.[12,18] In addition to skin and mucosal lesions, PNP can also affect other organs, such as the lungs and the gastrointestinal tract, leading to severe complications.

PV is more prevalent in females, with an estimated incidence of 1 to 5 cases per 1.000.000 population, and most commonly affects individuals in their fourth to sixth decade of life.[1,18] The Jewish population has a higher chance of being diagnosed.[8,14] Conversely, PNP does not show a gender preference and typically affects individuals in their fourth to seventh decade of life.[25] It can present with lesions on various oral sites, including the buccal or lingual mucosa, hard or soft palate, lips with vermillion border, or tongue. The lesions can be erythematous, vesicles, erosion, or ulcerations, typically accompanied by pain.[1,8,18] The buccal mucosa is the most commonly affected site, while the tongue is a less common site of involvement.[26] PV is characterized by a positive Nikolsky's sign, which is the slippage of the top layer of skin on gentle pressure.[14] Conversely, pemphigoid presents with ulceration and erosion in the oral mucosa, often mimicking oral lichenoid reaction.[12,18]

Approximately 45% of PNP patients show oral lesions as the first site.[12] The manifestations of the vermillion border with crusting and erosion are similar to other autoimmune diseases, such as erythema multiforme or Steven-Johnson syndrome.[25] Overall, dentists play a crucial role in identifying and recognizing oral manifestations of autoimmune diseases and performing a differential diagnosis to provide an accurate diagnosis and appropriate treatment plan.

The oral manifestation of pemphigus can be confused with oral lichen planus (OLP) and mucous membrane pemphigoid. Direct and indirect immuno-

fluorescence (DIF and IIF) can differentiate the differences. In DIF, IgG antibodies can be discovered with net-like features. The microscopy also shows a specific tombstone-like pattern resulting from separating the supra-epithelial layer and underlying connective tissue.[1,8,18] Desquamative gingivitis is one of the features of pemphigus vulgaris. The gingiva surface is without peels and is accompanied by erythema. However, 84% of mucous membrane pemphigoid cases can also exhibit desquamative gingivitis, while only 26% of pemphigus vulgaris cases have this characteristic.[22] Therefore, a biopsy is needed to confirm the diagnosis, as this feature is not specific to pemphigus.[12,27] Compared to pemphigus vulgaris, the histopathology of pemphigus foliaceus is mainly in the BMZ layer, with features of supra-epithelial blister and acantholysis.[18,25] In DIF, the deposition of IgG, IgM, and C3 can be discovered in the BMZ.[8,12,25]

Corticosteroids are commonly used in the management of pemphigus and PNP.[1,18] It is important to recognize the potential severity of these conditions and the need for prompt and appropriate treatment. Immunomodulatory therapies like rituximab and azathioprine are effective in managing pemphigus, while surgical removal of tumors may be necessary in cases of PNP.[8] Good oral hygiene is also crucial in managing these conditions.[8] It is concerning to hear that without treatment, the death rate of pemphigus can be as high as 90%.[14] However, appropriate medication therapy can significantly reduce the life-threatening fatal rate to 5-10%.[8,18,28] It is also important to consider the patient's age as a factor in their prognosis, with elderly patients potentially having a less favorable outcome.

Rheumatoid arthritis

Rheumatoid arthritis (RA) is another autoimmune disease with possible oral manifestation. It is a chronic inflammatory autoimmune disease. The clinical features mainly involved multiple joints bilaterally and were accompanied by morning stiffness that persisted for more than an hour.[2,7,29] The etiology of RA is still unknown. Still, smoking and infection can be environmental factors that trigger RA.[2] Cheng et al. described that dysbiosis of *Porphyromonas gingivalis* can result in the onset of RA. *P. gingivalis* induced the production of anti-citrullinated protein antibodies, which will be the essential factor in increasing the risk of RA.[30] 67.6% of RA individuals can be involved in the temporomandibular joint (TMJ).

The characteristics exhibit swelling of joints, pain, and limited jaw motion. If the young patient is diagnosed with juvenile RA, the TMJ could show underdeveloped mandible bone, ankylosis, and malocclusion with anterior open bite.[2,7,8] Approximately 70% of RA patients present with oral candidiasis; some show a lower salivary flow rate accompanied by halitosis.[31,32] The oral condition with xerostomia negatively affects Oral-Health-Related Quality of Life, especially in physical pain and psychological disability.[32] Compared to healthy individuals, a higher frequency of severe periodontal status, more significant bleeding, worsened gingival inflammation, and deeper pockets are detected in RA patients.[33]

In epidemiology, X-chromosomal is the risk factor for RA, so the female has a higher chance of RA. The incidence of RA in the 5th and 6th decades is the most frequent.[34] RA impacts more female; the ratio between female to male is 3:1 to 4:1.[2]

The diagnosis of rheumatoid arthritis often involves a combination of factors, including a positive result on the anti-citrullinated protein antibody test, joint involvement, and blood tests to measure inflammation (such as CRP and ESR).[2,29] However, it is important to note that other conditions can have similar symptoms and may need to be ruled out before a definitive diagnosis can be made. For example, skin lesions may be present in conditions such as psoriatic arthritis, systemic sclerosis, and systemic lupus erythematous. Muscle pain may suggest polymyalgia rheumatica or fibromyalgia, which can have similar symptoms to rheumatoid arthritis.[29]

In the RA treatment, NSAID and acetaminophen would be the suggestions for pain control.[2,8] Biological medication for immune suppression is used to manage hypersensitivity reactions.[8] Anti-TNF-α agents reduce immune-mediated inflammation and are also helpful in controlling the disorder.[31] Möller et al. noticed that dysbiosis of oral microbial in periodontitis patients is linked to RA, but periodontal treatment is no significant effect on RA.[33]

RA is associated with an increased risk of periodontal diseases, and controlling carbohydrate consumption and maintaining good oral hygiene is important for managing this risk in RA patients.[34] Regular dental appointments can also help with prophylaxis and monitoring the oral health of RA patients.[33] It's important for RA patients to work closely with their healthcare providers, including dentists, to manage both their RA and oral health.

Sjögren's syndrome

Sjögren's syndrome (SS) is an autoimmune rheumatic disease primarily affecting the exocrine gland responsible for producing tears and saliva. The local infiltration of B-lymphocytes can be found in the affected sites.[35-39] This damage can result in dry eyes and dry mouth and induces xerostomia and xerophthalmia.[1,2] The reduction in saliva production can increase caries rate and plaque accumulation, and long-term dry mouth can contribute to oral candidiasis.[36] While the etiology of Sjögren's syndrome is not fully understood, it is believed to be an autoimmune disorder where the body's immune system

mistakenly attacks its own tissues. Viral infections such as Epstein–Barr virus, HCV, and Coxsackie A virus have been suggested as possible environmental triggers for the disease. [36,40]

SS can cause salivary dysfunction, which reduces saliva production. This reduction in saliva flow can result in various oral health issues, including an increased risk of dental caries and periodontal diseases. Due to insufficient saliva, individuals with SS may also experience taste alteration, halitosis, and difficulties with chewing, swallowing, and speaking.[2,8] The condition may lead to xerostomia with taste alteration; rampant caries and advanced periodontitis are also one of the main reasons that decrease patients' living quality.[41-43]

Oral candidiasis is a fungal infection that can occur in individuals with SS due to reduced saliva flow and altered oral microbiota. It can present with erythematous or pseudomembranous features, and conditions such as median rhomboid glossitis and angular cheilitis may also be present.[1,2,43] Around 34% of individuals with SS may experience bilateral swelling or enlargement of the parotid glands, the most prominent salivary glands in front of the ears.[36] This swelling is often painless but can lead to discomfort and cosmetic concerns. SS is more prevalent in females than in men, with an estimated ratio of approximately 9:1.[1,2] It typically affects women in their 5th or 6th decades of life, although it can occur at any age.[7,43] The prevalence rate of SS is approximately 60.82 per 100,000 population, although this can vary depending on the population studied and the diagnostic criteria used.[36]

The autoantibodies anti-Sjögren A (SSA) and anti-Sjögren B (SSB) are commonly used in the diagnosis of SS.[1,44] These autoantibodies can be detected in the blood of individuals with SS and are considered a critical factor in diagnosing the condition. To confirm a diagnosis of SS, patients should meet two out of three conditions: xerostomia (dry mouth), keratoconjunctivitis sicca (dry eyes), and rheumatoid arthritis or another autoimmune disease.[1] Other tests may be performed to assess salivary function, such as salivary flow rate measurements or salivary gland biopsies.

The diagnosis of SS involves a combination of clinical evaluation, physical examination, and various diagnostic tests. Two common methods for diagnosis are biopsy of the minor salivary glands from lips and reduced salivary flow rate.[36,44] A labial salivary gland biopsy is included into SS diagnostic criteria and is based on the detection of focal lymphocytic sialadenitis. Although microscopic pattern of SS is representative for all minor as well as major salivary glands, currently only labial salivary gland biopsy is the SS criterium. It results from simply surgical approach of labial salivary glands.[45,46] Another diagnostic criterium is a positive Schirmer's test result, which is less than 5 mm in 5 minutes, indicates dry eyes.[2,8] The American-European Consensus Group SS criteria is widely used and include six criteria: 1) dry eyes, 2) dry mouth, 3) positive result of Schirmer's test, 4) labial minor salivary gland biopsy, 5) reduced salivary flow or diffuse sialectasias, and 6) positive result of autoantibodies SSA or SSB. These criteria help clinicians make an accurate diagnosis of Sjögren's syndrome.[38,39]

Xerostomia, or dry mouth, is a common symptom that can be caused by various conditions other than Sjögren's syndrome. Some systemic diseases, such as other autoimmune diseases, AIDS, and diabetes, can have similar characteristics to xerostomia. Additionally, certain medications can cause dry mouth as a side effect. Other conditions, such as HCV and amyloidosis, can also cause salivary gland enlargement and sialadenitis, which may mimic some of the symptoms of Sjögren's syndrome. Therefore, performing a differential diagnosis to rule out these conditions and accurately diagnose Sjögren's syndrome is important.[1,8]

Managing Sjögren's syndrome involves various approaches, depending on the symptoms and severity of the disease. Corticosteroids are commonly used to manage the symptoms of Sjögren's syndrome, while biological agents such as rituximab and azathioprine may be used for immunosuppression.[1,36,47] For xerostomia, cholinergic agonist drugs can be used to stimulate saliva production, while sugar-free gum and topical fluoride can help soothe the dry mouth and promote remineralization.[48] Antifungal medications can be used to treat oral candidiasis, and antimicrobial mouth rinse can help control bacterial growth in the mouth.[2,8] Hydroxychloroquine, a medication used to treat malaria, can also be used to treat autoimmune diseases such as Sjögren's syndrome by targeting innate immunity pathways.[47] Due to the risk of dental caries and periodontitis associated with xerostomia, patients with Sjögren's syndrome should maintain good oral hygiene, and dentists should recommend regular dental check-ups and caries prophylaxis.[1,48,49] In severe cases of Sjögren's syndrome, systemic manifestations such as renal failure, cardiovascular disease, lymphoma, and vasculitis can occur, which may increase the risk of mortality.[35,36] Therefore, early diagnosis and appropriate management of Sjögren's syndrome are essential to prevent these complications and improve patient outcomes.

IgG4-related diseases

Another group of autoimmune diseases are immunoglobulin G4 (IgG4)-related diseases (IgG4-RD). They constitute a group of immune-mediated entities that can affect many organs at nearly any anatomic site. However, head and neck area is the second most common site of IgG4-RD after the pancreas. The occurrence of IgG4-RD in the head and neck area is estimated at 20%.[50,51]

They are chronic, systemic, fibro-inflammatory diseases of unknown etiology. All IgG4-RD are characterized by the formation of infiltrations composed mainly of IgG4+ plasma cells, CD4+ and CD8+ T cells and by fibrosis in the organs that are affected. Depending on the organs involved, these infiltrations lead to organ dysfunction and a special clinical presentation. In the head and neck area Mikulicz's disease (MD) and Küttner's tumor (KT) are their main manifestations. The basic and entry criterion for both MD and KT diagnosis is the involvement of a set of salivary glands. KT is defined as less severe manifestation of the IgG4-RD in the head and neck area with the predominant fibrosis of affected salivary glands. KT is an enlargement and sclerosing sialadenitis of the submandibular glands (SMG). In turn, MD is usually a symmetrical enlargement of the lacrimal glands, parotid and SMG, and sometimes sublingual glands with the symptoms of dry mouth and ocular dryness. However, salivary secretion in MD is normal or decreased to a lesser extent. Both MD and KT as IgG4-RD may be confused with malignancy, infection or SS and vasculitis.[51] Moreover, in the past, MD was treated as a subtype of primary Sjögren's syndrome (pSS). Xerostomia is not the predominant symptom and the diagnostic criterion in MD contrary to SS. Furthermore, in contrast to pSS, the target of autoimmune attack in MD and KT is the parenchyma of the major salivary gland.[51,52] Microscopic examination and serum IgG4+ levels are diagnostic steps allow MD and KT to be distinguished from pSS. These entities also have different epidemiological characteristics. IgG4-RS, contrary to SS, mainly affects men in middle age. The median age of the onset of IgG4-RD is 58 years. The male–female ratio is approximately 5:1. The characteristic features of IgG4-RD are the slow progression of the disease and a good response to steroid therapy.[53] MD is characterized by idiopathic, bilateral, symmetrical and painless diffuse swelling of the lacrimal, parotid and SMG. This swelling lasts for at least 3 months. In MD, gland swelling is persistent, while gland swelling in SS is periodic. Enlargement of the salivary glands in MD is more frequent than in SS. KT is considered to be a subtype of MD. In KT, the swelling is well localized and limited mainly to the SMG. It presents with a firm, painless mass in the neck, which mimics the neoplastic process of SMG. Hard and painless salivary gland swelling is the predominant symptom. KT is characterized by asymptomatic bilateral swelling of the SMG. KT could be histopathologically diagnosed by strong lymphocytic infiltration and fibrosis in the SMG either with or without sialolith. Moreover, salivary duct obstruction, salivary stasis, sialolithiasis and secretory dysfunction play a role in the etiology of KT. Both KT and MD are characterized by increased serum IgG4+ levels > 135 mg/dl that has been defined as a general diagnostic criterion of all IgG4-RD. Lymphoplasmacytic infiltrations of uncertain etiology in the salivary glands fulfils the entry criterion for both MD or KT.[52-54]

Systemic lupus erythematous

Systemic lupus erythematosus (SLE) is an immune-mediated inflammatory disease that can affect multiple organs, with the skin being the most commonly involved. A characteristic skin feature of SLE is the butterfly rash, which appears on the face.[1,2] Recent research has suggested that changes in the gut microbiome may be associated with SLE and may offer an alternative method for diagnosing the disease.[55]

SLE patients are more likely to experience mental health issues, functional limitations, and physical pain compared to the general population, as evidenced by the oral-health-related quality of life questionnaire.[31,56] The most common oral manifestation of SLE is the presence of erythematous plaques with peripheral telangiectasia, which may occur on the buccal mucosa, gingiva, palate, and lips.[2,12] SLE patients may also experience recurrent oral aphthous ulcers, which can cause significant discomfort.

Periodontitis is a common oral health issue in SLE patients, with up to 70% of individuals diagnosed with the disease.[7] High CRP levels may be associated with periodontitis in SLE patients.[9] Xerostomia, or dry mouth, is also prevalent in SLE patients and can negatively impact their quality of life.[31,56] Spicy and hot foods may irritate oral lesions and trigger a burning sensation in SLE patients.[57]

SLE disproportionately affects black females in the United States and is most commonly diagnosed in individuals in their 4th decade of life.[1,2,57] The prevalence of SLE ranges from 12 to 50 in every 100.000 population and is more common in females than males, with a female-to-male ratio of approximately 4.5:1 to 8:1.[2,7,8,41,57]

Direct immunofluorescence is an important diagnostic tool for SLE as it can detect the deposition of immunoglobulin and complement proteins in the basement membrane, a characteristic feature of the disease.[2,13,28] Path histology can also examine tissue samples from affected organs or tissues to identify characteristic changes such as hyperkeratosis and thickening of the basal and spinous cell layers. However, it is important to note that the oral lesions associated with SLE, such as mucosal ulcers and recurrent aphthous stomatitis, can have a similar appearance to other oral lesions, such as lichenoid lesions or leukoplakia. Therefore, a thorough evaluation of the patient's clinical history and systemic symptoms, such as kidney dysfunction and joint pain, is necessary to make an accurate diagnosis of SLE.[2,18]

Corticosteroids are commonly used to manage oral lesions in SLE, including RAS and ulceration.[2,41] However, alternative treatments such as anti-malarial including

Table 1. Manifestations and management of selected autoimmune diseases

Disease	Diagnostic methods	Oral manifestation	Treatment
Behect's disease	HLA-B51 detection Criteria consist of oral ulcerations plus any 2 of the following recurrent genital ulcers, eye lesions, skin lesions Positive pathergy test	Recurrent oral aphthous ulcers	NSAID, Steroids: glucocorticoids (topical or systemic), Immunosuppressants: Azathioprine, Apremilast, TNF-α inhibitor Interferon-α
Mucous membrane pemphigoid and bullous pemphigoid	Direct immunofluorescence (Linear immunoglobulin G and complement C3 at basement membrane zone) Indirect immunofluorescence (Linear immunoglobulin G and/or immunoglobulin A at basement membrane zone)	Desquamative gingivitis, oral buccal and lingual mucosa, lips, and tongue Subepithelial separation of epithelium from connective tissue Positive Nikolsky sign	Steroids: topical steroids Systemic with dapsone and prednisone. Immunosuppressants: azathioprine, mycophenolate mofetil, cyclophosphamide, rituximab
Pemphigus vulgaris and paraneoplastic pemphigus	Direct immunofluorescence (Pemphigus vulgaris: IgG and C3 deposit in suprabasal layer; Paraneoplastic pemphigus: IgG and C3 deposit in BMZ) ELISA for the detection of antibodies HLA-DR4 detection	Desquamative gingivitis, superficial blisters and erosions on the palate, tongue, and labial/buccal mucosa Dyskeratosis, Acantholysis (Tzanck cells), Basal cells are separated from connective tissue with tombstones pattern Positive Nikolsky sign	Steroids: topical steroids Systemic with dapsone and prednisone. Immunosuppressants: Azathioprine, mycophenolate mofetil, cyclophosphamide, rituximab
Rheumatoid arthritis	Rheumatoid factor (RF) Anti-citrullinated peptide antibodies HLA-DRB1 detection	High risk of periodontitis TMJ dysfunction (surface erosion; ankylosis with an anterior open bite) Myofacial pain Reduced salivary flow rate (xerostomia) Oral candidiasis	NSAID: Ibuprofen, Naproxen Immunosuppressive medication: Rituximab, Azathioprine, TNF-α antagonists Antifungal drugs
Sjögren's syndrome	American-European Consensus Group criteria ANA, anti-SSA/Ro, anti-SSB/La Minor salivary gland biopsy and serology Schirmer's test Dry eyes Dry mouth Reduced salivary flow	Xerostomia with taste alterations Rampant caries Salivary gland pathology difficulty with chewing and swallowing oral candidiasis burning mouth complaints	Steroids: topical/systemic steroids Immunosuppresive drugs: Azathioprine, Cyclosporine, Hydroxychloroquine Antifungal drugs: nystatin Adjunctive therapy: topical fluoride, 2% Chlorhexidine mouth rinse
Mikulicz disease and Küttner tumor	Serum levels of IgG4 Histopathological examination	Billateral, painless swelling of parotid, submandibular and sublingual glands, lacrimal glands (Mikulicz disease) Severe fibrosis of submandibular gland, asymmetric swelling	Steroids: Systemic steroids Good response to steroids Immunosuppresive drugs
Systemic lupus erythematosus	ANA, anti-dsDNA, anti-Sm, Direct immunofluorescence: Depositions of IgG or IgM in the basement membrane zone Hyperkeratosis, lymphocytes infiltration in basal cell layer	Oral lesion common buccal mucosa, lips, gingival, Plaque or macules in red Oral aphthous ulceration Periodontitis xerostomia Possible transformation to squamous cell carcinoma	Steroids: topical/systemic steroids Immunosuppressants: Azathioprine, cyclophosphamide Adjunctive therapy: vitamin D and Ca^{2+} supplements Improvement of oral hygiene
Granulomatosis with polyangitis	Granuloma in the respiratory tract Necrosis and inflammation of the small vessels Glomerulonephritis induce kidney failure Anti-neutrophil cytoplasmic antibodies	Strawberry gingivitis Alveolar bone resorption Tooth loss Ulceration on oral mucosa, hard palate, soft palate and tongue.	Steroids: prednisone NSAID Immunosuppressive drugs : Cyclophosphamide, Rituximab
Lichen planus	Direct immunofluorescence (Deposition of immunoglobulin G, IgM, complement C3, or fibrinogen at basement membrane zone; deposition of immunoglobulin M in the colloid bodies),	Buccal and lingual mucosa, lips, tongue, and gingiva with erosions or ulcerations. Saw-tooth epithelial ridges, with desquamative gingivitis Striated white lesions (reticular variant) with or without erythematous	Corticosteroids: topical, systemic, Immunosuppressants: tretinoin cyclosporine, pimecrolimus, tacrolimus, TNF-alpha inhibitors Antimicrobials: Chlorhexidine, povidone-iodine, miconazole, nystatin Others: vitamin A

anti-malarial, NSAID drugs, and immunosuppressive medications such as azathioprine, mycophenolate, and cyclophosphamide can also be used to manage SLE.[8,58] Adjunctive therapies, such as vitamin D and calcium supplements, may also be recommended for SLE patients.[58] Additionally, isotretinoin can effectively manage skin lesions associated with SLE.[19] SLE patients need to be aware of the potential side effects of long-term corticosteroid use, including avascular necrosis and the risk of secondary infection after undergoing immunosuppressive therapy. SLE patients with chronic ulceration should also be monitored closely, as there is a potential risk of transformation into squamous cell carcinoma.[2,58] Therefore, it is important for SLE patients to have regular follow-up appointments with their healthcare provider and to report any changes or new symptoms.[2,8,58]

Granulomatosis polyangitis

Granulomatosis with polyangitis (GPA), previously known as Wegener's granulomatosis, is a chronic autoimmune disease characterized by necrosis of granulomatous lesions and inflammation of the vessels, particularly in the respiratory tract and kidneys.[59] The etiology of the disease is unknown, but environmental factors such as hydrocarbons and crystalline silica have been suggested as possible triggers.[60] The disease was first mentioned by Klinger in 1931, and Wegener provided a more detailed description of its symptoms in 1936.[41,61]

GPA is a rare disease; only a small percentage of affected individuals develop oral manifestations.[62] The most common oral manifestation is hyperplastic gingivitis, which is also known as "strawberry gingivitis."[59] This condition is characterized by gingiva enlargement, bleeding, and pain. In addition to hyperplastic gingivitis, ulcers on the oral mucosa, hard and soft palate, and tongue can also be seen in some patients.[59,60] However, the involvement of the parotid and submandibular glands is rare.[61,62] Periodontitis can also develop in GPA patients, leading to further bone resorption and tooth loss. Therefore, it is important for dentists to be aware of these oral manifestations and to refer patients for further evaluation and treatment if necessary.

GPA prevalence is around 5 per 100.000 population.[58] The disease is usually diagnosed in the 4th or 5th decade of life, and there is no gender predilection.[59,] These criteria include the presence of granuloma in the respiratory tract, necrosis, inflammation of small vessels, and glomerulonephritis that can lead to kidney failure.[27,58] Microscopy may reveal local infiltration of lymphocytes and neutrophils in the gingiva, which dentists must differentiate from malignant tumors or Hodgkin's lymphoma.[59,60] Cytoplasmic-staining ANCA (anti-neutrophil cytoplasmic antibodies) is crucial in distinguishing GPA from gingival hyperplasia, vascular proliferation, and squamous cell carcinoma.[63,64]

Immunosuppressive drugs such as cyclophosphamide are recommended to manage GPA, typically at 2 mg/kg per day.[27] This medication helps to reduce the immune system's response that is causing inflammation. Steroids such as prednisone (at the dose of 1 mg/kg per day) or NSAIDs (non-steroidal anti-inflammatory drugs) can also be used to alleviate pain.[27,29,62] Rituximab, a monoclonal antibody, is another effective treatment option for GPA cases.[60,64] It works by targeting and killing the abnormal immune cells that are attacking the body's tissues. Without treatment, the fatal rate of GPA is approximately 82%, with a 5-month survival time. Interrupted therapy can lead to the relapse of GPA and may result in multiple organ damage.[62,63] Therefore, diagnosing the disease early and beginning treatment promptly to prevent kidney failure and death is crucial (Table 1).[29]

Conclusion

As the dentist may be the first healthcare professional to identify these symptoms, they must provide an accurate diagnosis and appropriate treatment plan to improve the patient's quality of life. Autoimmune diseases can present with a wide range of oral symptoms, making the differential diagnosis a critical aspect of the dentist's role. In some cases, the characteristics of different disorders may overlap, further emphasizing the importance of clinical expertise in distinguishing between them. As an integrated multidisciplinary team member, the dentist can collaborate with other healthcare professionals to provide comprehensive care for patients with autoimmune diseases. Early disease management can help prevent further complications and improve the patient's overall outcome. Table 1 likely includes additional information regarding the specific autoimmune diseases that may present with oral manifestations and their respective characteristics, which can assist dentists in making a more accurate diagnosis and developing a tailored treatment plan.

