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Role of genetic modification of the *PNPLA3* gene in predicting metabolically unhealthy obesity and metabolic associated fatty liver disease in children

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ABSTRACT

Introduction and aim. Single nucleotide variants (SNV) of the patatin-like phospholipase domain-containing protein 3 (*PNPLA3*) gene play an important role in hepatic lipid remodeling and lipogenesis *de novo*, which is associated with the development of metabolically unhealthy obesity (MUO) and metabolic associated fatty liver disease (MAFLD). The aim of the study was to define the contribution of SNV *PNPLA3* gene to the development of MUO, complicated by MAFLD in children.

Material and methods. 200 obese children aged 6-18 years were examined. The main group (n=118) was represented by children with MUO. The control group (n=82) consolidated of children with metabolically healthy obesity (MHO). Whole genome sequencing (CeGat) was performed in 31 children of the main and 21 children of the control group.

Results. Among obese children, 14 variants of SNV *PNPLA3* (rs139051, rs34179073, rs2294918, rs139047, rs779127153, rs2076212, rs738409, rs738408, rs4823173, rs2072906, rs2076213, rs141106484, rs138736228) were identified, including SNV *PNPLA3* g.44322818, not described in the dbSNP core database. The role of the following SNV *PNPLA3* genotypes in the development of MUO complicated by MAFLD was revealed: rs738409 C/G (Relative risk (RR)=1.71); rs738408 C/T (RR=1.71); rs4823173 G/A (RR=1.57); rs2072906 A/G (RR=1.57) with Sensitivity (Se)=0.63 and Specificity (Sp)=0.72.

Conclusion. The contribution to the development of MUO complicated by MAFLD in children is made by the linked association of genotypes: rs738409 C/G, rs738408 C/T, rs4823173 G/A and rs2072906 A/G out of 14 *PNPLA3* SNVs diagnosed by us.

Keywords. children, metabolic associated fatty liver disease, obesity, patatin-like phospholipase domain-containing protein 3, single nucleotide variants

Introduction

The basis of metabolic associated fatty liver disease (MAFLD) is the accumulation of lipid droplets (LD) in more than 5% of hepatocytes, which are detected during histological examination, or an increase in the proton density of the fat fraction of more than 5.6% according to proton magnetic resonance spectroscopy in humans, who consume little or no alcohol, and in the absence of secondary causes of liver damage.[1] Currently, MAFLD is considered not as a primary liver disease, but as one of the components of the metabolic syndrome. An excess of nutrients entering the body causes the development of metabolically unhealthy obesity (MUO), which, unlike metabolically healthy obesity (MHO), is characterized by such changes as abdominal obesity, dyslipidemia, arterial hypertension, insulin resistance and impaired carbohydrate tolerance.[2,3]

Genome-wide association studies (GWAS) have demonstrated that single nucleotide variants (SNV) of the *Patatin-like phospholipase domain-containing protein*

3 (*PNPLA3*) gene, highly expressed in the liver (43 transcripts per million - TPM), skin (10 TPM), and adipose tissue (3 TPM)[4], highly associated with MUO and MAFLD phenotype.[5-12]

PNPLA3 gene, HGNC:18590, ENSG00000100344 (adiponutrin (ADPN), calcium independent phospholipase A2 epsilon (iPLA2epsilon), dJ796I17.1, C22orf20, FLJ22012) located on chromosome 22: 43,923,792-43,964,488 has 43,964,488 strand (0GR028) forward:CM 5 transcripts (splice variants), 303 orthologues, 4 paralogues. The *PNPLA3* gene encodes the LD transmembrane protein, adiponutrin. The PNPLA3 protein consists of 481 amino acids, has the enzymatic activity of triacylglycerol hydrolase 5 (TG5), lysophosphatidyl acyltransferase 6 (LPAAT6) and calcium-independent phospholipase A2 (iPLA2), play an important role in hepatic lipid remodeling and lipogenesis *de novo*, is involved in intracellular lipolysis of triacylglycerides (TG). Protein PNPLA3[148I] has hydrolase activity towards retinol esters, which leads to the formation of retinolic acid, which, being released from hepatic stellate cells, suppresses their activation and leads to inhibition of proliferation, migration and secretion of chemokines.[13]

However, the main function of PNPLA3 is considered to be the ability to inhibit the activity of adipose triglyceride lipase (ATGL), which is a key enzyme that controls the release of fatty acids from LD hepatocytes.[14] Activation of ATGL, which maintains an optimal LD size, is possible after ubiquitylation and proteasomal degradation of the PNPLA3 protein.

Replacing isoleucine with methionine at position 148 of the *PNPLA3* gene (rs738409 C>G) leads to the formation of a mutated PNPLA3[I148M] protein highly resistant to proteasomal degradation, inactivation of ATGL, and accumulation of fatty acids in LD of hepatocytes.[6]

Protein PNPLA3[I148M] is the target of transcription factor NF-kB[15] and has the selective ability to activate oxidative stress through the IRE-1α/JNK/c-Jun signaling pathway in the endoplasmic reticulum. Unlike the PNPLA3 protein, the PNPLA3[I148M] protein is localized in the cytoplasm, which promotes c-Jun-dependent expression of pro-inflammatory cytokines such as TNF-α, causing the development of steatohepatitis and liver fibrosis.[16]

Patients with hepatic steatosis (obese and non-obese) have been found to have an increased incidence of the rs738409 C/G genotype compared to a population of people with obesity but without hepatic steatosis.[17] It has been proven that the formation of the mutant protein PNPLA3[I148M] causes an increase in the level of the serum biomarker of microvesicular steatosis 3-methylglutarylcarnitine.[18]

The contribution of SNV rs738409 *PNPLA3* predicts the severity of MAFLD and the degree of activity of non-alcoholic steatohepatitis (NASH): $p=3.94\times10^{-8}$, in both adults ($p=9,73\times10^{-15}$) and children ($p=9,92\times10^{-6}$) and is the most significant.[19,20] The relative risk of MAFLD in individuals with minor variants is, according to different authors, from 1.58 to 2.29.[21,22] The role of rs738409 *PNPLA3* is especially significant in males and in children with elevated levels of basal insulinemia and hypertriglyceridemia.[23]

The results of GWAS meta-analyses demonstrated that SNV *PNPLA3* (rs139051, rs12483959 and rs2072907) also have a significant impact on childhood obesity; SNV rs4823173 *PNPLA3* determine excessive accumulation of LD in hepatocytes in adult patients and ALT increase ($p=3,44\times10^{-10}$), causing a high risk of developing hepatocellular carcinoma.[5,24-26]

The contribution of other SNVs of the *PNPLA3* gene, identified in our research work by whole genome sequencing, to the development of MUO in children complicated by MAFLD, as the most common liver disease worldwide, remains poorly understood.

Aim
The aim of the study was to define the contribution of SNV of the *PNPLA3* gene to the development of MUO, complicated by MAFLD in children.

Material and methods
Ethical approval
Participants provided written informed consent, and research protocols and procedures were approved according to the ethical standards of the Helsinki Declaration 2013 and by the Human Research Ethics Committee of Dnipro State Medical University (ethical approval DSMU/EC/19/1107). Time of data collection: January 2020 – August 2022.

Study design
Observational, analytical, longitudinal, cohort study.[27] Inclusion criteria: children with polygenic obesity (BMI≥97th percentiles) 6-18 years old. Exclusion criteria: children with monogenic and/or syndromic obesity, pregnancy.

To test the hypothesis about the association of the studied SNVs with obesity phenotypes, an analysis of the frequency of *PNPLA3* genetic variants, along with measurements of anthropometric and biochemical parameters, according to the recommendations of IDEFICS 2014, was carried out in a cohort of 200 obese children aged 6–18 years in the children's endocrinology department of the CNE Dnipro Clinical Hospital No. 9 of the Dnipro City Council (children from an urban obesity clinic).[28] For the examination of children, the consent of their parents was obtained. The main group (n=118) was represented by children with MUO. The control group (n=82) consisted of children with MHO. Each participant was identified by a code used in the database.

For inclusion in the main observation group, the presence of abdominal obesity and two of the presented criteria were taken into account: 1). Fasting glycemia ≥ 5.6 mmol/L; 2). High-density lipoprotein (HDL) ≤ 1.03 mmol/L or less than 10th percentile of the age norm; 3). TG ≥1.7 mmol/L or more than the 90th percentile of the age norm; 4) Systolic blood pressure (SBP) above the 90th percentile for a given age, gender and height; 5). Diastolic blood pressure (DBP) above the 90th percentile for a given age, gender and height.[29-32]

The abdominal type of obesity was determined according to the consensus of the International Diabetes Federation (IDF), based on the excess of the waist circumference over the 90th percentile for children.[33,34]

Anthropometric measurements were made in a child in underwear and without shoes. Height (cm) was measured using Heightronic Digital Stadiometer® to the nearest 0.1 cm. Weight (kg) and body fat (BF) percentage was measured using Tefal Bodysignal body composition analyzer (France). The calculation of the percentage of fat or BF in the body was performed automatically with a discreteness of 0.1%, according to the requirements of Tefal Bodysignal, with the evaluation of results according to the unified centile scales for children of this age.[35] Waist circumference (WC), hip circumference (HC) was measured using a standardized anthropometric tape, measuring the circumference at the midpoint between the top of the iliac crest and the lower part of the lateral rib cage to the nearest 0.1 cm.[36] BMI was converted to SDS by means of the current WHO growth references.[37]

Systolic and diastolic blood pressure (DBP) were measured using a digital oscillimetric device, Dinamap ProCare (GE Healthcare).

Laboratory examination for the formation of observation groups for obesity phenotypes included general clinical methods. Blood samples were obtained after an overnight fast by venipuncture in vacutainer gel tubes, and serum was separated from cells by centrifugation in a certified laboratory "Synevo" (Ukraine) using an analyzer and a Cobas 6000 test system; Roche Diagnostics (Switzerland). The analysis of serum glucose was carried out by the hexokinase method; the determination of triglycerides and high-density lipoproteins of blood plasma was carried out by the enzymatic – colorimetric method. The study of the levels of alanine aminotransaminase (ALT) and aspartate transaminase (AST) was performed by the kinetic method and assessed according to NASPGHAN guidelines.[38] The determination of the level of basal insulin was performed using the immunochemical testing method with electrochemiluminescent detection (ECLIA). The level of basal insulin in the venous blood was considered normal 2.6–24.9 mcU/ml.

Additionally, in the comparison groups, we assessed biochemical markers (AST/ALT ratio index, where an indicator of more than 1 was considered pathological; As-partate aminotransferase/platelet ratio index (APRI), where an indicator of more than 0,76 (Metavir F0-F1) was considered pathological; visceral adiposity index (VAI), according to Amato.[39-41] The threshold values determined for VAI in predicting MUO were 1.58, 1.30 and 1.78 for the general population, boys and girls, respectively.[42]

To study the role of pro-inflammatory markers in the development of meta-inflammation in children with obesity, the serum levels of interleukin-1β (IL-1β), interleukin-6 (IL-6), C-reactive protein (CRP). IL-1β was detected by the immunochemical method with chemi-luminescence immunoassay (CLIA). Analyzer and test – system: Immulite (Siemens AG), Germany. The reference value of IL-1β level was 0–5 pg/ml. IL-6 was determined by an enzyme-linked immunosorbent assay (ELISA) using a Cobas 6000/Cobas 8000 kit provided by Roche Diagnostics (Switzerland). The reference value of IL-6 level was 1.5–7.0 pg/ml. The level of CRP was measured using the turbidimetric immunoassay method. Analyzer and test – system: Cobas 6000 (with 501 modules), Roche Diagnostics (Switzerland). The CRP level of 0–5 mg/dl was considered the reference value. Leptin was determined using ELISA. Analyzer and test system: Tecan Sunrise, LDN (Germany). The reference value of leptin level for boys was 2–5.6 ng/ml, for girls – 3.7-11.1 ng/ml. Adiponectin was tested using ELISA. Analyzer and test system: Mediagnost GmbH (Germany). Interpretation of the results was carried out as follows: low cardiovascular risk - more than 10 μg/ml; moderate cardiovascular risk – 7-10 μg/ml; high cardiovascular risk – 4-7 μg/ml; very high cardiovascular risk – less than 4 μg/ml.

The sample population examined by whole genome sequencing (NGS, Illumina CSPro®, CeGat, Germany) consisted of 31 children of the main and 21 children of the control group and was qualitatively homogeneous in relation to the general population). Average amount of DNA (μg) in samples – 0.875. Library Preparation: Quantity used 50 ng. Library Preparation Kit: Twist Human Core Exome plus Kit (Twist Bioscience). Sequencing parameters: NovaSeq 6000; 2 x 100 bp.

Bioinformatic analysis – demultiplexing of the sequencing reads was performed with Illumina bcl2fastq (version 2.20). Adapters were trimmed with Skewer, version 0.2.2.[43] DNA-Seq: Trimmed raw reads were aligned to the human reference genome (hg19-cegat) using the Burrows-Wheeler Aligner, BWA – mem version 0.7.17-cegat.[44] ABRA, version 2.18 and Genotype-Harmonizer v.1.4.20 were used for local restructuring of readings in target regions to improve more accurate detection of indels in the genome during mutagenesis.[45,46]

All children underwent an ultrasound examination of the liver using the Simens Sonoline G 40 device (Japan) using of MAFLD ultrasound criteria according to Saverymuttu.[47]

To recognize the functional effects of SNV *PNPLA3* in the development of MUO complicated by MAFLD, statistical methods were used: analysis of variance, Odds Ratio (OR) with calculation of 95% Confidence Interval (CI), Spearman correlation analysis, sequential Wald analysis with calculation of Relative Risk (RR), Predictive Coefficient (PC), Sensitivity (Se), Specificity (Sp) and p-value for each variable.[48] Statistical processing of the results was performed using Microsoft Excel (Office Home Business 2KB4Y-6H9DB-BM47K-749PV-PG3KT) and STATISTICA 6.1 software (StatSoftInc, no. AGAR909E415822FA).

Results

The proportion of boys in the main group was 40% (47/118), in the control group – 50% (41/82). In this connection, RR MUO in girls was 1.2 times higher than in boys, p<0.05.

In the comparison groups, there was a statistically significant difference in anthropometric data, indicators of carbohydrate and fat metabolism, adipocytokine status of patients in the form of hyperleptinemia, adiponectinemia, increased levels of IL-6 and CRP among children with MUO. The results of clinical (Table 1) and paraclinical examinations (Table 2) among children with various obesity phenotypes allowed us to identify the most frequent clinical associations of markers of a complicated course of obesity (dyslipidemia, arterial hypertension, hyperglycemia).

Table 1. Average values of anthropometric and manometric examination of children with different phenotypes of obesity

Indicator	Children with MUO (M±m)	Children with MHO (M±m)	p
BMI in percentiles, %	99.54±0.31	98.74±0.39	0.12
Physical development in percentiles	74.9±4.85	55±6.86	0.02
BF in girls, %	38.2±2.3	28.9±0.8	0.0004
BF in boys, %	35.5±2.5	25.0±2.1	0.02
WC in girls, cm	97,6±4.2	79.9±3.1	0.001
WC in boys, cm	101.5±3.2	86.8±4.8	0.014
Correlation WC/H in girls	0.61±0.02	0.57±0.02	0.16
Correlation WC/H in boys	0.6±0.02	0.54±0.02	0.03
Correlation WC/HC in girls	0.9±0.04	0.88±0.1	0.85
Correlation WC/HC in boys	0.94±0.04	0.87±0.02	0.12
A body shape index (ABSI), %	0.072±0.001	0.071±0.02	0.96
SBP in percentiles	83.4±3.13	72.5±4	0.04
DBP in percentiles	87.1±2.81	67.15±4.22	0.0004

In the main group, the most frequent clinical association was a combination of abdominal obesity, dyslipidemia and arterial hypertension (33.3%), the presence of four markers of a complicated course of obesity was noted in every fifth patient with MUO (20%). Hyperglycemia was diagnosed only in the main group in the association of abdominal obesity with dyslipidemia

(6.7%) or in combination with abdominal obesity and arterial hypertension (6.7%). In the control group, every fourth patient (25%) was diagnosed with isolated abdominal obesity, in 85% of patients a combination of two markers of a complicated course of obesity was found, namely obesity with initial manifestations of arterial hypertension (SBP/DBP=85–90th percentiles) – in 35% of patients or associations of obesity with initial manifestations of dyslipidemia (HDL=11–25th percentile or TG=85–90th percentile) – in 20% of the examined.

Table 2. Features of carbohydrate and fat metabolism in children with different obesity phenotypes

Indicator	Children with MUO (M±m)	Children with MHO (M±m)	p
Fasting blood glucose, mmol/L	5.23±0.13	4.85±0.11	0.03
HbA1c,%	5.36±0.11	5.22±0.22	0.57
Basal insulin, mcU/ml	29.58±2.13	12.9±1.44	0.0001
HOMA-IR	6.76±0.5	2.61±0.22	0.0001
Leptin in girls, ng/ml	51.97±2.92	26.04±2.92	0.0001
Leptin in boys, ng/ml	43.1±2.92	12.51±2.92	0.0001
Adiponectin, mcg/ml	4.99±0.57	11.13±1.7	0.001
IL-6, pg/ml	4.36±0.82	1.97±0.22	0.007
IL-1β, pg/ml	3.89±0.63	3.3±0.92	0.59
CRP, mg/ml	5.67±0.96	2.57±0.57	0.007
HDL, mmol/L	1.34±0.1	1.29±0.04	0.64
HDL in percentiles	30.83±4.04	33.2±2.9	0.63
TAG, mmol/L	1.45±0.01	1.25±0.1	0.05
TAG in percentiles	88±1.27	84±1.5	0.04
VAI in girls	2.6±0.31	1.5±0.14	0.002
VAI in boys	1.48±0.31	0.81±0.14	0.05
ALT, UI/L	27.54±1.4	23.75±0.8	0.02
AST, UI/L	27.57±1.65	21.55±1.32	0.006
AST/ALT ratio index	1.12±0.06	0.8±0.03	0.0002
APRI	0.93±0.01	0.74±0.01	0.0002

Impaired fasting glycemia and/or carbohydrate tolerance during an oral glucose tolerance test was detected in 33.3% and 16.7% of patients in the main group and was not observed in patients in the control group.

The greatest contribution to the development of MVR was noted at: the value of the HOMA index exceeding the 95th percentile (RR=9.33); ultrasound signs of steatohepatosis (RR=6.33); extreme obesity (RR=6); AST/ALT ratio index>1 (RR=3.56); ultrasound signs of hepatomegaly according to Saverimutt (RR=3.33); DBP above 90th percentile (RR=3.07); SBP above 90th percentile (RR=2.27); HDL less than 25th percentile (RR=1.87) (Table 3).

MAFLD was diagnosed among children of the main group in 66.6%, and in the control group - in 10% of patients in the following association with other markers of a complicated course of obesity (Table 4).

Table 3. Relative risk of MUO calculation with 95% confidence interval*

Indicator	MUO, %	MHO, %	EER$_{MUO}$	CER$_{MHO}$	RR	S	95% CI	Se	Sp
HOMA-IR exceeding the 95th percentile	93.3	10	0.933	0.1	9.33	0.67	2.49-34.87	0.93	0.9
Ultrasound findings regarding steatohepatosis according to Saverymuttu	63	10	0.63	0.1	6.33	0.68	1.65-24.25	0.91	0.62
Extreme obesity (with body weight more than 120% from 95 percentile)	16.6	5	0.35	0.05	6	1.01	1-43.76	0.9	0.48
AST/ALT ratio index>1	53.3	15	0.53	0.15	3.56	0.56	1.19-10.64	0.84	0.55
Ultrasound findings regarding hepatomegaly	66.7	20	0.67	0.2	3.33	0.47	1.34-8.3	0.83	0.62
DBP exceeding the 90th percentile	76.7	25	0.77	0.25	3.07	0.4	1.4-6.71	0.82	0.68
SBP exceeding the 90th percentile	56	25	0.57	0.25	2.27	0.42	1-5.15	0.77	0.53
HDL less than 25 percentile	46	25	0.47	0.25	1.87	0.43	0.8-4.37	0.74	0.48

* Absolute risk in the main group: EER – experimental group event rate; absolute risk in the control group: CER – control group event rate; RR – relative risk; S – relative risk standard error; Se – sensitivity; Sp –specificity

Table 4. Types of association of metabolic markers and triglycerides in obesity phenotypes in children aged 6-18 years

Association types of metabolic markers	MUO, %	MHO, %
Isolated abdominal obesity + MAFLD	0	5
Abdominal obesity + Dyslipidemia + MAFLD	13.3	5
Abdominal obesity + Arterial hypertension + MAFLD	13.3	0
Abdominal obesity + Arterial hypertension + Dyslipidemia + MAFLD	13.3	0
Abdominal obesity + Hyperglycemia + Dyslipidemia + MAFLD	6.7	0
Abdominal obesity + Arterial hypertension + Hyperglycemia + Dyslipidemia + MAFLD	20	0

MAFLD was registered in 50% of girls and 33% of boys with various obesity phenotypes, p<0.05. We have established a correlation between an early indicator of nonspecific hepatocellular damage (ALT) and the fol-lowing clinical/biochemical parameters: APRI (r=0.77); AST (r=0.62); steatohepatosis on ultrasound examination (r=0.6); body weight (r=0.59); BMI (r=0.59); the presence of extreme obesity (r=0.47); SBP (r=0.47); hepatomegaly on ultrasound examination (r=0.45); impaired fasting glycemia (r=0.44); waist circumference (r=0.43); hip circumference (r=0.43); impaired tolerance to carbohydrates (r=0.35); AST/ALT ratio index<1 (r= -0.37); growth (r=0.3); hyperleptinemia (r=0.29); ABSI (r= -0.28); arterial hypertension (r=0.28); hyperuricemia (r=0.28) and age (r=0.28).

Among obese children, 14 variants of SNV *PNPLA3* (rs139051, rs34179073, rs2294918, rs139047, rs779127153, rs2076212, rs738409, rs738408, rs4823173, rs2072906, rs2076213, rs141106484, rs138736228) were identified, including SNV *PNPLA3* at position 44322818

Table 5. Characterization of the SNV *PNPLA*3 in obesity phenotypes in children*

Position	GnomAD_maxPOP	dbSNP	Ref/Alt	Zygosity (Proportion HOM^p/HET/HOM^n, %) MUO	Zygosity (Proportion HOM^p/HET/HOM^n, %) MHO	Consequence	BaseChange	CADD	RawScore	Clinical Significanse (gnomAD browser)
44324676	NFE	rs139051[a]	A/G	23.3/46.7/30	30/55/15	intronic	c.421-28A>G	4.31	0.103	Not Reported in ClinVar
44328832	NFE	rs34179073	C/T	73.3/26.7/0	75/25/0	synonymous	c.561C>T	10.46	0.62	Benign
44342116	AFR	rs2294918	A/G	10/50/40	10/55/35	missense	c.1300A>G	0.02	-0.67	Benign
44323074	EAS	rs139047	G/A	36.7/43.3/20	40/40/20	intronic	c.420+27G>A	2.91	0.02	Not Reported in ClinVar
44323036	SAS	rs779127153	G/A	100/0/0	95/5/0	missense	c.409G>A	29.8	4.23	Not Reported in ClinVar
44322970	AFR	rs2076212	G/T	86.7/13.3/0	100/0/0	missense	c.343G>T	0.19	0.19	Benign
44324727	AMR	rs738409[a]	C/G	50/43.3/0.67	55/35/10	missense	c.444C>G	15.73	1.4	Benign/ risk factor
44324730	AMR	rs738408[a]	C/T	50/43.3/0.67	55/35/10	synonymous	c.447C>T	1.13	-0.13	Benign
44328730	AMR	rs4823173	G/A	53.3/40/25	55/35/10	intronic	c.487-28G>A	0.22	-0.37	Not Reported in ClinVar
44333172	AMR	rs2072906	A/G	53.3/40/25	55/35/10	intronic	c.979+20A>G	0.69	-0.21	Not Reported in ClinVar
44322922	AMR	rs2076213	T/G	90/10/0	85/15/0	missense	c.295T>G	1.28	-0.11	Benign/VUS
44324767	AMR	rs141106484[a]	G/A	93.3/0.67/0	95/5/0	splice_region	c.484G>A	25.9	3.73	VUS
44336019	SAS	rs138736228	G/A	100/0/0	95/5/0	intronic	c.1112+14G>A	1.01	-0.15	Likely benign
44322818	–	–	A/G	96.7/0.33/0	100/0/0	missense	c.191A>G	0.01	-1.12	Variant is not available in the dbSNP core database

* GnomAD_maxPOP – the frequency distribution of *PNPLA3* mutations. AFR, AMR, EAS, FIN, NFE, SAS and OTH represent African, American, East Asian, Finnish, Non-Finnish European, South Asian and other populations; Ref – reference allele; Alt – alternative allele; Consequence –functional consequence of the variation in relation to the transcript. The nucleotide change and position relative to the coding sequence of the affected transcript in HGVS nomenclature: c. CDS Position Reference Base > Alternative Base. Example: c.223A> T. This column is empty if the variant is intergenic; CADD – combined annotation dependent depletion; VUS – variant of uncertain significance; [a] – SNV *PNPLA3* associated with MUO

(NC_000002.11:g.44322818C>A), not described in the dbSNP core database, Table 5.

The role of the following SNV *PNPLA3* genotypes in the development of MUO has been revealed: rs139051 G/G (RR=2; PC=3); rs141106484 A/G (RR=1.3; PC=1.2); rs738409 C/G (RR=1.2; PC=1); rs738408 C/T (RR=1.2; PC=1), p<0.05.

The CADD indicators calculated by us for SNV *PNPLA3* were characterized as follows: rs139051 GG – 4.31 (mutation in the intron region); rs141106484 AG – 25.9 (mutation in the intron region); rs738409 CG – 15.73 (missense); rs738408 CG – 1.13 (synonymous variant).

We have established a correlation between an early indicator of nonspecific hepatocellular damage (ALT) and the following genotypes (SNV *PNPLA3*): rs738409 CG/GG (r=0.43); rs738408 CT/TT (r=0.43); rs4823173 GA/AA (r=0.43); rs2072906 AG/GG (r=0.43); rs141106484 AG (r=0.38); rs139051AG/GG (r=0.25); rs34179073 CT (r= -0.24); rs2294918AG/GG (r=0.20); rs2076213 TG (r= -0.19); pos. 44322818 AG (r= -0.15); rs2076212 GT (r= -0.13), p<0.05. The risk of developing MAFLD among the examined cohort of children with MUO was observed with a combination of four SNV *PNPLA3*: rs738409 C/G (RR=1.71); rs738408 C/T (RR=1.71); rs4823173 A/G (RR=1.57); rs2072906 A/G (RR=1.57) with Sensitivity (Se)=0.63 and Specificity (Sp)=0.72 (Table 6).

Table 6. Relative risk of calculating MAFLD in children with SNV *PNPLA3* with 95% confidence interval

SNV PNPLA3	EER$_{MUO}$	CER$_{MHO}$	RR	S	95% CI	Se	Sp
rs139051	0.61	0.71	0.85	0.29	0.48–1.51	0.74	0.18
rs34179073	0.5	0.68	0.73	0.38	0.35–1.55	0.21	0.63
rs2294918	0.66	0.33	2	0.82	0.4–10.13	0.94	0.18
rs139047	0.53	0.81	0.64	0.26	0.39–1.07	0.52	0.18
rs779127153	–	0.63	–	–	–	–	1
rs2076212	0.25	0.0.69	0.36	0.87	0.06–2.01	0.05	0.73
rs738409	0.8	0.46	1.71	0.3	1–3.11	0.63	0.72
rs738408	0.8	0.46	1.71	0.3	1–3.11	0.63	0.72
rs4823173	0.8	0.5	1.57	0.28	1–2.75	0.63	0.72
rs2072906	0.8	0.5	1.57	0.28	1–2.75	0.63	0.72
rs2076213	1	0.59	1.68	0.16	1.23–2.3	0.16	1
rs141106484	1	0.6	1.65	0.15	1.22–2.22	0.11	1
rs138736228	–	0.63	–	–	–	–	1
g.44322818	1	0.62	1.61	0.15	1.21–2.14	0.05	1

The relative risk of developing MAFLD in the presence of a combination of these four SNV *PNPLA3* genotypes increased by 1.2 times among patients with MUO. A direct correlation was found between the association of SNV rs738409 C/G and the combination of rs738408 C/T, rs4823173 G/A, and rs2072906 A/G in patients with MAFLD (r=0.74; p<0.05).

Discussion

This work is devoted to the search for genetic determinants of MAFLD by carefully studying the target group of patients with MUO, in which, according to Murag the risk of this disease is 70-90%.[49] According to our results, MAFLD occurs in 66% of children with MUO. In previous studies, a stable association of the SNV *PNPLA3* gene with MAFLD (RR> 1.6) was found accompanied by an increase in TG and a decrease in HDL.[7,50] Unlike the study by Lee et al. our work demonstrates a greater likelihood of MAFLD occurrence among girls than among boys, and confirms the relationship of the onset of the disease with adolescence (RR=3.11), levels of basal insulinemia (RR=9.33); and to a lesser extent with a decrease in HDL levels (RR=1.87), p<0.05 compared with the results obtained by Gloudemans et al.[7,23]

In this work, we first determined the contribution of 14 SNV *PNPLA3* to the formation of various obesity phenotypes in children and the risk of MAFLD in MUO/MHO and presented SNV *PNPLA3* is not available in the dbSNP core database (NC_000002.11:g.44322818 C>A), whose role remains to be explored in a larger sample of patients.

We found that the RR of MUO occurrence doubled in the presence of the rs139051 A/G *PNPLA3* genotype and was higher compared to the rs141106484 A/G (RR=1.3), rs738409 C/G (RR=1.2), rs738408 C/G genotypes (RR=1.2).

At the same time, we did not reveal the contribution of SNV rs139051 A/G *PNPLA3* to the formation of MAFLD, as well as the research group of Ragab et al.[20] According to their results, this genetic variant was equally common in both MAFLD patients (82.5%) and healthy individuals (85%). And according to some authors, it even reduced the risk of MAFLD by 0.58 times (95% CI: 0.342–0.984; p=0.04).[51] At the same time, other researchers indicate that SNV rs139051 *PNPLA3* is significantly correlated with persistent meta-inflammation[51] and the level of basal insulinemia (p=0.04)[52] demonstrating its pathological role based on the modulation of the phospholipid metabolite profile and the formation of insulin resistance in MUO.

We also demonstrated the contribution of SNV rs141106484 of the *PNPLA3*^{B162M} gene to the formation of MAFLD in children, in contrast to the only work by Gerhard, which determined the pathogenic risk (0.974) of SNV rs141106484 of the *PNPLA3*^{B162M} gene in liver cirrhosis.[53]

Najafi et al. also considered the combined contribution of the G/C rs738409 and T/C rs738408 genotypes to the development of MAFLD (p=0.004).[52] The combination of these SNV *PNPLA3* was associated with an earlier onset of MAFLD in non-obese patients.[17] Qin Pang et al. also indicated a higher likelihood of developing MAFLD, NASH, and liver fibrosis in the presence

of a combination of the following SNVs: rs738409 G allele (OR=2.77, 95% CI: 1.18-6.54; p=0.02); rs4823173 A allele (OR=2.73, 95% CI: 1.16-6.44; p=0.02), and rs2072906 G allele (OR=3.05, 95% CI: 1.28-7.26; p=0.01) but in adult patients with chronic viral hepatitis B.[54]

We have for the first time revealed an increase in RR MAFLD in the presence of a combination of four SNV *PNPLA3* genotypes (rs738409 C/G, rs738408 C/T, rs4823173 G/A and rs2072906 A/G) by 1.2 times among children with MUO. We found a strong direct correlation between the SNV rs738409 C/G association and the combination of rs738408 C/T, rs4823173 G/A, and rs2072906 A/G genotypes in patients with MAFLD (r=0.74; p<0.05).

Conclusion

The presence of the following SNV *PNPLA3* genotypes predetermines the development of MUO: rs139051 G/G (RR=2; PC=3); rs141106484 A/G (RR=1.3; PC=1.2); rs738409 C/G (RR=1.2; PC=1); rs738408 C/T (RR=1.2; PC=1), p<0.05.

The combination of four SNV *PNPLA3* genotypes (rs738409 C/G, rs738408 C/T, rs4823173 G/A and rs2072906 A/G) among children with MUO increases the risk of development by 1.2 times.

The presence of SNV rs738409 and rs738408 *PNPLA3* affects both the occurrence of MUO and MAFLD in children.

Declarations

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

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ORIGINAL PAPER

Relationship between social media addiction and bodyweight

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ABSTRACT

Introduction and aim. Social media addiction is increasing rapidly due to the widespread use of smartphones and the availability of the internet. Also, social media addiction constitutes a risk factor for many physical and mental diseases. We aimed to determine the frequency of social media addiction among obese patients.

Material and methods. Adult patients who were admitted to the Diet Clinic of a tertiary healthcare hospital in order to lose weight enrolled in the study. A questionnaire including; Social Media Addiction Scale, body mass index (BMI), and demographic information (age, sex) was applied to all participants. Participants were grouped as obese, overweight, and normal weight.

Results. A total of 179 patients (mean age: 34.1±12.6 years) enrolled in the study. No difference in sex was found (45.2% women, 47.3% men). Approximately half of the study population was found to be social media addicts. Participants younger than 45 years of age were found to have higher rates of social media addiction (X^2= 4.26; p<0.05). The frequency of social media addiction was found to increase with increasing BMI (41.7%, 43.6%, and 48%; respectively).

Conclusion. Social media addiction is prevalent and it is also more frequent in younger adults. Learning more about social media addiction and its relationship with obesity can decrease its health consequences.

Keywords. addictive behavior, body mass index, obesity, social media

Introduction

Social media (SM) which was only a communication tool at first now occupies a very important place in our lives as a result of the increasing prevalence of smartphones and easy access to the internet. SM are web 2.0 applications where online content is produced, shared and individuals collaborate.[1] It includes; blogs, videos, photos, multimedia materials that do not require or use limited personal information, content communities where articles are shared, social networking sites where individuals interact with their environment by creating their profiles, and games played by many people at the same time by creating virtual characters.[2]

What differentiates SM, from traditional media is that it is interaction-based, sharing-themed, and user-based. SM, which has become the most effective way for people to express themselves, has 4.6 billion users today and 90% of whom access it through mobile applications.[3] Social media addiction can be defined as a behavioral addiction characterized by a constant urge to use SM and spending too much time on it which results in impairment of other daily activities of the individual.[4]

Today more than 1.9 billion adults aged 18 and over are overweight, and more than 600 million of them were obese. In another words, 39% of adult population are

overweight and 13% are obese worldwide.[5] The etiology of obesity is multifactorial but the most important mechanism is the storage of fat in the adipose tissue as a result of high caloric intake and low physical activity. Developing technology and our evolving social system facilitates sedentary lifestyles which causes to obesity. In recent years, studies on the relationship between the internet and SMA (Social Media Addiction) to mental and physical diseases have been increasing as they constitute a major role in our lives.[6,7]

Obesity is considered an epidemic disease with critical health implications and a major risk factor for non-communicable diseases, putting a burden on healthcare systems.[8,9] Learning more about its etiology can contribute to a decrease in its resultant health consequences and lessen the economic burden on the health care systems.

Aim
Therefore, in this study, we aimed to determine the frequency of SMA in different BMI groups and to evaluate whether a relationship exists between SMA and bodyweight.

Material and methods
Ethical approval
This study was approved by the local ethics committee (Ethics Committee of Health Sciences University Diskapi Yildirim Beyazit Training and Research Hospital-Ankara date: 27.11.2017 decision number: 43/16).

Study design
We conducted a cross-sectional study among patients admitted to the Diet Clinic of a tertiary healthcare hospital. The power analysis we carried out revealed that our study required minimum sample size of 170 participants to provide a statistical power of 0.80. All subjects voluntarily (i.e., they did not receive payment or other compensation) and anonymously participated in the present research and gave their written informed consent to take part in the study. The inclusion criteria included intending to lose weight or to have a controlled diet and being over 18 years old. Data were collected over a time period of 3 months. A total of 203 patients volunteered to participate in the study. Due to incomplete information, 24 patients were excluded and the study was performed with 179 patients. A questionnaire including Social Media Addiction Scale (SMAS) and demographic information (age, sex) was applied to all participants. All patients' body mass indexes (BMI) were calculated by measuring their height and weight. SMAS is a 5- point Likert-type questionnaire consisting of 41 items with a Cronbach's alpha value of 0.967 which emerged after all validity and reliability studies were conducted. The lowest score that can be obtained from

SMAS is 41 while the highest score is 205. Participants were grouped as followed according to their scores; 41-73: 'no SMA' and ≥74: 'have SMA'.[10]

BMI, a rough population measure of obesity, is calculated by dividing a person's weight (in kilograms) by their height (in meters) squared. The method of measurement does not differ according to sex or age. According to WHO; a person with a BMI of 30 or more is classified as obese, and a person with a BMI in the range of 25-29.99 is classified as overweight. The value of >18.5, which is accepted as the lower limit in BMI measurement, allows us to distinguish normal weight.[5]

Data were analyzed using Statistical Package for Social Sciences (SPSS version 21.0, IBM®, Chicago, IL, US). Descriptive statistical methods in the evaluation of demographic data; frequency, percentage, mean, and standard deviation were used. The normal distribution of the data was tested by the Shapiro-Wilks test. Quantitative variables were stated as mean ± standard deviation and categorical variables as number and percentage (%). In the examination of a statistically significant difference in categorical variables between the groups, the Chi-Square test was used. The correlation between BMI and SMAS score was assessed by Pearson's correlation analysis. The differences were considered statistically significant when the p-value was lower than 0.05.

Results
A total of 179 patients (124 (69.3%) women, 55 (30.7%) men) enrolled in the study. The mean age of the participants was 34.1±12.6 years. Twenty-four of the patients (13.4%) were normal weight, fifty-five (30.7%) were over-weight and one hundred patients were obese (55.9%). Analysis of the data revealed that; 45.8% of all patients were found to be SM addicts. When the relationship between sex and SMA was evaluated; it was determined that 47.3% of men and 45.2% of women were SM addicts. There is no significant relationship between sex and SMA ($X^2= 0.07$; $p>0.05$).