Declarations

Funding

This research received no external funding.

Author contributions

Conceptualization, K.B., S.Y.L. and Y.W.H; Methodology, S.Y.L. and Y.W.H.; Resources, S.Y.L. and Y.W.H.; Writing – Original Draft Preparation, S.Y.L. and Y.W.H.; Writing – Review & Editing, K.B.; Supervision, K.B.; Project Administration, K.B.

Conflicts of interest

The authors declare no conflict of interest.

References

1. Greco A, De Virgilio A, Ralli M, et al. Behçet's disease: new insights into pathophysiology, clinical features and treatment options. *Autoimmunity Reviews*. 2018;17(6):567-575. doi: 10.1016/j.autrev.2017.12.006
2. Saccucci M, Di Carlo G, Bossù M, Giovarruscio F, Salucci A, Polimeni A. Autoimmune Diseases and Their Manifestations on Oral Cavity: Diagnosis and Clinical Management. *J Immunol Res.* 2018;2018:6061825. doi: 10.1155/2018/6061825
3. Mays JW, Sarmadi M, Moutsopoulos NM. Oral Manifestations of Systemic Autoimmune and Inflammatory Diseases: Diagnosis and Clinical Management. *J Evid Based Dent Pract*. 2012;12(3):265-282. doi: 10.1016/S1532-3382(12)70051-9
4. Chen J, Yao X. A Contemporary Review of Behcet's Syn-

drome. *Clin Rev Allergy Immunol.* 2021;61(3):363-376. doi: 10.1007/s12016-021-08864-3
5. Özdede A, Hatemi G. An evaluation of apremilast for the treatment of adult patients with oral ulcers associated with Behçet's syndrome. *Expert Opin Pharmacother.* 2021;22(12):1533-1537. doi: 10.1080/14656566.2021.1939307
6. Davatchi F, Chams-Davatchi Ch, Shams H, et al. Behcet's Disease: Epidemiology, Clinical Manifestations, and Diagnosis. *Expert Rev Clin Immunol.* 2017;13(1):57-65. doi: 10.1080/1744666X.2016.1205486
7. Rios Scherrer MA, Barreto Rocha V, Campos Garcia L. Behçet´s disease: review with emphasis on dermatological aspects. *An Bras Dermatol.* 2017;92(4):452-464. doi: 10.1590/abd1806-4841.20177359
8. Rashid H, Lamberts A, Diercks GFH, et al. Oral Lesions in Autoimmune Bullous Diseases: An Overview of Clinical Characteristics and Diagnostic Algorithm. *Am J Clin Dermatol.* 2019;20(6):847-861. doi: 10.1007/s40257-019-00461-7
9. Alibaz-Oner F, Direskeneli H. Advances in the Treatment of Behcet's Disease. *Curr Rheumatol Rep.* 2021;23(6):47. doi: 10.1007/s11926-021-01011-z
10. Gualtierotti R, Marzano AV, Spadari F, Cugno M. Main Oral Manifestations in Immune-Mediated and Inflammatory Rheumatic Diseases. *J Clin Med.* 2018;8(1):21. doi: 10.3390/jcm8010021
11. Cheng YS, Gould A, Kurago Z, Fantasia J, Muller S. Diagnosis of oral lichen planus: a position paper of the American Academy of Oral and Maxillofacial Pathology. *Oral Surg Oral Med Oral Pathol Oral Radiol.* 2016;122(3):332-354. doi: 10.1016/j.oooo.2016.05.004
12. Schifter M, Yeoh SC, Coleman H, Georgiou A. Oral mucosal diseases: the inflammatory dermatoses. *Aust Dent J.* 2010;55(1):23-38. doi: 10.1111/j.1834-7819.2010.01196.x
13. Michael White C, Chamberlin K, Eisenberg E. Curcumin, a turmeric extract, for oral lichen planus: A systematic review. *Oral Diseases.* 2019;25:720-725. doi: 10.1111/odi.13034
14. Sankar V, Noujeim M. Oral Manifestations of Autoimmune and Connective Tissue Disorders. *Atlas Oral Maxillofac Surg Clin North Am.* 2017;25(2):113-126. doi: 10.1016/j.cxom.2017.04.004
15. Wei J, Ma D, Qiu M, et al. Medical treatments for pregnant patients with oral lichen planus. *Acta Odontol Scand.* 2017;75(1):67-72. doi: 10.1080/00016357.2016.1250944
16. Agha-Hosseini F, Barati H, Moosavi M-S. Aquaporin3 (AQP3) expression in oral epithelium in oral lichen planus. *Exp Mol Pathol.* 2020;115:104441. doi: 10.1016/j.yexmp.2020.104441
17. Mendes GG, Servato JP, Borges FC, et al. Differential metallothionein expression in oral lichen planus and amalgam-associated oral lichenoid lesions. *Med Oral Patol Oral Cir Bucal.* 2018;23 (3):e262-e268. doi: 10.4317/medoral.22144
18. Sen S, Sen S, Dutta A, Abhinandan, KV, Singh AK. Oral manifestation and its management in postmenopausal women: an integrated review. *Menopause Rev.* 2020;19(2):101-103. doi: 10.5114/pm.2020.97867
19. Chu S, Michelle L, Ekelem C, Sung CT, Rojek N, Mesinkovska NA. Oral isotretinoin for the treatment of dermatologic conditions other than acne: a systematic review and discussion of future directions. *Arch Dermatol Res.* 2021;313(6):391-430. doi: 10.1007/s00403-020-02152-4
20. Magliocca KR, Fitzpatrick SG. Autoimmune Disease Manifestations in the Oral Cavity. *Surg Pathol Clin.* 2017;10(1):57-88. doi: 10.1016/j.path.2016.11.001
21. Bagan J, Jiménez Y, Murillo J, Bagan L. Oral mucous membrane pemphigoid: A clinical study of 100 low-risk cases. *Oral Dis.* 2018;24(1-2):132-134. doi: 10.1111/odi.12744
22. Kuriachan D, Suresh R, Janardhanan M, Savithri V. Oral Lesions: The Clue to Diagnosis of Pemphigus Vulgaris. *Case Rep Dent.* 2015; 2015:593940. doi: 10.1155/2015/593940
23. Munhoz EA, Cardoso CL, Barreto JA, Soares CT, Damante JH. Severe manifestation of oral pemphigus. *Am J Otolaryngol.* 2011;32(4):338-342. doi: 10.1016/j.amjoto.2010.03.005
24. Paolino G, Didona D, Magliulo G, et al. Paraneoplastic Pemphigus: Insight into the Autoimmune Pathogenesis, Clinical Features and Therapy. *Int J Mol Sci.* 2017;18(12):2532. doi: 10.3390/ijms18122532
25. Sultan AS, Villa A, Saavedra AP, Treister NS, Woo SB. Oral mucous membrane pemphigoid and pemphigus vulgaris- a retrospective two-center cohort study. *Oral Dis.* 2017;23(4):498-504. doi: 10.1111/odi.12639
26. Said S, Golitz L. Vesiculobullous Eruptions of the Oral Cavity. *Otolaryngol Clin N Am.* 2011;44(1):133-160. doi: 10.1016/j.otc.2010.09.00544
27. McNamara KK, Kalmar JR. Erythematous and Vascular Oral Mucosal Lesions: A Clinicopathologic Review of Red Entities. *Head Neck Pathol.* 2019;13(1):4-15. doi: 10.1007/s12105-019-01002-8
28. Mays JW, Carey BP, Posey R, et al. World Workshop of Oral Medicine VII: A systematic review of immunobiologic therapy for oral manifestations of pemphigoid and pemphigus. *Oral Dis.* 2019;25(1):111-121. doi: 10.1111/odi.13083
29. Wasserman AM. Diagnosis and management of rheumatoid arthritis. *Am Fam Physician.* 2011;84(11):1245-1252.
30. Cheng Z, Do T, Mankia K, et al. Dysbiosis in the oral microbiomes of anti-CCP positive individuals at risk of developing rheumatoid arthritis. *Ann Rheum Dis.* 2021;80(2):162-168. doi: 10.1136/annrheumdis-2020-216972
31. Manzano BR, da Silva Santos PS, Bariquelo MH, Merlini NRG, Honório HM, Rubira CMF. A case-control study of oral diseases and quality of life in individuals with rheumatoid arthritis and systemic lupus erythematosus. *Clin Oral Investig.* 2021;25(4):2081-2092. doi: 10.1007/s00784-020-03518-8
32. Radwan-Oczko M, Duś-Ilnicka I, Richards P, Thomsen AM, Rasmussen C. Rheumatoid arthritis patients' oral health and disease activity. *Int J Rheum Dis.* 2019;22(8):1538-1543. doi: 10.1111/1756-185X.13590
33. Möller B, Kollert F, Sculean A, Villiger PM. Infectious

Triggers in Periodontitis and the Gut in Rheumatoid Arthritis (RA): A Complex Story about Association and Causality. *Front Immunol.* 2020;11:1108. doi: 10.3389/fimmu.2020.01108
34. Rodrigues-Fernandes CI, Migliorati CA, Gueiros LAM, et al. Adalimumab-induced sarcoidosis-like reaction involving oral cavity in rheumatoid arthritis: a case-based review. *Clin Rheumatol.* 2021;40(9):3833-3839. doi: 10.1007/s10067-021-05654-2
35. Vivino FB, Carsons SE, Foulks G, et al. New Treatment Guidelines for Sjögren's Disease. *Rheum Dis Clin North Am.* 2016;42(3):531-551. doi: 10.1016/j.rdc.2016.03.010
36. Both T, Dalm VA, van HPM, van DPL. Reviewing primary Sjögren's syndrome: beyond the dryness - From pathophysiology to Diagnosis and treatment. *Int J Med Sci.* 2017;14(3):191-200. doi: 10.7150/ijms.17718
37. Maślińska M, Przygodzka M, Kwiatkowska B, Sikorska-Siudek K. Sjögren's syndrome: still not fully understood disease *Rheumatol Int.* 2015; 35:233-241.
38. Shiboski CH, Shiboski SC, le Seror, et al. American College of Rheumatology/European League Against Rheumatism Classification Criteria for Primary Sjögren's Syndrome. A Consensus and Data-Driven Methodology Involving Three International Patient Cohorts *Ann Rheum Dis.* 2017;76:9-16.
39. Shiboski CH, Shiboski SC, Seror R. 2016 America College of Rheumatology/European League Against Rheumatism Classification Criteria for primary Sjögren's syndrome. *Arthritis Rheumatol.* 2017;69:35-45.
40. Maślińska M. The role of Epstein-Barr virus infection in primary Sjögren's syndrome. *Curr Opin Rheumatol.* 2019; 31(5):475-483. doi: 10.1097/BOR.0000000000000622
41. Schmalz G, Patschan S, Patschan D, Ziebolz D. Oral-Health-Related Quality of Life in Adult Patients with Rheumatic Diseases—A Systematic Review. *J Clin Med.* 2020;9(4):1172. doi: 10.3390/jcm9041172
42. Błochowiak KJ, Olewicz-Gawlik A, Trzybulska D, et al. Serum ICAM-1, VCAM-1 and E-selectin levels in patients with primary and secondary Sjögren's syndrome. *Adv Clin Exp Med.* 2017 ;26(5):835-842. doi: 10.17219/acem/61434
43. Błochowiak K, Olewicz-Gawlik A, Polańska A, Nowak-Gabryel M, Kocięcki J. Oral mucosal manifestations in primary and secondary Sjögren's syndrome and dry mouth syndrome. *Post Derm Alerg,* 2016;33(1):23-27.
44. Stefanski AL, Tomiak C, Pleyer U, Dietrich T, Burmester GR, Dörner T. The Diagnosis and Treatment of Sjögren's Syndrome. *Dtsch Arztebl Int.* 2017;114(20):354-361. doi: 10.3238/arztebl.2017.0354
45. Kamiński B, Guzera Z. The unknown face of labial salivary glands. Labial salivary gland biopsy as a diagnostic tool. *Medical Studies.* 2019;35:172-179.
46. Błochowiak K, Sokalski J. Labial salivary gland biopsy in the diagnosis of Sjögren's syndrome. *Eur J Clin Exp Med.* 2019;2:162-168. doi: 10.15584/ejcem.2019.2.10
47. Negrini S, Emmi G, Greco M, et al. Sjögren's syndrome: a systemic autoimmune disease. *Clin Exp Med.* 2022;22(1):9-25. doi: 10.1007/s10238-021-00728-6
48. Zablotskyy O, Tomczyk M, Błochowiak K. Current recommendations for treatment and diagnosing of xerostomia in Sjögren's syndrome. *Eur J Clin Exp Med.* 2019;17(4):356-363. doi: 10.15584/ejcem.2019.4.12
49. Baer AN, Walitt B. Update on Sjögren Syndrome and Other Causes of Sicca in Older Adults. *Rheum Dis Clin North Am.* 2018;44(3):419-436. doi: 10.1016/j.rdc.2018.03.002
50. Sebastian A, Dionizy P, Wiland P. *IgG4-Related Disease and the Spectrum of Mimics in Rheumatology. Chronic autoimmune epithelitis. Sjögren's syndrome and other autoimmune diseases of the exocrine glands*. M. Maslinska (red.) IntechOpen Book Series Rheumatology, London; 2019: 73–85.
51. Kamiński B, Błochowiak K. Mikulicz's disease and Küttner's tumor as manifestations of IgG4-related diseases: A review of the literature. *Reumatologia*, 2020;58(4):243-250.
52. Wallace ZS, Naden RP, Chari S, et al. The 2019 American College of Rheumatology/European League against rheumatism classification criteria for IgG4-related disease. *Ann Rheum Dis,* 2020;79(1):77-87.
53. Umehara H, Okazaki K, Masaki Y, et al. A novel clinical entity, IgG4-related disease (IgG4RD): general concept and details. *Mod Rheumatol* 2012;22:1-14. doi: 10.1007/s10165-011-0508-6
54. Umehara H, Okazaki K, Masaki Y, et al. Comprehensive diagnostic criteria for IgG4related disease (IgG4RD), 2011. *Mod Rheumatol,* 2012;22(1):21-30.
55. Tunnicliffe DJ, Singh-Grewal D, Kim S, Craig JC, Tong A. Diagnosis, Monitoring, and Treatment of Systemic Lupus Erythematosus: A Systematic Review of Clinical Practice Guidelines. *Arthritis Care Res (Hoboken).* 2015;67(10):1440-1452. doi: 10.1002/acr.22591
56. Benli M, Batool F, Stutz C, Petit C, Jung S, Huck O. Orofacial manifestations and dental management of systemic lupus erythematosus: A review. *Oral Dis.* 2021;27(2):151-167. doi: 10.1111/odi.13271
57. Li BZ, Zhou HY, Guo B, et al. Dysbiosis of oral microbiota is associated with systemic lupus erythematosus. *Arch Oral Biol.* 2020;113:104708. doi: 10.1016/j.archoralbio.2020.104708
58. Bolton C, Chen Y, Hawthorne R, et al. Systematic Review: Monoclonal Antibody-Induced Subacute Cutaneous Lupus Erythematosus. *Drugs RD.* 2020;20(4):319-330. doi: 10.1007/s40268-020-00320-5
59. Greco A, Marinelli C, Fusconi M, et al. Clinic manifestations in granulomatosis with polyangiitis. *Int J Immunopathol Pharmacol.* 2016;29(2):151-159. doi: 10.1177/0394632015617063
60. Szczeklik K, Włudarczyk A, Wawrzycka-Adamczyk K, et al. Oral manifestations of granulomatosis with polyangiitis - Clinical and radiological assessment. *J Dent Sci.* 2019;14(1):54-60. doi: 10.1016/j.jds.2018.10.004
61. Almouhawis HA, Leao JC, Fedele S, Porter SR. Wege-

ner's granulomatosis: a review of clinical features and an update in Diagnosis and treatment. *J Oral Pathol Med.* 2013;42(7):507-516. doi: 10.1111/jop.12030

62. Faizan A, Bridget ES, Temitope O, Misha R. Oral Granulomatous Disease *Dermatol Clin.* 2020;38(4):429-439. doi: 10.1016/j.det.2020.05.004

63. Apoita-Sanz M, Blanco-Jauset P, Polis-Yanes C, et al. Granulomatosis with Poliangeitis (Wegener's Granulomatosis): Orofacial Manifestations. Systematic Review and Case Report. *Oral Health Prev Dent*. 2020;18(1):929-943. doi: 10.3290/j.ohpd.a45433

64. Barrett. AW. Wegener's granulomatosis of the major salivary glands. *J Oral Pathol Med.* 2012;41(10):721-727. doi: 10.1111/j.1600-0714.2012.01141.x

Wydawnictwo UR 2023
ISSN 2544-1361 (online)
doi: 10.15584/ejcem.2023.3.28

http://www.ejcem.ur.edu.pl
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2023; 21 (3): 639–647

REVIEW PAPER

Effectiveness of novel iron regulators in the treatment of diabetic nephropathy

Dekai Banerjee [1], Ginpreet Kaur [1], Bappaditya Chatterjee [1], Hemant Joshi [2], Seema Ramniwas [3], Hardeep Singh Tuli [4]

[1] SVKM's Narsee Monjee Institute of Management Studies, Shobhaben Pratapbhai Patel School of Pharmacy and Technology Management, Mumbai, India
[2] School of Biotechnology, Jawaharlal Nehru University, New Delhi, India
[3] University Centre for Research and Development, University Institute of Pharmaceutical Sciences, Chandigarh University, Gharuan, Mohali, India
[4] Department of Bio-Sciences and Technology, Maharishi Markandeshwar Engineering College, Maharishi Markandeshwar (Deemed to be University), Mullana-Ambala, India

ABSTRACT

Introduction and aim. The novel advancements of upcoming iron regulators used to treat diabetic nephropathy have implicated a common manifestation of combination chelation therapy used to eliminate end-stage renal disease associated with inflammation and iron imbalance that is altered by renal iron absorption. However, iron accumulation in the clustered kidneys that filter blood may cause problems that affect diabetic blood sugar regulation.

Material and methods. A well-designed method was employed to discover relevant research publications on iron chelators and their potential to treat diabetic nephropathy. "Iron chelators", "diabetic nephropathy", "end-stage renal disease", and "chelation therapy" were searched in Google Scholar, Web of Science, PubMed, and EMBASE.

Analysis of literature. Although the specific etiology and development have not been fully explored, emerging evidence on iron pathophysiology helps comprehend the pathogenesis of acute kidney damage and chronic kidney disease, which crucially provides novel iron chelation therapy techniques. Ferroptosis and hepcidin marker proteins increase oxidative/nitrifying stress and kidney injury. Iron chelator medicines including deferoxamine, deferasirox, and deferiprone were tested as prophylactic strategies.

Conclusion. This article covers both preclinical and clinical aspects of iron chelators to avoid diabetic nephropathy, including novel iron therapies that must be reviewed when selecting dosing regimens.

Keywords. acute kidney injury, chronic kidney disease, end-stage renal disease, iron chelators, renal iron handling

Introduction

Diabetic nephropathy (DN), the most common complication of type II diabetes mellitus and the leading cause of end-stage renal disease (ESRD) globally, are caused by the microvascular barriers of diabetes and causes kidney injury that is significantly more likely to cause morbidity and mortality.[1] It was discovered ten years ago that 40% of diabetic patients have type II diabetes mellitus which is characterized by declined glomerular filtration rate (GFR) and persistent presence of albuminuria (or an albuminuria excretion rate of >300 mg/d or 200 g/min), which eventually results in ESRD.[2]

Corresponding author: Ginpreet Kaur, e-mail: ginpreet.kaur@nmims.edu

Received: 2.07.2023 / Revised: 15.07.2023 / Accepted: 17.07.2023 / Published: 30.09.2023

Banerjee D, Kaur G, Chatterjee B, Joshi H, Ramniwas S, Tuli HS. *Effectiveness of novel iron regulators in the treatment of diabetic nephropathy.* Eur J Clin Exp Med. 2023;21(3):639–647. doi: 10.15584/ejcem.2023.3.28.

It is also predicted that $4.39x10^8$ individuals by 2030 will get diabetes mellitus worldwide, which has a varied etiology and is characterized by hyperglycemia resulting from abnormalities in insulin action, insulin secretion, or both. Many organs, particularly the eyes, nerves, feet, blood vessels, kidneys, and heart, are related to damage, dysfunction, and failure in chronic diabetes. Microvascular problems like retinopathy, neuropathy, and nephropathy have been linked to long-term diabetes.[3] Globally there has been an increase in the burden of chronic diseases like hypertension and diabetes. With a frequency of 3.8% and 11.8% in rural adults and in urban adults, respectively, India has the highest cases of diabetes worldwide. Between 25 and 40 % of them go on to acquire ESRD, often known as chronic kidney disease (CKD). Type I and type II diabetes both result in ESRD which raises the risk of acute kidney infection (AKI). Unlike polycystic kidney disease, when their size is reduced, glomeruli and kidneys are often larger in diabetic nephropathy.[4]

For improved disease control, it is essential to cure individuals that have more chance to develop DN. Many variables and mechanisms are associated to the onset and progression of DN. Renal damage and elevated iron (Fe) content have been linked and it has been fundamentally seen that people with diabetic nephropathy are more frequently found to have CKD and AKI which alters iron control for absorption.[5] In a previous study, it was observed that diabetes increased the levels of Fe regulating proteins and elevated the Fe concentration in kidney of type I and II diabetic mouse models. The increased renal Fe induced the renin-angiotensin system in the kidney leading to the development of diabetic nephropathy which can be overcome by the use of Fe chelator deferiprone.[6] Additionally, this contributes to illnesses like anemia, nephrotic syndrome, lupus nephritis, and Wegner's disease. In conclusion, Fe deposition occurs frequently in CKD but less frequently in acute kidney disease, which may be due to changed molecular levels of Fe management that induce renal damage.[7] The term "renal Fe handling" basically refers to the process by which circulating Fe can be filtered by the kidney's glomerulating function and almost reabsorbed by tubules of epithelial tissue to prevent urinary Fe waste.[8] One key obstacle in the pathogenesis of many kidney disorders, including diabetic nephropathy is related with the association of hepcidin serum concentrations along with C-reactive proteins (CRP) wherein the high level of hepcidin concentrations in the body produced by inflammation.[9]

Aim

It is a narrative review that reveals the therapeutic potential of novel iron chelators to treat diabetic nephropathy.

Material and methods

A well-designed method was employed to discover relevant research publications on iron chelators and their potential to treat diabetic nephropathy. "Iron chelators", "diabetic nephropathy", "end-stage renal disease", and "chelation therapy" were searched in Google Scholar, Web of Science, PubMed, and EMBASE.

Analysis of literature

Molecular pathways

The Fe transporter protein transferrin transports Fe from the plasma, where it is bound, to the majority of bodily tissues and cells. Afterward, Fe-free transferrin is exposed to extracellular fluid for a subsequent cycle of Fe binding and administration by selective absorption of Fe transferrin via hepcidin and ferroportin.[10] Less than 0.1 percent, or about 2-3 mg, of the body's total Fe, is associated with transferrin. The key factor causing the transferrin and its associated Fe to shift after two to three hours is nearly 20 or about 25 mg of Fe absorption. Fe is accumulated within cells as ferritin and Fe-regulated processes control each cell's Fe requirements are met via a reciprocal relationship between the quantity of Fe-storing ferritin and the number of transferrin receptors.[11]

Iron abnormalities in acute kidney injury

No intervention has been found to certainly and consistently prevent AKI despite years of investigation. Increasing our knowledge of AKI pathophysiology is therefore required in order to find novel treatment targets.[12] AKI could be a significant public health concern that makes a variety of hospital admissions more challenging globally. The ability of Fe to catalyze the Fenton and Haber-Weiss reactions, which result in the application of oxidative damage to cell membranes, proteins, and DNA, makes Fe essential for many physiological processes but harmful to the kidneys and other organs when present in excess.[13] AKI is majorly responsible for higher patient death. Fe's crucial role in AKI is supported by a large body of evidence from diagnostic models.[14] Fe content is noticeably elevated within the kidneys of animals exposed to a variety of toxic stimuli, exogenous Fe infusion exacerbates nephritic injury, and most importantly specialized Fe chelation in medicine is protective. Additionally, exogenous hepcidin administration, the chief regulator of overall Fe equilibrium protects against AKI conditions while genetic engineering of essential proteins associated with the regulation, transport, and metabolism of Fe (such as heme oxygenase-1, ferritin, and ferroportin) has an impact on these processes.[15] A small number of human researchers have looked into the viability of using such animal models in therapeutic settings, and the early findings are undeniably positive.