The analysis of the SMA status of the participants according to BMI groups showed that 41.7% of the participants in the normal weight group, 43.6% of the overweight participants, and 48% of the obese participants were SM addicts. When the overweight and obese participants were evaluated together, it was determined that 46.5% of them were SM addicts. There is no significant relationship between the BMI group and SMA ($X^2= 0.46$; $p>0.05$), (Table 1).

The relationship between age groups and SMA was evaluated by dividing the participants into two groups; under 45 years and over 45 years. It was determined that 50% of the participants under the age of 45 and 31.7% of the participants aged 45 and above were SM addicts. The SMA rate is higher in participants under the age of 45 ($X^2= 4.26$; $p<0.05$), (Table 2).

Table 1. Relationship between body mass index and social media addiction

			Social Media Addiction		Total	X^2	p
			Absent	Present			
Body Mass Index Groups	Normal	n	14	10	24		
		%	58.3%	41.7%	100%		
	Overweight	n	31	24	55		
		%	56.4%	43.6%	100%	0.46	0.79
	Obese	n	52	48	100		
		%	52%	48%	100%		
Total		n	97	82	179		
		%	54.2%	45.8%	100%		

Table 2. The relationship between age groups and social media addiction

			Social Media Addiction		Total	X^2	p
			Absent	Present			
Age Groups	<45 years	n	69	69	138		
		%	50%	50%	100%		
	≥45 years	n	28	13	41		
		%	68.3%	31.7%	100%	4.26	0.04
Total		n	97	82	179		
		%	54.2%	45.8%	100%		

When the correlation between BMI and SMA scores in the group under the age of 45 is looked into; it is understood that there is a low-level positive and statistically insignificant relationship between BMI and SMA scores (r=0.052; p>0.05). When the results are evaluated in general without considering the age groups, it is seen that there is a low-level positive and statistically insignificant relationship between BMI and SMA scores (r=0.041; p>0.05).

Discussion

SMA is mostly investigated as a risk factor for psychiatric diseases with children, adolescents, and young people mainly chosen as the target group.[6,7] Problematic use of SM is found to be associated with depression and anxiety, as well as eating disorders, self-harm, and suicidal ideation.[6-11] The striking point in these studies is that in addition to its relationship with psychiatric diseases SM also causes isolation and a decrease in daily physical activities of the individuals. This situation has brought the possibility of SMA being a cause of obesity on our agenda. However, there is a limited number of published studies investigating the relationship between SMA and obesity. On the other hand, the internet addiction obesity relationship has managed to be the subject of many studies in the last decade.

SMA and internet addiction are closely related topics with the latter being a more popular topic of research. Similar problems arise as a result of problematic use. The increase in internet and SM usage time causes a decrease in the time allocated for physical activity.

When the studies on internet addiction are reviewed, it is seen that internet addiction triggers obesity. One of the studies showed that moderate/severe internet addiction is associated with a 22-fold increased risk of obesity.[12]

In a study conducted by Bozkurt H. et al. in 2018; the frequency of internet addiction and its relationship with BMI was investigated in children and adolescents aged 8 to 17 years. A total of 437 people were sampled, of which 268 were obese and 169 were healthy control groups. They found the prevalence of internet addiction at 24.6% in the obese group and 11.2% in the control group. In conclusion, a significant relationship between internet addiction and BMI was found.[13]

In a few studies about SM, it has been claimed that it can cause decreased physical activity and eating disorders which are risk factors for obesity and concluded that, exceeding two hours of daily use of any electronic media was found to be associated with increased risk of being overweight.[14]

In our study, the SMA rate was higher in the age group below 45 years. We found this grouping appropriate as according to age classification young age is considered to be up to 44. Although it was not significant, as BMI increases in this age group, SMA scores also increase and there is a higher positivity as compared to the general study population. When the literature is reviewed, it is seen that similar results are supporting this data. In a recent study that examined the relationship between SMA and psychiatric diseases, a negative relationship was found between SMA and age.[15] Another study involving 556 women found that Facebook addiction decreased with age.[16] The higher prevalence of SMA in the younger age group can be attributable to their higher levels of digital literacy and understanding.[17]

The recent articles mostly studied the effects of SM on the younger population. Considering the effect of time in our study, we found it appropriate to include adults as well instead of limiting SMA to young people. There is no doubt that the use of the internet and SM will gradually become widespread in the adult population and there will be an increase in studies on adults. In particular, the effects of this addiction on human health will be better understood with studies to be conducted on the 18–45 age group which has a high addiction rate.

When SMA was evaluated according to sex, our data revealed no significant relationship. In the literature, several previous types of research have pointed out the same finding. In a study conducted with 194 social network users, no statistically significant difference was found between social network addiction and sex.[18] In another study in Turkey, where 447 students were examined in terms of Facebook addiction, it was found that there was no significant relationship between sex and Facebook addiction.[19] In a study investigating the

psychological risk factors of SMA, in which smartphone users between the ages of 18 and 40 participated in China, it was found that there was no significant relationship between sex and SMA.[20] On the contrary several researches in recent years had findings that show a sex tendency. Some studies have revealed that males were more addicted to SM.[21-23] While other studies have results where females have a higher addiction rate than males.[24] Another interesting sex-related data in our study was that the female participants were much more in number than the male participants. Since we conducted our study in a diet clinic, the reason for this may be that women have higher body dissatisfaction as compared to male.[25]

The weak correlation in our study may have been caused by insufficient sample size and/or uneven sample distribution. In future studies, stronger results can be obtained if the distribution of the obese and control groups is kept equal and the sample size is increased. Another limitation of our study can be the 'self-assessment bias' which arises when participants do not accurately judge their level of social addiction while filling out the survey. Also, being single, having mental illnesses such as depression and anxiety, dietary factors, level of physical activity, and owning smart devices could be other factors that may affect SMA and complicate its potential relationship with BMI. Unfortunately, we did not evaluate those factors in our study. Finally, the prevalence of SMA in the general population itself might be too high so it does not show a statistically important difference between obese and normal-weight individuals in the society. The latest research by Global WebIndex revealed that 57.6% of the world's population uses SM.[26]

Conclusion

Obesity is a major health concern worldwide and most importantly it is a modifiable one. The result of this study provides an insight into the unexplored relationship between SMA and BMI. There is no doubt that SMA is prevalent. Future studies should be conducted to investigate the effects of SMA, which is becoming a major concern for human health. More accurate results can be obtained if the sample size is increased and equal distribution of the groups can be ensured for comparative analyses.

Declarations

Author contributions
Conceptualization, S.T., S.M. and C.A.; Methodology, S.T., S.M. and C.A.; Software, S.T. and C.A.; Validation, S.T. and C.A.; Formal Analysis, S.T. and C.A.; Investigation, S.T., A.Ö., Z.S.A.; Resources, S.T., S.M. and C.A.; Data Curation, S.T. and C.A.; Writing – Original Draft Preparation, S.T., A.Ö., Z.S.A. and C.A.; Writing – Review & Editing, S.T., A.Ö., Z.S.A. and C.A.; Visualization, S.T., A.Ö., Z.S.A. and C.A.; Supervision, C.A.; Project Administration, S.T. and C.A.; Funding Acquisition, S.T. and C.A.

Conflicts of interest
No conflict of interest was 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 Health Sciences University Diskapi Yildirim Beyazit Training and Research Hospital-Ankara date: 27.11.2017 decision number: 43/16).

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ORIGINAL PAPER

Nurses' COVID-19 fears and patient safety attitudes in the pandemic

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ABSTRACT

Introduction and aim. The fear experienced by nurses during the COVID-19 pandemic may threaten patient safety. This study was conducted with the aim of examining nurses' COVID-19 fears and attitudes to patient safety in the pandemic.

Material and methods. The research had a descriptive and cross-sectional design. The research sample consisted of 245 nurses working at a teaching and research hospital in Istanbul, Turkey's most populous province. Research data were collected in June–July 2021. A Nurse Characteristics Form, the Fear of COVID-19 Scale (FCV-19S) and the Patient Safety Attitude Questionnaire were used to collect data. The data evaluation was performed using descriptive statistics, Student t test, one-way variance (ANOVA), and Pearson correlation analysis.

Results. The nurses' mean score for fear of COVID-19 was 16.67±6.88, and their mean score for patient safety attitude was 141.70±27.78. Their COVID-19 fear levels and mean patient safety attitude scores were found to differ according to their intention to leave the job, their education on COVID-19 and their age.

Conclusion. Nurses' experiencing of physical, social and psychological problems relating to the COVID-19 pandemic should be followed up in the long term.

Keywords. COVID-19, nurses, patient safety

Introduction

Patient safety is an integral part of quality health care.[1] For this reason, provision of sustainable, safe and optimal care for patients causes concern.[2,3] Patient safety is the actions taken to prevent and remove problems which may affect patients and their families during health services provided by health professionals.[4] The World Health Organization has reported that in hospitals in low and middle income countries, 134 million adverse events occur annually, and that of these, 2.6 million result in death. At the same time, it is reported that four out of ten people who are treated in hospital suffer adverse effects from some situation or event.[2] Since the first days of the nursing profession, nurses have assumed a vital role in ensuring and improving patient safety, because of the nature of their work.[1,5,6] In every environment in which they work, they are charged with protecting patients from possible dangers and preventing unwanted results of interventions.[7] A wrong or negative attitude to patient safety may make the implementation of policies relating to patient safety more difficult. Therefore, it is necessary to know attitudes to patient safety and the factors affecting them in order to create a safe environment.[8]

COVID-19 has threatened the lives of many people,[9] and in particular has been the cause of stress, exhaustion, depression, anxiety, fear and sleeplessness in health workers who are fighting the disease.[10-13] A high level of fear of COVID-19, particularly in nurses caring for patients affected by it, increases stress and negative feelings.[14,15] Work-related stress is a potential cause of concern for health professionals. Nurses' experience of anxiety, depression, fear and exhaustion, can pose a risk for a safe health care service.[8,11,16] A time of pandemic can disrupt wellbeing and work efficiency in times of crisis.[17] Therefore, nurses should maintain their psychological health to ensure the quality of care for patients.[11,12] Since the beginning of the pandemic, many studies have been conducted on the effects of COVID-19 on the mental health of health workers, but it is important to know how it affected how nurses fulfilled their roles, and the attitudes and behaviors which they displayed.[6,10,11,18,19]

Attitude is a concept which includes beliefs and behaviors which can affect individuals' decisions and shape their behaviors.[20] Patient safety attitude refers to the extent to which the healthcare staff perceives the safety culture of the department or hospital.[21] Showing a negative attitude to patient safety has a negative effect on interventions relating to patient safety.[22] During the pandemic, nurses have put their lives at risk in order to perform their duties, and have felt the fear of becoming infected or of infecting others.[6] A feeling of uncertainty in relation to COVID-19 may cause fears concerning coronavirus.[17] Fear is a feeling which shows itself with a high level of emotional avoidance of the reason for certain stimuli, and which affects individuals' physical reactions, cognitive skills and mental states. An increase in the level of fear increases intolerance of an uncertain situation, triggers various negativities and can make the situation worse.[13] Nurses are at high risk of occupational exposure while caring for patient with COVID-19. The severity of an illness, mortality and the risk of infection cause worry and fear in nurses. This can affect nurses' health and quality of care during the pandemic.[17,18] To the best of our knowledge, there are no studies in the literature that have reported on the relationship between patient safety attitudes and COVID-19 fear levels in nurses.

Aim

This study was conducted with the aim of examining the fears of COVID-19 and attitudes to patient safety of nurses on the front line of the fight against the illness during the pandemic.

Material and methods
Ethical approval
In this study, all procedures were performed in accordance with the ethical standards, and by the Helsinki Declaration. This study was approved by Ç University's institutional review body (Approval No. 2021-YÖNP-0378). The researcher explained to each nurse participant what the study was about as well as their rights and roles as study participants. Participation was voluntary, anonymous, and did not involve any compensation. Informed consent was obtained from all participants.

Study design and participants
This study used a descriptive cross-sectional research design. The population of the study consisted of 557 nurses working at a teaching and research hospital in Istanbul, Turkey's most populous province. Nurses who had been on active duty in patient care and who accepted to participate were included in the research. Nurses who were on leave or on sick leave on the dates when the research was performed (n=90), who had not been on active duty in patient care during the pandemic (n=124), who filled the data collection forms incompletely (n=28) or, who not accepted to participate (n=70) were not included in the study. The final study population consisted of 245 (73.1%) nurses.

Data collection tools
After the necessary explanations about the research were given to the nurses included in the research, data collection tools were applied to the nurses who volunteered to participate. Research data were collected in June-July 2021. A Nurse Characteristics Form, the Fear of COVID-19 Scale (FCV-19S) and the Patient Safety Attitude Questionnaire were used to collect data.

Nurse Characteristics Form: This form contained 21 questions dealing with the nurses' sociodemographic and other descriptive characteristics such as gender, age, marital status, the clinic where they worked, and total professional experience.

Fear of COVID-19 Scale (FCV-19S): The Fear of COVID-19 Scale was developed by Ahorsu et al. (2020) and adapted to Turkish language by Bakioğlu et al. (2021).[17,18] The FCV-19S is a 7-item self-report instrument to measure the severity of COVID-19 fear. The scores that can be obtained from the scale are between 7 and 35. As the score obtained from the scale increases, the fear of COVID-19 increases. Cronbach's alpha internal consistency coefficient of the original scale was 0.82. Cronbach's alpha for Turkish version was 0.88. The Cronbach's a for the current total sample was 0.71.

Patient Safety Attitude Questionnaire: The Safety Attitude Questionnaire (SAQ) was developed by Sexton et al (2006) and adapted to Turkish language by Baykal and Altuntas (2010).[21,23] The questionnaire includes 46 items and six subscales; job satisfaction, teamwork climate, safety climate, perceptions of management, stress recognition, and working conditions. The scores that can be obtained from the scale are between 46 and 230. An increase in total subscales of overall score indicates a

positive attitude toward patient safety; as the total score increases, patient safety attitude increases; and as the total score decreases, patient safety attitude decreases. The Cronbach alpha value of the scale was 0.93, and those of the sub-dimensions were between 0.72 and 0.86. In this study, the Cronbach's alpha value was found to be 0.71, and those of the sub-dimensions were between 0.64 and 0.72.

Statistical analysis

Continuous variables are expressed as means ± SD, and categorical variables are expressed as percentages. The data was examined for conformity to normal distribution with the Kolmogorov-Smirnov test, and evaluated using the Student t test and one-way variance (ANOVA) analysis. Post-Hoc analyses were performed where appropriate using Bonferroni correction. The correlation between the nurses' coronavirus fear levels and their attitudes to patient safety was determined with Pearson correlation analysis. For all tests, two-sided P values of <.05 were considered as significant. Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) version 20.0 for Windows (SPSS Inc., Chicago, Illinois, USA).

Results

The mean age of the nurses included in the study was 28.39±4.57 years, and their total professional experience was 70.40±50.05 months. A majority of the participants (51%) lived with their families, 80.4% were female, 74.3% were university graduates, and 65% were single. Similarly, 75.5% had no intention of leaving the profession, 82.2% had no chronic health problem, 81.6% had cared for COVID-19 patients, and 58.4% had not had a COVID-19 infection. The proportion of nurses who had had a problem with patient safety but who had not reported it was 54.9% (Table 1).

The nurses' mean score for coronavirus (COVID-19) fear levels was 16.67±6.88, and it was found that they experienced coronavirus fear at a low level (Table 1). Coronavirus fear levels were high in those who intended to leave the profession (p=0.000), those who had a chronic illness (p=0.02), and those who had had no training on COVID-19 (p=0.003). Coronavirus fear levels were higher in those aged 26–30 than in those aged 21–25 (p=0.01, Table 2).

The nurses' mean total score on the Patient Safety Attitude Questionnaire was 141.70±27.78, showing that they had a positive attitude to patient safety. Total scores related to the subscales show that the highest score was obtained from teamwork climate (39.84±8.94) and that the lowest score obtained was from stress recognition (15.28±4.88) (Table 1). Mean patient safety attitude scores were high in nurses who were aged 31 or above (p=0.000), those with a total professional experience of

Table 1. Nurses' socio-demographic and descriptive characteristics

		n (%)
Age	21-25 years	45(18.4)
	26-30 years	151 (61.6)
	31 years or more	49 (20)
Total professional experience	1-5 years	163 (66.5)
	6-10 years	49 (20)
	11 years or more	33 (13.5)
Do you live alone at home?	Yes	83 (33.9)
	No. With my family	125 (51)
	No. With a friend	37 (15.1)
Gender	Female	197 (80.4)
	Male	48 (19.6)
Educational status	Health vocational high school	18 (7.3)
	Ordinary degree	30 (12.2)
	Honours degree	182 (74.3)
	Postgraduate (Masters/Doctorate)	15 (6.1)
Marital status	Married	85 (34.7)
	Single	160 (65.3)
Place of work	Ward	73 (29.8)
	ICU	139 (56.7)
	Operating theater	16 (6.5)
	Other	17 (6.9)
Work status	Clinician/Ward nurse	228 (93.1)
	Nurse manager	17 (6.9)
Working arrangement	Always daytime	38 (15.5)
	Shift (night/day)	194 (79.2)
	Always night	13 (5.3)
Average weekly working hours	≤ 40 hours	87 (35.5)
	> 40 hours	158 (64.5)
Intention to leave the profession	Yes	60 (24.5)
	No	185 (75.5)
Do you have a chronic illness problem?	Yes	44 (18)
	No	201 (82)
Have you cared for COVID-19 patients?	Yes	200 (81.6)
	No	45 (18.4)
Have you had a COVID-19 infection?	Yes	102 (41.6)
	No	143 (58.4)
Have you had training on COVID-19?	Yes	179 (73.1)
	No	66 (26.9)
Have you had psychological support because of COVID-19?		
	Yes	6 (2.4)
	No	239 (97.6)
Have you had training on patient safety?	Yes	219 (89.4)
	No	26 (10.6)
Have you had any problems with patient safety?	Yes	71 (29)
	No	174 (71)
If you had a patient safety problem, did you report it?	Yes	32 (45.1)
	No	39 (54.9)
Fear of COVID-19 Scale score	(7−35)	16.67±6.88 (min.7−max.35)
Patient Safety Attitude Questionnaire total score		
(46−230)		141.70±27.78 (min.69−max.225)
Work Satisfaction (11−55)		29.42±9.75 (min.11−max.55)
Teamwork climate (12−60)		39.84±8.94 (min.15−max.59)
Safety climate (5−25) Perceptions of management (7−35)		16.68±4.54 (min.5−max.25)
Stress recognition (5−25)		22.82±16.53 (min.7−max.25)
Working conditions (6−30)		15.28±4.88 (min.5−max.25)
		18.69±3.94 (min.8−max.30)

Table 2. Factors affecting nurses' coronavirus fear levels[a]

		M±SD	p
Age	21-25 years	14.2±5.75	
	26-30 years	17.53±7.37	**0.01***
	31 years or more	16.31±5.7	
Intention to leave the profession	Yes	19.4±8.61	
	No	15.79±5.99	**<0.001**
Chronic health problem	Yes	18.72±7.21	
	No	16.22±6.74	**0.02***
COVID-19 training	Yes	15.88±6.33	
	No	18.82±7.85	**0.003***

[a] Significant difference at p<0.05; value in bold: significant; ANOVA, Students t test

Table 3. Factors affecting nurses' attitudes to patient safety[a]

		Patient Safety Attitude Questionnaire												
		Work Satisfaction		Teamwork climate		Safety climate		Perceptions of management		Stress recognition		Work conditions		Total	
		M±SD	p	M±SD	p	M±SD	p	M±SD	p	M±SD	p	M±SD	p	M±SD	p
Age	21-25 years	30.24±10.11		40.60±9.72		16.33±5.13		22.22±7.52		14.18±4.68		18.13±4.39		141.64±28.39	
	26-30 years	27.51±9.34		38.60±8.93		16.18±4.56		22.22±20.39		15.18±4.91		18.18±3.52		136.24±26.01	
	31 years or more	34.57±8.88	<0.001	42.98±7.40	0.009*	18.51±3.38	0.006*	25.20±5.51	0.53	16.61±4.76	0.04*	20.77±4.15	<0.001	158.59±26.19	<0.001
Total professional experience	1-5 years	27.40±9.41		38.22±8.99		15.75±4.69		21.75±19.85		15.19±4.88		18.15±3.55		134.93±25.94	
	6-10 years	31.00±8.65		41.90±7.84		18.00±3.52		25.51±5.54		14.73±4.57		18.61±4.14		147.75±23.72	
	11 years or more	37.09±8.90	<0.001	44.79±7.92	<0.001	19.30±3.65	<0.001	27.06±4.24	0.23	16.54±5.26	0.23	21.45±4.42	<0.001	166.15±27.00	<0.001
Living alone Yes		29.82±9.52		39.48±8.44		16.91±4.54		24.55±26.78		15.53±4.71		18.35±3.24		141.88±24.47	
No. With family		30.26±9.92		40.72±8.98		16.89±4.51		22.61±6.56		15.38±4.64		19.18±4.13		145.15±28.21	
No. With friend		25.70±9.07	0.03*	37.67±9.66	00.17	15.43±4.58	0.19	19.62±7.28	0.31	14.38±5.98	0.46	17.78±4.56	0.10	129.65±30.59	0.01*
Marital status	Married	32.15±9.16		41.52±8.75		17.70±3.76		23.88±5.71		16.19±4.71		19.60±4.35		151.05±27.49	
	Single	27.97±9.78	0.001*	38.95±8.93	0.03*	16.13±4.83	0.01*	22.25±20.03	0.46	14.80±4.92	0.03*	18.21±3.63	0.008*	136.74±26.72	<0.001
Place of work	Ward	31.53±10.01		41.38±10.53		17.12±5.44		23.78±7.15		14.94±4.69		19.59±3.86		148.01±28.42	
	ICU	27.32±9.34		38.47±7.99		16.10±4.19		21.80±21.17		14.91±4.91		17.92±3.52		134.76±25.30	
	Operating theater	35.44±10.68		45.87±6.91		19.06±3.19		27.00±5.82		17.94±5.36		21.97±5.93		167.75±31.98	
	Other	31.94±6.23	0.001*	38.70±7.66	0.004*	17.23±3.27	0.06	23.06±2.77	0.61	17.29±4.04	0.03*	18.59±3.79	0.001*	146.82±18.69	<0.001
Work status	Clinician/Ward nurse	28.78±9.38		39.39±8.85		16.41±4.50		22.49±17.06		15.17±4.84		18.52±3.94		139.57±26.64	
	Nurse manager	38.06±10.84	<0.001	45.88±8.00	0.004*	20.29±3.51	0.001*	27.18±4.05	0.26	16.82±5.39	0.17	21.00±3.26	0.01*	170.23±27.83	<0.001
Working arrangement	Always day	34.34±10.70		43.16±8.55		18.95±3.49		24.16±6.32		16.05±4.80		19.92±4.38		156.50±27.79	
	Shift (night/day)	28.30±9.22		39.16±8.77		16.27±459		22.58±18.26		15.08±4.92		18.44±3.78		138.55±26.54	
	Always night	31.77±10.61	0.001*	40.31±10.96	0.07	16.08±4.99	0.003*	22.46±7.90	0.86	16.08±4.63	0.44	18.85±4.58	0.10	145.54±33.33	0.001*
Weekly average working hours	≤ 40 hours	31.92±10.18		42.46±8.56		18.23±4.04		26.17±26.01		16.00±4.94		19.16±4.42		151.45±27.77	
	> 40 hours	28.05±9.26	0.003*	38.40±8.84	0.001*	15.82±4.59	<0.001	20.97±6.67	0.01*	14.89±4.82	0.08	18.43±3.64	0.16	136.33±26.37	<0.001
Intention to leave the profession	Yes	25.95±9.78		37.40±9.02		16.55±4.80		18.88±7.40		13.78±5.32		17.05±3.85		129.28±26.83	
	No	30.55±9.50	0.001*	40.63±8.79	0.01*	16.72±4.71	0.80	24.09±18.39	0.03*	15.77±4.64	0.006*	19.22±3.84	<0.001	145.73±26.95	<0.001
Caring for COVID-19 patients	Yes	28.07±9.49		39.35±8.74		16.34±4.59		22.27±18.06		15.06±4.89		18.32±3.56		138.18±25.92	
	No	35.44±8.65	<0.001	42.00±9.56	0.07	18.18±4.02	0.01*	21.22±5.69	0.28	16.27±4.78	0.13	20.31±5.07	0.002*	157.35±30.56	<0.001
COVID-19 training	Yes	30.68±9.71		40.67±9.10		16.95±4.73		24.11±18.82		15.43±4.71		18.98±3.95		145.42±27.90	
	No	26.01±9.11	0.001*	37.59±8.11	0.01*	15.94±3.93	0.12	19.30±6.22	0.04*	14.86±5.33	0.41	17.91±3.85	0.06	131.62±24.99	<0.001
Patient safety training	Yes	30.05±9.48		40.26±8.94		16.77±4.49		23.47±17.19		15.45±4.68		18.83±3.93		143.68±27.18	
	No	24.15±10.64	0.003*	36.31±8.24	0.03*	15.88±5.01	0.34	17.31±7.40	0.07	13.88±6.26	0.12	17.50±3.89	0.10	125.04±27.70	0.001*

[a] Significant difference at p<0.05; value in bold: significant; ANOVA, Students t test

Table 4. Correlation between nurses' COVID-19 fear levels and attitudes to patient safety[a]

	Patient Safety Attitude Questionnaire												
	Work Satisfaction		Teamwork climate		Safety climate		Perceptions of management		Stress recognition		Work conditions		Total	
Fear of COVID-19	r	p	r	p	r	p	r	p	r	p	r	p	r	p
	0.02	0.78	-0.10	0.10	0.04	0.60	-0.04	0.58	-0.18	**0.005**	-0.23	**<0.001**	-0.07	0.27

[a] r: correlation coefficient; using Pearson's correlation analyses

≥11 years (p=0.000), those who were married (p=0.001), those who were nurse managers (p<0.001), those whose mean weekly working hours were ≤40 (p<0.001), those who did not plan to leave the profession (p<0.001), those who were not caring for COVID-19 patients (p<0.001), those who had had training on COVID-19 (p<0.001), and those who had had training on patient safety (p=0.001). Also, mean scores on patient safety attitudes were higher in those who lived with their families compared with those living with friends (p=0.01), in those working in the operating theater compared with those working on the ward or in the intensive care unit (ICU) (p=0.000), and in those permanently working in the daytime compared with those doing shift work (p=0.001) (Table 3).

There was respectively a very weak and a weak negative correlation between the nurses' patient safety attitudes sub-dimensions of stress recognition (r=-0.18) and working conditions (r=-0.23) and their level of fear of coronavirus (Table 4).

Discussion

Nurses have an important role in ensuring patient safety, because they provide care directly to patients.[6] During the pandemic, feelings such as stress, anxiety, depression, fear and exhaustion threatened the maintenance of patient safety practices.[11,24] No studies were found in the literature examining the relation between patient safety attitudes and COVID-19 fear levels in nurses. In this study, nurses' COVID-19 fears and patient safety attitudes during the pandemic were examined. In the literature, it is known that nurses' attitudes to patient safety are positive.[25-28] The results of this study are similar to the literature. However, it is noticeable that the nurses' attitudes to patient safety were not at a high level. A positive patient safety attitude is important to prevent unwanted events from occurring.[29] Health professionals' training on patient safety is an important factor affecting patient safety.[30] Ünver and Yeniğün reported that the attitudes to patient safety of nurses who stated that they had received patient safety training were higher.[28] This study reached similar conclusions.

The study found that the most positive attitude regarding patient safety pertained to teamwork climate and that the most negative attitude pertained to stress recognition. Similar conclusions have been reached in different studies.[27,28,31] Teamwork among health workers is necessary not only to ensure patient safety but also to improve work satisfaction and the stress levels of personnel.[32,33] It is also reported in different studies examining the attitudes of nurses to patient safety that the lowest score was obtained on the sub-dimension of stress recognition.[31,34] Stress recognition is related to the recognition of the effect on work performance of stress factors relating to nurses' work.[21] In this study, it can be said that nurses who were aged 31 or above, were married, worked in the operating theater, and did not intend to leave the profession had better mechanisms to recognize stress in the workplace, and thought that their daily work performance was affected in stressful situations.

Work satisfaction is defined as a person's positivity in relation to their work experience.[21] In this study, work satisfaction had the second highest score in nurses. Labrague et al. found that the coronavirus fear levels of nurses in the Philippines were 19.92±6.15, and that higher COVID-19 fear levels were correlated with increasing psychological stress, lower work satisfaction, decreasing health perceptions and an increasing intention to leave the profession.[6] Baysal et al. found that during the pandemic, the COVID-19 fears of nurses in Turkey, Brazil, Spain and Italy were above average, and that they had a negative effect on the quality of their professional life.[35] Factors such as inadequate personnel and resources, lack of administrative support and teamwork, low job satisfaction and an excessive workload have a negative effect on patient safety.[33] It has been reported in previous studies that nurses working excessive hours had lower scores on patient safety than those working fewer hours.[32,36,37] Similar conclusions were reached in this study.

Hu et al. found that nurses working in China in the city of Wuhan during the COVID-19 outbreak reported a high level of fear (30.41±7.60).[11] In other studies, mean scores of the level of fear of coronavirus in nurses have been found to be 20.23±5.87 and 25.09±7.29.[14,15] Similarly, coronavirus fear levels in health workers have been found to be high.[16,38] In contrast to these findings, the coronavirus fear level of nurses in this study was low (16.67±6.88). Gaining knowledge and experience on the patient group which they encountered during the pandemic may have helped nurses to cope with stress. It is reported in the literature that COVID-19 fear increased in nurses who had not taken part in training on COVID-19.[6,15] Having better information and knowledge to be able to cope in emergency situations can minimize nurses' COVID-19 fears.[39] In this study also,

coronavirus fear levels were high in nurses who had not had training in COVID-19. In addition, coronavirus fear levels were low and the total score of patient safety attitude and the scores on all sub-dimensions except security climate were found to be high in nurses who did not intend to leave the profession compared with ones who did. Nurses who were not content with their work have a greater tendency to make medical errors.[36] Identifying and reporting medical errors is one of the most significant steps toward the adoption of measures to increase patient safety.[40] For this reason, not reporting or under-reporting negative events obstructs measures to ensure patient safety. In the study, a significant proportion of the nurses who had experienced a problem relating to patient safety stated that they had given a false report or had not reported it. In other studies, the proportion of nurses giving false reports was found to be low.[29,30,41]

Study limitations
A limitation of this study is that selection was biased because of the use of a questionnaire. The fact that this study was conducted at a single center was another limitation. Apart from these, the cross-sectional nature of the current study precludes the causal effects. The results obtained did not express the exact causal relationships between the variables.

Conclusion
It was found in this study that the nurses' fear of coronavirus was low and their attitudes to patient safety were positive, but that the correlation between them was not significant. The proportion of nurses who had experienced a problem concerning patient safety and had nevertheless reported it was not at the desired level. It is thought that there is a need for studies to improve the conditions whereby unwanted incidents occur which are a risk to patient safety but which are not reported. Adaptation of nurses working in COVID-19 wards must be ensured, and the necessary training should take place regularly with the benefit of up-to-date information. Nurses' experiencing of physical, social and psychological problems relating to the COVID-19 pandemic should be followed up in the long term. In addition, an assessment should be made of the reflection in health care service of these problems which may occur. Nurses' working hours should be adjusted, taking these problems into account.

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Declarations

Author contributions
Conceptualization, S.E., S.T. and E.D.; Methodology, S.E., S.T. and E.D.; Software, S.E. and S.T.; Validation, S.E. and S.T.; Formal Analysis, S.E. and S.T.; Investigation, S.E., S.T. and E.D.; Resources, S.E., S.T. and E.D.; Data Curation, S.E., S.T. and E.D.; Writing Original Draft Preparation, S.E. and S.T.; Writing – Review & Editing, S.E., S.T. and E.D.; Visualization, S.E., S.T. and E.D.; Supervision, S.E. and S.T.; Project Administration, S.E. and S.T.

Conflicts of interest

Data availability

Ethical approval

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ORIGINAL PAPER

Attitudes towards complementary medicine practices among patients admitted to a primary care unit during the COVID-19 pandemic in Turkey

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ABSTRACT

Introduction and aim. The use of complementary medicine applications is increasing due to the interest of society. We aimed to assess the attitudes, behaviors, and awareness of the patients who applied to a primary care unit towards complementary medicine practices.

Material and methods. This cross-sectional study was conducted with the voluntary participation of 562 individuals who admitted in a primary care unit. The data was collected through face-to-face interviews through a research questionnaire form and the Complementary, Alternative, and Conventional Medicine Attitudes Scale between 20 June-20 August 2021. The sample size was calculated with the OpenEpi v3.01program and statistical analyzes were performed using the SPSS-24 package program.

Results. Their mean age was 39.73±12.95 years and women accounted for 55.5% (n=312) of all participants. The most known complementary medicine methods were phytotherapy, acupuncture, and cup therapy. The information sources of the participants about Complementary Medicine were mostly people around them; only 31.1% of them consulted a doctor. The mean total scale score was 111.45±19.08. Those with chronic diseases, employees, who had COVID-19 disease, high educational status, and those who evaluated their health status as good had more positive attitudes towards complementary medicine practices. A weak negative correlation was found between age and total scale score.

Conclusion. Although positive attitudes towards complementary medicine practices are exhibited, health professionals are consulted at very low rates as a source of information. Health care providers must make arrangements and plans to provide this increasing interest from reliable sources.

Keywords. attitudes, complementary medicine, primary care

Introduction

Complementary medicine (CM) refers to holistic practice. It focuses on health promotion and disease prevention by combining evidence-based and traditional medicine.[1] According to the World Health Organization (WHO), CM is a set of knowledge, skills, and practices based on theories, beliefs, and experiences specific to different cultures, which can or cannot be explained. It is used to diagnose, treat, and prevent physical and mental diseases and promote health.[2]

From the past to the present, many people resort to traditional and CM practices for treating diseases, preventive and cultural purposes. CM preference varies according to country, geographical region, ed-

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ucation level, beliefs, and social and cultural factors.[3] Some people prefer modern medical practices and have a negative attitude towards CM, while others value CM because it is compatible with their health standards and beliefs.[4]

According to the World Health Organization 2019 report, 170 (88%) of the 194 member countries have officially accepted the use of CM through the development of national policies, laws, regulations, and programs.[5] More than 100 million Europeans are currently CM users. One-fifth of these regularly use CM, and the same number of people prefer health services that include CM.[5,6] It has been reported that there are much more CM users in Africa, Asia, Australia, and North America.[7] It has been reported that more than 40% of the population in countries such as the USA, Germany, Switzerland, Cuba, Japan, and Chile; 86% in Korea and 60% in China use CM.[8-9] Studies conducted in Turkey demonstrated that, the use of CM is between 37% and 76%.[10] According to 2018 WHO survey, States in the Western Pacific Region reported that CM was used by 93% of their communities.[5]

In a study conducted in the United States, it was concluded that healthy adults and people with chronic diseases have the potential for additional benefits of CM alongside modern medicine, treatment beyond the scope of modern medicine, the avoidance of unwanted side effects of treatments, and the relatively lower cost of some CM treatments, use CM therapies.[11]

The widespread use of CM in Africa and some developing countries can be attributed to its easy availability.[12] For example, while the ratio of traditional healers to the population in Africa is 1:500, the ratio of medical doctors to the population is 1:40 000.[13] In some countries where the conventional healthcare system is fairly well established, such as Singapore and the Republic of Korea, 76% and 86% of the relevant populations still commonly use CM due to cultural and historical influences.[14] Studies have reported that CM users have positive effects.[15-16]

Positive factors that lead individuals to CM are: The perception that CM is safer and more natural, the feeling of keeping the treatment under control with the active role of the patient, the absence of invasive methods, the treatment practitioners dedicating much time to the patient, a view that is compatible with their lifestyles and belief systems and an effort to seek a holistic approach to medical care.[4]

Other reported factors leading to the use of CM treatments are; inadequate doctor-patient relationship, long waiting time, limited time to be allocated to the patient, dissatisfaction with medical health services, fear of the side effects of medical treatments, rejection of medicine, insecurity, and being desperate for recovery of the disease.[7]

In many countries, modern and CM treatment methods have been applied together for years. The traditions, customs, and beliefs of the society, easy access to CM treatment products, and health needs that cannot be met in modern medicine are among the factors that cause people to turn to CM. In addition, orientation towards the natural, suspicion of current care and treatment methods, fear of possible side effects, cognitive, emotional, and sociocultural characteristics, behaviors, and attitudes are included. Inadequacies in treating cancer and some chronic diseases, helplessness, hopelessness, different expectations, and ignorance psychologically overwhelmed the patients and led them to these practices.[16]

In the United States, it is estimated that between 28 and 94 percent of rheumatic disease patients, and about 90% of cancer patients, have tried CM treatment.[17] In France, patients with chronic musculoskeletal disease accounted for a more significant proportion of visits to CM practitioners.[18] Many patients with multiple sclerosis resort to CM treatments.[18] Based on data from national monitoring of CM services in China, the top five diseases admitted to CM hospitals in 2008 were cerebrovascular disease, slipped intervertebral disc, hemorrhoids, ischemic heart disease, and essential hypertension, and this can be interpreted as people with diseases tend to use CM more.[19]

Determining attitudes and behaviors towards CM practices at individual and social levels will facilitate health care planning.[20]

Aim

This study aimed to evaluate the attitudes, behaviors, and awareness of patients who applied to a primary care unit towards CM practices. Another goal was to investigate whether the participants were intellectually compatible with CM, their dissatisfaction with modern medicine, and their holistic view of health.

Material and methods
Ethical approval
Patients were provided with detailed information about the procedures and they signed written consent forms. The approval of the ethics committee was obtained before initiation of the study (meeting date; 18/06/2021, decision number; 2021/3309). All procedures performed in this study involving human participants were in accordance with the ethical standards specified by the institutional and national research committee and with the Helsinki Declaration and its later amendments or comparable ethical standards.

Study design and participants
In this cross-sectional study volunteers over 18 years old, who applied to a primary care unit for any reason but with no psychiatric disease, were included.

The sample size was calculated with the OpenEpi v3.01 program based on the registered population of 3900 adult people served by the primary care unit, and it was found to be at least 558 with a 5% significance level, 95% confidence interval, and 99% power. To exceed this number, the study was completed with 562 participants.