The majority of animal studies on the therapeutic effects of Fe chelation mainly focused on preventing AKI. The mechanism of actions of different Fe chelators is shown in Figure 1. In particular, Fe chelators were typically given before (or concurrently with) the injury to the urinary organ.[16] Contrarily, only a little amount of research has been done on the usage of Fe chelators as a kind of therapeutic for the cure of AKI after the harm to the urinary organ has already occurred.[17] The therapeutic effects of DFP which was used alone or combined with deferoxamine (DFO) were seen within 30 minutes of the onset of urinary organ injury in a mouse model of AKI caused by aluminium chloride. However, no study has looked into how well Fe chelators work to treat AKI if their administration is put off for many hours or more.[18] Idiopathic reactions exist regarding the mechanisms supporting DFO's preventive benefits in AKI-prone animals. One apparent justification, however, is that virulent forms of non-transferrin safe Fe are removed from the circulation and chelated by DFO which prevents downstream detrimental effects including supermolecule Fe-dependent oxidative deaths such as peroxidation and ferroptosis.[19] Additionally, DFO inhibits lysosomal Fe-mediated death induced by H_2O_2 and artesunate via the ferroptosis freelance pathway, indicating that it may have completely diverse effects on various Fe pools (such as lysosomal, mitochondrial, cytosolic, or extracellular Fe), which remains partly active in triggering different death phenotypes in response to different lethal stressors. DFO may also affect cell survival and proliferation by scavenging superoxide free radicals, among other impacts on cells.[20]

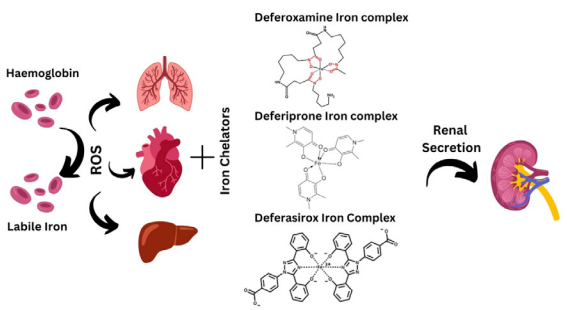

Fig. 1. Mechanism of actions of iron chelators to sequester the free iron and combat the kidney injury

Iron abnormalities in chronic kidney disease

Multiple pathways seriously decrease Fe metabolism in advanced renal disease. Fe deficiency is now discovered in the vast majority of individuals along with dialysis co-dependent CKD, despite the fact that a thorough review of each patient's development over time has not been conducted.[21] High hepcidin concentrations may result in reduced Fe absorption and elevated levels of Fe loss, especially in cases of gastrointestinal bleeding, in Fe insufficiency (Fig. 2). It has been shown that serum hepcidin concentrations correlate with CRP, which measures the correlation between hepcidin and the estimated glomerular filtration rate, and shows that high concentrations of hepcidin are partially caused by inflammation linked to the development of many kidney disorders.[22] Hepcidin levels are also elevated after hemodialysis or peritoneal dialysis by additional inflammatory stimuli such as intermittent infection and blood interaction with foreign objects like catheters or dialysis membranes.[23] Hepcidin is expelled via the membrane and its concentration in the plasma declines during hemodialysis, however, because of the rapid hepcidin formation, the concentration of hepcidin is returned within an hour of the procedure being finished.[24]

Inflammation-induced regulation of hepcidin secretion

Hepcidin synthesis is greatly boosted in infections and other inflammatory situations, which causes distinctive inflammatory hypoxia.[25] Hepcidin transcription is typically induced by inflammation through a second inflammatory mechanism that stimulates hepcidin via activin B, the BMP receptor, and the Smad signaling pathway and it also relies on the interleukin-6,14 receptor and the JAK2-STAT3 pathway (Figure 2).[25] The JAK2-STAT3 pathway and hepcidin have been implicated in the development and progression of diabetic nephropathy. The JAK2-STAT3 pathway is activated in response to cytokines and growth factors, including interleukin-6 (IL-6), which is known to be upregulated in diabetic nephropathy. It works by signaling and transcriptional regulation.[26]

Signaling: Upon ligand binding, JAK2 is activated, leading to the phosphorylation of STAT3.[27]

Transcriptional Regulation: Phosphorylated STAT3 forms dimers and translocates to the nucleus, where it acts as a transcription factor, regulating the expression of target genes.[28] The JAK2-STAT3 pathway is associated with inflammation and fibrosis, which are prominent features of diabetic nephropathy. Activation of the pathway leads to the production of pro-inflammatory cytokines and the promotion of fibrotic processes.[29]

- renal injury: The activation of JAK2-STAT3 pathway in diabetic nephropathy contributes to renal injury by promoting oxidative stress, inflammation, and fibrosis, ultimately leading to kidney dysfunction.[30]
- hepcidin and iron metabolism: Hepcidin dysregulation in diabetic nephropathy affects iron metabolism. Increased hepcidin levels lead to decreased iron absorption and release, resulting in disrupted iron homeostasis. Altered iron metabolism has been implicated in the pathogenesis of diabetic nephropathy.[31]

Therapeutic Implications:

- targeting JAK2-STAT3 Pathway: Modulating the JAK2-STAT3 pathway has emerged as a potential

therapeutic strategy for various diseases, including diabetic nephropathy. Inhibitors targeting JAK2 or STAT3 have shown promise in preclinical studies, but further research is needed to assess their efficacy and safety.[32]

– hepcidin manipulation: Considering the involvement of hepcidin dysregulation in diabetic nephropathy, therapies aimed at modulating hepcidin levels or iron metabolism might have therapeutic potential. However, specific interventions targeting hepcidin in diabetic nephropathy are still under investigation.[33]

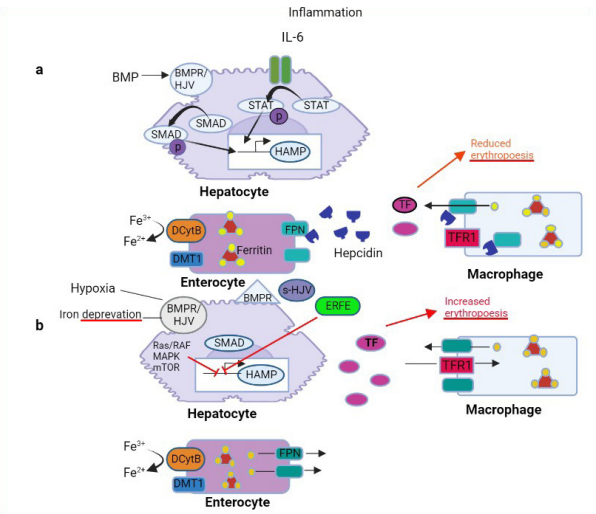

Fig. 2. Hepcidin regulation in diabetic nephropathy; BMP – bone morphogenetic proteins; SMAD –suppressor of mothers against decapentaplegic; DMT1-divalent metal (Ion) transporter 1; ERFE – erythroferrone; hepcidin antimicrobial peptide; IL-6 – interleukin-6; DCYTB – duodenal cytochrome b

Therapeutic approaches to treat diabetic nephropathy

For acute kidney injury

In individuals with diabetic nephropathy, AKI can occur as a complication. Several factors can contribute to AKI in these cases, including infections, medications (e.g., certain antibiotics or contrast agents), and dehydration. Diabetic nephropathy weakens the kidneys over time, making them more susceptible to acute injury. Severe or recurrent episodes of AKI can accelerate the progression of diabetic nephropathy.[34] AKI episodes may cause additional damage to the already compromised kidneys, leading to a decline in kidney function and worsening of the underlying diabetic nephropathy.[35] Both AKI and diabetic nephropathy are commonly associated with diabetes. Diabetic nephropathy develops in individuals with diabetes, particularly those with poorly controlled blood sugar levels. Prompt recognition and management of AKI are crucial to prevent further kidney damage. Treatment focuses on addressing the underlying cause, maintaining fluid and electrolyte balance, and providing supportive care. In severe cases, dialysis may be necessary.[36] Intracellular and systemic systems help to keep the equilibrium of Fe under normal circumstances. Fe excess may exist when this intricate homeostatic mechanism malfunctions. It is challenging to identify Fe excess. Even though it is intrusive and risky, liver biopsies continue to be the gold standard. With little danger to the patient, the ability to identify Fe using non-invasive approaches let researchers better grasp the rate of Fe overload in various organs.[37]

Even though it may not be a precise procedure, estimating serum ferritin (mg/L) is the simplest and, as a result, the most frequently used diagnostic tool for determining body Fe reserves. Myelodysplastic syndromes, sickle cell disease, and thalassemia are the most prevalent haematological conditions that lead to Fe overload. Deferiprone, deferoxamine, and deferasirox are the three medications that have been authorised for the cure of Fe excess in all of these disorders.[38] These chelators have been shown to improve event-free survival (EFS) by lowering tissue Fe levels and preventing Fe overload problems. "The U.S. Food and Drug Administration (FDA) have given the go-ahead for the use of DFO, deferasirox (DFX), and diisopropyl fluorophosphate (DFP) as three Fe chelators for the treatment of acute kidney disease". These medications need to vary in terms of pharmacokinetics, methods of administration, and side effects.[39] The novel Fe chelators used frequently for AKI has been discussed further and the upcoming Fe chelators are mentioned in Table 1.

Deferoxamine (DFO)

DFO has the longest clinical history because previously it was the main Fe chelator that was initially tested and approved by the FDA.[40] Compared to DFP or DFX, DFO has a greater affinity for binding to Fe, and it was employed in large quantities of animal models of acute kidney injury and extrarenal organ injury. In a chronic situation, DFO is supplied parenterally via a blood vessel infusion or an infinite hypodermic pump (in the acute setting).[41] The DFO-Fe advanced (ferrioxamine) is removed through the feces and urine when DFO binds to Fe first and then the binding takes place. Ocular toxicity and hypotension are the most severe possible side effects of DFO, and they virtually exclusively affect people who get extremely high dosages (for example, 60 mg/kg).[42]

Deferasirox (DFX)

A second Fe chelator, DFX, wasn't approved for almost 40 years (between 1968 and 2005). With a half-life of 8 to 16 hours, DFX, an oral Fe chelator, enables a simple once-daily dosage. Moreover, DFX causes a 38% increase in blood serum creatinine. As a result, DFX is not

recommended for people with adequate to advanced chronic kidney disease (eGFR, 40 ml/min).[43]

Deferiprone (DFP)

DFP is another oral Fe chelator that is given three times daily and has a $t_{1/2}$ of about two hours. DFP has an advantage over other Fe chelators in that it has a higher lipophilicity and higher intracellular penetrance, which makes it easier to chelate intracellular Fe [33]. This DFP characteristic has been used to increase cardiac Fe chelation in patients with hypochromic anemia.[44]

Outcomes from evaluation of the iron chelators

DFO was found to be a good candidate for use in clinical trials due to a variety of qualities for the prevention of AKI. The logistical difficulties that can arise due to enteral injection of DFP in the context of acute disorders, as well as problems with aspiration and reduced gastrointestinal absorption, are avoided with epithelial duct administration of DFO.[45] DFO has been used by both people and animals for the longest time. Even at doses as high as 32 mg/kg, it is also well tolerated acutely. So, small to adequate variance doses of DFO (10–20 mg/kg) would probably be enough to sustain the action because Fe chelation therapy in this scenario primarily aims to eliminate the current chemical change in Fe.

Treatment for chronic kidney disease

CKD and DN are closely related conditions, and the hormone hepcidin plays a role in their connection. Diabetic nephropathy is a type of kidney disease that occurs as a complication of diabetes. It is characterized by damage to the small blood vessels in the kidneys, resulting in impaired kidney function. Over time, this can lead to chronic kidney disease.[46] Hepcidin is a hormone primarily produced in the liver. Its main function is to regulate iron levels in the body. However, emerging research suggests that hepcidin may also play a role in kidney disease, including CKD and DN. In CKD, hepcidin levels are often increased. This leads to a condition known as "anemia of chronic kidney disease" (CKD-associated anemia). Hepcidin acts by inhibiting the release of iron from cells, including the iron required for the production of new red blood cells.[47] As a result, CKD patients with high levels of hepcidin may experience lower red blood cell counts and anemia.

In the case of diabetic nephropathy, hepcidin may contribute to the development and progression of the disease. Research suggests that hepcidin levels are increased in diabetic nephropathy, and this increase is associated with impaired iron metabolism and kidney damage. Elevated hepcidin levels can lead to increased iron retention within the kidneys, promoting inflammation and oxidative stress, which are factors contributing to the progression of DN.[48]

Oral administration of iron

It is advised to begin treatment with oral Fe in CKD individuals who are not presently following dialysis or peritoneal dialysis. The optimal time to provide 200 mg of elemental Fe to an adult patient is during a fast (ferrous salts are suggested for better absorption). The most prevalent issues with oral Fe therapy in CKD- which may need hospitalisation to get Fe intravenously – involve gastrointestinal intolerance, intestinal absorption issues, or a non-compliance.[49]

Iron infusion

In individuals with CKD who are not receiving dialysis, intravenous Fe therapy is advised if:

- After three months of oral Fe therapy, or in cases of unable to tolerate oral Fe or malabsorption, Fe parameter targets are not fulfilled.
- In patients who need a rapid Hb response and have severe anaemia and Fe shortage.[50]

Considerations for treatment strategies currently applied to CKD

Ferrous citrate (FC): The negative effects of bronzed diabetes, which are a problem with IV formulations, may not occur when Fe replacement is done orally with preparations like FC since there may be additional physiological constraints on Fe absorption. FC is permitted to be used in ESRD patients as a phosphate binder. In more recent times, the FDA authorised FC for the treatment of CKD patients who weren't receiving chemical analysis. Recent studies without the use of chemical analysis show that FC increases Hgb in CKD patients while also lowering phosphorus (stage 3–5).[51]

Ferric maltol: In the United Kingdom and the United States, ferric maltol is authorised for the cure of people with inflammatory bowel disease and has a low risk of side effects. It has shown quick anemia repair. Individuals with CKD stages 3–4 performed phase three of the study with oral ferrous maltol for the cure of United Nations agency in subjects with CKD in order to compare the budding implications of ferrous maltol on Hgb vs. placebo (AEGIS-CKD). The outcomes of the experiment are still being finalized.[52]

Ferric salt change state (FPC): In 2015, the FDA gave the drug its green light to be utilized in individuals undergoing chemical analysis. It is a complex, carbohydrate-free, soluble Fe salt that is given to hemodialysis patients through the dialysate. It prevents Fe sequestration inside the reticuloendothelial macrophages and gives Fe to transferrin. In the continuous replacement exploitation Fe soluble equivalents (CRUISE) one and two trials, FPC was compared to placebo in individuals with ESRD receiving hemodialysis. When compared to a placebo, FPC administered by dialysate was analysed to well preserve Hgb, TSAT, and protein while significantly reducing the need for ESA dose.[53]

Liposomal/Sucrosomial Fe: A lipid bilayer encircles the ferrous salt core in sucrosomial Fe, which also has a layer of sucrosomes (sucrester, a chemical agent, and extra amyloid compounds). By doing this, Fe can dodge the effects of hepcidin's downregulation and be consumed by microfold cells through the systema lymphaticum, limiting any adverse consequences that could otherwise occur. According to preliminary research, liposomal Fe increases Hgb in CKD individuals while lowering the chance of toxic effects. Sucrosomial Fe has also been demonstrated to lower the anaemia in individuals suffering from cancer, disturbed patients, and people having bariatric surgery. The effectiveness and safety of sucrosomial Fe in CKD patients have not been studied.[54]

All the novel Fe supplements and their effective doses are discussed in Table 1.

Table 1. Upcoming novel iron therapies used for treatment of DN

Therapeutic molecule	Chemical class	Development phase	Exposure time	Hb, g/dl	Hepcidin concentration, µg/l	Refs
Daprodustat	HIF-PH inhibitor	Phase II	24 weeks	~0.1	–20.6	56
		Phase III	24 weeks	Increased 0.79	Decreased –55.67%	
Desidustat	HIF-PH inhibitor	Phase II	6 weeks	Increased 1.57 to 2.92	Decreased –59.24 to –91.36	57
Enarodustat	HIF-PH inhibitor	Phase II	6 weeks	Increased	Decreased	58
Molidustat	HIF-PH inhibitor	Phase II	16 weeks	Increased 1.4 to 2.0	Decreased –18	59
Roxadustat	HIF-PH inhibitor	Phase II	4 weeks	Increased 0.4 to 1.8	Decreased –225 to –70	60
		Phase III	8 weeks	Increased 1.9	Decreased –56.14	
Vadadustat	HIF-PH inhibitor	Phase II	20 weeks	Increased	Decreased	61
PRS-080#22	Hepcidin antagonist	Phase I	Single dose	Unaffected	Decreased	62
LY2928057 (monoclonal antibody against ferroportin)	Abrogated interaction with hepcidin	Phase I	6 weeks	–	Increased	
LY3113593 (monoclonal Antibody against BMP6)	Decreased expression of hepcidin	Phase I	Single dose	Increased	Decreased	
Vitamin D2	Decreased expression of hepcidin	Phase IV	6 months	–	–	63
Vitamin D3	Decreased expression of hepcidin	Phase I	6 weeks	Stable	Stable	

Future prospects

The essential and upcoming future prospect treatment strategies used for the treatment for CKD and AKI are described here in Table 1 and Table 2. Renal Vitamin D dysregulations, oxidative stress, inflammation, and apoptosis were all linked to Fe-induced nephrotoxicity. Although, DFX decreased the amount of systemic Fe in the body, vitamin D3 monotherapy demonstrated very low renal Fe concentrations and tissue destruction. The co-therapy strategy however demonstrated the greatest therapeutic benefits through increased variation of renal Fe-homeostatic molecules.[55] Daprodustat boosts HIF-levels by inhibiting HIF-PH, stabilising HIF-1 which raises EPO levels and lowers hepcidin levels (Table 1). An assay using recombinant ferroportin-expressing HEK 293 cells and the novel humanised IgG4 monoclonal antibody LY2928057 demonstrates strong inhibition of hepcidin activity. It inhibits ferroportin-hepcidin binding with high affinity towards human ferroportin.

Table 2. Novel iron supplements used for CKD

	Formulations	References
Ferrous Compounds (Fe^{2+})		
Ferroglycine sulfate	Ferro Sanol®: 100 mg (capsule), Glutaferro Drops: 30 mg/mL	64
Fe gluconate	Losferron: 80 mg (tablet)	65
Fe latate	Cromatonbic Ferro: 37.5 mg (drinkable vial)	66
Fe sulfate	FeroGradumet: 105 mg (tablet); Tradyferon: 80 mg (tablet)	66
Ferric compounds (Fe^{3+})		
Ovalbumin	Kilor, Syron 600, Profer, Ferroprotin: 80 mg (packet)	67
Ferrimannitol	Kilor, Syron 300, Profer, Ferroprotin: 40 mg (packet)	68
Ferrocolinate	Podertoinc adults: 112 mg (packet)	69
Succinylcasein Fe	Lactoferrin, Ferrocurir, Ferplex: 40 mg (drinkable vial)	69

Conclusion

In conclusion, the hepcidin level presents a complex and significant clinical challenge in the treatment of diabetic nephropathy associated with CKD and AKI. Hepcidin dysregulation can contribute to iron metabolism abnormalities, leading to iron overload or iron deficiency, both of which can adversely affect kidney function and overall health. Dysregulation of hepcidin levels during AKI and CKD could potentially impact renal iron handling, oxidative stress, and inflammation, further exacerbating kidney injury. Managing the interplay between diabetic nephropathy, CKD, AKI, and hepcidin requires a multifaceted approach. Additionally, this review discusses about the interventions to modulate hepcidin levels and iron metabolism which may hold promise in improving outcomes. Further research is needed to elucidate the precise mechanisms underlying hepcidin's role in the context of diabetic nephropathy, CKD and AKI, with the ultimate goal of developing targeted therapies and personalized treatment plans for affected individuals. In summary, the association between diabetic

nephropathy, CKD, AKI, and hepcidin underscores the intricate interplay between various pathological processes. In individuals with CKD and in cases of AKI, Fe scarcity and Fe overload is a frequent and treatable cause. Given the limits of hepcidin and ferritin in determining Fe shortage and Fe overload in patients with anaemia of CKD, innovative therapy is weighed against the possible advantages of circumventing or limiting blood transfusions. For the treatment of CKD and AKI, a variety of approved medications are available, including both oral and intravenous formulations are suitable for patients with stable CKD, and Fe chelation therapy for AKI is advised globally leading to a wealth of clinical data. DFP, DFO, and DFX are just a few of the Fe chelators medications that are commonly used to treat acute kidney injury. Additional medications that are currently undergoing clinical trials may also be combined with Fe chelators to treat diabetic nephropathy. Fe modulators can demonstrate their therapeutic effects by acting on the hepcidin and ferritin modulators. Understanding these relationships can contribute to the development of novel therapeutic approaches and improve patient outcomes in this complex clinical scenario. So, for novel approach Fe modulators play an important role in treating CKD and AKI by enhancing good renal properties used in the treatment of renal disease.

Declarations

Funding
No funding was received to carry out this work.

Author's contributions
Conceptualization, G.K. and D.B.; Methodology, B.C.; Software, H.J. and S.R.; Validation, G.K., H.S.T., B.C. and S.R.; Formal Analysis, G.K.; Investigation, D.B.; Resources, H.j.; Data Curation, S.R.; Writing – Original Draft Preparation, D.B.; Writing – Review & Editing, G.K.; Visualization, G.K.; Supervision, G.K.

Conflicts of interest
The authors declare no conflicts of interest.

Data availability
Not applicable.

References

1. van Raaij S, van Swelm R, Bouman K, et al. Tubular iron deposition and iron handling proteins in human healthy kidney and chronic kidney disease. *Sci Rep*. 2018;8(1):9353. doi: 10.1038/s41598-018-27107-8
2. Magee C, Grieve DJ, Watson CJ, Brazil DP. Diabetic Nephropathy: a Tangled Web to Unweave. *Cardiovasc Drugs Ther*. 2017;31(5-6):579-592. doi: 10.1007/s10557-017-6755-9
3. Oltean S, Coward R, Collino M, Baelde H. Diabetic Nephropathy: Novel Molecular Mechanisms and Therapeutic Avenues. *Biomed Res Int*. 2017;2017:1-1. doi: 10.1155/2017/3146524
4. Murphy F, Bennett L, Jenkins K. Managing anaemia of chronic kidney disease. *British Journal of Nursing*. 2010;19(20):1281-1286. doi: 10.12968/bjon.2010.19.20.79681
5. Besarab A, Coyne DW. Iron supplementation to treat anemia in patients with chronic kidney disease. *Nat Rev Nephrol*. 2010;6(12):699-710. doi: 10.1038/nrneph.2010.139
6. Chaudhary K, Chilakala A, Ananth S, et al. Renal iron accelerates the progression of diabetic nephropathy in the HFE gene knockout mouse model of iron overload. *American Journal of Physiology-Renal Physiology*. 2019;317(2):512-517. doi: 10.1152/ajprenal.00184.2019
7. Ganz T, Nemeth E. Iron Balance and the Role of Hepcidin in Chronic Kidney Disease. *Semin Nephrol*. 2016;36(2):87-93. doi: 10.1016/j.semnephrol.2016.02.001
8. Ganz T. Systemic Iron Homeostasis. *Physiol Rev*. 2013;93(4):1721-1741. doi: 10.1152/physrev.00008.2013
9. Abbaspour N HRKR. Review on iron and its importance for human health. *J Res Med Sci*. 2014;19(2):164-174.
10. Donovan A, Lima CA, Pinkus JL, et al. The iron exporter ferroportin/Slc40a1 is essential for iron homeostasis. *Cell Metab*. 2005;1(3):191-200. doi: 10.1016/j.cmet.2005.01.003
11. Coyne DW. Hepcidin: clinical utility as a diagnostic tool and therapeutic target. *Kidney Int*. 2011;80(3):240-244. doi: 10.1038/ki.2011.141
12. Walker VJ, Agarwal A. Targeting Iron Homeostasis in Acute Kidney Injury. *Semin Nephrol*. 2016;36(1):62-70. doi: 10.1016/j.semnephrol.2016.01.003
13. Schrier RW, Wang W, Poole B, Mitra A. Acute renal failure: definitions, diagnosis, pathogenesis, and therapy. *J Clin Investig*. 2004;114(1):5-14. doi: 10.1172/JCI22353
14. Jacobs A. Serum ferritin and iron stores. *Fed Proc*. 1977;36(7):2024-2027.
15. Sharma S, Leaf DE. Iron Chelation as a Potential Therapeutic Strategy for AKI Prevention. *J Am Soc Nephrol*. 2019;30(11):2060-2071. doi: 10.1681/ASN.2019060595
16. Pavkov ME, Knowler WC, Bennett PH, Looker HC, Krakoff J, Nelson RG. Increasing incidence of proteinuria and declining incidence of end-stage renal disease in diabetic Pima Indians. *Kidney Int*. 2006;70(10):1840-1846. doi: 10.1038/sj.ki.5001882
17. Hoste EAJ, Bagshaw SM, Bellomo R, et al. Epidemiology of acute kidney injury in critically ill patients: the multinational AKI-EPI study. *Intensive Care Med*. 2015;41(8):1411-1423. doi: 10.1007/s00134-015-3934-7
18. Selim M. Deferoxamine Mesylate. *Stroke*. 2009;40(3),1:90-91. doi: 10.1161/STROKEAHA.108.533125
19. Kianian F, Seifi B, Kadkhodaee M, Sadeghipour HR, Ranjbaran M. Nephroprotection through Modifying the Apoptotic TNF-α/ERK1/2/Bax Signaling Pathway and Oxidative Stress by Long-term Sodium Hydrosulfide Administration in Ovalbumin-induced Chronic Asthma. *Immunol Invest*.