Data collection

In the socio-demographic characteristics form, the participants' age, gender, marital status, education level, income, chronic diseases, whether they smoked, whether they had the COVID-19 disease, and the COVID-19 vaccination were questioned. In addition, which of the mentioned CM applications they knew, which ones they used and found effective, their information sources, for what purpose they used CM methods, and whether they wanted to receive consultancy on this issue were questioned.

Complementary, Alternative, and Conventional Medicine Attitude Scale (CACMAS): CACMAS was developed to measure how health care recipients' attitudes affect their use of complementary medicine treatment methods. The Turkish validity and reliability study of the CACMAS, which was developed by McFadden et al.[21] in 2010, was conducted by Köse et al.[22] in 2018. CACMAS includes three sub-dimensions: an intellectual view towards CM, dissatisfaction with modern medicine, and a holistic view of health. It is arranged in the form of a seven-point Likert scale. The scale consists of twenty-two positive items, and five (1, 4, 8, 9, 26) are negative statements. Items with negative statements were scored in reverse (7-6-5-4-3-2-1) when analyzing. Increasing subdimensional scores indicate a more positive attitude towards CM (for example, higher scores show more dissatisfaction with modern medicine). The minimum score on the scale is 27, and the maximum score is 189 points. The scale does not have a cut-off value, and people have a positive attitude towards CM as the score increases.

Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Sciences version 24 (IBM, Armonk, NY) software. Participants with missing values in an outcome variable were excluded from any analysis of that variable. Descriptive statistics were expressed as mean, standard deviation, minimum-maximum values, frequency, and percentile. Student's t-test and One-Way ANOVA tests were used to evaluate the relations between scores and socio-demographics. Pearson correlation and Logistic regression analysis were used. A p-value less than 0.05 was considered statistically significant with a 95% confidence level.

Results

A total of 562 participants, 312 women (55.5%) and 250 men (44.5%) were included in the study. The mean age

of the participants was 39.73±12.95 years old. There was no statistically significant difference between the genders in terms of age (p=0.172). Of the participants, 54.4% were married, 55.0% had a chronic disease, and 63.9% had regular health checks even if they had no complaints. The socio-demographic characteristics of the participants are shown in Table 1.

Table 1. The socio-demographic characteristics of the 562 participants who admitted to a primary care unit during the COVID-19 pandemic in Turkey

	n	%
Gender		
Female	312	55.5
Male	250	44.5
Marital Status		
Married	306	54.4
Single	256	45.6
Chronic disease		
Present	253	45
Absent	309	55
Smoking Status		
Smoking	169	30.1
Never smoked	177	31.5
Quitted smoking	216	38.4
Education level		
Illiterate	23	4.1
Primary school	99	17.6
High school	238	42.3
University	135	24.1
Master degree	67	11.9
Income status		
Income less than expenses	154	27.4
Income equal to expenses	304	54.1
Income more than expenses	104	18.5
Working status		
Still working	352	62.6
Not working	210	37.4
Self-Assessment of Health Status		
Perfect	202	35.9
Good	228	40.6
Not bad	98	17.4
Bad	34	6.1
Having Regular Check-Up Examinations		
Yes	359	63.9
No	203	36.1
Having had COVID-19 disease		
Yes	317	56.4
No	245	43.6
COVID-19 vaccination status		
Vaccinated	401	71.4
Unvaccinated	161	28.6
Using CM for COVID-19		
Yes	159	28.3
No	403	71.7

* CM – complementary medicine; COVID-19 – coronavirus disease 19

The rates of participants knowing that the given CM treatment methods exist, believing that the method is effective, and using the method are presented in Table 2.

Table 2. Awareness, beliefs, and experiences of 562 patients applying a primary care unit in Turkey, about CM treatment methods during the COVID-19 pandemic*

	Knew the method exists		Believed that the method is effective		Used the method	
	n	%	n	%	n	%
Acupuncture	407	72.4	330	58.7	67	11.9
Phytotherapy	479	85.2	459	81.7	464	82.6
Hirudotherapy	344	61.2	178	31.7	103	18.3
Ozone Therapy	153	27.2	128	22.8	54	9.6
Cup Therapy	435	77.4	384	68.3	198	35.2
Hypnosis	129	23.0	102	18.1	21	3.7
Homeopathy	55	9.8	40	7.1	32	5.7
Prolotherapy	45	8	45	8	27	4.8
Chiropractic	29	5.2	29	5.2	12	2.1
Osteopathy	44	7.8	44	7.8	19	3.4
Larvae treatment	14	2.5	14	2.5	0	0
Apitherapy	76	13.5	70	12.5	39	6.9
Musicotherapy	112	19.9	96	17.1	49	8.7

* CM – complementary medicine; COVID-19 – coronavirus disease 19; more than one method was ticked

Those who consulted a doctor for information and counseling about CM methods and use, comprised 31.1% of the participants (n=175). Among them, 39.4% (n=69) could not receive counseling because the doctor stated that he/she did not know these methods.

The most common source of information about CM was the people around them, such as friends and relatives, with 75.4% (n=424), secondly television and the internet with 64.4 % (n=362). Only a few, such as 37.9% (n=213), received information from health personnel, and 22.2% (n=125) stated that they obtained information by reading a book about CM methods.

Of the participants, 73.8% thought traditional and CM practices were reliable due to no side effects or complications. The reasons why they use these methods are shown in Table 3. Participants who experienced CM treatment methods were more likely to believe that the method was effective (p=0.005 for acupuncture, p=0.001 for phytotherapy, and p=0.001 for cupping therapy).

The total and sub-dimensional scores of the CACMAS are listed in Table 4. CACMAS total and subdimensional scores were compared with marital status, and no statistically significant difference was found (p=0.910, p=0.235, p=0.517, p=0.473, respectively). The scores of the employees were found to be higher than those who were not working (p=0.001). Total scale scores and holistic view of health sub-dimension scores of those with any chronic disease were higher than those with no chronic disease (p=0.001, p=0.003, respectively).

Table 3. Indications of CM treatment methods in 562 participants in a primary care unit during the COVID-19 pandemic in Turkey*

	n	%
To relieve muscle and joint pain	87	15.5
Lose weight	62	11
According to religious belief	166	29.5
Common cold	445	79.2
Skin defects	69	12.3
Physician advice	59	10.5
No other option for treatment	49	8.7
For a rapid recovery	391	69.6
Failure to benefit from medications	142	25.3
Having side effects of drugs	122	21.7
To prevent disease progression	68	12.1

* CM – complementary medicine; COVID-19 – coronavirus disease 19; more than one method was ticked

Table 4. Total and sub-dimensional CACMAS scores of the 562 participants who admitted to a primary care unit during the COVID-19 pandemic in Turkey*

CACMAS	Mean ± SD
Intellectual Perspective on Complementary Medicine	30.91±7.79
Dissatisfaction with Modern Medicine	40.16±9.05
Holistic View of Health	40.37±8.28
Total score	111.45±19.08

* SD – standard deviation; CACMAS – Complementary, Alternative, and Conventional Medicine Attitudes Scale; COVID-19 – coronavirus disease 19

Total scores of the CACMAS of those who had COVID-19 disease and those who used CM methods to protect or treat COVID-19 were found to be statistically significantly higher (p=0.048, p=0.010, respectively). There was no significant relationship between the scores obtained from the scale and whether or not to have the COVID-19 vaccine. Table 5 shows the comparison of the scores obtained from the scale with related conditions.

A statistically significant relationship was found between the education level of the participants and the total and sub-dimension scores of the CACMAS (p<0.001). A strong positive correlation was found between education level and total scale score (Pearson correlation coefficient: 0.751, p<0.001). It was determined that those with master's degrees received higher scores on the scale.

Income status had no effect on the total scale score and sub-dimension scores (p>0.05). According to the health status self-assessment scale, those who evaluated their health status as bad, had statistically significantly lower scores from the CACMAS scale (p=0.003, p=0.018, p=0.002, p=0.002, respectively).

The intellectual perspective on CM and dissatisfaction with modern medicine sub-dimension scores of those who have never smoked were significantly lower

Tablo 5. Relation of CACMAS scores and some socio-demographic characteristics in 562 participants admitting to a primary care unit during the COVID-19 pandemic in Turkey*

	Intellectual Perspective on Complementary Medicine Score (mean±SD)	p	Dissatisfaction with Modern Medicine Score (mean±SD)	p	Holistic View of Health Score (mean±SD)	p	Total score (mean±SD)	p
Gender								
Female	30.34±7.24	0.056	39.36±8.18	0.018	40.48±8.40	0.721	110.19±17.97	0.082
Male	31.61±8.39		41.17±9.95		40.23±8.14		113.02±20.30	
Working Status								
Working	31.88±8.30	0.001	41.59±8.98	0.001	41.05±8.63	0.012	114.52±19.83	0.001
Not working	29.28±6.56		37.77±8.67		39.23±7.54		106.30±16.55	
Chronic disease								
Present	31.85±8.57	0.009	40.30±8.84	0.739	42.11±8.56	0.001	114.28±20.15	0.001
Absent	30.13±7.01		40.05±9.23		38.94±7.76		109.13±17.85	
Having regular check-ups								
Yes	31.16±7.93	0.306	40.11±9.29	0.869	40.91±8.41	0.041	112.19±19.20	0.221
No	30.46±7.53		40.25±8.62		39.42±7.96		110.14±18.82	
Having had the COVID-19 disease								
Yes	31.35±7.71	0.126	40.54±8.72	0.256	40.89±7.98	0.090	112.79±18.56	0.048
No	30.33±7.88		39.67±9.45		39.70±8.62		109.71±19.62	
Having had the COVID-19 vaccine								
Vaccinated	30.92±7.97	0.936	40.20±8.68	0.862	40.55±8.55	0.415	111.69±19.68	0.639
Unvaccinated	30.86±7.36		40.06±9.94		39.92±7.55		110.85±17.53	
Using CAM methods for COVID-19								
Yes	31.38±7.84	0.367	41.03±9.55	0.155	42.35±8.61	0.001	114.76±20.46	0.010
No	30.72±7.77		39.82±8.83		39.59±8.02		110.14±18.36	

* SD – standard deviation; CM – complementary medicine; COVID-19 – coronavirus disease 19; CACMAS – Complementary, Alternative, and Conventional Medicine Attitudes Scale

than those who smoked and quitted (p<0.001, p<0.001, respectively). The Holistic View of Health sub-dimension scores and total scale scores of current smokers were higher than non-smokers (p=0.026, p<0.001, respectively). There was a weak negative correlation between age and total scale score (Pearson correlation coefficient: -0.187, p=0.001).

Discussion

This study focuses on the participants' awareness of the CM methods and attitudes towards CM practices among patients presenting to a primary care unit for any reason. Most of the participants (85%) had heard about at least one CM method and had positive attitudes towards it. Nearly three-quarters stated that CM information sources were the people around them. Only a quarter had obtained information from a healthcare professional. Those with chronic diseases, those who recovered from COVID-19 disease, and employees had more positive attitudes towards CM methods.

In the present study gender did not have any significant effect on CACMAS scores. Studies show that women use CM methods more than men.[21-23] It has been reported that low income, low education level, poor health status, depressed mood, and presence of chronic disease are associated with a positive attitude towards CM in women.[24-25] It has been reported that female medical students thought physicians should have knowledge about traditional and CM methods at a higher rate than males and had more positive attitudes towards CM methods.[26]

It has been found that traditional medicine users are older than those who prefer modern medicine, religion is more important in their lives, and their economic situation and health are worse.[27] The current study presented a weak negative correlation between age and total scale score. The American population aged between 45-54 years old had significantly increased trust and belief in CM. However, it has been found that confidence in some CM methods is lower in the elderly because of being unfamiliar with their culture.[28-29]

Among the participants who consulted a doctor, more than one-third stated that the doctor did not know these methods, and this may be because CM methods are still not included in the medical school curriculum. It was found that first-year medical school students were more willing to receive CM training than third-year students. The education received in medical faculties affects physicians' attitudes towards CM.[30]

Lack of communication between physicians and patients about CM practices has been emphasized in many studies.[31] Similarly, in the present study, the information sources of the participants about CM are mostly the experiences of the people around them. The rate of getting information from a health professional on this subject

is only one-third. In a study conducted with cancer patients, patients with pain due to chronic inflammatory diseases, and chronic dialysis patients, it was found that patients and healthcare professionals did not have sufficient information about CM methods, and better communication is needed.[32-33] Physicians who evaluate the individual as a whole with all organ systems need to increase their level of knowledge in order to direct their patients to accurate and reliable information about these methods, which are becoming increasingly popular.[34]

Traditional medicine methods and public health services are expected to be evidence-based. However, scientific explanations and evidence were less important for the personal use of these methods. There are insufficiently illuminated gaps in understanding risk and individual and structural perspectives between CM users and medical practitioners, which may cause health risks and uncertainties associated with CM. It affects communication between doctors and CM users and may adversely affect CM users' access to community health services.[35] Knowing the indications for CM and guiding the patients in their decisions will accelerate the access to the correct information and the recovery.[36] Despite their limited knowledge, the rate of those who find the CM methods safe was about 78%, which is high. This can be considered as the limited side effects of the methods and the fact that the users have achieved targeted benefits.

Total scores of the CACMAS of those who had the COVID-19 disease and those who used CM methods to prevent or treat COVID-19 were higher. COVID-19 epidemic, which can be fatal, and prognosis, residual effects are unpredictable besides substandard treatment, increased the sense of uncertainty in people. High scores may be associated with this.

Having a healthy lifestyle or evaluating the state of health as good and religious and ideological views are closely related to the use of CM and belief in CM. Personal factors and satisfaction with traditional medicine do not affect the level of belief in CM.[24] In the current study, approximately 30% of participants preferred CM methods due to their religious beliefs. The total scores and holistic view of health sub-dimension scores of those who used a CM method for any reason were higher. It was determined that as the education level increased, positive attitudes towards CM increased, and those who evaluated their health status as good got higher scores on the scale. In addition, those with higher education levels had higher total and sub-dimension scores and more information about CM methods. Although some CM methods require high cost, no relationship was found between income levels and scale scores.

Side effects of medical treatments, fatigue, and joint pain are the most common reasons for CM use.[32] Pain, depression, and fatigue are the most common reasons

for CM use and the symptoms that most benefit from CM methods.[37]

The most common reason for using CM was to get rid of cold and flu, with a high rate of 80% in the present study. The rate of those who stated that they used CM methods because they had a disease that there was no other remedy with modern medical methods was approximately 1/10. Only ten percent of the participants used the CM method under the advice of a physician. Two third of the participants experienced these methods to achieve a rapid recovery, and one-fourth preferred them as they did not benefit from the drugs recommended by modern medical methods.

The holistic view of the health sub-dimension scores of those with any chronic disease was higher, and this can be since those with chronic diseases tend to search more on CM methods.

In a study among physicians, the most known CM methods were acupuncture, aloe vera, and high-dose vitamin C.[36] In a palliative care center, it was found that there was a very high prevalence of CM use, and the most frequently used methods were aromatherapy, homeopathy, and vitamins, respectively.[38] Cancer patients' most frequently used CM methods were acupuncture, homeopathy, herbal medicine, and traditional Chinese medicine.[39] The present study found that homeopathy is among the least known and used CM methods. In a study conducted with nurses, the most well-known methods were massage, herbal therapy, acupuncture, and prayer therapy. The least known methods were osteopathy and homeopathy.[40] Similarly, in the present study, the most known methods were phytotherapy, cupping, and acupuncture, while the least known were larval treatment, chiropractic, and osteopathy, followed by homeopathy.

In a study conducted in Turkey, it was reported that visual analog scale scores decreased after the use of CM for pain complaints.[15] In this study, the participants who used the mentioned methods believed that the method was highly effective.

This study has some limitations. The fact that the participation was voluntary may have resulted in more participation from those interested in CM. Those who were previously diagnosed with a psychiatric illness were not included in the study as they might have difficulty filling out the questionnaire and scale used in the study. Considering that these patients may have a great interest in CM, it may have caused a lack of results. Further studies can be planned by developing appropriate scales for these patients. As another limitation, there may be some CM practices that participants do not remember or treatments they do not consider as CM.

Conclusion

There is an increasing interest in CM treatment methods in society. Being older, female, having chronic diseas-es, having a high level of education, and having diseases with inadequate treatment methods have a higher positive effect on attitudes towards CM. Patients' awareness and information resources are insufficient. There is a need for new informative approaches targeting interested patients and physicians to access accurate information from reliable sources. The increasing belief and demand for CM should be taken into account.

Declarations

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ORIGINAL PAPER

Salivary tumour necrosis factor-alpha and receptor for advanced glycation end products as prognostic and predictive markers for recurrence in oral squamous cell carcinoma – a pilot study

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ABSTRACT

Introduction and aim. Tumour necrosis factor-alpha (TNF-α) belongs to the cytokine family TNF/TNFR. As a multifunctional cytokine, TNF-α plays a significant role in diverse and a variety of cellular events such as cell survival, proliferation, differentiation, and death. As a pro-inflammatory cytokine, TNF-α acts as a bridge between inflammation and carcinogenesis. Receptor for advanced glycation end products (RAGE) are cellular receptors belonging to the immunoglobulin superfamily. As one of the primary mediators of innate immunity, acute and chronic inflammatory disorders, and certain cancers, RAGE signaling plays an important role. The aim of the present study is to analyze the prognostic significance of salivary TNF-α and RAGE in oral squamous cell carcinoma.

Material and methods. A study was conducted testing saliva samples collected from ten patients with well-differentiated and moderately differentiated oral squamous cell carcinomas. To determine the levels of TNF-α and RAGE in unstimulated saliva from patients, an ELISA kit from RAY BIOTECH was used for the study, and the readings were read at 450 nm. Statistical analysis was performed using SPSS software. Version 23 of SPSS was used to plot the standard curve. Statistical comparisons were done using Mann-Whitney U test and ROC analysis.

Results. Salivary TNF-α and RAGE in patients were considered to be induced by radiotherapy at a higher level in moderately differentiated squamous cell carcinoma when compared to well differentiated squamous cell carcinoma. Thus, there is an increase in the induced Salivary TNF-α and RAGE levels by radiotherapy with increase in the histological stages of oral squamous cell carcinoma. The statistical analysis also proved the same.

Conclusion. Hence salivary TNF-α and RAGE may be used as a biomarker for oral cancer to predict the prognosis.

Keywords. biomarker, oral squamous cell carcinoma, prognosis, RAGE, saliva, TNF-α

Introduction

Oral squamous cell carcinoma (OSCC) is a malignant tumor that is located in the upper part of the digestive system. 54010 new cases of oropharyngeal cancer were recorded in 2021 based on cancer statistics. Most of these cancers arise in the mouth, on the tongue, in the tonsils and oropharynx, in the gums, on the floor of the mouth, and elsewhere in the oral cavity.[1] They can be found in the lips and minor salivary glands of the oral cavity. Most people diagnosed with these cancers are older than 63,

though some younger people can also be affected. About 20% of cases occur in people younger than 40. Whites are slightly more likely to be affected than Blacks. According to the National Cancer Institute, the lifetime risk of developing oral cavity and oropharyngeal cancer is about 1 in 60 (1.7%) for men and 1 in 140 (0.71%) for women. The risk of mouth and throat cancer is also influenced by a number of other factor.[2] During the past 20 years, the overall rate of new cases of oral cavity and oral squamous cell carcinoma has only slightly increased. Yet during this same time, both women and men have seen an increase in OSCC associated with human papillomavirus (HPV) infection. Over the last 30 years, these cancers have experienced a decline in death rates.[3]

The assay for salivary biomarkers has developed tremendously in recent years. Many omics technologies, including proteomics and transcriptomics, have been applied to analyze salivary constituents.[4] Among the newer additions to the omics field, metabolomics examines biofluids, cells, and tissues for metabolites. Molecular substances such as these are substrates, intermediates, or products of biochemical reactions.[5] Cells, tissues, and organisms respond physiologically or pathologically to such molecules depending on their genetic properties and environmental exposure.[6]

Tumour necrosis factor – alpha (TNF-α) is a member of the TNF/TNFR cytokine family.[7] TNF-α is mainly involved in the maintenance of the source and homeostasis of the basic immune system, inflammation, and host defense.[8] It is a type II transmembrane protein with an intracellular N terminus. It has a particular signaling potential both as a membrane-integrated protein and as a soluble cytokine released after proteolytic cleavage.[9-11] TNF-α has regulatory soluble functions on various crucial physiological processes such as synaptic plasticity, learning and memory, and astrocyte-induced total synaptic strengthening TNF-α is a multifunctional cytokine that plays an important role in diverse and a variety of cellular events such as cell survival, proliferation, differentiation, and death.[12,13] As a particular pro-inflammatory cytokine family, Tumor necrosis factor is secreted by the inflammatory cells, which may be involved in inflammation and leads to carcinogenesis.

As a major proinflammatory cytokine, TNF-α is able to act as an endogenous tumor promoter to bridge inflammation and carcinogenesis. In regard to cancer, TNF-α plays a double role.[14] On one hand, TNF-α could be an endogenous tumor promoter, because TNF stimulates cancer cells' growth, proliferation, invasion and metastasis, and tumor angiogenesis. On the other hand, TNF-α could be a cancer killer.[15] The property of TNF-α in inducing cancer cell death makes it potential cancer therapeutic, although much effort is needed to decrease its toxicity for systematic TNF-α administration. It is also aimed at sensitizing cancer cells to TNF-α induced apoptosis by inhibiting survival signals such as NF-κB, by combined therapy.[16]

Receptor for advanced glycation end-products (RAGE) is a cell surface receptor that belongs to the immunoglobulin (Ig) superfamily. RAGE signaling plays a central role within the inflammatory response, mediating aspects of innate immunity, acute and chronic inflammatory conditions, and certain cancers.[17-20] Activated RAGE recruits extracellular signal-regulated kinase–1 and –2 (ERK-1/2), leading to downstream activation of NF-κB via the MAP kinase pathway.[21]

The human RAGE gene has a short 3' UTR, eleven exons, and a 5' flanking region that regulates its transcription. It is found on chromosome 6 in the class III area of the major histocompatibility complex. RAGE mediates physiological and pathological consequences by interacting with a number of ligands, each of which is linked to a specific illness.[22] RAGE plays multiple roles in cancer cells; RAGE-HMGB1 interaction stimulates tumor invasiveness and growth.[20] Moreover, HMGB1-DNA complexes trigger the association of RAGE with Toll-like receptor 9, which is important for the immune reaction to pathogens.[18] The whole saliva represents a posh balance among local and systemic sources. This enables the appliance of saliva within the diagnosis not just for exocrine gland disorders but also for oral diseases and systemic conditions. The role of saliva as a diagnostic tool in detecting oral squamous cell carcinoma is currently under research, the prognostic value of RAGEs in head and neck cancers looks promising.

The role of TNF- α and RAGEs in head and neck cancers and their prognostic use is now being studied off-lately, but the expression of RAGEs within the saliva in post-radiation therapy is not yet elucidated as immediate cellular damage after irradiation is supposed to result in cytokine-mediated multicellular interactions with induction and progression of inflammatory and fibrotic tissue reactions.

Aim

Thus, this study aims is to analyze the prognostic significance of radiation induced salivary TNF- α and RAGE in oral squamous cell carcinoma with the following objectives: i) to elucidate the expression and levels of TNF-α and RAGE expression within the salivary samples of post-radiation oral squamous cell carcinoma of various stages, ii) to determine if levels increase with increasing OSCC histological stages after radiotherapy treatment, and iii) to know the efficacy of the TNF-α and RAGE as salivary biomarkers in OSCC.

Material and methods
Ethical approval
Prior to the initiation of the study, clearance was obtained by the Scientific Review Board with the Ethical approval number IHEC/SDC/BDS/1977/01.

Study design

An *in vivo* comparative study was conducted on saliva samples from randomly selected ten (n=10) patients who presented with oral squamous cell carcinomas, well-differentiated (5) and moderately differentiated (5), at the tertiary cancer care center for radiotherapy over a period of 3months from February to May 2021. A saliva sample was collected and tested after the patient underwent five rounds of regular radiotherapy treatment. The study was non-invasive and easy to perform without much inconvenience to patients. However, the sample size was limited, the test validation was done by an expert pathologist.

Criteria for selection of study subjects

Patients who were diagnosed to have well-differentiated and moderately differentiated oral squamous cell carcinoma, had completed all five cycles of radiotherapy treatment for the same were included in the study. As the objective of the study includes the histological staging alone, the clinical staging was not included in the present study . It was also ensured that patients with systemic comorbidities or terminally ill patients were not taken up for the study. The patients who developed radiation induced mucosal changes were excluded. As the expression of salivary TNF-α and RAGE (Ghrelin) in normal healthy individuals stated in the previous studies as at a non-detectable amount, we excluded the healthy controls from our study.[23]

All the subjects included in the study belonged to the same ethnic group. Informed consent was obtained from the study subjects for inclusion in the study and it was also ensured that the subject's anonymity was maintained. All the participants completed a questionnaire covering medical, residential, and occupational history.

Sample collection

10 saliva samples were collected from patients with post-radiotherapy oral cancer. Unstimulated saliva from the patients was collected in Eppendorfs for a volume of 1ml. Then it was stored at -20ºC. During the procedure, it was thawed and centrifuged. Samples were collected during the timeframe of 4 months in the year 2021.

Principle of the test

ELISA is based on the competitive binding technique in which the TNF-α and RAGE (Ghrelin) present in the sample competes with a fixed amount of horseradish peroxide (HRP)-labeled TNF-α and RAGE (Ghrelin) on a human monoclonal antibody. Standards and samples are pipetted into the wells and TNF-α and RAGE (Ghrelin) present in a sample is bound to the wells by the immobilized antibody. The wells are washed and a biotinylated anti-human TNF-α and RAGE (Ghrelin) antibody was added.

After washing away unbound biotinylated antibody, HRP conjugated streptavidin is pipetted into the wells. The wells were washed again, a TMB substrate solution is added to the wells and color develops in proportion to the amount of TNF-α and RAGE (Ghrelin) bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.

Reagent preparation

All reagents and samples were brought to room temperature (18-25°C) before use. Also, Assay Diluent B (Item E) should be diluted to 5-fold with deionized or distilled water before use. For dilution of samples, Assay Diluent A (Item D) should be used for dilution of serum and plasma samples. The suggested dilution for normal serum/plasma is 2 – 20 fold. For the preparation of the standard, a vial of Item C was briefly spun. 400 µL of Assay Diluent A (for serum/ plasma samples) was added to Item C vial to prepare the 50ng/ml standard. The powder was dissolved thoroughly by a gentle mix.

15 µL TNF-α and RAGE (Ghrelin) standard (50 ng/ml) was added from the vial of Item C, into a tube with 485 µL Assay Diluent A or 1X Assay Diluent B to prepare a 1,500 pg/ml standard solution. 400 µL Assay Diluent A or 1X Assay Diluent B was pipetted into each tube. 1,500 pg/ml standard solution was used to produce a dilution series (shown below). Each tube was mixed thoroughly before the next transfer. Assay Diluent A or 1X Assay Diluent B served as the zero standards (0 pg/ml). If the Wash Concentrate (20X) (Item B) contained visible crystals, it was warmed to room temperature and mixed gently until they dissolved.

20 ml of Wash Buffer Concentrate was diluted into deionized or distilled water to yield 400 ml of 1X Wash Buffer. Detection Antibody vial (Item F) was briefly spun before use. 100 µL of 1X Assay Diluent B (Item E) was added to the vial to prepare a detection antibody concentrate. This was then pipetted up and down to mix gently (the concentrate can be stored at 4°C for 5 days). The detection antibody concentrate should be diluted 80-fold with 1X Assay Diluent B (Item E) and used in relevant prior steps. The HRP-Streptavidin concentrate vial (Item G) was briefly spun and pipetted up and down to mix gently before use, as precipitates may form during storage. HRP-Streptavidin concentrate should be diluted 200-fold with 1X Assay Diluent B (Item E).

Assay procedure

All reagents and samples were brought to room temperature (18-25°C) before use. It is recommended that all standards and samples be run at least in duplicate. Removable 8-well strips were labeled as appropriate for the experiment. 100 µL of each standard and sample was added to appropriate wells. These wells were then covered and incubated for 2.5 hours at room tempera-

ture with gentle shaking. The solution was discarded and washed 4 times with 1X wash solution. Each well was filled and washed with Wash Buffer (300 μl) using a Pipette. Complete removal of the liquid at each step is essential for good performance. After the last wash, any remaining Wash Buffer was removed by aspirating or decanting. The plate was inverted and blotted with clean paper towels. 100 μl of 1X prepared biotinylated antibody was added to each well. This was then incubated for 1 hour with gentle shaking. The solution was discarded and the wash was repeated. 100 μL of prepared Streptavidin solution was added to each well. This was then incubated for 45 minutes at room temperature with gentle shaking. The solution was discarded and the wash was repeated. 100 μL of TMB One-Step Substrate Reagent (Item H) was added to each well and incubated for 30 minutes at room temperature in the dark with gentle shaking. 50 μl of Stop Solution (Item I) was added to each well and read at 450 nm immediately.

Calculation of results

The mean absorbance was calculated for each set of duplicate standards, controls, and samples, and the average zero standard optical density was subtracted. The standard curve was plotted using SPSS software version 23, with standard concentration on the x-axis and absorbance on the y- axis. The best-fit straight line was drawn through the standard points.

Sensitivity

The minimum detectable dose of Human TNF-α and RAGE (Ghrelin) was determined to be 3 pg/ml. The minimum detectable dose is defined as the analyte concentration resulting in an absorbance that is 2 standard deviations higher than that of the blank (diluents buffer).

Statistical analysis

Statistical analysis was performed using SPSS software (IBM, Armonk, New York, United States). As the same size was less than 30, Mann-Whitney U test was performed to compare the results. Dependent variables include oral cancer while independent variables include age and sex.

Result

Demographic data

The study included a total of n=10 participants who were divided into two groups. Group I consists of well differentiated squamous cell carcinoma (WDSCC) n=5 and Group II consists of moderately differentiated squamous cell carcinoma (MDSCC) n=5,

Statistics

Mann-Whitney U test was performed to compare the results and $p<0.05$ was considered significant and $p>0.05$

was considered not significant. Also ROC curve analysis was done with significance level <0.05.

Salivary TNF-α in patients with WDSCC

Among the 5 samples analyzed, all 5 showed the presence of TNF-α in salivary samples. The mean value of salivary TNF-α among Group I was 7.87±0.26 pg/dl with a median of 8 pg/dl.

Salivary TNF-α in patients with MDSCC

Among the 5 samples analyzed, all 5 showed the presence of TNF-α in salivary samples. The mean value of salivary TNF-α among Group II was 12.81±0.724 pg/dl with a median of 12.42 pg/dl.

Salivary RAGE (Ghrelin) in patients with WDSCC

Among the 5 samples of the saliva of patients with WDSCC analyzed, all 5 samples showed the presence of RAGE in the tissue samples. The mean value of RAGE among the WDSCC group a mean value of 0.684±0.296 pg.dl with a median of 0.83 pg/dl.

Salivary RAGE (Ghrelin) in patients with MDSCC

Among the 5 samples analyzed, all 5 samples showed the presence of RAGE in the tissue samples. The mean value of RAGE among the MDSCC group was 1.32±0.258 pg/dl with a median of 1.3 pg/dl.

Comparison of TNF-α and RAGE (Ghrelin) among salivary samples of WDSCC and MDSCC

The comparison between the groups shows statistical significance. A higher salivary TNF-α and RAGE (Ghrelin) was found among MDSCC patients when compared to those of WDSCC patients.

Table 1. Table showing the mean of the significance of the observations at the levels of p<0.05

Salivary Markers	Group	Mean (pg/dl)	Std. Deviation (pg/dl)	Median (pg/dl)	Quartiles (Q1,Q3) (pg/dl)	p value (Non parametric analysis and ROC)
TNF-α (pg/dl)	WDSCC	7.878	0.265	8	7.66,8.03	0.009
	MDSCC	12.81	0.72	12.42	12.31,13.50	(<0.05)
RAGE (Ghrelin) (pg/dl)	WDSCC	0.684	0.296	.83	0.40,0.895	0.009 (<0.05)
	MDSCC	1.32	0.258	1.3	1.1,1.55	

Both TNF-α and RAGE (Ghrelin) levels were high in patients with MDSCC.

The median value of TNF-α in well differentiated carcinoma (8 pg/dl) were significantly lower than the values of moderately differentiated carcinoma (12.42 pg/dl).
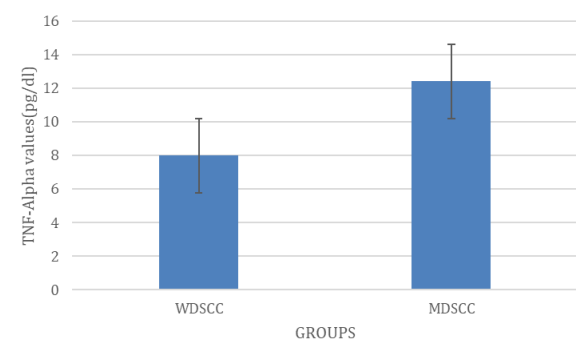
Fig. 1. Bar graph showing salivary TNF-α levels in WDSCC and MDSCC. The X-axis represents the type of squamous cell carcinoma and Y-axis represents concentrations of salivary TNF-α.
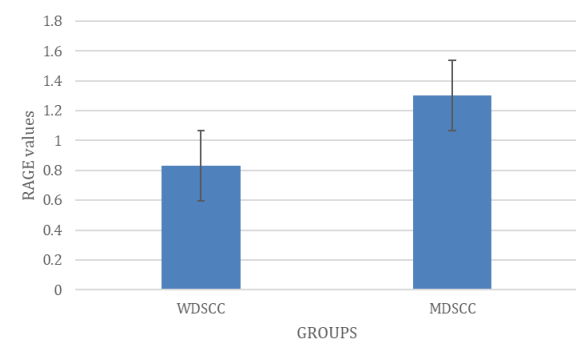
Fig. 2. The bar chart shows the median concentration of RAGE in WDSCC samples was 0.83 pg/dl and in MDSCC was 1.3 pg/dl. The graph indicates that there is a significant increase in the salivary levels of RAGE in MDSCC when compared to WDSCC (p<0.05).

Discussion

According to our study, the majority of OSCC patients (50%) are between the ages of 41-50 years, 30% are between the ages of 51-60 years, and 20% are between the ages of 30- 40 years. It was found that the male study population (70%) was higher than the female study population (30%). On comparing the TNF-α and RAGE (Ghrelin) levels in saliva of the patients with squamous cell carcinoma, it was found that both TNF-α and RAGE (Ghrelin) were induced by radiation at high levels with increasing the stage of OSCC. The difference was found to be significant at p<0.05.

Salivary TNF-α is a proinflammatory cytokine that is released by macrophages. This type of pro-inflammatory cytokine has a special role in the regulation of the immune response and has a prognostic significance. Enhanced expression of salivary TNF-α has been found in patients with rheumatoid arthritis, chain smokers, and chronic obstructive pulmonary disease.[11] And also, most of the salivary cytokines are majorly correlated with the periodontal status and oral inflammatory issues in recent days.[24] On the other hand, neoplastic lesions also influence salivary inflammatory cytokine in their advanced stages. Mostly the anti-tumor therapy may lead to changes like tumor lysis and that leads to an increase in the salivary cytokines levels.[25]

Salivary diagnostics is a dynamic and emerging research field of molecular technology and diagnostics to aid in the diagnosis of oral and other systemic diseases. Saliva sample collection is easily done and is stored for the early detection of diseases as it contains specific biological markers.[23,26] These days, the saliva sample collection is majorly preferred as it is easily taken without any invasive procedures, and the need for a blood draw would become unnecessary. TNF-α also activates the transcription of NF-κB factor, which helps in the stimulation of cell proliferation, and also mainly increases the secretion of inflammatory cytokines.[27] TNF-α is usually not detectable in normal healthy individuals but increases in the serum and tissue levels are found in inflammatory and infectious conditions and the serum levels well correlate with the severity of the infection. A recent survey done, reported that the medical professionals accepted that screening for medical conditions is important and they were ready to participate when the sample collection was saliva rather than a finger price.[28-30]

In recent days, higher levels of baseline TNF-α is associated with more aggressive behavior of disease and poor survival in patients with immunotherapy resistance.[31] A lower TNF-α in saliva may be related to significantly higher TNF-α levels in serum in a similar group of acute lymphoblastic leukemia children. In the present study, the salivary TNF-α levels were induced at higher rate due to radiation in the case of MDSCC. In contrast to the present study, the study conducted by Deepti G et al., the author concludes that the salivary TNF-α levels were majorly increased in the oral leukoplakia and OSCC as the OSCC patients were majorly having tobacco/smoking habits.[32] In the previous studies done, there was a significant correlation between IL-lβ and sTNF-RI. The mean values of SCC were also elevated as a whole at a particular concentration.[28] IL-6 and TNF-α were considered to be the most sensitive parameters in the early stages of cancer and may be used as an additional biomarker in oral cancer.[33]

Saliva samples of breast cancer patients concluded that when comparing low-stage samples with high-stage cancerous tissues, our results demonstrated that the expression levels of RAGE, at the mRNA and protein levels, were significantly increased in the high-stage samples compared to the low-stage samples explained that two factors are important in progression: inflammation and angiogenesis and RAGE have a significant role in these processes and they found and concluded that mRNA level of RAGE was significantly higher in cancerous tissues compared with that of normal tissues and they also used immunohistochemistry analysis

to confirm and validate of the increased expression of RAGE.[34,35] A certain article explained that RAGEs help regulate cell migration.[36] Few more articles have explained RAGE overexpression of colon cancer, and also the cancer of the stomach, mouth, and tongue through several characteristics like metastasis, invasion, and angiogenesis.[37]

In the present study, the levels of TNF-α and RAGE (Ghrelin) was induced at higher rate due to radiotherapy in MDSCC. As the sample were collected from the cancer patients who attended regularly for all the cycles of the radiotherapy and the expression of salivary TNF-α and RAGE (Ghrelin) in normal healthy individuals stated in the previous studies as at a non-detectable amount, we had a limited sample size and excluded healthy controls respectively.[23] Also, other limitations include systemic illnesses and their treatments among the study population were not taken into account, i.e, whether they take insulin tablets or shots or any other mode of treatment. With further research and a greater sample size, salivary TNF-α and RAGE (Ghrelin) can be used as very good biomarkers of oral cancer.