2022;51(3):602-618. doi: 10.1080/08820139.2020.1858860
20. Molina-Jijón E, Zarco-Márquez G, Medina-Campos ON, et al. Deferoxamine pretreatment prevents Cr(VI)-induced nephrotoxicity and oxidant stress: Role of Cr(VI) chelation. *Toxicology*. 2012;291(1-3):93-101. doi: 10.1016/j.tox.2011.11.003
21. Gafter-Gvili A, Schechter A, Rozen-Zvi B. Iron Deficiency Anemia in Chronic Kidney Disease. *Acta Haematol*. 2019;142(1):44-50. doi: 10.1159/000496492
22. National Collaborating Centre for Chronic Conditions (UK). Anaemia Management in Chronic Kidney Disease: National Clinical Guideline for Management in Adults and Children. London: Royal College of Physicians (UK); 2006. PMID: 20945579. Published online June 2015.
23. Agarwal AK. Iron metabolism and management: focus on chronic kidney disease. *Kidney Int Suppl*. 2021;11(1):46-58. doi: 10.1016/j.kisu.2020.12.003
24. Sheetz M, Barrington P, Callies S, et al. Targeting the hepcidin–ferroportin pathway in anaemia of chronic kidney disease. *Br J Clin Pharmacol*. 2019;85(5):935-948. doi: 10.1111/bcp.13877
25. Brosius FC, He JC. JAK inhibition and progressive kidney disease. *Curr Opin Nephrol Hypertens*. 2015;24(1):88-95. doi: 10.1097/MNH.0000000000000079
26. Babitt JL, Lin HY. Molecular Mechanisms of Hepcidin Regulation: Implications for the Anemia of CKD. *Am J Kidney Dis*. 2010;55(4):726-741. doi: 10.1053/j.ajkd.2009.12.030
27. Hu J, Fan X, Meng X, Wang Y, Liang Q, Luo G. Evidence for the Involvement of JAK/STAT/SOCS Pathway in the Mechanism of Tangshen Formula-Treated Diabetic Nephropathy. *Planta Med*. 2014;80(08/09):614-621. doi: 10.1055/s-0034-1368454
28. Berthier CC, Zhang H, Schin M, et al. Enhanced Expression of Janus Kinase–Signal Transducer and Activator of Transcription Pathway Members in Human Diabetic Nephropathy. *Diabetes*. 2009;58(2):469-477. doi: 10.2337/db08-1328
29. Zhu M, Wang H, Chen J, Zhu H. Sinomenine improve diabetic nephropathy by inhibiting fibrosis and regulating the JAK2/STAT3/SOCS1 pathway in streptozotocin-induced diabetic rats. *Life Sci*. 2021;265:118855. doi: 10.1016/j.lfs.2020.118855
30. Berthier CC, Zhang H, Schin M, et al. Enhanced Expression of Janus Kinase–Signal Transducer and Activator of Transcription Pathway Members in Human Diabetic Nephropathy. *Diabetes*. 2009;58(2):469-477. doi: 10.2337/db08-1328
31. Nemeth E, Ganz T. Hepcidin-Ferroportin Interaction Controls Systemic Iron Homeostasis. *Int J Mol Sci*. 2021;22(12):6493. doi: 10.3390/ijms22126493
32. Sun M, Bu W, Li Y, et al. Danzhi Jiangtang Capsule ameliorates kidney injury via inhibition of the JAK-STAT signaling pathway and increased antioxidant capacity in STZ-induced diabetic nephropathy rats. *Biosci Trends*. 2018;12(6):595-604. doi:10.5582/bst.2018.01255
33. Saboor M, Zehra A, Hamali H, Mobarki A. Revisiting Iron Metabolism, Iron Homeostasis and Iron Deficiency Anemia. *Clin Lab*. 2021;67:10.7754/Clin.Lab.2020.200742. doi: 10.7754/Clin.Lab.2020.200742
34. Yu SMW, Bonventre J V. Acute Kidney Injury and Progression of Diabetic Kidney Disease. *Adv Chronic Kidney Dis*. 2018;25(2):166-180. doi: 10.1053/j.ackd.2017.12.005
35. Allison SJ. Mechanism of AKI sensitivity in diabetic nephropathy. *Nat Rev Nephrol*. 2014;10(9):484-484. doi: 10.1038/nrneph.2014.125
36. Lei Q, Xu F, Liang S, et al. Clinical Acute Kidney Injury and Histologic Acute Tubular-Interstitial Injury and Their Prognosis in Diabetic Nephropathy. *Nephron*. 2022;146(4):351-359. doi: 10.1159/000520944
37. Mishra J. Amelioration of Ischemic Acute Renal Injury by Neutrophil Gelatinase-Associated Lipocalin. *J Am Soc Nephrol*. 2004;15(12):3073-3082. doi: 10.1097/01.ASN.0000145013.44578.45
38. Mo M, Gao Y, Deng L, Liang Y, Xia N, Pan L. Association Between Iron Metabolism and Acute Kidney Injury in Critically Ill Patients With Diabetes. *Front Endocrinol (Lausanne)*. 2022;13. doi: 10.3389/fendo.2022.892811
39. Rouault TA. Biogenesis of iron-sulfur clusters in mammalian cells: new insights and relevance to human disease. *Dis Model Mech*. 2012;5(2):155-164. doi: 10.1242/dmm.009019
40. Ismail THB, Ali BH, Bashir AA. Influence of iron, deferoxamine and ascorbic acid on gentamicin-induced nephrotoxicity in rats. *Gen Pharmacol*. 1994;25(6):1249-1252. doi: 10.1016/0306-3623(94)90145-7
41. Cui HJ, He H yu, Yang AL, et al. Efficacy of Deferoxamine in Animal Models of Intracerebral Hemorrhage: A Systematic Review and Stratified Meta-Analysis. *PLoS One*. 2015;10(5):e0127256. doi: 10.1371/journal.pone.0127256
42. Choi CW, Lee J, Lee HJ, Park HS, Chun YS, Kim B Il. Deferoxamine Improves Alveolar and Pulmonary Vascular Development by Upregulating Hypoxia-inducible Factor-1α in a Rat Model of Bronchopulmonary Dysplasia. *J Korean Med Sci*. 2015;30(9):1295. doi: 10.3346/jkms.2015.30.9.1295
43. Ghaith MM, El-Boshy M, Almasmoum H, et al. Deferasirox and vitamin D3 co-therapy mitigates iron-induced renal injury by enhanced modulation of cellular anti-inflammatory, anti-oxidative stress, and iron regulatory pathways in rat. *J Trace Elem Med Biol*. 2022;74:127085. doi: 10.1016/j.jtemb.2022.127085
44. Zou C, Liu X, Liu R, et al. Effect of the oral iron chelator deferiprone in diabetic nephropathy rats. *J Diabetes*. 2017;9(4):332-340. doi: 10.1111/1753-0407.12420
45. Zou C, Xie R, Bao Y, et al. Iron chelator alleviates tubulointerstitial fibrosis in diabetic nephropathy rats by inhibiting the expression of tenascin C and other correlation factors. *Endocrine*. 2013;44(3):666-674. doi: 10.1007/

s12020-013-9907-0
46. Wada J, Makino H. Inflammation and the pathogenesis of diabetic nephropathy. *Clin Sci*. 2013;124(3):139-152. doi: 10.1042/CS20120198
47. Meza Letelier CE, San Martín Ojeda CA, Ruiz Provoste JJ, Frugone Zaror CJ. Pathophysiology of diabetic nephropathy: a literature review. *Medwave*. 2017;17(01):e6839--e6839. doi: 10.5867/medwave.2017.01.6839
48. Doshi SM, Friedman AN. Diagnosis and Management of Type 2 Diabetic Kidney Disease. *Clin J Am Soc Nephrol*. 2017;12(8):1366-1373. doi: 10.2215/CJN.11111016
49. Batchelor EK, Kapitsinou P, Pergola PE, Kovesdy CP, Jalal DI. Iron Deficiency in Chronic Kidney Disease: Updates on Pathophysiology, Diagnosis, and Treatment. *J Am Soc Nephrol*. 2020;31(3):456-468. doi: 10.1681/ASN.2019020213
50. Cases A, Egocheaga MI, Tranche S, et al. Anemia en la enfermedad renal crónica: protocolo de estudio, manejo y derivación a Nefrología. *Aten Primaria*. 2018;50(1):60-64. doi: 10.1016/j.aprim.2017.09.007
51. Li L, Zheng X, Deng J, Zhou J, Ou J, Hong T. Ferric citrate for the treatment of hyperphosphatemia and anemia in patients with chronic kidney disease: a meta-analysis of randomized clinical trials. *Ren Fail*. 2022;44(1):1113-1123. doi: 10.1080/0886022X.2022.2094273
52. Gasche C, Ahmad T, Tulassay Z, et al. Ferric Maltol Is Effective in Correcting Iron Deficiency Anemia in Patients with Inflammatory Bowel Disease. *Inflamm Bowel Dis*. 2015;21(3):579-588. doi: 10.1097/MIB.0000000000000314
53. Umanath K, Jalal DI, Greco BA, et al. Ferric Citrate Reduces Intravenous Iron and Erythropoiesis-Stimulating Agent Use in ESRD. *J Am Soc Nephrol*. 2015;26(10):2578-2587. doi: 10.1681/ASN.2014080842
54. Elli L, Ferretti F, Branchi F, et al. Sucrosomial Iron Supplementation in Anemic Patients with Celiac Disease Not Tolerating Oral Ferrous Sulfate: A Prospective Study. *Nutrients*. 2018;10(3):330. doi: 10.3390/nu10030330
55. Ribeiro M, Fonseca L, Anjos JS, et al. Oral iron supplementation in patients with chronic kidney disease: Can it be harmful to the gut microbiota? *Nutr Clin Pract*. 2022;37(1):81-93. doi: 10.1002/ncp.10662
56. Singh AK, Cizman B, Carroll K, et al. Efficacy and Safety of Daprodustat for Treatment of Anemia of Chronic Kidney Disease in Incident Dialysis Patients. *JAMA Intern Med*. 2022;182(6):592. doi: 10.1001/jamainternmed.2022.0605
57. Dhillon S. Desidustat: First Approval. *Drugs*. 2022;82(11):1207-1212. doi: 10.1007/s40265-022-01744-w
58. Fujikawa R, Nagao Y, Fujioka M, Akizawa T. Treatment of anemia associated with chronic kidney disease with the HIF prolyl hydroxylase inhibitor enarodustat: A review of the evidence. *Therapeutic Apheresis and Dialysis*. 2022;26(4):679-693. doi: 10.1111/1744-9987.13820
59. Akizawa T, Macdougall IC, Berns JS, et al. Iron Regulation by Molidustat, a Daily Oral Hypoxia-Inducible Factor Prolyl Hydroxylase Inhibitor, in Patients with Chronic Kidney Disease. *Nephron*. 2019;143(4):243-254. doi: 10.1159/000502012
60. Li ZL, Tu Y, Liu BC. Treatment of Renal Anemia with Roxadustat: Advantages and Achievement. *Kidney Diseases*. 2020;6(2):65-73. doi: 10.1159/000504850
61. Eckardt KU, Agarwal R, Farag YM, et al. Global Phase 3 programme of vadadustat for treatment of anaemia of chronic kidney disease: rationale, study design and baseline characteristics of dialysis-dependent patients in the INNO2VATE trials. *Nephrol Dial Transplant*. 2021;36(11):2039-2048. doi: 10.1093/ndt/gfaa204
62. Renders L, Budde K, Rosenberger C, et al. First-in-human Phase I studies of PRS-080#22, a hepcidin antagonist, in healthy volunteers and patients with chronic kidney disease undergoing hemodialysis. *PLoS One*. 2019;14(3):e0212023. doi: 10.1371/journal.pone.0212023
63. Cardoso MP, Pereira LAL. Native vitamin D in pre-dialysis chronic kidney disease. *Nefrología*. 2019;39(1):18-28. doi: 10.1016/j.nefro.2018.07.004
64. Akarsu S, Taskin E, Yilmaz E, Yilmaz H, Kilic M, Aygun AD. Treatment of Iron Deficiency Anemia with Intravenous Iron Preparations. *Acta Haematol*. 2006;116(1):51-57. doi: 10.1159/000092348
65. Shepshelovich D, Rozen-Zvi B, Avni T, Gafter U, Gafter-Gvili A. Intravenous Versus Oral Iron Supplementation for the Treatment of Anemia in CKD: An Updated Systematic Review and Meta-analysis. *Am J Kidney Dis*. 2016;68(5):677-690. doi: 10.1053/j.ajkd.2016.04.018
66. Ohira Y, Chen CS, Hegenauer J, Saltman P. Adaptations of Lactate Metabolism in Iron-Deficient Rats. *Exp Biol Med*. 1983;173(2):213-216. doi: 10.3181/00379727-173-41633
67. Gutiérrez L, Morales MP, Lázaro FJ. Magnetic and structural study of the state of iron in the oral haematinic ferrimannitol ovoalbumin. *J Inorg Biochem*. 2006;100(3):413-417. doi: 10.1016/j.jinorgbio.2006.01.006
68. Bailie GR, Larkina M, Goodkin DA, et al. Data from the Dialysis Outcomes and Practice Patterns Study validate an association between high intravenous iron doses and mortality. *Kidney Int*. 2015;87(1):162-168. doi: 10.1038/ki.2014.275
69. Fishbane S, Block GA, Loram L, et al. Effects of Ferric Citrate in Patients with Nondialysis-Dependent CKD and Iron Deficiency Anemia. *J Am Soc Nephrol*. 2017;28(6):1851-1858. doi: 10.1681/ASN.2016101053

Wydawnictwo UR 2023
ISSN 2544-1361 (online)
doi: 10.15584/ejcem.2023.3.29

http://www.ejcem.ur.edu.pl
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2023; 21 (3): 648–653

CASUISTIC PAPER

Analysis of dysarthria in a 55-year-old female patient with multiple sclerosis by means of an IT tool based on respiratory and phonatory examination – a case study

Wojciech A. Warmbier [1,2], Małgorzata Popiel [1], Agnieszka Guzik [3], Mariusz Drużbicki [3], Halina Bartosik-Psujek [1,4]

[1] Clinic of Neurology with the Stroke Unit, Clinical Hospital No. 2 in Rzeszow, Rzeszow, Poland
[2] Subcarpathian Center for Neurorehabilitation, Rzeszow, Poland
[3] Department of Physiotherapy, Institute of Health Sciences, College of Medical Sciences, University of Rzeszow, Rzeszow, Poland
[4] Department of Neurology, Institute of Medical Sciences, College of Medical Sciences, University of Rzeszow, Rzeszow, Poland

ABSTRACT

Introduction and aim. There are no available objective tools for assessing dysarthric disorders in the course of multiple sclerosis (MS). The aim of the study is to analyse a case of mixed dysarthria in a 55-year-old female patient in the course of secondary progressive MS and to present the severity of the patient's dysarthric disorder in contrast with the control group as evaluated by means of an IT tool based on respiratory and phonatory examination.

Description of the case. A 55-year-old patient was admitted to the Clinic of Neurology with the Stroke Unit due to a worsening condition in the course of MS. She was examined with an objective tool for respiratory and phonatory disorders. Her results were, then, compared to those of 24 healthy individuals from the control group, matched in terms of sex and age. Following speech parameters were analysed: phonetics of utterances, number of produced syllables per breath during the execution of individual texts, sound quality, intonation, and the total performance time of each.

Conclusion. The analysed case indicates that the developed objective IT tool is a promising diagnostic method that can facilitate diagnosis and can be used in clinical practice.

Keywords. dysarthria, multiple sclerosis, respiratory and phonatory disorders

Introduction

Multiple sclerosis (MS) is a demyelinating, inflammatory disease of the central nervous system. It is one of the most common causes of disability in young adults.[1] In Poland, MS incidence and prevalence are higher than previously reported. In 2019 it amounted to 6.6, currently 131.2/100,000 inhabitants.[2] Globally, the disease affects a total of 2.8 million people – 35.9 per 100,000 population.[3] The most common clinical symptoms include retrobulbar optic neuritis, spastic paresis, cerebellar ataxia, sensory disturbances.[4]

In the course of MS, especially in its primary and secondary progressive forms, the most common disorder that affects the quality of verbal communication is dysarthria, which due to the nature of MS, most often occurs in a mixed form and affects up to 45% of patients.[5] At the same time, dysarthria is the least accurately described clinical symptoms in the course of

Corresponding author: Agnieszka Guzik, e-mail: aguzik@ur.edu.pl

Received: 9.06.2023 / Revised: 6.07.2023 / Accepted: 9.07.2023 / Published: 30.09.2023

Warmbier WA, Popiel M, Guzik A, Drużbicki M, Bartosik-Psujek H. *Analysis of dysarthria in a 55-year-old female patient with multiple sclerosis by means of an IT tool based on respiratory and phonatory examination – a case study.* Eur J Clin Exp Med. 2023;21(3):648–653. doi: 10.15584/ejcem.2023.3.29.

MS.[5-7] Speech-related symptoms in the course of MS may be caused or aggravated by weakness, spasticity of the tongue muscles, jaw muscles, soft ligament, diaphragm, and vocal cords. Movement coordination disorders of the above organs may also occur.[8] Impaired communication may result from difficulties in voice control and articulation of words due to the impaired function of the muscles responsible for speech and insufficient subglottic pressure.[9]

The most frequently reported speech disorders include comprehension problems pertaining to understanding the content of the message, slowed down rate of speech, hoarseness, volume and tone control complications, disfluent speech and problems with swallowing.[9-12]

The motivation for undertaking the study was the lack of available, objective tools for the assessment of dysarthric disorders in the course of MS. So far, in Poland, dysarthric disorders have most often been assessed on the basis of patient's interview and by using scales, e.g. the Speech Pathology Specific Questionnaire for people with MS.[13] However, the subjectivity of these tools carries the risk of error, therefore we decided to create an objective tool for the assessment of dysarthric disorders in MS patients in the form of an IT tool (software with elements of machine learning) based on the examination of respiratory and phonatory disorders, which aims to diagnose and track the dynamics of dysarthric changes.

Aim

The aim of the paper is to analyse the case of mixed dysarthria in a 55-year-old female patient with a secondary progressive type of multiple sclerosis. We attempt to present the severity of the patient's dysarthric disorder in comparison with the control group of 24 healthy individuals based on the output data obtained by means of an IT device examining respiratory and phonatory disorders.

Description of the case

Presentation

A 55-year-old female patient was admitted to the Clinic of Neurology with the Stroke Unit due to a worsening condition in the course of MS (Fig. 1). She was diagnosed with relapsing remitting MS in 2015. In 2015-2020 she was treated with immunomodulation as part of drug programs (initially INFb, then from 2019 with dimethyl fumarate). In 02.2021, due to the transition of the disease to a secondary progressive form, the patient began treatment with Mitoxantrone, which was discontinued after the 4th dose in 08.2021.

On neurological examination the patient was conscious, in logical verbal contact, presenting dysarthric speech, dysphagia, shallow right nasolabial fold, spastic paresis of the right upper limb of medium degree, exaggerated reflexes in both upper limbs, tremor of the left upper limb, significant spastic paresis of the right lower limb, exaggerated deep reflexes in both lower limbs, bilateral Babinski sign, painful paraesthesia in the right half of the body, positive Romberg test with eyes open. She could pass several meters with bilateral support, a walker (the Expanded Disability Status Scale EDSS-6.5).

On speech examination the patient communicated verbally and performed suggested verbal tasks. Aphasia was not observed. She also denied experiencing problems of such type. The examination revealed mixed dysarthric disorders, chanted speech, slightly hoarse voice with increased muscle tension. The motility of the articulation apparatus was slightly reduced with preserved praxis of the articulation organs. Facial muscle tone was slightly reduced on the right side with the soft palate evenly toned. In terms of phonetics irregularities were observed in spontaneous speech including rare elisions of fricatives, especially the final ones, and irregular and rare substitution of fricatives with plosives.

During her stay at the hospital ward the patient was assessed with an objective tool for examining respiratory and phonatory disorders (Figure 1).

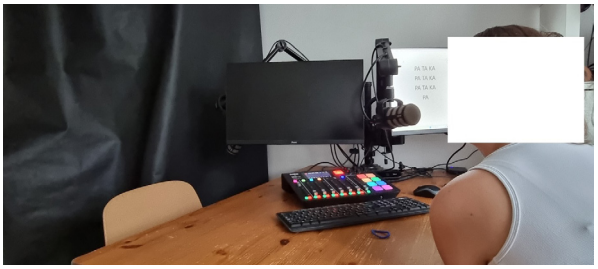

Fig. 1. Taking patient's voice sample

Methods

The results were then compared with those of 24 persons from the control group matched in terms of sex and age. The characteristics of the control group are presented in Table 1.

Table 1. Baseline characteristics of the control group

	Control group (n=24)
Age [years], mean	41.25
Sex [female/male]	12/12
Age < 40 years	12
Age > 40 years	12

The assessment involved reading 3 suggested texts in Polish:

1. A poem: 16 lines 10 syllables each – a poem in verse 2 lines each;
2. A text in prose: including simple and complex sentences; containing all sounds of the Polish language, as well as phonetic phenomena indicating potential dysarthric disorders, for instance consonantal clus-

ters provoking phonetic mistakes, 4 sentences, 3 complex and 1 simple: 1st sentence – 24 words, 2nd sentence – 4 words, 3rd sentence- 21 words, 4th sentence – 23 words;

3. The diction exercise: required accurate (without phonetic mistakes) realization of 10 syllables in the fastest possible manner).
   PA TA KA PA TA KA PA TA KA PA

Following speech parameters were subject to analysis:

- Phonetics: whether there were errors in terms of phonetic realization, elisions of fricatives and their substitution with plosives, phonetic simplifications concerning the manner and place of articulation not attributable to articulatory inaccuracy;
- Number of syllables produced per breath during the realization of each text;
- Sound quality assessment with GRBAS (0-3) scale
- G (grade of hoarseness)
- R (rough- ness)
- B (breathiness)
- A (asthenic)
- S (strained)
- Intonation – changes in relation to falling intonation (applies to affirmative sentences);
- Performance time of particular texts – in contrast with the results obtained in the control group

All speech parameters shown in the manuscript were first analyzed by a specialist, and then the results of the examination were entered into the IT tool. For the purposes of machine learning, a scale of 0-5 has been introduced, where 0 means no speech disorders and 5 extreme disorders in relation to the results of healthy people.

Results

In the control group there were no disorders connected to an improper intonation or phonetic errors due to dysarthria. In terms of voice quality according to GRBAS scale the result was 0 (G0 R0 B0 A0 S0). The average performance time was: a text in prose 46.41s , a poem – 36.21s, a diction exercise – 1.61s. The average number of breaths needed for producing individual texts was: a text in prose – 7.5 , a poem – 8.21, a diction exercise – 1. The average number of syllables produced per breath: a text in prose – 29.4, a poem – 19.5, a diction exercise – 10.

Whereas, in the analysed case of the MS patient with dysarthric disorder presenting itself with improper intonation and phonetic errors the GRBAS scale result was 6 (G1 R2 B0 A0 S3). The average performance time was: 90.57s – a text in prose, 58.93s – a poem, 2.99s – a diction exercise. The average number of breaths needed for producing individual texts was: a text in prose – 24, a poem – 16, a diction exercise – 1. The average number of syllables produced per breath: a text in prose – 9.01, a poem – 10, a diction exercise – 10. Table 2 shows the results in comparison with the control group.

Table 2. Speech parameters compared between the MS patient and the control group

Control group				MS patient
Performance time (PT)				
Performance time in seconds	mean	maximum	minimum	**PT**
PROSE	46.41	66.016	32.89	90.57
POEM	36.21	43.63	28.33	58.93
DICTION EXERCISE	1.61	2.91	1.08	2.99
Average number of breaths needed for reading the text	mean	maximum	minimum	**Average number of breaths needed for reading the text**
PROSE	7.5	12	4	24
POEM	8.21	14	6	16
DICTION EXERCISE	1	1	1	1
Average number of syllables per breath:				
PROSE	29.4	90	21	9.01
POEM	19.5	60	20	10
DICTION EXERCISE	10	10	10	10
GRBAS SCALE				
grade of hoarseness		0		1
roughness		0		2
breathiness		0		0
aesthetic		0		0
strained		0		3
Number of phonetic errors				
POEM		0		0
PROSE		0		8

In addition to a much longer performance time than the average obtained in the control group, the patient has an ascending intonation, which indicates, among others, the increased utterance effort in each presented speech sample, as shown in Figure 2. All graphs present intonation during the execution of declarative sentences.