Conclusion

According to our study, the majority of OSCC patients (50%) are between the ages of 41-50 years, 30% are between the ages of 51-60 years, and 20% are between the ages of 30- 40 years. It was found that the male study population (70%) was higher than the female study population (30%). Within the limitations of the present study, Salivary TNF-α and RAGE in patients were considered to be induced by radiotherapy at a higher level in MDSCC when compared to WDSCC. Thus, there is an increase in the induced Salivary TNF-α and RAGE levels by radiotherapy with increase in the histological stages of OSCC. This difference was found to be significant and this study further insists that analysis of salivary TNF-α and RAGE (Ghrelin) is very useful for the prediction of prognosis and recurrence rate among patients treated for OSCC.

Declarations

Author contributions
Conceptualization, M.P.B. and S.R.S.; Methodology, M.P.B.; Software, M.P.B.; Validation, M.P.B., S.R.S. and N.R.; Formal Analysis, M.P.B.; Investigation, M.P.B.; Resources, M.P.B. and N.R.; Data Curation, M.P.B.; Writing – Original Draft Preparation, M.P.B.; Writing – Review & Editing, M.P.B.; Visualization, M.P.B.; Supervision, M.P.B.; Project Administration, M.P.B.; Funding Acquisition, N.R.

Data availability
Datasets analyzed during the current study are available from the corresponding author after submitting a reasonable request.

Ethics approval
Prior to the initiation of the study, clearance was obtained by the Scientific Review Board with the Ethical approval number IHEC/SDC/BDS/1977/01.

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ORIGINAL PAPER

The relationship between the professional values and care behaviors of surgical nurses in Turkey

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ABSTRACT

Introduction and aim. The professional values and the care behaviors of surgical nurse's play an important role in providing quality patient care and increasing patient satisfaction. The aim of this study was evaluated the relationship between the professional values and care behaviors of surgical nurses.

Material and methods. This descriptive and correlational study consisted of 245 surgical nurses working in Turkey. Data were collected using a Personal Information Form, the Nurses Professional Values Scale, and the Caring Behaviors Inventory-24.

Results. The NPVS total mean score of the surgical nurses was 118.26. The CBI-24 total mean score of the surgical nurses was 5.25. The nurses obtained high mean scores in both the Nurses Professional Values Scale and subscales, and the Caring Behavior Inventory-24 and subscales. A weak, positive and significant relationship was found between the professional values and care behaviors of the surgical nurses (p<0.001). Both the individual and professional characteristics of the surgical nurses affected their perceptions of professional values and care behaviors (p<0.05).

Conclusion. The study showed that they had high perceptions of professional values and caring behaviors and adopted at most human dignity from professional values. Personal and organizational activities should be carried out to improve the professional values of surgical nurses and have them internalize care behaviors.

Keywords. care behaviors, professional values, surgical nursing

Introduction

Nursing is an integral part of healthcare, contributing to both protection and improvement of the health of healthy, sick and disabled individuals of all ages, families and societies, as well as finding solutions for their health issues.[1,2] Professionalism plays an important role in having nurses perform these functions.[3] Professionalism is defined as a set of attitudes and behaviors considered suitable for a particular profession.[4] Professionalism develops and is shaped through individual values and professional trainings received over time.[5] Professional values are the core element of a profession, which is important for strengthening one's professional identi-

ty and performance.[4,6,7] Professionalism requires a high level of education, caring for the work done, motivation with the desire to help others, organization, having ethical principles, taking responsibility and decision-making abilities.[4,8]

It is important to better know and understand the professional values of nurses who constitute the largest working group in the healthcare system.[7] In this context, the American Nurses Association (ANA) and the International Nurses Association (ICN) have identified seven core values in nursing: altruism, aesthetics, equality, freedom, human dignity, justice and truth, which constitute the basis of the professional values

of nurses.[9,10] Today, globalization, migration, nursing insufficiency, new diseases, aging population and demand for high quality care are complex issues that cause ethical problems for nurses.[7] Professional values play an important role for nurses in advocating social behaviors and attitudes and making decisions in cases of ethical dilemmas.[6,10]

The care provided by surgical nurses constitutes the largest part of healthcare services provided to surgical patients.[11] Surgical branches play an important role in hospitals. Many patients with different diseases may need to have surgery. In such cases, patients experience emotions that can negatively affect their treatment. Surgical nurses' awareness of these conditions and care behaviors help patients to recover easily.[12] Care behaviors include actions to ensure the well-being of sick individuals, such as sensitivity, relaxation, careful listening, honesty and acceptance without judgment.[11] The care behaviors of surgical nurses may be affected by perceptions and individual/cultural characteristics of both nurses and patients, disease diagnosis, institutional structure and nursing workload. Their care behaviors may also be affected by the methods they use in caring for different patients, lack of time and support and personal characteristics such as conscience, religious belief system, personal philosophy, sense of responsibility and sacrifice.[11,13,14] Surgical nurses are healthcare professionals who are in contact with patients undergoing surgery for the longest time. Therefore, their care behaviors play an important role in providing quality patient care and increasing patient satisfaction.[15,16] Surgical nurses are the health professionals who have the longest contact with patients who have had surgery. Therefore, care behaviors play an important role in providing quality patient care and increasing patient satisfaction.[15,16] There are few studies examining the relationship between nurses' professional values and care behaviors. Most of the studies have been conducted to determine the professional values or care behaviors of nursing students and nurses working outside of surgical clinics. However, surgical nurses play an important role in the diagnosis and treatment process. Therefore, this study was carried out to determine the relationship between the professional values and care behaviors of surgical nurses in Turkey.

Aim

This study was conducted to determine the relationship between the professional values and care behaviors of surgical nurses in Turkey.

Material and methods

Ethical approval

Ethical approval was obtained from the Human Research Ethics Committee of Zonguldak Bulent Ecevit University (decision no:373, decision date: 29.05.2018) and an institutional permission from the hospitals; Zonguldak Bulent Ecevit University Health Practice and Research Hospital (decision no: 53137, decision date: 08.11.2018) Zonguldak provincial directorate of health (decision no: 39330677-799, decision date: 21.02.2019). Informed consents were obtained from the surgical nurses who participated in the study, after they were informed about the purpose and content of the study and the use of data only for scientific purposes. The nurses were told that they could leave the study at any time. Their identifying information was kept confidential.

Design and sample

This descriptive and correlational study was conducted with surgical nurses from five public hospitals and a university hospital that have a service score above 90 in Turkey and serve in accordance with the Health Quality Standards. According to the health statistics, 227.292 nurses work in Turkey.[17] However, there is no statistical data on the number of surgical nurses working in Turkey. The sample calculations made with Power = 0.80, β = 0.05 and α = 0.05, the minimum number of people to be included was determined as 216. The sample consisted of 245 surgical nurses who could speak and write in Turkish, had no communication problems, were actively on duty and agreed to participate in the study. No sample selection was made in the study, and maternity leave, annual leave with report, etc. 40 nurses who did not work for reasons and 55 nurses who did not agree to participate in the study were not included in the sample.

Instruments

The data were collected using a Personal Information Form, the Nurses Professional Values Scale (NPVS) and the Caring Behaviors Inventory-24 (CBI-24).

Personal Information Form: This form consisted of 17 closed-ended questions about the nurses' introductory information.[18,19]

Nurses Professional Values Scale (NPVS): This scale was first developed by Weis and Schank.[5] This is a five-point Likert-type scale with 44 items and five subscales. Its Turkish validity and reliability study was performed by Orak and Alpar.[10] The scale has two Turkish versions, where one with 44 items is used to measure the perspective of nurses on ethical rules, and one with 33 items is used to examine the professional values of nurses. The 31-item Turkish version of the scale was used in this study. The total scale score varies between 31 and 155. High scores in the scale suggest that nurses attach more importance to professional values.[10] The internal consistency of the scale (Cronbach's alpha) was reported as 0.95 for the entire scale, and it ranged between 0.65 and 0.87 for its subscales.[10]

Caring Behaviors Inventory-24 (CBI-24): The scale was first developed by Wu et al. and is the short form of the Caring Behaviors Inventory-42.[20] Its Turkish validity and reliability study was conducted by Kurşun and Kanan.[21] It is used to evaluate the nursing care process. This 24-item scale consists of four subscales: assurance, knowledge and skills, respectfulness and connectedness. The subscale scores vary between 1 and 6 points. The internal consistency of the scale (Cronbach's alpha) for both patients and nurses was reported as 0.96 for the entire scale, and it ranged between 0.82 and 0.92 for its subscales.[21]

Data collection

The data were collected between November 30, 2018 and February 28, 2019. The nurses were asked to fill out the data collection tools in person, when appropriate. While the nurses were filling out the data collection tools, the researcher waited in the nurse room for answering their possible questions. It lasted around 15-20 minutes to complete the data collection tools.

Data analysis

The data were analyzed using the Statistical Package for the Social Sciences 22.0 (SPSS, IBM, Armonk, New York, United States). Descriptive statistics including frequency, percentage and mean-standard deviation were used to evaluate the nurses' sociodemographic data obtained from their personal information forms. Data distribution was assessed using the Kolmogorov-Smirnov test. The Nonparametric Mann Whitney U test and Kruskal Wallis test were used to analyze the non-normally distributed data. Pearson's correlation analysis was used to examine the relationship between the total mean scores of two scales with normal distribution. A p-value smaller than 0.05 was considered statistically significant.

Results

The characteristics of surgical nurses

The mean age of the surgical nurses was 33.63 ± 7.53 years, 82.9% of them were female, 68.2% had bachelor's degrees. Additionally, 22.9% worked at emergency surgery units, and 17.1% worked at general surgery services. Approximately half of them (47.8%) had a total work experience of 11 years or longer. More specifically, 35.9% of the nurses had a work experience of 3 years or shorter, and 34.7% had an experience of 10 years or above at surgical units. Moreover, 71.4% served as clinical nurses. Of those at the surgical unit, 53.5% cared for 11 or more patients per day.

Additionally, 27.3% of the nurses were satisfied with their profession. Among the participants, 44.9% chose the profession of nursing willingly, and 35.1% were members of a professional association. Moreover, 57.1% had ethics training.

Table 1. Characteristics of surgical nurses

Characteristics		n (%) or mean (SD)
Age		33.63 (7.53)
Gender	Female	203 (82.9)
	Male	42 (17.1)
Educational level	Health vocational high school	23 (9.4)
	Associate degree	37 (15.1)
	Bachelor degree	167 (68.2)
	Master degree	18 (7.3)
Department	General surgery	42 (17.1)
	Operating room	26 (10.6)
	Orthopedics and traumatology	20 (8.2)
	Neurosurgery	12 (4.9)
	Surgery intensive care unit	25 (10.2)
	Coronary surgery	11 (4.5)
	Thoracic surgery	11 (4.5)
	Emergency surgery	56 (22.9)
	Plastic and reconstructive surgery	6 (2.4)
	Eye surgery	3 (1.2)
	Urology	19 (7.8)
	Otolaryngology-head and neck surgery	9 (3.7)
	Pediatric surgery	5 (2)
Working experience (year)	≤5	70 (28.6)
	6-10	58 (23.7)
	11 ≤	117 (47.8)
Working experience in surgical units (year)	≤ 3	88 (35.9)
	4-6	39 (15.9)
	7-9	33 (13.5)
	10 ≤	85 (34.7)
Task	Clinical nurse	175 (71.4)
	Operating room nurse	24 (9.8)
	Intensive care unit nurse	33 (13.5)
	Other (administration etc)	13 (5.3)
Working hour (weekly)	≤40	11 (4.5)
	40-48	144 (58.8)
	49 ≤	90 (36.7)
Number of patients given daily care	1-5	41 (16.7)
	6-10	66 (26.9)
	11 ≤	131 (53.5)
	No	7 (2.9)
Satisfied with their profession	Not be satisfied	45 (18.4)
	Partially be satisfied	133 (54.3)
	To be satisfied	67 (27.3)
Choosing nursing profession willingly	Yes	40 (16.3)
	Partially	95 (38.8)
	No	110 (44.9)
Attended the scientific conference	Yes	107 (43.7)
	No	138 (56.3)
Member of a professional association	Yes	86 (35.1)
	No	159 (64.9)
Have ethics education	Yes	140 (57.1)
	No	105 (42.9)

Nurses professional values scale and caring behaviors inventory-24 scores

The NPVS total mean score of the surgical nurses was 118.26. Their mean scores were found as 42.24, 26.03, 18.81, 15.67, and 15.49 for the NPVS subscales of human dignity, sense of responsibility, activism, security and autonomy, respectively. The CBI-24 total mean score of the surgical nurses was 5.25. Their mean scores were found as 5.21, 5.21, 5.50, and 5.13 for the CBI-24 subscales of assurance, respectfulness, knowledge and skills and connectedness, respectively (Table 2).

Table 2. The scores of NPVS and CBI-24

NPVS and subscales	Mean (SD)	Min-Max
Human dignity	42.24 (7.91)	19-55
Sense of responsibility	26.03 (5.21)	15-35
Activism	18.81 (3.91)	8-25
Security	15.67 (2.84)	8-20
Autonomy	15.49 (3.10)	8-20
Total mean score	118.26 (21.10)	31-155
CBI-24 and subscales		
Assurance	5.21 (0.72)	2.5-6
Respectfulness	5.21 (0.73)	3-6
Knowledge and skills	5.50 (0.63)	3-6
Connectedness	5.13 (0.73)	3-6
Total mean score	5.25 (0.66)	3-6

Comparison of the nurses' characteristics and NPVS scores

The nurses with master's degrees had significantly higher mean scores in both NPVS (c^2=15.073; p=0.002) and its subscales of human dignity (c^2=13.754;p=0.003); activism (c^2=13.246;p=0.004) and autonomy (c^2=24.723;p=0.000) The nurses working at plastic and reconstructive surgery units had significantly higher mean scores, in both NPVS (c^2=22.475; p=0.033) and its subscales of sense of responsibility (c^2=28.033;p=0.005) and security(c^2=24.428;p=0.018), whereas those working at operating rooms had a significantly higher mean score, in activism only (c^2=28.626;p=0.004).

The surgical nurses with work experience of 6-10 years had a significantly higher mean score on NPVS (c^2=8.948; p=0.011), whereas those with a work experience of less than five years had significantly higher mean scores in the subscales of human dignity (c^2=6.494; p=0.039), sense of responsibility (c^2=12.595; p=0.002), activism (c^2= 8.572;p=0.014) and security (c^2=6.094;p=0.048). The nurses who were members of professional associations had a significantly higher mean score in the autonomy subscale (U=5612.500; p=0.020). The nurses who received ethics training had significantly higher mean scores in both NPVS and subscales, except for sense of responsibility (p<0.05). There was no statistically significant difference between the nurses' NPVS total and subscale mean scores with respect to their other descriptive characteristics (p> 0.05)

Comparison of the nurses' characteristics and CBI-24 Scores

The surgical nurses who worked 40-48 hours a week had significantly higher mean scores in both CBI-24 and all its subscales (p<0.05). The female surgical nurses who worked at plastic and reconstructive surgery units had significantly higher mean scores in both CBI-24 and subscales, except for knowledge and skills (p <0.05). The nurses who were satisfied with their profession had significantly higher mean scores in CBI-24 and the sub-

scales of assurance and respectfulness (p <0.05). The nurses who chose their profession willingly had significantly higher mean scores in the subscales of assurance and knowledge-skills, whereas those who chose their profession reluctantly had significantly higher mean scores in CBI-24 and the subscales of respectfulness and connectedness (p <0.05). There was no statistically significant difference between the nurses' CBI-24 total and subscale mean scores with respect to their other descriptive characteristics (p> 0.05). Work experience in surgical units caused a significant difference only in the subscale of knowledge and skills, whereby the nurses with a work experience of 10 years or higher at surgical units had significantly higher mean scores in the knowledge and skills subscale (c^2=9.474; p= 0.024).

The relationship between surgical nurses' professional values and care behaviors

A weak, positive and significant relationship was found between the professional values and care behaviors of the surgical nurses (p<0.001) (Table 3).

Table 3. The relationship between nurses' professional values and care behaviors

Care Behaviors Scale-24	Nurses' Professional Values Scale					
	Human dignity	Sense of responsibility	Activism	Security	Autonomy	Total mean score
Assurance	r=0.398 p=0.000	r=0.295 p=0.000	r=0.357 p=0.000	r=0.323 p=0.000	r=0.333 p=0.000	r=0.381 p=0.000
Respectfulness	r=0.385 p=0.000	r=0.321 p=0.000	r=0.369 p=0.000	r=0.344 p=0.000	r=0.331 p=0.000	r=0.387 p=0.000
Knowledge and skills	r=0.392 p=0.000	r=0.277 p= 0.000	r=0.347 p=0.000	r=0.346 p=0.000	r=0.378 p=0.000	r=0.382 p=0.000
Connectedness	r=0.388 p=0.000	r=0.313 p=0.000	r=0.361 p=0.000	r=0.321 p=0.000	r=0.327 p=0.000	r=0.381 p=0.000
Total mean score	r=0.420 p=0.000	r=0.324 p=0.000	r=0.385 p=0.000	r=0.356 p=0.000	r=0.364 p=0.000	r=0.411 p=0.000

Discussion

Nurses professional values scale and caring behaviors inventory-24 scores

This study determined that surgical units give importance to professional values. It is noteworthy that studies on the professional values of nurses are mostly conducted with nurse managers, pediatric nurses, cardiac surgery nurses, oncology nurses and nursing students.[3,18,22,23] These studies report that nurses have high professional values.[3,7,8,24,25] The result of this study was consistent with those of previous studies. Additionally, this result was significant in terms of showing that surgical nurses, who work in a complex healthcare structure, adopt the professional values of nursing.

In this study, the surgical nurses obtained high NPVS scores on the desired level. Additionally, the surgical nurses had the highest and lowest mean scores on the human dignity and autonomy subscales, respective-

ly. The humanistic view argues that the primary value of humanity for a sustainable social life is "human dignity".[26] However, Al-Banna conducted a study with cardiac surgery nurses in Iraq, and they stated that they obtained the highest and lowest mean scores on the autonomy and human dignity subscales, respectively.[22] Also, Other study has emphasized human dignity as the most important professional value of nurses.[27,28]

This study found that the surgical nurses had high perceptions of care behaviors. Several studies conducted in the world and Turkey have found that nurses and nursing students have high perceptions of care behaviors.[23,27] The high perceptions of care behaviors in surgical nurses who provide multi-faceted care at an intensive tempo is an important indicator in terms of reflecting that they provide good quality healthcare.

In this study, the surgical nurses obtained the highest mean score in the knowledge and skills subscale. Several studies conducted in the world and Turkey have reported that nurses have high knowledge-skills scores.[15,23,29] Nursing is a profession consisting of science and art. This result is a reflection of the nature of the profession of nursing. Additionally, the emphasis on practices for increasing knowledge and skills in nursing education in recent years might have been effective in achieving this result.