Discussion

The fact that the subject of dysarthric disorders in MS has so far been neglected became the main motivation for the study. Another reason was the lack of available, objective tools for the assessment of dysarthric disorders in the course of MS. Therefore, an IT tool was developed. It aims to diagnose and track the dynamics of dysarthric changes in, among others, MS patients. Consequently, the purpose of the study is to analyse the case of mixed dysarthria in a 55-year-old patient in the course of MS of the secondary progressive form, to present the severity of the dysarthric disorders in contrast with the control group, which consisted of 24 healthy people, matched in terms of sex and age, using an IT tool examining of respiratory and phonatory disorders.

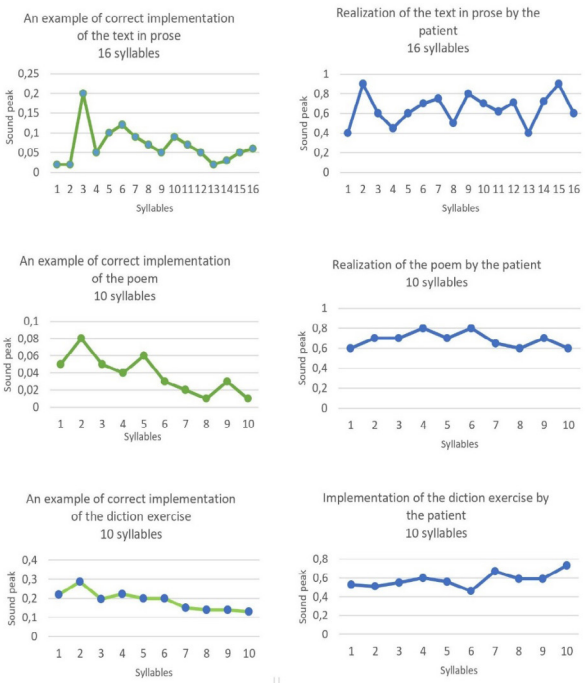

Fig. 2. Intonation graphs of texts fragments and diction exercises

On analysing individual speech parameters, it was shown that in the control group of healthy people assessed using the aforementioned IT tool, there were no disorders that could be associated with incorrect intonation and phonetic errors resulting from dysarthric disorders. Comparing these with the results of the MS patient, it was shown that not only was the performance time (reading a text in prose, a poem and the diction exercise) much longer than the average time in control group but also the average number of breaths needed to complete individual texts increased, except for diction exercise, where it was the same as in the control group (1 breath). The patient's effort and fatigue in the implementation of the above-mentioned texts was much larger. Her articulation and, above all, speech distribution abilities were definitely weaker than in the case of the control group.

The average number of syllables per breath proved to be significantly reduced, except for the diction exercise, where, as in the case of the average number of breaths, it was the same as in the control group, i.e. 10 syllables. This may be caused, among other things, by the lack of adequate amount of air necessary for speaking, increased effort, lack of proper respiratory and phonatory coordination and changes in muscle tension. In addition, the patient presented rising intonation, indicating, among others, the increased utterance effort in each presented speech sample. It is assumed that this is the result of excessive muscle tone during speech production.

It was not possible to find published sources that would allow a direct discussion between the results of this work. The studies by Rusz et al. and Rodgers et al. are an examination of speech rate and articulation rate. However, the measurement method differs due to a different unit of measurement (expressions/min, syllables/sec) as well as phonetic differences of the analysed languages.[14,15] Rodgers et al. studied the impact of cognitive functions and their relationship with speech and articulation rate in MS, which was also beyond our interest. Nevertheless, participants in those studies uttered syllables identical or similar to our diction exercise (/pa ta ka/ Rusz; /puh puh kuh/ Rodgers).[15] Moreover, in the work of Rusz et al. the longest realization of the /a/ sound per breath and reading an 80-word text were taken into account and analysed acoustically. The results of the study are consistent with ours, as the differences between the control group and MS patients are clearly visible. We share the conclusion regarding the encouragement of clinicians to pay special attention during therapy to coordination, including respiratory and phonatory coordination and its improvement, and not to increase muscle strength if the goal of therapy is to improve vocal/speech efficiency.[14]

In the work of Hartelius et.al. recordings of Australian and Swedish speakers were analysed. The subjects read the texts in their respective languages which were then subjectively cross-analysed by speech therapists from both countries. The study noted difficulties with unequivocal assessment of the depth of dysarthric disorders by listening to recordings by specialists – there was a lack of consistency in the assessment in many parameters.[16] Therefore, we believe that the assessment of dysarthria should primarily consider the objective features of speech. Hartelius et al. note the need for further interlinguistic work on the analysis of dysarthric disorders, which in our opinion is also interesting and important as it introduces a better understanding of such symptoms in the course of various neurological diseases.[16]

Though the results cannot be directly compared, the conclusions drawn from the cited works are consistent with our results. Sechidis et al. conducted an objective assessment of speech using machine learning modelling in patients with Parkinson's disease. The aim of their work was to find a correlation between speech and emotion. The researchers used the Mixture-of-Experts architecture for speech.[17] Primarily our studies differ in terms of the disease in question. We also do not study speech changes under the influence of emotions. In our work, we are looking for the most objective features of speech that will allow us to accurately determine the level of dysarthria and track the dynamics of changes to aid therapeutic process. For us, the acoustic parameters are an addition to the full picture of the disorder, while the main parameters are the performance time, the number of breaths needed to complete the text/phrase and the number of syllables spoken per breath. For the study,

we also used longer texts, which in our opinion increases the objectivity of the assessment. None of the cited works analysed the realization of a text in verse form.

Summing up the above considerations, it can be concluded that based on the analysed case the IT device is a promising tool for the diagnosis of dysarthric disorders, and the method of examination on which it is based is an innovative approach to the analysis of dysarthric speech disorders and can be the basis for a very thorough study of their changes also in the course of other neurological diseases.

Conclusion

The presented case involved speech disorders of the type of ataxic dysarthria in comparison to the control group of healthy people. The analysed case indicates that the developed objective IT tool, which aims to diagnose and track the dynamics of dysarthric changes in patients with MS, is a promising diagnostic method that can facilitate diagnosis and can be used in clinical practice. However, further studies on a larger group of MS patients are needed to draw far-reaching conclusions.

Declarations

Funding

This research received external funding.

The IT tool created in the course of the project run by BD Center Ltd.: Innovative IT tool aiding diagnostic process, prognosis and tracking of change dynamics in neurological patients – development work and its implementation' No RPPK.01.02.00-18-0004/20 co-financed by the European Regional Development Fund carried out within Regional Operational Programme of Podkarpackie Voivodeship 2014-2020 – Priority Axis 1: Competitive and Innovative Economy.

Author contributions

Conceptualization, W.A.W. and M.P.; Methodology, W.A.W.; Software, W.A.W. and M.P.; Validation, W.A.W. and M.P.; Formal Analysis, W.A.W., M.P. and A.G.; Investigation, W.A.W, M.P. and M.D.; Resources, W.A.W., M.P. and H.B.P.; Data Curation, W.A.W., H.B.P. and A.G.; Writing – Original Draft Preparation, W.A.W., M.P., M.D. and A.G ; Writing – Review & Editing, W.A.W., H.B.P. and A.G; Visualization, W.A.W; Supervision, W.A.W. and H.B.P.; Project Administration, W.A.W., M.P. and H.B.P.

Conflicts of interest

The authors declare no competing interests.

Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval

All subjects gave their informed consent for inclusion before they participated in the study. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Bioethical Committee at the University of Rzeszów (approval no. 3/01/2020).

References

1. Browne P, Chandraratna D, Angood C, et al. Atlas of Multiple Sclerosis 2013: A growing global problem with widespread inequity. *Neurology*. 2014;9;83(11):1022-1024. doi: 10.1212/WNL.0000000000000768
2. Morawska J, Niebudek-Bogusz E, Stasiołek M, Świderek-Matysiak M, Pietruszewska W. Speech Pathology-Specific Questionnaire for Persons with Multiple Sclerosis (SMS): adaptation, validation and preliminary assessment of the diagnostic potential. *Mult Scler Relat Disord*. 2021;49:102796. doi: 10.1016/j.msard.2021.102796
3. Wallin MT, Culpepper WJ, Campbell JD, et al. The prevalence of MS in the United States: A population-based estimate using health claims data. *Neurology.* 2019;5;92(10):e1029-e1040. doi: 10.1212/WNL.0000000000007035
4. Filippi M, Danesi R, Derfuss T, et al. Early and unrestricted access to high-efficacy disease-modifying therapies: a consensus to optimize benefits for people living with multiple sclerosis. *J Neurol*. 2022;269(3):1670-1677. doi: 10.1007/s00415-021-10836-8
5. De Biagi F, Heikkola LM, Nordio S, Ruhaak L. Update on Recent Developments in Communication and Swallowing in Multiple Sclerosis. *Int J MS Care*. 2020;22(6):270-275. doi: 10.7224/1537-2073.2020-023
6. Kappos L, Wolinsky JS, Giovannoni G, et al. Contribution of Relapse-Independent Progression vs Relapse-Associated Worsening to Overall Confirmed Disability Accumulation in Typical Relapsing Multiple Sclerosis in a Pooled Analysis of 2 Randomized Clinical Trials. *JAMA Neurol*. 2020;1;77(9):1132-1140. doi: 10.1001/jamaneurol.2020.1568
7. Fazeli M, Moradi N, Soltani M, et al. Dysphonia Characteristics and Vowel Impairment in Relation to Neurological Status in Patients with Multiple Sclerosis. *J Voice.* 2020;34(3):364-370. doi: 10.1016/j.jvoice.2018.09.018
8. Merson RM, Rolnick MI. Speech-language pathology and dysphagia in multiple sclerosis. *Phys Med Rehabil Clin N Am*. 1998;9(3):631-641.
9. Chiara T, Martin D, Sapienza C. Expiratory muscle strength training: speech production outcomes in patients with multiple sclerosis. *Neurorehabil Neural Repair*. 2007;21(3):239-249. doi: 10.1177/1545968306294737
10. El-Wahsh S, Heard R, Bogaardt H, Kumfor F, Ballard KJ. Variables Associated with Self-reported Language Impairment in Multiple Sclerosis: A Regression Analysis. *Int J MS Care.* 2021;23(2):85-92. doi: 10.7224/1537-2073.2020-096

11. De Pauw A, Dejaeger E, D'hooghe B, Carton H. Dysphagia in multiple sclerosis. *Clin Neurol Neurosurg.* 2002;104(4):345-351. doi: 10.1016/s0303-8467(02)00053-7
12. Fischer MT, Wimmer I, Höftberger R, Gerlach S, et al. Disease-specific molecular events in cortical multiple sclerosis lesions. *Brain*. 2013;136(Pt6):1799-17815. doi: 10.1093/brain/awt110
13. Guzy A. Voice emission diagnosis using a structured interview: practical implications. *Logopaedica Lodziensia*, 2019;(3):79-91. doi: 10.18778/2544-7238.03.06
14. Rusz, J, Benova, B, Ruzickova, H, et al. Characteristics of motor speech phenotypes in multiple sclerosis. *Mult Scler Relat Disord*. 2018;19:62-69. doi: 10.1016/j.msard.2017.11.007
15. Rodgers JD, Tjaden, K, Feenaughty L, Weinstock-Guttman B, Benedict RH. Influence of cognitive function on speech and articulation rate in multiple sclerosis. *J Int Neuropsychol Soc.* 2013;19:173-180. doi: 10.1017/S1355617712001166
16. Hartelius L, Theodoros D, Cahill L, Lillvik M. Comparability of perceptual analysis of speech characteristics in Australian and Swedish speakers with multiple sclerosis. *Folia Phoniatr Logop*. 2003;55(4):177-188. doi: 10.1159/000071017
17. Sechidis K, Fusaroli R, Orozco-Arroyave JR, Wolf D, Zhang YP. A machine learning perspective on the emotional content of Parkinsonian speech. *Artif Intell Med.* 2021;115:102061. doi: 10.1016/j.artmed.2021.102061

Wydawnictwo UR 2023
ISSN 2544-1361 (online)
doi: 10.15584/ejcem.2023.3.7

http://www.ejcem.ur.edu.pl
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2023; 21 (3): 654–658

CASUISTIC PAPER

Unilateral double maxillary paramolars in a non-syndromic child – a rare case report

Sonu Acharya [1], Bismay Singh [1], Alkananda Sahoo [2], Brinda Suhas Godhi [3]

[1] Department of Pediatric and Preventive Dentistry, Institute of Dental Sciences, SOA (Deemed to be University), Bhubaneswar, Odisha, India
[2] Department of Oral Pathology and Microbiology, Institute of Dental Sciences, SOA (Deemed to be University), Bhubaneswar, Odisha, India
[3] Department of Pediatric and Preventive Dentistry, JSS Dental College and Hospital, JSS Academy of Higher Education and Research, Mysuru, Karnataka, India

ABSTRACT

Introduction and aim. Extra teeth are the teeth which are more than the normal number of teeth in jaws. They can be present on a single side or both sides, singular or many, maxillary or mandibular. Paramolars are uncommon supernumerary molars which can be present on buccal or palatal side of molars. Very few reports of supernumerary teeth in maxilla or mandible being paramolars has been stated. No literature on unilateral twin paramolars in maxilla has been reported in literature yet. This is a first case report of unilateral twin paramolars in maxilla in a child.

Description of the case. This case report describes the presence of unilateral double maxillary paramolars and their handling in a thirteen-year-old boy, whose primary concern was that of severe pain in maxilla on both sides. After an identification of double maxillary paramolars was put forward and established by cone beam computer tomography, both the paramolars were extracted.

Conclusion. Supernumerary teeth have to be removed as soon as detected otherwise they can lead to malocclusion and pain due to caries after pulp involvement.

Keywords. children, double, maxillary, nonsyndromic, paramolar

Introduction

The most usual tooth defect in primary and secondary dentition is the variation in the number of teeth in excess of twenty in deciduous dentition and in excess of thirty two in permanent dentition.[1] Extra teeth or hyperdontia is the extra number of teeth present in either primary or permanent dentition. The extra teeth might be present on both sides or even in variables but usually occur on a single side.[2] Isolated supernumerary teeth occur in seventy six-eighty six percent of cases, dual supernumerary teeth in twelve-twenty three percent instances, and many superfluous teeth in less than one percent instances.[3] Multiple supernumerary teeth is rare in individuals without any disease or syndromic condition. The presence of multiple supernumerary teeth may be associated with various developmental disorders.[4] The universality of supernumeraries in secondary dentition ranges from 0.04 to 2.26% while in primary teeth it goes from 0.3 to 0.6%. In secondary teeth, the occurrence of extra teeth is two times as prevalent in men as in women.[5]

Corresponding author: Sonu Acharya, e-mail: sonu_ain@yahoo.com

Received: 27.03.2023 / Revised: 3.05.2023 / Accepted: 6.05.2023 / Published: 30.09.2023

Acharya S, Singh B, Sahoo A, Godhi BS. *Unilateral double maxillary paramolars in a non-syndromic child – a rare case report.* Eur J Clin Exp Med. 2023;21(3):654–658. doi: 10.15584/ejcem.2023.3.7.

Supernumerary teeth arise in the maxillary region rather than mandibular region. Most of the superfluous teeth are present in the pre maxilla area i.e. in the mid incisal area especially between the incisors (Table 1).[6] In rare cases, they can be located in the upper disto-molar zone, lower pre-molar, upper pre-molar, lower disto-molar, upper cuspid zone, and lower incisal region.[7,8]

Table 1. Frequency and prevalence of different types of supernumerary teeth [9]

	Mesiodens	Premolars	Distomolars	Paramolars	Laterals	Canines
Frequency (%)	47–67	8–9	26–27	15–16	2.05	0.40
Prevalence (%)	0.15–1.9	0.09–0.9	0.13–0.6	0.08–0.5	0.01–0.08	0.002–0.2

The likeliness of non-syndromic numerous superfluous teeth has been a rare entity. There have been reports of multiple supernumerary teeth which varies between 1 to 11% but mostly studies suggest that multiple supernumerary are less in percentage.[10] Additionally, the type of teeth, location, size, tooth series are very much variable. These are mostly seen in teenagers. Mostly multiple supernumerary teeth are present in both jaws. In these individuals, these are more often seen in incisor canine region but rarely in pre-molar molar region. The maximum of multiple extra teeth are present bilaterally. It is a rarity to find many extra teeth without any disease or syndromic condition though handful of cases have been cited.[11,12]

Aim

Here we discuss a case of unilateral twin paramolars in maxilla in a child of 13 years. This case report is the first to report such finding.

Description of the case

A thirteen years-old South-Asian male patient reported at the outpatient department of Pedodontics with a chief concern of severe pain in the top posterior teeth area on right side for one week. The child's medico-dental report was non involving with no concern of any systemic involvement or syndromic condition. The intra-oral clinical scrutiny disclosed a class one dental occlusion with thoroughly aligned teeth except the upper right second molar which was slightly palatably shifted. Apart from the full set of permanent teeth except third molars, there were two buccally placed extra teeth between the upper right first and second molar (Fig. 1).

These were diagnosed as paramolars because of the location of these supernumerary teeth. The mesial paramolar had deep dentinal caries. The mesial paramolar had two cusps but one cusp was destroyed due to caries. The distal paramolar was conical in morphology. The guardians were told about the existence of supernumerary teeth and was told to get complete X-ray check-up. The cone beam computed tomography (CBCT) radiograph further confirmed the presence and location of supernumerary (Fig 2).

Fig. 1. Pre-operative picture showing double paramolars

Fig. 2. CBCT showing two extra teeth adjacent to molar

The CBCT was done to rule out fusion of the second molar to the distal supernumerary and for better understanding of the tooth morphology. The parents were informed about the extra teeth and removal of paramolars. The possible occurrence of meals getting between teeth, pain, cavities in first and second molars and worsening of the adjacent periodontal well-being. The parents agreed for extraction of both the paramolars. The required blood tests were performed before extraction and both the paramolars were extracted (Fig. 3 and 4).

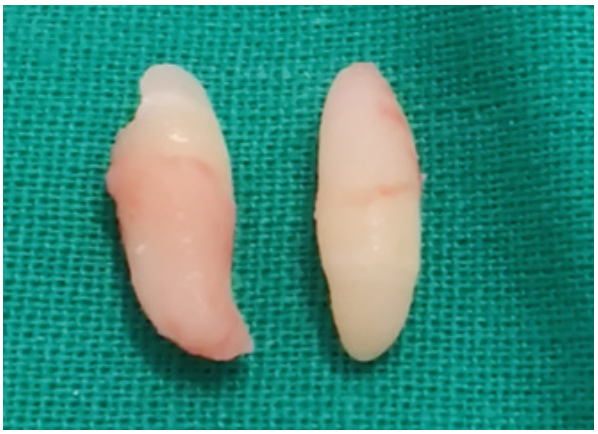

Fig. 3. Extracted paramolars

Fig. 4. Post extraction view

Before the procedure, a local anesthetic solution (2% Lignocaine with 1:100000 adrenalin) was given. A small incision was made to access the paramolars, then with the help of elevator (Coupland elevators) and extraction forceps these were removed. In some cases, the tooth may need to be sectioned and removed in pieces to make the procedure easier. Medications (analgesics) were provided for control of post-operative pain. Informed and written consent was taken by parent of the child for the procedure being carried out as well as for publishing the case report as and where required. The patient is being followed up. The healing was uneventful and the second molars were being followed up to be in place.

Discussion

The paramolars are relatively uncommon in occurrence. The correct cause of this dental anomaly is not yet known with certainty. Multiple ideas been put forward for their existence.[13]

A thorough literature review disclosed a few of cases cited of para-molars (Table 2). Para-molars are very less seen in maxillary arch, less often bilaterally, almost rare in deciduous dentition and till date only one case in primary dentition has come to light. They are often rudimental, most commonly situated in buccal region through the 2nd and 3rd molars, although a rarity of cases have been reported of paramolars being present between 1st and 2nd molars.[14-16] Joining of the para-molar with the respective normal tooth is a highly rarity.[17]

Table 2. Reported cases of paramolars[9]

Arch/side Maxilla	Researcher	Year	Study group	Location
Unilateral	Puri et al.	2013	Indian	Buccal placed between second and third molars
Unilateral	Nayak et al.	2012	Indian	Palatal placed between left first and second molars
Unilateral	Nagaveni et al	2010	Indian	Buccal placed between right first and second molars
Unilateral	Ghogre and Gurav	2014	Indian	Fused with the second molar
Unilateral	Venugopal et al	2013	Indian	Fused with the right second molar
Unilateral	Salem et al.	2010	Iran	Fused with the left second molar
Mandible	Rosa et al.	2010	Brazil	Fused with the right first molar
Mandible	Ballal et al.	2007	Indian	Fused with the second molar
Mandible	Ghoddusi et al.	2006	Iran	Fused with the left second molar
Mandible	Dubuk et al.	1996	Japanese	Mesial to the right second molar
Mandible	Kumasaka et al.	1988	Japanese	Two impacted paramolar on the right side

Supernumerary teeth might show normal eruption, can show impaction, inversion, or may be having an abnormality in the outgoing path. Extra teeth showing a normal way of erupting will show easy eruption. But, usually thirteen-thirty four percent of all secondary extra teeth erupt normal in comparison with seventy three percent of deciduous extra teeth.[18]

The various complications might be impediment or late eruption of related secondary teeth; deranged erupting nearby teeth; migration, or turning of nearby teeth; crowding because of space problems; mal-occlusion because of a decrease of area; spaces between molars; trauma to cheek mucosal membrane when buccal para-molars cause tears; interference when undergoing orthodontic treatment; abnormality in root growth of erupting secondary tooth; cystic creation; excess new growth; tri-geminal neural pain due to the para-molar compresses ion of the trigeminal neural tissue, pulpal degeneration, and root absorption of nearby teeth because of force exertion; tooth decay because of bacterial retention in non-cleansing regions; and gingivitis and local periodontal inflammation in the tissues.[19,20] As reported here in our report, the existence of para-molars lead to dental caries due to food/bacteria deposition and showed inflamed adjacent periodontium. The second molar is palatably placed because of these paramolars in our case.

The care of individuals with para-molar generally is associated with the area involved and on the effect on surrounding teeth and cardinal relevant structure enti-

ties. Management variations for para-molar just like any other extra teeth may be either to observe or extract. Observation means no treatment but regular monitoring of the individual with clinical and radiographical features. This should be done if the para-molar is asymptomatic. Although if complications occur, it is deemed necessary to remove the para-molar.[21,22] In this report, removal of the decayed para-molar as well as the normal paramolar was done to relieve pain and provide great per-oral cleanliness maintenance.

The case report here is the only case of double paramolars reported in maxilla that too on same side. Paramolars have been reported in maxilla bilaterally as well as unilateral double paramolars in the mandible but no case report has been published on double paramolars on same side in maxilla.[23] There is no case reported as such in children yet. The paramolars were extracted in this case to prevent further complications. The treatment protocol described to surgically remove the supernumerary teeth after advising a CBCT. The CBCT clearly identified the supernumerary as being two different entities and also clearly delineated the teeth to be not fused to the permanent molar.

Conclusion

The clinician should have a good knowledge on supernumerary teeth and the consequences the patient might face due to these teeth. Adequate investigations have to be made to diagnose these cases. Once a diagnosis is made the most appropriate treatment has to be rendered to the patient.

Declarations

Funding

No funds were received.

Author contributions

Conceptualization, S.A. and B.S.; Methodology, S.A.; Software, A.S.; Resources, B.S.G.; Data Curation, X.X.; Writing – Original Draft Preparation, S.A., B.S., A.S. and B.S.G.; Writing – Review & Editing, S.A., B.S., A.S. and B.S.G.

Conflicts of interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

Data availability

The datasets used and/or analyzed during the current study are open from the corresponding author on reasonable request.

Ethics approval

All subjects gave their informed consent for inclusion before they participated in the study.

References

1. Omer RS, Anthonappa RP, King NM. Determination of the optimum time for surgical removal of unerupted anterior supernumerary teeth. *Pediatr Dent.* 2010;32(1):14-20.
2. Zhu JF, Marcushamer M, King DL, Henry RJ. Supernumerary and congenitally absent teeth: a literature review. *J Clin Pediatr Dent.* 1996;20(2):87-95.
3. LecoBerrocal MI, Martín Morales JF, Martínez González JM. An observational study of the frequency of supernumerary teeth in a population of 2000 patients. *Med Oral Patol Oral Cir Bucal.* 2007;12(2):134-138.
4. Hattab FN, Yassin OM, Rawashdeh MA. Supernumerary teeth: Report of three cases and review of the literature. *ASDC J Dent Child.* 1994;61:382-393. doi: 10.1155/2012/745265
5. Bodin I, Julin P, Thomsson M. Hyperodontia. I. Frequency and distribution of supernumerary teeth among 21,609 patients. *Dentomaxillofac Radiol.* 1978;7(1):15-17. doi: 10.1259/dmfr.1978.0002
6. Dhull KS, Acharya S, Ray P, Yadav S, Prabhakaran SD. Bilateral maxillary paramolars: a case report. *J Dent Children.* 2012;79(2):84-87. doi: 10.5005/jp-journals-10005-1231
7. Dubuk AN, Selvig KA, Tellefsen G, Wikesjö UM. Atypically located paramolar. Report of a rare case. *Eur J Oral Sci.* 1996;104(2):138-140. doi: 10.1111/j.1600-0722.1996.tb00058.x
8. Anthonappa RP, King NM, Rabie AB. Aetiology of supernumerary teeth: a literature review. *Eur Arch Paediatr Dent.* 2013;14(5):279-288. doi: 10.1007/s40368-013-0082-z
9. Mahto RK, Dixit S, Kafle D, Agarwal A, Bornstein M, Dulal S. Nonsyndromic Bilateral Posterior Maxillary Supernumerary Teeth: A Report of Two Cases and Review. *Case Rep Dent.* 2018;2018:5014179. doi: 10.1155/2018/5014179
10. Sulabha AN, Sameer C. Unusual Bilateral Paramolars Associated with Clinical Complications. *Case Rep Dent.* 2015;2015:851765. doi:10.1155/2015/851765
11. Von Arx T. Anterior maxillary supernumerary teeth: A clinical and radiographic study. *Aus Dent J.* 1992;37:189-195. doi: 10.1111/j.1834-7819.1992.tb00741.x
12. Batra P, Duggal R, Parkash H. Non-syndromic multiple supernumerary teeth transmitted as an autosomal dominant trait. *J Oral Pathol Med.* 2005;34:621-625. doi: 10.1111/j.1600-0714.2005.00271.x
13. Parolia A, Kundabala M. Bilateral maxillary paramolars and endodontic therapy: a rare case report. *J Dent (Tehran).* 2010;7(2):107-111.
14. Nagaveni NB, Umashankara KV, Radhika NB, Reddy PB, Manjunath S. Maxillary paramolar: report of a case and literature review. *Arch Orafac Sci.* 2010;5:24-28.
15. Liu DG, Zhang WL, Zhang ZY, Wu YT, Ma XC. Three-dimensional evaluations of supernumerary teeth using cone-beam computed tomography for 487 cases. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.* 2007;103(3):403-411. doi: 10.1016/j.tripleo.2006.03.026

16. Proff P, Fanghänel J, Allegrini S Jr, Bayerlein T, Gedrange T. Problems of supernumerary teeth, hyperdontia or dentes supernumerarii. *Ann Anat.* 2006;188(2):163-169. doi: 10.1016/j.aanat.2005.10.005
17. Ghoddusi J, Zarei M, Jafarzadeh H. Endodontic treatment of a supernumerary tooth fused to a mandibular second molar: a case report. *J Oral Sci.* 2006;48(1):39-41. doi: 10.2334/josnusd.48.39
18. Liu JF. Characteristics of premaxillary supernumerary teeth: a survey of 112 cases. *ASDC J Dent Child.* 1995;62(4):262-265.
19. Palikaraki G, Vardas E, Mitsea A. Two Rare Cases of Non--Syndromic Paramolars with Family Occurrence and a Review of Literature. *Dent J.* 2019;7(2):38. doi: 10.3390/dj7020038
20. Ata-Ali F, Ata-Ali J, Peñarrocha-Oltra D, Peñarrocha-Diago M. Prevalence, etiology, diagnosis, treatment and complications of supernumerary teeth. *J Clin Exp Dent.* 2014;6(4):e414-e418. doi: 10.4317/jced.51499.
21. Subasioglu A, Savas S, Kucukyilmaz E, Kesim S, Yagci A, Dundar M. Genetic background of supernumerary teeth. *Eur J Dent.* 2015;9(1):153-158. doi: 10.4103/1305-7456.149670
22. Díaz A, Orozco J, Fonseca M. Multiple hyperodontia: report of a case with 17 supernumerary teeth with non syndromic association. *Med Oral Patol Oral Cir Bucal.* 2009;14(5):E229-E231.
23. Kumasaka S, Hideshima K, Shinji H, Higasa R, Kubota M, Uchimura N. A case of two impacted paramolar in lower right molar dentition. *Kanagawa Shigaku.* 1988;23(3):417-423.

Wydawnictwo UR 2023
ISSN 2544-1361 (online)
doi: 10.15584/ejcem.2023.3.8

http://www.ejcem.ur.edu.pl
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2023; 21 (3): 659–662

CASUISTIC PAPER

A patient with overlap syndrome: systemic lupus erythematosus, dermatomyositis, and Sjögren's syndrome – a rare overlapping diseases case report

Aldona Sokołowska [1], Mateusz Iwański [1], Piotr Dąbrowski [2]

[1] Student Scientific Association at the Department of Human Immunology, Institute of Medical Sciences, Medical College of Rzeszow University, University of Rzeszow, Rzeszow, Poland
[2] Department of Rheumatology, Institute of Medical Sciences, Medical College of Rzeszow University, University of Rzeszow, Rzeszow, Poland

ABSTRACT

Introduction and aim. Autoimmune rheumatic diseases are a group of disorders with similar clinical, laboratory and immunological manifestations. Connective tissue diseases include systemic scleroderma, dermatomyositis or polymyositis, Sjögren's syndrome, rheumatoid arthritis, and systemic lupus erythematosus. If the patient meets the diagnostic criteria for at least two of these diseases and has specific serologic markers, a diagnosis of overlap syndrome is possible.

Description of the case. This case describes a 27-year-old man who had a history of paroxysmal fever, night sweats, erythema-like skin lesions on the forearms and lower legs, a feeling of progressive muscle weakness especially in the proximal muscles, and dry mouth. The patient was diagnosed with an overlap syndrome: systemic lupus erythematosus, dermatomyositis, and Sjögren's syndrome.

Conclusion. Overlap syndrome is difficult to treat due to its multisystem nature, requiring a symptomatic therapeutic approach and careful control of medication doses to reduce side effects while controlling disease activity.

Keywords. autoimmune diseases, overlap syndrome, self-reactive

Introduction

Overlap syndrome is an inflammatory rheumatic disease in which the patient has clinical features of various autoimmune rheumatic diseases.[1-3] Mixed connective tissue disease is a rare autoimmune disease characterized by the presence of anti-RNP antibodies.[4,5] The most common diseases included in overlap syndrome are rheumatoid arthritis, systemic lupus erythematosus, systemic scleroderma, dermatomyositis, polymyositis, and Sjögren's syndrome.[6-8] Overlap syndrome can be diagnosed if the patient meets at least both diagnostic criteria and has specific serologic markers.[1,3] The pathogenesis is based on the development of an excessive, antigen-driven, self-reactive immune response that is a consequence of genetic predisposition and environmental factors.[2]

Systemic lupus erythematosus (SLE) is a complex, chronic autoimmune disease with variable clinical manifestations associated with the presence of multiple autoantibodies, such as anti-dsDNA and anti-Sm, which cause the deposition of immune complexes.[9-11]

Sjögren's syndrome is a chronic inflammatory autoimmune disease of unknown etiology with ANA> 1:80 and anti-Ro and anti-La antibodies. The disease specif-

Corresponding author: Aldona Sokołowska, e-mail: aldonasokolowskaa@gmail.com

Received: 7.08.2022 / Revised: 30.04.2023 / Accepted: 11.05.2023 / Published: 30.09.2023

Sokołowska A, Iwański M, Dąbrowski P. *A patient with overlap syndrome: systemic lupus erythematosus, dermatomyositis, and Sjögren's syndrome – a rare overlapping diseases case report.* Eur J Clin Exp Med. 2023;21(3):659–662. doi: 10.15584/ejcem.2023.3.8.

ically affects the lacrimal and salivary glands with lymphocyte infiltration resulting in impaired function.[12,13]

Dermatomyositis is a rare idiopathic inflammatory myopathy accompanied by dermatitis. Autoimmune mechanisms play a major role in the pathogenesis of dermatomyositis, among which anti-Jo-1, anti-SRP and anti-Mi-2 autoantibodies play an important role.[14,15]

Aim

The aim of this study was to present this unique disease entity with typical clinical features, detected in a 27-year-old man.

Description of the case

A 27-year-old Caucasian man, he reported paroxysmal fever, night sweats, erythema-like skin lesions on the forearms and lower legs, a feeling of progressive muscle weakness, especially proximal muscles, and dry mouth, which was confirmed by a physical examination. The appearance of these ailments was associated with the consumption of large amounts of alcohol during sertalinum treatment.

During the patient's stay in August 2020 on the ward, the alanine aminotransferase level was monitored, which was initially 138 U/L (norm: 10–49) and eventually decreased to 76 U/L (norm: 10–49), the creatine kinase level was initially 822 U/L (norm: 46–171) and then decreased to 84 U/L (norm: 46–171), gamma glutamyltranspeptidase value was 209 U/L (norm: <73), immunoglobulin G 20.33 g/L (norm: 6.59–16.00), interleukin-67 (IL-67) 16 pg/mL (norm: <3.4), lactate dehydrogenase 521 U/L (norm: 120–246). Laboratory investigations revealed antibody titer of 1: 5120, granular and nuclear staining of 1: 10240, ANA 3 present in high titer of RNP, Sm, SSA and SSB as well as dsDNA, decreased levels of complement components C3 and C4, leukopenia and lymphopenia, mild anemia, thrombocytopenia, liver damage and hypergammaglobulinemia. The parotid glands are heterogeneous, with reduced echogenicity, with the presence of small hypoechogenic areas up to 2-3 mm in diameter. Under general anesthesia, a section of the mucosa of the lower lip along with the salivary gland was taken for histopathological examination.

The patient was diagnosed with overlap syndrome: systemic lupus erythematosus, dermatomyositis and Sjögren's syndrome in August 2020. Intravenous methylprednisolone was administered, followed by oral steroids, and cyclosporine when the transaminase levels decreased. The treatment resulted in improvement of the patient's general condition and improvement of the myopathy symptoms. The treatment included pulses of methylprednisolone, followed by continued systemic intravenous steroid therapy, and cyclosporine was added after transaminase levels decreased. After the treatment, the patient felt better and the symptoms of myopathy resolved. On follow-up, leukopenia and thrombocytopenia persisted, responding poorly to the previous treatment. After hematological consultation trepanobiopsy was suggested, but the patient did not agree. After improvement of the patient's condition, he was discharged home with recommendations to follow a liver diet, follow-up examinations were ordered and the patient was declared unfit for work. In treatment, he received 6 pulses of methylprednisolone at 500 mg, then oral prednisone at a dose of 60 mg/d and cyclosporine at a dose of 200 mg/d (body weight 88.7 kg).

On September 25, 2020, he was re-admitted to assess the tolerance and effectiveness of treatment, the concentration of cyclosporine was determined (58.6 ng/ml) and due to non-therapeutic levels (> 80), the dose of cyclosporine was increased to 250 mg/day, the dose of prednisolone was reduced to 40 mg/day.

In March 2021, the patient was hospitalized due to the intensification of skin lesions such as erythema of the neckline, face, arms and hands. Additionally, significant leukopenia was found – WBC $2*10^3$/μl (norm: $4*10^3$–$10*10^3$), and vitamin B12 deficiency was found – 189 pg/ml (norm: 211–911). The patient was given 5 infusions of methylprednisolone, 125 mg each, and injections of vit. B12 (3 x 1000j each). The level of white blood cells was normalized: WBC $7.14*10^3/mm^3$ and skin changes disappeared. Still in treatment: prednisolone 20 mg/d with slow reduction and cyclosporine 250 mg/d.

The patient was hospitalized in 2022 for parenchymal liver injury. The patient developed hypertension as a result of taking steroid medication. On admission, the patient reported no significant complaints, no signs of muscle weakness, and skin lesions on the hands were still present on physical examination. Chronic myopathic changes were found in the right quadriceps muscle. Recordings from the rectus muscle of the right anterior and tibial right thigh demonstrating electrical silence at rest in the muscles. There is no abnormality in the conduction parameters of the examined nerves. Laboratory tests of alanine aminotransferase were initially 263 U/L, and after two weeks the level dropped to 136 U/L, creatine kinase value was 96 U/L, rheumatolophrenic factor in IgM caliber was 29.4 IU/ml (norm: ≥ 20), gamma glutamyltranspeptidase was initially 204 U/L, and after two weeks decreased to 105 U/L.

Laboratory tests performed showed leukopenia, no thrombocytopenia, hypertransaminasemia with slightly elevated cholestasis parameters. Autoimmune tests revealed the presence of anti-nuclear granular fluorescence antibodies 1: 320 and cytoplasmic fluorescence antibodies 1: 640, in ANA 3 highly positive RNP, SS-A, Sm, Ro-52, ribosomal protein P, complement components normal. After gastrointestinal consultation, it was

recommended to consider liver biopsy due to the triple digit transaminase values found in 2020.

In 2022, leukopenia with lymphopenia appeared again (WBC 2.5*10^3/µl, Lymph 17%), which disappeared after infusions of methylprednisolone (from 1 to 3 a 250 mg) and immunoglobulins – 80 g/month – 2 infusions of 40 g, every 4 weeks 6 times, starting from March 2022, a total of 480 g.

In April 2022 there was an attempt to include Mycophenolate Mofetil, but it ended with a 3-fold increase in transaminases (administration stopped). Regular monthly supplementation of vitamin B12 (a 1000 units) was ordered.

With such a therapeutic regimen, the patient's development to date was characterized by marked clinical improvement, which allowed him to significantly improve his functional capacity. The patient was classified as unfit for work. The patient was discharged home with recommendations to follow a liver diet, photoprotection, and to take medications such as prednisone, vitamin B12, proton pump inhibitor, and bisoprolol. A visit to the rheumatology department was scheduled to evaluate disease activity and to continue immunoglobulin treatment.

Table 1. Comparison of patient outcomes in disease exacerbation and in remission after intravenous administration of methylprednisolone

	Exacerbation of disease 2021	Remission of disease 2021 after infusions of methylprednisolone
WBC (4–10*10^3/µl)	2.0	7.14
	Exacerbation of disease 2022	**Remission of disease 2022 after infusions of methylprednisolone**
WBC (4–10*10^3/µl)	2.5	3.7

Discussion

Overlap syndromes are very rare and affect people with systemic lupus erythematosus who also have features of another rheumatologic condition, including rheumatoid arthritis, Sjögren's syndrome, vasculitis, and/or myositis.[1-3] Identifying patients with overlapping syndromes is important as these patients may require different monitoring and treatment regimens.[1,2] Clinically, as seen in this case, the most characteristic symptomatology is the presence of erythema-like skin lesions on the forearms and lower legs, a feeling of progressive muscle weakness, especially proximal muscle weakness, dry mouth and night sweats. Due to the overlap of the abovementioned diseases, the patient was treated with steroids and immunoglobulin infusions.

The treatment of the overlap syndrome depends on the symptoms predominant in the clinical picture and involves the treatment of the diseases that are part of it. The results of the study by Balbir-Gurman et al. indicate a relatively high incidence of scleroderma overlap syndrome with Sjgogren's syndrome or myositis, and the overlap of scleroderma with SLE is quite rare, the frequent use of steroids, cyclophosphamide and DMARDs, as well as IVIG in patients with overlapping scleroderma is observed.[3] Ramya et al. described a case of a patient with overlapping symptoms of SLE, systemic scleroderma, and secondary Sjögren's syndrome who received immunosuppressive and corticosteroid therapy.[16] Recently, biologic medications have been used in refractory cases of overlap syndrome, but the ill effect of such medications is the high likelihood of disease exacerbation in these patients.[16-20] Itikyala et al. described a syndrome of overlap between systemic lupus erythematosus and vasculitis associated with a cytoplasmic anti-neutrophil antibody; the patient is doing well after treatment with rituximab, but this entity should be recognized and requires appropriate treatment.[10] On the other hand, we describe a rare case of overlap between three diseases: SLE, dermatomyositis and Sjögren's syndrome, which are currently well controlled by treatment with corticosteroids and immunoglobulin infusions, while controlling the patient's general condition.

Conclusion

Due to the multisystem nature of the overlap syndrome, it is difficult to treat. Clarification of each patient's condition can lead to improved patient care.

Declarations

Funding

This research received no external funding.

Author contributions

Conceptualization, A.S., M.I., and P.D.; Methodology, P.D.; Validation, A.S., and M.I.; Formal Analysis, A.S., M.I., and P.D.; Investigation, A.S., and M.I.; Writing – Original Draft Preparation, A.S.; Writing – Review & Editing, A.S., and M.I.; Visualization, A.S.; Supervision, P.D.; Project Administration, P.D. All authors have read and agreed to the published version of the manuscript.

Conflicts of interest

The authors declare no conflict of interest.

Data availability

The data sets used and/or analysed during the current study are available from the corresponding author upon reasonable request.

Ethics approval

Consent for publication was obtained from participant.

References

1. Parente J, Mathurdas P, Wandschneider L, Aranha J, Siopa L. Síndroma de overlap [Overlap syndrome]. *Acta Med Port.* 2011;24(S3):719-724.

2. Jury EC, D'Cruz D, Morrow WJW. Autoantibodies and overlap syndromes in autoimmune rheumatic disease. *J Clin Pathol.* 2001;54:340-347. doi: 10.1136/jcp.54.5.340
3. Balbir-Gurman A, Braun-Moscovici Y. Scleroderma overlap syndrome. *Isr Med Assoc J*. 2011;13(1):14-20.
4. Gunnarsson R, Hetlevik SO, Lilleby V, Molberg Ø. Mixed connective tissue disease. *Best Pract Res Clin Rheumatol*. 2016;30(1):95-111. doi: 10.1016/j.berh.2016.03.002
5. Pepmueller PH. Undifferentiated Connective Tissue Disease, Mixed Connective Tissue Disease, and Overlap Syndromes in Rheumatology. *Mo Med*. 2016;113(2):136-140.
6. Sapkota B, Al Khalili Y. Mixed Connective Tissue Disease. In: *StatPearls*. Treasure Island (FL): StatPearls Publishing; 2022.
7. Abdelgalil Ali Ahmed S, Adam Essa ME, Ahmed AF, et al. Incidence and Clinical Pattern of Mixed Connective Tissue Disease in Sudanese Patients at Omdurman Military Hospital: Hospital-Based Study. *Open Access Rheumatol*. 2021;13:333-341. doi: 10.2147/OARRR.S335206
8. Alves MR, Isenberg DA. "Mixed connective tissue disease": a condition in search of an identity. *Clin Exp Med*. 2020;20(2):159-166. doi: 10.1007/s10238-020-00606-7
9. Justiz Vaillant AA, Goyal A, Varacallo M. Systemic Lupus Erythematosus. In: *StatPearls*. Treasure Island (FL): StatPearls Publishing; 2023.
10. Itikyala S, Pattanaik D, Raza S. Systemic Lupus Erythematosus (SLE) and Antineutrophil Cytoplasmic Antibody-Associated Vasculitis (AAV) Overlap Syndrome: Case Report and Review of the Literature. *Case Rep Rheumatol.* 2019;2019:5013904. doi: 10.1155/2019/5013904
11. Basta F, Fasola F, Triantafyllias K, Schwarting A. Systemic Lupus Erythematosus (SLE) Therapy: The Old and the New. *Rheumatol Ther*. 2020;7(3):433-446. doi: 10.1007/s40744-020-00212-9
12. Thorne I, Sutcliffe N. Sjögren's syndrome. *Br J Hosp Med (Lond)*. 2017;78(8):438-442. doi: 10.12968/hmed.2017.78.8.438
13. Stefanski AL, Tomiak C, Pleyer U, Dietrich T, Burmester GR, Dörner T. The Diagnosis and Treatment of Sjögren's Syndrome. *Dtsch Arztebl Int*. 2017;114(20):354-361. doi: 10.3238/arztebl.2017.0354
14. Volc-Platzer B. Dermatomyositis - update [Dermatomyositis-update]. *Hautarzt*. 2015;66(8):604-610. doi: 10.1007/s00105-015-3659-0
15. DeWane ME, Waldman R, Lu J. Dermatomyositis: Clinical features and pathogenesis. *J Am Acad Dermatol*. 2020;82(2):267-281. doi: 10.1016/j.jaad.2019.06.1309
16. Ramya R, Swarnalakshmi R, Preethi A, Pradeep R. A case of progressive systemic sclerosis/lupus overlap syndrome: Presenting with parotid swelling. *J Oral Maxillofac Pathol*. 2021;25(2):372. doi: 10.4103/0973-029X.325259
17. Jantarat A, Muangchan C. Epidemiology and clinical characteristics of systemic sclerosis overlap syndrome (SSc--OS), and the factors significantly associated with SSc-OS in Thai patients with systemic sclerosis. *Mod Rheumatol*. 2022;32(5):899-907. doi: 10.1093/mr/roab079
18. Pope JE. Scleroderma overlap syndromes. *Curr Opin Rheumatol*. 2002;14(6):704-710. doi: 10.1097/00002281-200211000-00013
19. Iaccarino L, Gatto M, Bettio S, et al. Overlap connective tissue disease syndromes. *Autoimmun Rev*. 2013;12(3):363-373. doi: 10.1016/j.autrev.2012.06.004
20. Pakozdi A, Nihtyanova S, Moinzadeh P, Ong VH, Black CM, Denton CP. Clinical and serological hallmarks of systemic sclerosis overlap syndromes. *J Rheumatol*. 2011;38(11):2406-2409. doi: 10.3899/jrheum.101248

Wydawnictwo UR 2023
ISSN 2544-1361 (online)
doi: 10.15584/ejcem.2023.3.17

http://www.ejcem.ur.edu.pl
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2023; 21 (3): 663–668

CASUISTIC PAPER

Management challenges and therapeutic strategies for metastatic melanoma – a case report

Andrea M. Aglio [1], Salvatore Cracchiolo [1], Giuseppe Impellizzeri [1], Michał Górecki [2]

[1] Department of Biomedicine, Neuroscience and Advanced Diagnostics, University of Palermo, Palermo, Italy
[2] Department of Clinical Oncology, University Clinical Hospital, Rzeszów, Poland

ABSTRACT

Introduction and aim. This case report focuses on a 26-year-old female with metastatic melanoma. It highlights the diagnostic process, initial immunotherapy, disease progression, and successful response to second-line therapy. Emphasizing the importance of early detection, personalized treatment, and adaptive strategies, it provides valuable insights into managing this aggressive form of skin cancer.

Description of the case. A 26-year-old Caucasian female presented with a suspicious pigmented lesion on her thigh in 2013. The lesion was confirmed as superficial skin melanoma. No lymph node biopsy was performed. In 2021, she had abdominal pain and imaging revealed melanoma metastasis in the peritoneum, lungs and brain. Genetic testing showed BRAF V600E mutation and PD-L1 expression in tumor cells. She received immunotherapy and radiation for a central nervous system metastases but developed a brain hematoma. Follow-up imaging showed disease progression. She started second-line therapy with iBRAF/iMEK, and her condition rapidly improved with regression of metastatic lesions. Follow-up imaging confirmed significant positive changes and almost complete regression of neoplastic lesions. She continues to receive the targeted therapy and shows a positive response.

Conclusion. Early diagnosis improves outcomes in metastatic melanoma. Peritoneal metastases should be considered in patients with abdominal symptoms. The combination of gamma knife radiosurgery with immunotherapy or targeted therapy shows promise for managing brain metastases, but careful patient selection and monitoring are vital due to potential risks. Treatment responses in advanced melanoma vary, with this case highlighting a favorable response to BRAF/MEK inhibitor therapy in a patient with a BRAF gene mutation. Further research and clinical trials are needed to refine treatment approaches and improve outcomes in metastatic melanoma.

Keywords. immunotherapy, metastatic melanoma, peritoneal metastases

The list of abbreviations:

CNS – central nervous system, **CT** – computed tomography, **GKRS** – gamma-knife radiosurgery, **ICI** – immune checkpoint inhibitor, **IT** – immune therapy, **MMM** – metastatic malignant melanoma, **MM** – metastatic melanoma, **MRI** – magnetic resonance imaging, **PET** – positron emission tomography, **PM** – peritoneal metastases/metastasis, **SRS** – stereotactic radiosurgery, **SSM** – superficial skin melanoma, **TT** – targeted therapy

Introduction

Metastatic melanoma (MM) is an aggressive form of skin cancer associated with a high mortality rate.[1] Early de-

Corresponding author: Andrea M. Aglio, e-mail: aglio.andream@gmail.com

Received: 20.05.2023 / Revised: 9.06.2023 / Accepted: 14.06.2023 / Published: 30.09.2023

Aglio AM, Cracchiolo S, Impellizzeri G, Górecki M. *Management challenges and therapeutic strategies for metastatic melanoma – a case report.* Eur J Clin Exp Med. 2023;21(3):663–668. doi: 10.15584/ejcem.2023.3.17.

tection and prompt intervention play a crucial role in improving patient outcomes. However, the progression of melanoma to metastatic disease poses significant challenges in treatment and management.[2] In this case report, we present the case of a 26-year-old Caucasian female patient who initially presented with superficial skin melanoma and subsequently developed metastatic nodules in the peritoneal cavity, lungs, and central nervous system (CNS). We discuss the diagnostic findings, first-line immunotherapy with Nivolumab and Ipilimumab, subsequent disease progression, and the successful response to therapy with Encorafenib and Binimetinib.

Aim

The aim of this case report is to highlight the diagnosis and management of MM in a young female patient. The report focuses on the initial diagnosis of superficial skin melanoma, followed by the detection of metastatic nodules in the peritoneal cavity and CNS, eight years after the initial excision. The report also describes the patient's treatment journey, including first-line treatment with Nivolumab and Ipilimumab, which was ineffective in controlling the disease, and the subsequent successful use of second-line therapy with BRAF/MEK inhibitors Encorafenib and Binimetinib. This case report aims to provide insight into the use of various treatment options for MM and to emphasize the importance of early diagnosis and timely treatment for this aggressive form of cancer.

Description of the case

A 26-year-old Caucasian female patient initially presented with a suspicious pigmented lesion on the medial portion of her right thigh in 2013. The lesion measured approximately 8 mm, and an excisional biopsy was performed. The biopsy results confirmed the presence of superficial skin melanoma (SSM) with a Breslow thickness of 2 mm and no ulceration. The staging of the melanoma was classified as pT2a, according to the 7th Edition of the TNM classification. Following the biopsy result wide excision was performed. At that time, no sentinel lymph node biopsy was performed. Previously described steps took place in a hospital different than the current.

In July 2021, the patient was admitted to the surgical ward due to pain in the right iliac fossa, initially suggestive of acute appendicitis. An ultrasound of the abdominal cavity was conducted, revealing a hypoechoic area along the ascending colon and a hypoechoic lymph node measuring 18x10 mm. During the surgical procedure, soft tissue nodules associated with the peritoneal lymphatic network were discovered, while the appendix appeared normal. Histopathological examination of the peritoneal nodules confirmed melanoma metastasis.

Further diagnostic investigations were carried out to assess the extent of the disease. CT, PET-CT, and MRI of the head were performed, revealing multiple metastatic nodules ranging in size from 2 to 8 mm in the lungs' parenchyma. Additionally, neoplastic implants measuring up to 24 mm in diameter were observed in the peritoneal cavity. A metastatic focus in the left temporal lobe of the CNS, measuring 10 mm, was also confirmed. Genetic testing of the histopathological sample revealed the presence of BRAF V600E mutation, indicating sensitivity to BRAF kinase inhibitors. The testing also identified PD-L1 antigen expression in approximately 1–49% of tumor cells (TPS about 5%).

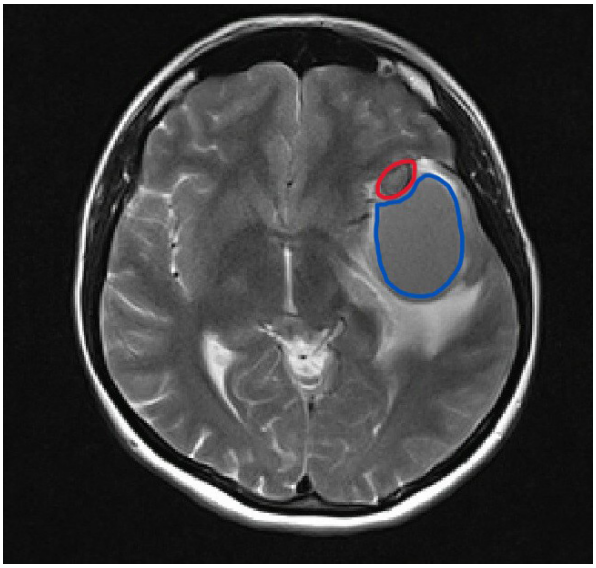

Fig. 1. Hematoma (blue circle) and metastatic lesion (red circle) following GKRS

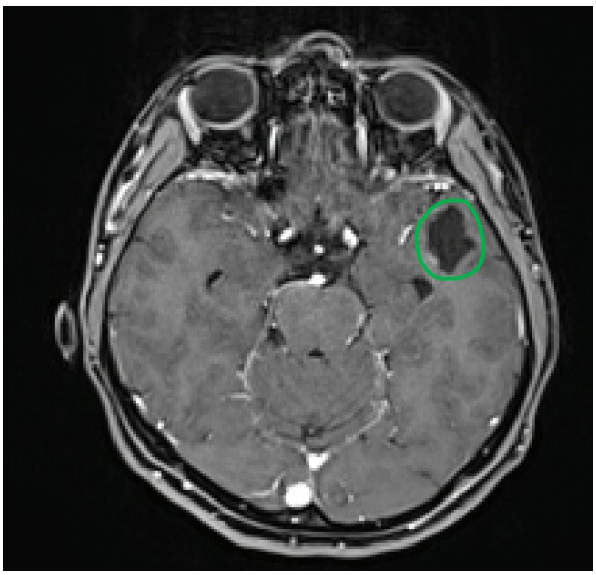

Fig. 2. Shrunken hematoma (green circle)

First-line treatment with Nivolumab (1 mg/kg) and Ipilimumab (3 mg/kg) immunotherapy was initiated on September 10th, 2021. In addition to immunotherapy, Gamma Knife stereotactic radiotherapy was adminis-

tered to the metastatic lesion in the left temporal lobe, delivering a radiation dose of 20 Gy in a single fraction. Following CNS irradiation, the patient experienced headaches accompanied by nausea and vomiting. An MRI of the head revealed a 46 x 33 mm hematoma at the site of the irradiated metastatic focus (Fig. 1). Conservative treatment, including a 2-week course of steroid therapy with Dexamethasone (6 mg/die for week 1, and 1 mg/die for week 2), was initiated, leading to the resolution of neurological symptoms and shrinkage of the hematoma (Fig. 2).

After completing four courses of immunotherapy, follow-up imaging showed disease progression according to RECIST 1.1 criteria, correlated with a rapid deterioration of the patient's clinical condition. Notably, the ineffectiveness of immunotherapy was evident in the abdominal cavity, with the presence of larger and more numerous metastatic nodules in the peritoneum (Fig. 3). The presacral region exhibited the largest focus measuring 90 mm. The metastatic lesions in the lungs remained similar in size and number compared to the baseline CT scan performed before the initiation of immunotherapy. Gastrointestinal symptoms, including abdominal pain, painful constipation, and intestinal transit disorders, developed due to the pressure of tumor implants on the intestines.

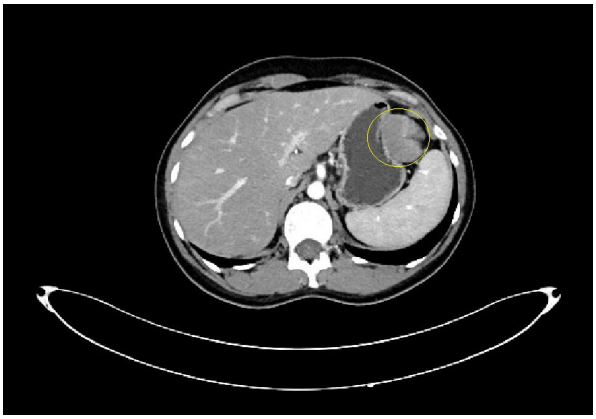

Fig. 3. Peritoneal metastases following four courses of Ipilimumab and Nivolumab

As a result of disease progression, the patient was urgently qualified for second-line therapy with BRAF/MEK inhibitors. Specifically, she started treatment with Encorafenib (450 mg) and Binimetinib (45 mg). Shortly after commencing this targeted therapy, the patient's clinical condition rapidly improved, and all gastrointestinal complaints subsided. After a month of treatment, the patient returned to a normal performance status (ECOG-0) and was able to resume her work activities.

On March 3rd, 2022, CT scans of the chest, abdomen, and pelvis, both before and after intravenous contrast administration, were performed to assess the treatment response. The imaging findings revealed several significant positive changes. The metastatic nodules in the lungs' parenchyma had completely disappeared. The pleural cavities and mediastinal organs appeared free from any pathological findings. The pathological masses surrounding the uterus had regressed, and there was almost complete regression of the observed peritoneal tissue masses (Fig. 4). The tissue densities in the gallbladder and integuments had also regressed. Importantly, there were no signs of pathological destruction in the bones. The follow-up imaging tests conducted after three months of therapy showed almost complete regression of all neoplastic lesions, consistent with RECIST 1.1 criteria.

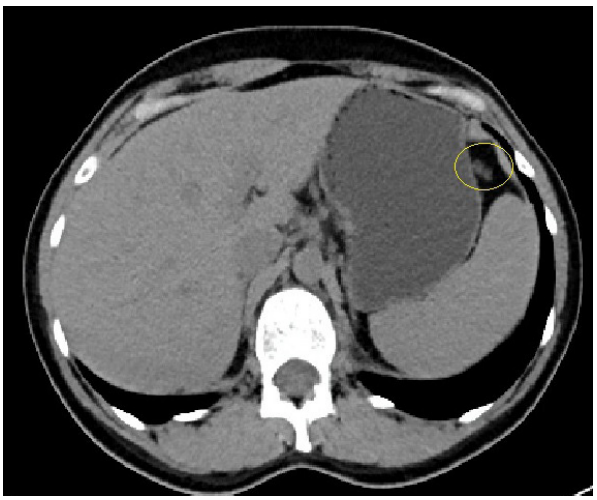

Fig. 4. Peritoneal metastases regression following three months of Encorafenib and Binimetinib therapy.

Throughout the course of treatment with Encorafenib and Binimetinib, no serious adverse events were recorded. The patient tolerated the therapy well, with the only noticeable consequence being mild alopecia

To date (May 2023), the patient continues to receive treatment with Encorafenib and Binimetinib, maintaining a highly positive therapeutic effect and experiencing minimal treatment-related toxicity.

Discussion

Metastatic malignant melanoma (MMM) is a highly aggressive form of skin cancer characterized by the spread of melanoma cells to other parts of the body beyond the skin. Historically, the prognosis for patients with metastatic cutaneous melanoma has been poor. However, recent advancements in cancer therapeutics, particularly the introduction of immune checkpoint inhibitors (ICIs) and small-molecule targeted drugs, have significantly improved patient outcomes, revolutionizing the field of melanoma therapeutic management.[1]

ICIs, such as Ipilimumab and Nivolumab, have demonstrated remarkable success in various cancer types, particularly melanoma. Nevertheless, a significant proportion of patients exhibit resistance to these therapies, often due to intrinsic factors. In the case presented here, the patient's peritoneal metastases (PM) displayed high resistance to immunotherapeutic treatment, raising questions about the mechanisms of resistance specific to PM in melanoma.

While PM typically originates from abdominal primary cancers, cases of PM secondary to MMM are relatively uncommon, and the true incidence remains unknown. Despite the limited number of reported cases in the literature, it has been observed that melanoma ranks as the third most common extra-abdominal cancer to metastasize to the peritoneum. This highlights the importance of recognizing and understanding PM as a potential manifestation of MM.[2]

Although existing literature on resistance to ICIs in PM primarily focuses on gastric cancer and colorectal cancer, it is plausible to consider that some of the resistance mechanisms observed in those metastatic cancers may also apply to MM. A study conducted by Küçükköse et al. utilizing a humanized mice model found that peritoneal metastases derived from colorectal cancer with high microsatellite instability exhibited insensitivity to ICIs. Furthermore, the presence of elevated levels of immunosuppressive cytokines in ascitic fluid, observed in the experimental model, offers a potential explanation for the refractory nature of peritoneal metastases to ICIs.[3]

Further investigation into the mechanisms underlying resistance in PM secondary to MM is warranted. Understanding the unique characteristics and factors contributing to therapeutic resistance in this specific context could potentially guide the development of novel treatment strategies to overcome resistance and improve patient outcomes. Additionally, exploring the potential role of microsatellite instability in MM may provide valuable insights into immunotherapy response and the resistance mechanisms involved.[4]

Concurrent treatment with stereotactic radiosurgery (SRS) and targeted therapy (TT) or immunotherapy (IT) in the management of brain metastases has been an area with limited safety data. However, Gamma Knife radiosurgery (GKRS) has emerged as a valuable modality for delivering a high dose of radiation to the lesion while minimizing radiation exposure to the surrounding normal brain parenchyma, resulting in high tumor control rates.

In this case, although the patient developed a hemorrhage following GKRS, it is important to note that several studies have reported no significantly increased risk associated with concurrent immunotherapeutic treatments.[5–8] The occurrence of hemorrhage or radiation reaction/necrosis after GKRS did not show any statistically significant differences in relation to IT/TT.[9]

Furthermore, patients treated with anti-PD-1, anti-CTLA-4, or a combination of anti-CTLA-4/PD-1 demonstrated a significantly longer time to new brain metastasis after GKRS compared to patients treated with other forms and combinations of oncological therapy. This finding highlights the potential synergistic effects of immunotherapeutic agents and GKRS in preventing the development of new brain metastases.[9]

The existing literature emphasize the importance of considering GKRS as a viable treatment option in the management of brain metastases in patients receiving immunotherapy or targeted therapy for malignant melanoma.[10] The combination of GKRS and immunotherapeutic agents has the potential to enhance treatment outcomes by controlling both local disease and systemic progression.

However, it is essential to recognize the potential risks associated with GKRS, such as hemorrhage, and weigh them against the potential benefits in each individual case. Close monitoring and appropriate patient selection are crucial to ensure the safety and efficacy of this treatment approach.

In the management of advanced melanoma with a mutation in the BRAF gene, two different treatment modalities exist as options: targeted therapy utilizing BRAF and MEK inhibitors, and immunotherapy, which involves checkpoint inhibition.[11]

The combination of BRAF and MEK inhibitors has emerged as the standard of care in the first-line treatment of patients with unresectable or metastatic BRAF-mutated melanoma. This therapy has shown promising results, with response rates ranging from 68% to 76%, median progression-free survival of 11–15 months, and a 3-year overall survival rate of approximately 40%.[12–16]

A case report by Stagno et al. highlighted the effectiveness of this combination therapy in two patients. One patient, heavily pretreated, achieved a partial response lasting 36 months with local treatment for oligoprogression disease. The second patient had a partial response lasting 10 months. However, the report also described a third patient with multisite visceral disease and high serum levels of lactate dehydrogenase, who experienced a short-lived clinical benefit followed by rapid disease progression. The fourth patient currently on treatment with BRAF/MEK inhibitors showed clinical benefit and radiological stable disease for over 3 months.[17]

Furthermore, the 5-year update of part 1 of the COLUMBUS trial further supports the use of combination treatment with BRAF and MEK inhibitors for advanced BRAF V600-mutant melanoma. This update demonstrated benefits in terms of progression-free survival and overall survival, reaffirming the role of this combination therapy as a standard of care.[18]

In this case, the patient demonstrated a more favorable response to BRAF/MEK inhibitors therapy compared to anti-CTLA-4 and anti-PD-1 immunotherapy. This finding is noteworthy and further shows the efficacy of such a therapy. Nevertheless, a study published by Van Breeschoten et al. reported that patients with MM treated with anti-PD-1 monotherapy as the first-line treatment exhibited a higher 2-year survival rate compared to those treated with first-line BRAF/MEK inhibitors. The median overall survival (OS) in the anti-PD-1 monotherapy cohort was 42.3 months, while patients receiving BRAF/MEK inhibitors as the first-line treatment had a median OS of 19.8 months.[19]

It is important to acknowledge that the selection of first-line therapy for advanced melanoma should be based on a comprehensive evaluation of available clinical evidence and, consideration of potential side effects and long-term benefits.

Conclusion

Early diagnosis plays a crucial role in improving patient outcomes for MM. As demonstrated in this case, the initial detection and excision of a superficial skin melanoma (SSM) in the patient provided an opportunity for intervention. However, despite the initial excision, the disease progressed underscoring the aggressive nature of melanoma and the importance of vigilant follow-up and surveillance.

PM as a manifestation of MM is relatively uncommon, but recognizing its potential occurrence is vital. This case report highlights the need to consider PM as a possibility in patients with MM, particularly in those presenting with abdominal symptoms. Further research is required to understand the unique characteristics and mechanisms of resistance specific to PM, as this knowledge can inform the development of effective treatment strategies tailored to this clinical context.

The combination of GKRS with immunotherapy or targeted therapy shows promise in managing brain metastases in patients with malignant melanoma. However, it is important to note that the occurrence of a hemorrhage following GKRS highlights the need for careful patient selection and monitoring.

Furthermore, this case emphasizes the diversity of treatment responses seen in advanced melanoma. Although immunotherapy using anti-CTLA-4 and anti-PD-1 agents has shown remarkable efficacy in different types of cancer, including melanoma, the standard of care for patients with BRAF V600 mutations remains the use of BRAF and MEK inhibitors. It is crucial to conduct further research and clinical trials to enhance our knowledge of treatment outcomes in this patient population.

In summary, this case report shows the multifaceted nature of MMM management, including the importance of early diagnosis, the challenges of PM, the potential benefits and risks of combining GKRS with immunotherapy or targeted therapy for brain metastases, and the variability of treatment responses. It emphasizes the need for further research, patient-specific considerations, and ongoing clinical trials to refine treatment approaches and improve outcomes in the complex landscape of MM. By continuing to advance our understanding of this aggressive form of cancer, we can work towards personalized and effective treatment strategies that maximize patient survival and quality of life.

Declarations

Funding

This research did not receive any specific grant from funding agencies.

Author contributions

Conceptualization, A.M.A., S.C., G.I. and M.G.; Methodology, A.M.A., S.C. and G.I.; Software, A.M.A., S.C. and G.I.; Validation, A.M.A., S.C., G.I. and M.G.; Formal Analysis, A.M.A., S.C. and G.I.; Investigation, A.M.A., S.C. and G.I.; Resources, A.M.A., S.C. and G.I.; Data Curation, A.M.A., S.C., G.I. and M.G.; Writing – Original Draft Preparation, A.M.A., S.C. and G.I.; Writing – Review & Editing, A.M.A., S.C. and G.I.; Visualization, A.M.A., S.C. and G.I.; Supervision, A.M.A. and M.G.; Project Administration, A.M.A. and M.G.

Conflicts of interest

The authors declare no competing interests.

Data availability

Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

Ethics approval

Informed consent for publication was obtained from the patient. We complied with the policy of the journal on ethical consent.

References

1. Pasquali S, Hadjinicolaou AV, Chiarion Sileni V, Rossi CR, Mocellin S. Systemic treatments for metastatic cutaneous melanoma. *Cochrane Database Syst Rev*. 2018;2018(2):CD011123. doi: 10.1002/14651858.CD011123.pub2
2. Flanagan M, Solon J, Chang KH, et al. Peritoneal metastases from extra-abdominal cancer – A population-based study. *Eur J Surg Oncol*. 2018;44(11):1811-1817. doi: 10.1016/j.ejso.2018.07.049
3. Heesters BA, Villaudy J, Verheem A, et al. Modeling resistance of colorectal peritoneal metastases to immune checkpoint blockade in humanized mice. *J Immunother Cancer*. 2022;10(12):e005345. doi: 10.1136/jitc-2022-005345

4. Kubecek O, Trojanova P, Molnarova V, Kopecky J. Microsatellite instability as a predictive factor for immunotherapy in malignant melanoma. *Med Hypotheses*. 2016;93:74-76. doi: 10.1016/j.mehy.2016.05.023
5. Ahmed KA, Abuodeh YA, Echevarria MI, et al. Clinical outcomes of melanoma brain metastases treated with stereotactic radiosurgery and anti-PD-1 therapy, anti-CTLA-4 therapy, BRAF/MEK inhibitors, BRAF inhibitor, or conventional chemotherapy. *Ann Oncol Off J Eur Soc Med Oncol*. 2016;27(12):2288-2294. doi: 10.1093/annonc/mdw417
6. Silk AW, Bassetti MF, West BT, Tsien CI, Lao CD. Ipilimumab and radiation therapy for melanoma brain metastases. *Cancer Med*. 2013;2(6):899-906. doi: 10.1002/cam4.140
7. Patel KR, Shoukat S, Oliver DE, et al. Ipilimumab and Stereotactic Radiosurgery Versus Stereotactic Radiosurgery Alone for Newly Diagnosed Melanoma Brain Metastases. *Am J Clin Oncol*. 2017;40(5):444-450. doi: 10.1097/COC.0000000000000199
8. Diao K, Bian SX, Routman DM, et al. Stereotactic radiosurgery and ipilimumab for patients with melanoma brain metastases: clinical outcomes and toxicity. *J Neurooncol*. 2018;139(2):421-429. doi: 10.1007/s11060-018-2880-y
9. Gatterbauer B, Hirschmann D, Eberherr N, et al. Toxicity and efficacy of Gamma Knife radiosurgery for brain metastases in melanoma patients treated with immunotherapy or targeted therapy—A retrospective cohort study. *Cancer Med*. 2020;9(11):4026-4036. doi: 10.1002/cam4.3021
10. Mathieu D, Kondziolka D, Cooper PB, et al. Gamma knife radiosurgery in the management of malignant melanoma brain metastases. *Neurosurgery*. 2007;60(3):471. doi: 10.1227/01.NEU.0000255342.10780.52
11. Ahmed M, Abdullah HM, Ali M, et al. Effectiveness of immunotherapy versus BRAF/MEK inhibitors in treatment-naïve BRAF-mutant advanced melanoma: A single institution retrospective analysis. *J Clin Oncol*. 2022;40(16):e21515-e21515. doi: 10.1200/JCO.2022.40.16_suppl.e21515
12. Robert C, Grob JJ, Stroyakovskiy D, et al. Five-Year Outcomes with Dabrafenib plus Trametinib in Metastatic Melanoma. *N Engl J Med*. 2019;381(7):626-636. doi: 10.1056/NEJMoa1904059
13. Dreno B, Ascierto PA, McArthur GA, et al. Efficacy and safety of cobimetinib (C) combined with vemurafenib (V) in patients (pts) with BRAFV600 mutation–positive metastatic melanoma: analysis from the 4-year extended follow-up of the phase 3 coBRIM study. *J Clin Oncol*. 2018;36(15):9522-9522. doi: 10.1200/JCO.2018.36.15_suppl.9522
14. Dummer R, Ascierto PA, Gogas HJ, et al. Encorafenib plus binimetinib versus vemurafenib or encorafenib in patients with BRAF-mutant melanoma (COLUMBUS): a multicentre, open-label, randomised phase 3 trial. *Lancet Oncol*. 2018;19(5):603-615. doi: 10.1016/S1470-2045(18)30142-6
15. Liszkay G, Gogas H, Mandalà M, et al. Update on overall survival in COLUMBUS: A randomized phase III trial of encorafenib (ENCO) plus binimetinib (BINI) versus vemurafenib (VEM) or ENCO in patients with BRAF V600–mutant melanoma. *J Clin Oncol*. 2019;37(15):9512-9512. doi: 10.1200/JCO.2019.37.15_suppl.9512
16. Ugurel S, Röhmel J, Ascierto PA, et al. Survival of patients with advanced metastatic melanoma: The impact of MAP kinase pathway inhibition and immune checkpoint inhibition - Update 2019. *Eur J Cancer Oxf Engl 1990*. 2020;130:126-138. doi: 10.1016/j.ejca.2020.02.021
17. Stagno A, Vari S, Annovazzi A, et al. Case Report: Rechallenge With BRAF and MEK Inhibitors in Metastatic Melanoma: A Further Therapeutic Option in Salvage Setting? *Front Oncol*. 2021;11:645008. doi: 10.3389/fonc.2021.645008
18. Dummer R, Flaherty KT, Robert C, et al. COLUMBUS 5-Year Update: A Randomized, Open-Label, Phase III Trial of Encorafenib Plus Binimetinib Versus Vemurafenib or Encorafenib in Patients With BRAF V600–Mutant Melanoma. *J Clin Oncol*. 2022;40(36):4178-4188. doi: 10.1200/JCO.21.02659
19. Van Breeschoten J, Wouters MWJM, Hilarius DL, et al. First-line BRAF/MEK inhibitors versus anti-PD-1 monotherapy in BRAFV600-mutant advanced melanoma patients: a propensity-matched survival analysis. *Br J Cancer*. 2021;124(7):1222-1230. doi: 10.1038/s41416-020-01229-1

Wydawnictwo UR 2023
ISSN 2544-1361 (online)

http://www.ejcem.ur.edu.pl
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2023; 21 (3): 669–676

Instructions for Authors

About the Journal

The European Journal of Clinical and Experimental Medicine (Eur J Clin Exp Med) is an open access journal, and all articles are free to access, download, share, and re-use. The Eur J Clin Exp Med is a peer-reviewed, international scientific journal that publishes full-length articles on topics within medical science. The journal welcomes submissions of articles on current advances in life and health sciences, clinical and experimental medicine, and related disciplines.

Open access and creative commons

All articles are published with **free** open access under the CC-BY Creative Commons attribution license (the current version is CC-BY, version 4.0. If you submit your paper for publication by the Eur J Clin Exp Med, you agree to have the CC-BY license applied to your work. Under this Open Access license, you, as the author, agree that anyone may download and read the paper for free. In addition, the article may be reused and quoted provided that the original published version is cited. This facilitates freedom in re-use and also ensures that Eur J Clin Exp Med content can be mined without barriers for the research needs.

Article processing charges

The Eur J Clin Exp Med is an open access journal and does **not** levy an article processing charge. There are no submission, color, or page charges for all article types.

Copyright Statement

Authors of articles published in the Eur J Clin Exp Med retain copyright on their articles, except for any third-party images and other materials added by the Eur J Clin Exp Med which are subject to copyright of their respective owners. Authors are therefore free to disseminate and re-publish their articles, subject to any requirements of third-party copyright owners and subject to the original publication being fully cited. Visitors may also download and forward articles subject to the citation requirements. The ability to copy, download, forward or otherwise distribute any materials is always subject to any copyright notices displayed. Copyright notices must be displayed prominently and may not be obliterated, deleted or hidden, totally or partially.

Ethics in publishing

The Eur J Clin Exp Med takes responsibility of enforcing rigorous peer-review together with strict ethical policies and standards to ensure to add high quality scientific works to the field of scholarly publication. Unfortunately, cases of plagiarism, data falsification, inappropriate authorship credit, and the like, do arise. The Eur J Clin Exp Med takes such publishing ethics issues very seriously and our editors are trained to proceed in such cases with a zero tolerance policy. The Eur J Clin Exp Med is a member of and subscribes to the principles of the Committee on Publication Ethics (COPE).

Editorial and submission policies

When you submit a manuscript to the Eur J Clin Exp Med we will take it to imply that the manuscript has not already been published or submitted elsewhere. If similar or related work has been published or submitted elsewhere, then you must provide a copy of this work with the submitted manuscript. You may not submit your manuscript elsewhere while it is under consideration in the Eur J Clin Exp Med. If the manuscript includes personal communications, please provide a written statement of permission from any person who is quoted. Permission by email is acceptable.

We reserve the right to reject a paper even after it has been accepted if it becomes apparent that there are serious problems with its scientific content, or our publishing policies have been violated.

Author responsibilities

Authorship provides credit for a researcher's contributions to a study and carries accountability. Authors are expected to fulfil the criteria below (adapted from McNutt et al., Proceedings of the National Academy of Sciences, 2018, 201715374; DOI: 10.1073/pnas.1715374115):

— **Each author** is expected to have made substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data; or the creation of new software used in the work; or have drafted the work or substantively revised it
— **AND** to have approved the submitted version (and any substantially modified version that involves the author's contribution to the study);
— **AND** to have agreed both to be personally accountable for the author's own contributions and to ensure that questions related to the accuracy or integrity of any part of the work, even ones in which the author was not personally involved, are appropriately investigated, resolved, and the resolution documented in the literature.

The Eur J Clin Exp Med does not require all authors of a research paper to sign the cover letter upon submission, nor do they impose an order on the list of authors. Submission to the Eur J Clin Exp Med is taken by the publication to mean that all the listed authors have agreed to all of the contents. The corresponding (submitting) author is responsible for having ensured that this agreement has been reached, and for managing all communication between the publication and all co-authors, before and after publication.

Author contributions statements

Authors are required to include a statement of responsibility in the manuscript (at the end of the main text, before the 'References' section) that specifies the contribution of every author. For articles with several authors, a short paragraph specifying their individual contributions must be provided. The following statements should be used "Conceptualization, X.X. and Y.Y.; Methodology, X.X.; Software, X.X.; Validation, X.X., Y.Y. and Z.Z.; Formal Analysis, X.X.; Investigation, X.X.; Resources, X.X.; Data Curation, X.X.; Writing – Original Draft Preparation, X.X.; Writing – Review & Editing, X.X.; Visualization, X.X.; Supervision, X.X.; Project Administration, X.X.; Funding Acquisition, Y.Y.".

Corresponding author – responsibilities

The corresponding (submitting) author is solely responsible for communicating with the Eur J Clin Exp Med and for managing communication between co-authors. Before submission, the corresponding author ensures that all authors are included in the author list, its order has been agreed by all authors, and that all authors are aware that the paper was submitted.

A confidential process

The Eur J Clin Exp Med treats the submitted manuscript and all communication with authors and referees as confidential. Authors must also treat communication with the Eur J Clin Exp Med as confidential: correspondence with the Eur J Clin Exp Med, referee reports and other confidential material must not be posted on any website or otherwise publicized without prior permission from the Eur J Clin Exp Med publishing team, regardless of whether or not the submission is eventually published. Our policies about posting preprints and post prints, and about previous communication of the work at conferences or as part of a personal blog or of an academic thesis, are described in the Confidentiality section.

Referee suggestions

During the submission process, please suggest three potential reviewers (names and institutional e-mail addresses) with the appropriate expertise to review the manuscript, but please keep in mind that we are not obliged to follow these recommendations. The proposed referees should neither be current collaborators of the co-authors nor have published with any of the co-authors of the manuscript within the last five years. Proposed reviewers should be from different institutions to the authors. You may suggest reviewers from among the authors that you frequently cite in your paper. You may also name a limited number of scientists who should not review your paper (up to 3 named individuals or laboratories); these exclusions will be honored. The decision of the Editorial Board Member on the choice of referees is final.

Ethics, use of experimental animals, and human participants

For articles in the Eur J Clin Exp Med reporting experiments on live vertebrates and/or higher invertebrates, the methods section must include a statement: (i) identifying the institutional and/or licensing committee approving the experiments, including any relevant details; (ii) confirming that all experiments were performed in accordance with relevant guidelines and regulations.

For research involving human participants, authors must identify the committee that approved the research, confirm that all research was performed in accordance with relevant guidelines/regulations, and include in their manuscript a statement confirming that informed consent was obtained from all participants and/or their legal guardians.

Authors may be required to submit, on request, a statement from the research ethics committee or institutional review board indicating approval of the research.

Competing interests policy

In the interests of transparency and to help readers to form their own judgements of potential bias, authors must declare any competing financial and/or non-financial interests in relation to the work described. For the purposes of this policy, competing interests are defined as financial and non-financial interests that could directly undermine, or be perceived to undermine, the objectivity, integrity and value of a publication, through a potential influence on the judgements and actions of authors with regard to objective data presentation, analysis and interpretation. Examples of potential competing interests include employment, consultancies, stock ownership, honoraria, paid expert testimony, patent applications/registrations, and grants or other funding.

Competing interests statement format guidelines

The statement included in the article file must be explicit and unambiguous, describing any potential competing interest (or lack thereof) for EACH contributing author.

Examples of declarations are:

— Competing interests: The author(s) declare no competing interests.
— Competing interests: Dr X's work has been funded by A. He has received compensation as a member of the scientific advisory board of B and owns stock in the company. He also has consulted for C and received compensation. Dr Y and Dr Z declare no potential conflict of interest.
— Competing interests: "This work was supported by the [Funding Agency] under Grant [number]."

Peer-reviewers

The Eur J Clin Exp Med invites peer-reviewers to exclude themselves in cases where there is a significant conflict of interest, financial or otherwise. However, just as financial interests need not invalidate the conclusions of an article, nor do they automatically disqualify an individual from evaluating it. We ask peer-reviewers to inform the editors of any related interests, including financial interests as defined above that might be perceived as relevant. Editors will consider these statements when weighing peer-reviewers' recommendations.

Availability of materials and data

In order to maintain the integrity, transparency and reproducibility of research records, authors are encouraged to make their experimental and research data openly available either by depositing into data repositories or by publishing the data and files as supplementary information in this journal.

Data may be deposited with specialized service providers or institutional/subject repositories, preferably those that use the DataCite mechanism. Large data sets and files greater than 60 MB must be deposited in this way. For a list of other repositories specialized in scientific and experimental data, please consult databib.org or re3data.org. The data repository name, link to the data set (URL) and accession number, doi or handle number of the data set must be provided in the paper. The journal Data also accepts submissions of data set papers.

Data availability statement format guidelines

The statement should be provided as a separate section (titled 'Data Availability') at the end of the main text, before the 'References' section. Data availability statements should include, where applicable, accession codes, other unique identifiers and associated web links for publicly available datasets, and any conditions for access of non-publicly available datasets. Where figure source data are provided, statements confirming this should be included in data availability statements. Depending on the data described in the manuscript, data availability statements commonly take one of the following forms, or can be a composite of the statements below:

— The datasets generated during and/or analyzed during the current study are available in the [NAME] repository, [PERSISTENT WEB LINK TO DATASETS].
— The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.
— All data generated or analyzed during this study are included in this published article (and its Supplementary Information files).
— The datasets generated during and/or analyzed during the current study are not publicly available due to [REASON(S) WHY DATA ARE NOT PUBLIC] but are available from the corresponding author on reasonable request.
— No datasets were generated or analyzed during the current study.
— The data that support the findings of this study are available from [THIRD PARTY NAME] but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. Data are however available from the authors upon reasonable request and with permission of [THIRD PARTY NAME].

Correction and retraction policy

The Eur J Clin Exp Med operates the following policy for making corrections to its peer-reviewed content.

Publishable amendments must be represented by a formal online notice because they affect the publication record and/or the scientific accuracy of published information. Where these amendments concern

peer-reviewed material, they fall into one of four categories: Publisher Correction (formerly Erratum), Author Correction (formerly Corrigendum), Retraction or Addendum.

Publisher Correction (formerly Erratum). Notification of an important error made by the journal that affects the publication record or the scientific integrity of the paper or the reputation of the authors or the journal.

Author Correction (formerly Corrigendum). Notification of an important error made by the author(s) that affects the publication record or the scientific integrity of the paper, or the reputation of the authors or the journal.

Retraction. Notification of invalid results. All co-authors must sign a Retraction specifying the error and stating briefly how the conclusions are affected, and submit it for publication. In cases where co-authors disagree, the in-house editors may seek advice from independent referees and impose the type of amendment that seems most appropriate, noting the dissenting author(s) in the text of the published version.

Addendum. Notification of additional information. Addenda are published when the in-house editors decide that the addendum is crucial to the reader's understanding of a significant part of the published contribution.

Peer-review process

Initial checks

Once submitted, your manuscript will be assigned to a member of our Editorial Board, who will read the paper and decide whether it is appropriate for the journal. Manuscripts that are within scope and seem, on initial assessment, to be technically sound and scientifically valid, will be sent to external reviewers. Copies of any papers containing similar or related work under consideration or in press at other journals must be included with the submission.

Manuscripts that do not fit the journal's ethics policy or do not meet the standards of the journal will be rejected before peer-review. Manuscripts that are not properly prepared will be returned to the authors for revision and resubmission.

Peer review

Once a manuscript passes the initial checks, it will be assigned to at least two independent experts for peer-review. Reviewers will be able to access your manuscript securely using our online system, whilst maintaining referee anonymity. A double-blind review is applied, where authors' identities are unknown to reviewers and vice versa. Peer review comments are confidential and will only be disclosed with the express agreement of the reviewer.

Editorial Decision

After considering the reviewer reports the Editorial Board Member will make one of the following decisions:

— Accept outright,
— Request a minor revision, where authors revise their manuscript to address specific concerns,
— Request a major revision, where authors revise their manuscript to address significant concerns and perhaps undertake additional work,
— Reject outright.

The final decision is made by the Editor-in-Chief.

Revisions

In cases where the referees or Editorial Board Member has requested changes to the manuscript, you will be invited to prepare a revision. The decision letter will specify a deadline for submission of a revised manuscript. Once resubmitted, the manuscript may then be sent back to the original referees or to new referees, at the Editorial Board Member's discretion.

A revised manuscript should be submitted via the revision link provided in the decision letter, and not as a new manuscript. The revision should also be accompanied by a point-by-point response to referees explaining how the manuscript has been changed. Please ensure that all issues raised have been addressed in the first round of revision. Where the authors disagree with a reviewer, they must provide a clear response.

Final submission and acceptance

When all editorial issues are resolved, your paper will be formally accepted for publication. Once accepted, the manuscript will undergo professional copy-editing, English editing, final corrections, pagination, and, publication on the http://www.ejcem.ur.edu.pl/. The Eur J Clin Exp Med reserves the right to make the final decision about matters of style and the size of figures.

Appeals

Even in cases where the Eur J Clin Exp Med does not invite resubmission of a manuscript, some authors may ask the Editorial Board to reconsider a rejection decision. These are considered appeals, which, by policy, must take second place to the normal workload. In practice, this means that decisions on appeals often take several weeks. Only one appeal is permitted for each manuscript, and appeals can only take place after peer review. Final decisions on appeals will be made by the Editorial Board Member handling the paper.

Decisions are reversed on appeal only if the relevant Editorial Board Member is convinced that the original decision was a serious mistake. Consideration of an appeal is merited if a referee made substantial errors of fact or showed evidence of bias, but only if a reversal of that referee's opinion would have changed the original decision. Similarly, disputes on factual issues need not be resolved unless they were critical to the outcome.

If an appeal merits further consideration, the Editorial Board Member may send the authors' response and the revised paper out for further peer review.

ORCID

The Eur J Clin Exp Med supports the use of ORCID. The Eur J Clin Exp Med mandates ORCID iDs for all submitting authors; this is published on the final article to promote discoverability and credit. Please provide the ORCID iDs of the authors in the title page.

Submission guidelines

Submission Process

Manuscripts for the Eur J Clin Exp Med should be submitted online at https://mc04.manuscriptcentral.com/pmur. The submitting author, who is generally the corresponding author, is responsible for the manuscript during the submission and peer-review process. The submitting author must ensure that all eligible co-authors have been included in the author list (read the criteria to qualify for authorship) and that they have all read and approved the submitted version of the manuscript. To submit your manuscript, register and log in to the submission website. All co-authors can see the manuscript details in the submission system, if they register and log in using the e-mail address provided during manuscript submission.

Cover letter

A cover letter must be included with each manuscript submission. It should be concise and explain why the content of the paper is significant, placing the findings in the context of existing work and why it fits the scope of the journal. Confirm that neither the manuscript nor any parts of its content are currently under consideration or published in another journal. The names of proposed and excluded reviewers should be provided in the submission system, not in the cover letter.

Accepted File Formats

Authors must use Microsoft Word to prepare their manuscript. Please insert your tables, graphics (schemes, figures, etc.) in the main text after the paragraph of its first citation.

In most cases, we do not impose strict limits on word count or page number. However, we strongly recommend that you write concisely and stick to the following guidelines:

— We encourage not exceeding 20 pages for original and review papers, and 8 pages for case reports of standard computer text (1800 signs on a page).
— The main text should be no more than 4,500 words (not including Abstract, Methods, References and figure legends).
— The title should be no more than 20 words.
— The abstract should be no more than 250 words.
— Recommended font: Times New Roman, 12 points.
— Manuscript text should be double-spaced. Do not format text in multiple columns.

Types of Publications

Manuscripts submitted to the Eur J Clin Exp Med should neither be published previously nor be under consideration for publication in another journal. The main article types are as follows:

Original research manuscripts. The journal considers all original research manuscripts provided that the work reports scientifically sound experiments and provides a substantial amount of new information.

Reviews. These provide concise and precise updates on the latest progress made in a given area of research. Systematic reviews should follow the PRISMA guidelines.

The Eur J Clin Exp Med accepts also the following types of submissions: case reports, letters to the editor, commentaries, book reviews, and reports from scientific meetings and conferences.

Reporting guidelines

The guidelines listed below should be followed where appropriate. Please use these guidelines to structure your article. Completed applicable checklists, structured abstracts and flow diagrams should be uploaded with your submission; these will be published alongside the final version of your paper.

Please refer to existing guidelines for reporting methodology; e.g.:

— AGREE guidelines for clinical practice guidelines
— ARRIVE guidelines for *in vivo* animal studies
— CARE guidelines for clinical case reports
— CONSORT guidelines for clinical trials
— PRISMA guidelines for systematic reviews and meta-analyses
— SPIRIT for clinical trials
— STARD guidelines for studies of diagnostic accuracy
— STROBE guidelines for observational studies

Manuscript Preparation

Your paper should consist of the following parts. Title page should be supplied as a **separate** file.

Research manuscripts should comprise:

— Title page: Title, Author list, Affiliations, Abstract, Keywords.
— Research manuscript sections: Introduction, Aim, Materials and Methods, Results, Discussion, Conclusions.
— Back matter: Supplementary Materials, Acknowledgments, Funding Statement, Author Contributions,

Conflicts of Interest, Data Availability, Ethics Approval, References.

Research manuscript sections:

— *Introduction*

State the objectives of the work and provide an adequate background, avoiding a detailed literature survey or a summary of the results.

— *Material and methods*

Provide sufficient details to allow the work to be reproduced by an independent researcher. Methods that are already published should be summarized, and indicated by a reference. If quoting directly from a previously published method, use quotation marks and also cite the source. Any modifications to existing methods should also be described.

— *Results*

Results should be clear and concise. The section may be divided into subsections, each with a concise subheading. Tables and figures central to the study should be included in the main paper. Do not use the term "significant" unless p-values are provided. Show p-values to 2 or 3 decimal places. The Results section should be written in past tense.

— *Discussion*

This should explore the significance of the results of the work, not repeat them. Avoid extensive citations and discussion of published literature.

— *Conclusions*

Summarize the work's findings, state their importance, and possibly recommend further research.

Review manuscripts should comprise:

— Title page: Title, Author list, Affiliations.
— Abstract, Keywords, Literature review sections.
— Back matter: Supplementary Materials, Acknowledgments, Funding Statement, Author Contributions, Conflicts of Interest, Data Availability, References.

Structured reviews and meta-analyses should use the same structure as research articles and ensure they conform to the PRISMA guidelines.

Case reports should comprise:

— Title page: Title, Author list, Affiliations.
— Abstract, Keywords. Case reports should include a succinct introduction about the general medical condition or relevant symptoms that will be discussed in the case report; the case presentation including all of the relevant de-identified demographic and descriptive information about the patient(s), and a description of the symptoms, diagnosis, treatment, and outcome; a discussion providing context and any necessary explanation of specific treatment decisions; a conclusion briefly outlining the take-home message and the lessons learned.
— Back matter: Supplementary Materials, Acknowledgments, Funding Statement, Author Contributions, Conflicts of Interest, Data Availability, Ethics Approval, References.

Requirements for case reports submitted to Eur J Clin Exp Med:

— Patient ethnicity must be included in the Abstract under the Case Presentation section.
— Consent for publication is a mandatory journal requirement for all case reports. Written informed consent for publication must be obtained from the patient (or their parent or legal guardian in the case of children under 18, or from the next of kin if the patient has died).

Language Style

Manuscripts must be submitted in English (American or British usage is accepted, but not a mixture of these).

Title page

These sections should appear in all manuscript types:

Title: The title of your manuscript should be concise and informative. It should identify if the study reports (human or animal) trial data, or is a systematic review, meta-analysis or replication study. When gene or protein names are included, the abbreviated name rather than full name should be used.

Author List and Affiliations: Authors' full first and last names must be provided. For each affiliation provide the details in the following order: department, institution, city, country. If available, the e-mail address of each author should also be provided. At least one author should be designated as *corresponding author*, and his or her email address and other details should be included at the end of the affiliation section.

Abstract: The abstract should be a total of about 250 words maximum. The abstract should be a single paragraph and should follow the style of structured abstracts: *Introduction and aim:* Place the question addressed in a broad context and highlight the purpose of the study; *Material and methods*: Describe briefly the main methods or treatments applied. Include any relevant preregistration numbers, and species and strains of any animals used. *Results:* Summarize the article's main findings; and *Conclusion*: Indicate the main conclusions or interpretations.

Keywords: Three to six pertinent keywords need to be added after the abstract in alphabetical order. We recommend that the keywords are specific to the article, yet reasonably common within the subject discipline.

Back Matter

Supplementary Materials: Describe any supplementary material published online alongside the manuscript (figure, tables, video, spreadsheets, etc.). Please indicate the name and title of each element as follows Figure S1: title, Table S1: title, etc.

Acknowledgments: Thank all of the people who helped with the research but did not qualify for authorship. Acknowledge anyone who provided intellectual assistance, technical help, or special equipment or materials.

Funding Statement: All sources of funding of the study should be disclosed.

Author Contributions: Authors must supply an Author Contribution Statement as described in the *Author contributions statements* section.

Conflicts of Interest: Authors must supply a competing interests statement. For more details please see *Competing interests policy*.

Data Availability: Authors must include a Data Availability Statement in all submitted manuscripts; see *Availability of materials and data* section for more information.

Ethics approval: Example of an ethical statement: "All subjects gave their informed consent for inclusion before they participated in the study. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of XXX (Project identification code)."

References: References must be numbered in order of appearance in the text (including table captions and figure legends) and listed individually at the end of the manuscript. We recommend preparing the references with a bibliography software package, such as EndNote, Reference Manager or Zotero to avoid typing mistakes and duplicated references.

References style

In-text citations and references should be prepared according to the American Medical Association (AMA) style. Each item should be listed in numerical order.

In-Text Citations

Each reference should be cited in the text using superscript arabic numerals. These superscript numbers should be outside periods. If you are citing sequential references, these should be indicated with a hyphen. Nonsequential references should be separated with commas. There should not be a space between numbers. For example: The degree of respiratory muscles fatigue depends on the applied exercise protocol and the research group's fitness level.[1,2] The greatest load with which a patient continues breathing for at least one minute is a measure of inspiratory muscles strength.[3] Diabetes mellitus is associated with a high risk of foot ulcers.[4-6]

Sample Reference

In listed references, the names of all authors should be given unless there are more than 6, in which case the names of the first 3 authors are used, followed by "et al.". If the source does not have any authors, the citation should begin with the title.

To find the proper abbreviation of journal go to the National Library of Medicine PubMed Journals Database at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=Journals.

Page number(s) should be inserted in full (for example: use 111–112, not 111–2).

The following are examples of individual citations made according to the required rules of editing and punctuation:

— **Article from a journal, number of authors from 1 to 6**

Author AA, Author BB, Author CC. Title of article. *Accepted Abbreviated Journal Title*. Year;Volume(Issue):Page-Page. doi (if available)

Lee JC, Seo HG, Lee WH, Kim HC, Han TR, Oh BM. Computer-assisted detection of swallowing difficulty. C*omput Methods Programs Biomed*. 2016;134(2):72-78. doi: 10.1016/j.cmpb.2016.07.010

Morris A. New test for diabetes insipidus. *Nat Rev Endocrinol*. 2019;15(10):564-565. doi: 10.1038/s41574-019-0247-x

— ***Article from a journal, number of authors* more *than 6***

Author AA, Author BB, Author CC, et al. Title of article. *Accepted Abbreviated Journal Title*. Year;Volume(Issue):Page-Page. doi (if available)

Gonzalez ME, Martin EE, Anwar T, et al. Mesenchymal stem cell-induced DDR2 mediates stromal-breast cancer interactions and metastasis growth. *Cell Rep*. 2017;18:1215-1228. doi: 10.1016/j.celrep.2016.12.079

Jordan J, Toplak H, Grassi G, et al. Joint statement of the European Association for the Study of Obesity and the European Society of Hypertension: obesity and heart failure. *J Hypertens*. 2016;34:1678-1688. doi: 10.1097/HJH.0000000000001013

— **Websites**

Author AA (if indicated). Webpage title. Name of Website. URL. Published or Updated date. Accessed date.

Cholera in Haiti. Centers for Disease Control and Prevention Web site. http://www.cdc.gov/haiticholera/. Published October 22, 2010. Updated January 9, 2012. Accessed February 1, 2012.

Address double burden of malnutrition: WHO. World Health Organization site. http://www.searo.who.int/mediacentre/releases/2016/1636/en/. Accessed February 2, 2017.

— **Book**

Author AA, Author BB. *Title of Work*. Location: Publisher; Year:Page-Page

Doane GH, Varcoe C. *Family Nursing as Relational Inquiry: Developing Health– Promoting Practice*. Philadelphia, PA: Lippincott Williams & Wilkins; 2005:25-28.
London ML, Ladewig PW, Ball JW, et al. *Maternal & Child Nursing Care*. Upper Saddle River, NJ: Pearson Education; c2011:101-103.
— Chapter in a book
Chapter Author AA. Title of chapter. In: *Name of Book*. Edition Number. Editor AA, ed. Location: Name of Publisher; Year:Page-Page.
Grimsey E. An overview of the breast and breast cancer. In: *Breast Cancer Nursing Care and Management*. 2nd ed. Harmer V, ed. Chichester, UK: Wiley-Blackwell; 2011:35-42.
NOTE: The Editorial Board requires consistent and carefully made references prepared according to the above-mentioned AMA standards. Otherwise, the work will be sent back to the authors.

Preparing Figures, Schemes and Tables

File for Figures and Schemes must be provided during submission and at a sufficiently high resolution (minimum 1000 pixels width/height, or a resolution of 300 dpi or higher). Common formats are accepted, however, TIFF, JPEG, EPS and PDF are preferred.

Please ensure the figures and the tables included in the single file are placed next to the relevant text in the manuscript, rather than at the bottom or the top of the file. The corresponding caption should be placed directly below the figure (not on the figure itself) or above the table. All figures, schemes, and tables should be numbered following their number of appearance (Figure 1, Scheme 1, Figure 2, Scheme 2, Table 1, etc.).

Tables should present new information rather than duplicating what is in the text. Readers should be able to interpret the table without reference to the text.

All table columns should have an explanatory heading. To facilitate the copy-editing of larger tables, smaller fonts may be used, but no less than 8 pt. in size. Tables must be provided in an editable format in appropriate place in the main text. Tables provided as jpeg/tiff files will not be accepted. Do not submit your tables in separate files.

Abbreviations

The journal requires using only standard abbreviations. Abbreviations should be defined in parentheses the first time they appear in the abstract, main text and in figure or table captions and used consistently thereafter. Ensure consistency of abbreviations throughout the article. Keep abbreviations to a minimum.

SI Units

SI Units (International System of Units) should be used. Imperial, US customary and other units should be converted to SI units whenever possible.