Comparison of the nurses' characteristics and NPVS scores

This study found no statistically significant difference between the surgical nurses' professional values according to their descriptive characteristics including their work experience at surgical units, current place of duty, professional satisfaction and status of willingly choosing the profession. One study on the professional values of nurses in Turkey reported that gender and work experience at surgical units do not significantly affect professional values in nurses.[8] In the literature, similar studies have been mostly conducted in Middle Eastern countries. Studies conducted in Saudi Arabia and Iran have reported no relationship between the descriptive characteristics and professional values of nurses.[25,30] Another study conducted with surgical nurses in Iran reported that female nurses obtained significantly higher NPVS scores.[31]

This study found a statistically significant difference between the surgical nurses' human dignity, security and activism values, but it determined no statistically significant difference between their responsibility values based on their educational levels. Additionally, having master's degrees significantly increased their professional value levels. Erkus and Dinc reported a significant relationship between the educational level and professional values of Turkish nurses.[6] One systematic review study stated that nurses with higher education adopt more professional values to provide quality nurs-

ing care.[32] This suggests that access to current information by having undergraduate and graduate education affects professional values in nurses. However, there are also studies showing that the level of education does not affect the professional values of nurses.[11,19]

In this study, the surgical nurses who were members of professional associations had significantly higher mean scores in the autonomy subscale, and those who attended scientific conferences had significantly higher mean scores in the activism, security and autonomy subscales. Additionally, those with ethical training had significantly higher mean scores in all professional values subscales, except for sense of responsibility. These results suggested that being a member of professional associations, having scientific studies, attending conferences and receiving ethics training, which are important indicators of professionalization in nursing, have a positive effect on the professional values of surgical nurses. Monroe reported that having ethics education positively affects the professional values of nurses.[5] Torabizadeh et al. also reported that operating room nurses with ethics training obtained significantly higher NPVS mean score.[31] On the other hand, the low number of nurses (36%) who were members of professional associations was a remarkable result suggesting that awareness of associations among surgical nurses is still not on the desired level. Schank and Weis reported that most nurses are not members of professional associations.[5] They also stated that nurses do not have sufficient awareness of the role of professional care associations in shaping healthcare policies. Another study found a significantly low number of nurses who were members of professional associations, suggesting that being a member of a professional association did not affect the professional values of nurses.[33] Increasing the awareness of nurses on professional organization based on volunteerism is important for them to adopt professional nursing values. In this respect, it is important for nursing professional associations to set active, continuous and sustainable goals and take an active role in development of nurses' professional values.

This study found a significant relationship between the nurses' professional work experience and all professional values, except for autonomy. Some studies reported that professional work experience does not affect the professional values of nurses.[33] However, other studies reported a positive significant relationship between nurses' work experience and their professional values.[6,7,33] Unlike other studies, this study determined that, as the work experience of the surgical nurses decreased, their NPVS mean scores increased significantly. This result may have been due to the changes in individual, social and cultural characteristics of the participants depending on generational changes and the institutional features of the hospitals they worked for.

This study also determined that the surgical nurses who worked less than 40 hours at surgical units obtained the highest NPVS mean score. Additionally, the surgical nurses without night shifts had significantly higher mean scores in the security subscale. As another important result of this study, the surgical nurses who were satisfied with their profession had a higher NPVS mean score, although the difference was not statistically significant. Studies have reported that weekly working hours positively affect the professional satisfaction and values of nurses.[34] One study on the professional values of nurses found that nurse managers had higher professional values than those who had lower work experience and were actively involved in patient care.[18] Kantek and Kaya also reported significantly higher NPVS mean scores for nurse managers who were satisfied with their profession. In this study, the fact that nurse managers without night shifts undertake important tasks such as determining the clinical organization and the standard of patient care and following and applying innovative approaches and practices in this regard might have had a positive impact on their professional values.[35]

This study found that the surgical nurses who worked at operating rooms, general surgery units and plastic and reconstructive surgery units adopted higher levels of professional values. However, one study in Turkey found no statistically significant relationship between the professional values of nurses based on their work units.[36] Unlike the previous study results, the result of this study showing higher professional values in surgical nurses suggested that the treatment and care approaches of patients at these surgical units were being carried out in line with team spirit and work, and therefore, all employees including nurses who worked at these units adopted the organizational culture, causing them to have higher levels of professional values.

Comparison of the nurses' characteristics and CBI-24 scores

In this study, a statistically significant difference was found between the surgical nurses' perceptions of care behaviors with respect to gender, except for knowledge and skills subscale, whereby the female nurses had higher perceptions of care behaviors than the male nurses. Unlike this study, previous studies have reported that gender has no effect on the care behaviors of nurses.[14,16] The higher perception of the care behaviors in the female nurses in this study may be attributed to the lower number of the male nurses in the study. The lack of a significant difference between the knowledge and skills subscale mean scores of the male and female nurses in the study may be attributed to adoption of a patient-centered care approach at surgical units.

The fact that surgical nurses have adequate knowledge and skills of critical thinking and quick decision-making for planning and maintaining patient care

due to the health conditions of surgical patients and are willing to do their jobs affects the quality of patient care. This study found that the surgical nurses who chose the profession of nursing willingly and were satisfied with their job had significantly higher perceptions of care behaviors. One study, which compared the caring behaviors of American and Turkish nursing students, found that choosing the profession willingly increased the perceptions of care behaviors.[24]

This study found no statistically significant relationship between the surgical nurses' educational levels and their perceptions of care behaviors. However, it was also found that the nurses who attended scientific conferences had significantly higher perceptions of care behaviors. Previous studies have reported different results regarding the relationship between nurses' educational levels and their care behavior perceptions. Some studies found no statistically significant difference between nurses' care behaviors with respect to their educational levels.[16] On the other hand, one study reported that the educational level of nurses positively affected their perceptions of care behaviors.[14] This suggests that, besides basic nursing education, participation in scientific activities and in-service trainings, which lead to professional development of nurses in their work life, increase their perceptions of care behaviors.

This study also found no statistically significant relationship between the surgical nurses' work experience in the profession and surgical units and their perceptions of care behaviors. However, it determined that the nurses with higher work experience at surgical units obtained significantly higher mean scores in the knowledge and skills subscale. It found a statistically significant difference between the caring behaviors of nurses based on work experience in two studies.[15,16] It cannot be denied that professional experience and concurrent trainings increase the professional knowledge and skills of nurses. This was a significant result in terms of showing that surgical nurses care about their professional development.

In this study, the nurses who worked 40-48 hours a week at surgical units had significantly higher perceptions of care behaviors. Nurses who work 40-48 hours a week can maintain an effective communication with their patients every day. Additionally, this period is important for nurses to identify, follow up and meet the needs of patients regularly. Therefore, this period also has a positive effect on nurses' job satisfaction and perception of care behaviors. This result was consistent with those of a previous study.[34]

The relationship between surgical nurses' professional values and care behaviors

This study found a positive relationship between the nurses' professional values and care behaviors. Professionalism is very important to determine the professional standards

in providing quality care.[16] Therefore, the high NPVS and CBI-24 mean scores of the nurses in this study suggested that surgical nurses adopt professional nursing values and provide good quality care for their patients. Geyer et al. reported that activation of professional values positively affects all dimensions of care behaviors and job performance.[37] Aktas and Karabulut found a significant positive relationship between the professional values and caring behaviors of Turkish nursing students.[38] The result of this study was consistent with those in the literature.

Study limitations
This study had some limitations. First of all, it had a small sample size. So, its results may not be generalized to all surgical nurses in Turkey. Secondly, the female nurses constituted the majority of the sample. Therefore, the results cannot be generalized to both genders.

Conclusion
This study showed that surgical nurses had high perceptions of professional values and care behaviors, and they adopted at most human dignity from professional values. Both the individual and professional characteristics of the surgical nurses affected their perceptions of professional values and care behaviors. Additionally, there was a positive significant relationship between the nurses' professional values and care behaviors.

According to the these results, it is recommended (i) to conduct in-service trainings to increase the professional values of surgical nurses in coordination with their work life, (ii) to review institutional policies to provide surgical nurses with both professional and job satisfaction, (ii) to make organizational and social arrangements and organize scientific activities that will increase surgical nurses' adoption of organizational culture at their work units and (iv) to perform further comprehensive studies of the professional values and care behavior perceptions of surgical nurses.

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Ethics approval

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ORIGINAL PAPER

Newborn blood spot screening – knowledge of genetic testing among mothers

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ABSTRACT

Introduction and aim. Newborn blood spot screening (NBS) uses genetic technology to screen for selected genetic, endocrine, and metabolic disorders. The purpose of the study was to assess the knowledge of newborn blood spot genetic screening among expectant mothers.

Material and methods. Between October 2015 and January 2016, a 20 question, multiple-choice questionnaire was administered to expectant mothers presenting for a pre-natal ultrasound at the Maternal Fetal Care Center in Milwaukee, Wisconsin Froedtert Hospital. Statistical analysis used Chi-Square or Fisher's exact test for categorical variables.

Results. 103 women completed the survey; 34% believed that education regarding screening is incomplete and 39% believed that it needs improvement. 27% knew the purpose of newborn screening.

Conclusion. Many mothers lack general and specific knowledge about NBS and the diseases screened for. Health education that provides accurate and complete information on the newborn blood spot screening should be provided to all parents prior to the administering of any genetic testing. Key areas that should be targeted include: purpose of NBS, screened diseases and how to interpret the results of the test.

Keywords. newborn, newborn blood spot screening, screening, perinatal care

Introduction

Newborn screening is the most widely used form of genetic technology. Newborn screenings uses a few drops of blood from the newborn's heel to screen for the presence of genetic, endocrine, and metabolic disorders. These screening tests identify infants at increased risk for selected genetic disorders, allowing for diagnoses and treatment to occur after follow-up tests. Currently in the United States, screening tests are mandated and are available for over 60 different disorders.[1,2] The benefits of such testing include early intervention and appropriate management to prevent intellectual and physical disabilities As NBS is mandatory, targets large popula-

tions, and offers preventative interventions. The widespread adoption of NBS, albeit beneficial, also poses a question of informed decision making.[2,3]

The paternalistic nature of medicine often overshadows patient autonomy. When this happens, patients are denied the ability to make informed decisions for themselves. In this modern era of medicine, with advanced technologies developing each day, a discussion of informed consent is crucial. It is notable that a report by the Committee for the Study of Inborn Errors of Metabolism of the National Academy of Sciences recommended that "participation in a genetic screening program should not be made mandatory by law, but should be

left to the discretion of the person tested or, if a minor, of the parents or legal guardian".[4] Physicians must present information accurately and sensitively, including the diagnosis, the nature and purpose of the recommended interventions, and the burdens, risks and benefits of all options including no treatment. Informed consent for newborn screening should involve the discussion of guidelines established by the American College of Obstetricians and Gynecologists and the Royal College of Paediatric and Child Health[5] that require information on the condition for which the test is being offered, purpose of the test, likelihood of positive and negative results, possibility of false positive and false negatives, and the risks and implications of test results.[6,7]

Literature concerning NBS suggests that there is a discrepancy in the informed consent process between healthcare professionals and parents.[8-10] Parents report that they feel that although screening is a routine test, they are not aware they even have a choice in the matter.[11]

Despite efforts to decrease the knowledge gap between healthcare professionals and expectant and new mothers, a gap remains.[12] Healthcare professionals may not be providing consistent and reliable information to parents regarding newborn screening, which affects patient decision-making and informed consent. Informed consent creates an open dialogue between patient and provider. It allows patients to take an active role in their health care by providing autonomy and allows for trust in the health care relationship.

This study sought to investigate the current understanding of newborn screening among expectant mothers. We hypothesize that there is a lack of knowledge among mothers regarding newborn screening. The results of the study will help develop targeted health education for expectant mothers whose children are undergoing NBS so that they can be fully informed of the process.

Aim

The purpose of the study was to assess the knowledge of newborn blood spot genetic screening among expectant mothers.

Material and methods

Design

Cross-sectional descriptive study was used.

Ethical approval

The authors declare that the research was carried out in accordance with the Declaration of Helsinki of 1964 and its last revision of 2013. This study was approved by the Medical College of Wisconsin IRB as well as the Office of Clinical Research and Innovative Care Compliance (1/06/2015). All patients enrolled in the study were informed of the aims and intentions of the study, that their participation was anonymous and voluntary.

Data collection

Data were collected between October 2015 and January 2016, 103 expectant mothers were approached for enrollment during their outpatient visits in the Maternal and Fetal Care Center in Milwaukee, Wisconsin Froedtert Hospital. Inclusion criteria included: pregnant women of legal age who speak and read English. Members of the study team as well as ultrasound technologists approached women who met inclusion criteria to participate in the study.

Expectant mothers were approached during a waiting period while in the ultrasound suites of the Maternal and Fetal Care Center. Participants received the questionnaire, which included an informational cover letter stating the purpose of the study, the voluntary nature of their participation, a description of the procedures, and a statement assuring their anonymity.

Twenty questions multiple choice survey collected quantitative information regarding the patients' experiences with newborn screening and the informed consent process in an urban hospital in Milwaukee, Wisconsin. It was developed by the last author. Participants were encouraged to answer all questions and were also allowed to leave answers blank if they did not know the answer. The survey took approximately 10 minutes to complete and was collected soon after completion. Participants were allowed to leave answers blank if they did not know the answer. Blank answers and multiple answers for certain questions about facts of NBS were counted as incorrect.

The questionnaire asked about basic facts about newborn screening, perceptions of informed consent, experiences of obtaining medical knowledge, and mothers' general opinions regarding newborn screening. Demographic information including age, parity, education level, and residence were also included on the questionnaire.

Statistical analysis

Analysis included Chi-Square or Fisher's exact test for categorical variables. Univariate analyses were used for continuous variables, and only complete data were used. All statistical analyses were performed using SAS version 9.4 (SAS Institute, Cary, North Carolina) software. And $p \leq 0.05$ was considered significant.

Results

A total of 103 expectant mothers completed the questionnaire. The response rate for this study was 100%. The average age was 29 years, with a range of 18 to 33 years. The majority of women (68%) had a college education or higher (Table 1).

Table 1. Survey sample characteristics (n=103)

Item	n	%
Age		
18-25	29	39%
26-30	17	23%
31-35	32	27%
>35	8	11%
Level of eduction		
Elementary School	0	0%
Less than High School	1	1%
Completed High School	28	28.3%
College or Higher	70	70.7%
Place of Residence		
Urban	87	87.9%
Rural	12	12.1%
Number of Children		
0	18	18%
1	33	33%
2	29	29%
3	13	13%
4+	7	7%

Table 2. Knowledge of mothers on newborn blood spot screening

Purpose of Newborn Screening	n	%
Diagnosis of disease	41	40.59
Confirmation of the possibility of disease in children in the future	27	26.73
Confirmation of carriers of certain diseases in children	29	28.71
Combination of answers	4	3.96
Total	101	100
Necessity of Screening	**n**	**%**
Necessary	83	80.58
Not Necessary	4	3.88
No Opinion	16	15.53
Total	103	100
Knowledge of which diseases are screened	**n**	**%**
Yes-Detailed	18	17.65
Yes-Incomplete	45	44.12
No	39	38.24
Total	102	100
Medical knowledge of diseases that are screened	n	%
Yes-Detailed	18	17.82
Yes-Incomplete	41	40.59
No	42	41.58
Total	101	100
Source of Knowledge	n	%
Internet	8	12.12
Medical Staff	28	42.42
Books, Magazines	5	7.58
Family, Friends	3	4.55
Other	9	13.64
Combination of answers	13	19.72
Total	66	100
When does NBS occur?	n	%
24 hours after birth	76	75.25
Day 2	14	13.86
Between 4-6 days	9	8.91

Combination of answers	2	1.98
Total	101	100
Do you think there is a need for consent?	n	%
Yes	66	64.71
No	17	16.67
I don't know	19	18.63
Total	102	100
When did you learn about NBS?	n	%
Before Current Pregnancy	37	38.14
During Current Pregnancy	55	56.70
After birth	5	5.15
Total	97	100
Has your child already had screening?	n	%
Yes	31	30.10
No	53	51.46
I don't know	19	18.45
Total	103	100
If YES, did you give consent?	n	%
Yes	29	61.70
No	7	14.89
I don't remember	11	23.40
Total	47	100
Did you have enough time to make a decision?	n	%
Yes	50	86.21
No	8	13.79
Total	58	100
Did you receive information on the collection procedure?	n	%
Yes - Detailed	15	15.15
Yes - Incomplete	16	16.16
No information	68	68.69
Total	99	100
Did you receive information on when and how the results are interpreted?	n	%
Yes	23	23.23
No	76	76.77
Total	99	100
Is education for newborn screening sufficient?	n	%
Sufficient	24	25.53
Incomplete	32	34.04
Needs improvement	37	39.36
Combination of answers	1	1.06
Total	123	100
Preferred Method of Receiving Information	n	%
Internet	6	6
Medical Staff	80	80
Books, Magazines	3	3
Family, Friends	1	1
Other	14	14
Total	100	100

As shown in Table 2, many women did not know the purpose of newborn screening, with only 26.73% of women correctly answering that it was a confirmation of the possibility of a disease. 38.24% of women also reported having no knowledge of what diseases were screened with only 17.65% of women having detailed knowledge. Many women received no information on

the process of newborn screening (68.69%) as well as when and how the results will be interpreted (76.77%).

Presented with the question on whether education for women on newborn screening is sufficient, only 25.53% say it is sufficient, with 34% reporting that education is incomplete and the other 39.36% reporting that the current education needs improvement. The majority of mothers (80%) also report their preferred method of receiving medical knowledge would be through medical staff with only 6% preferring the Internet for their source.

Analyses demonstrated that the effect of demographics or age does not influence correct answers. There is also no statistically significant difference in number of correct answers between rural and urban mothers.

Discussion

Our findings indicate that the majority of women believe that newborn screening is mandatory, yet education regarding newborn screening to be insufficient. Although the majority of women do have knowledge about the diseases that are screened for, their perception of their knowledge is often incomplete and lacking. The majority of women also incorrectly answered fact, based questions on newborn screening such as purpose of the test and methods of collection. The questionnaires also addressed sources of knowledge and preferred sources on knowledge in order to gauge potential points of intervention. The two most highly desired sources were medical staff and the Internet.

It is evident through these findings that there is still a knowledge gap regarding what patients know about newborn screening and how they understand the informed consent process. This study shows that education on newborn screening is lacking and as such, the informed consent process is incomplete. Patients are not receiving adequate medical knowledge for an important genetic test such as newborn screening. This study also presents us with an insight into the mothers' perceived understanding of newborn screening as well as a point of intervention in terms of current education.

Perhaps two of the most important discoveries in this study were the current method of receiving information among mothers, and their preferred method of receiving information. These data relate to informed choice stemming from education that should be given by the healthcare provider during the current pregnancy. The education can be in a written form but should also be communicated directly to the patient by the healthcare provider. Many of the women were divided between medical staff and the Internet, in terms of receiving knowledge about newborn screening. Ideally, the percentage of mothers receiving information from medical staff should be higher. Studies have shown that written information regarding screenings do not provide substantial informed choices.[11] Rather, information given by health care professionals was more beneficial.[9]

It has been shown that parental knowledge regarding newborn screening as well as other genetic testing such as prenatal testing is often low and incomplete.[15] The results of this study are consistent with earlier studies. General knowledge about testing is lacking, as well as knowledge about which diseases are screened for and knowledge about those specific diseases.[16-21] Regarding the demographics of the women surveyed, there was no significant difference in knowledge across ages or place of residence. This shows that healthcare professionals should be wary of assuming that older women, or women who live in cities as opposed to rural areas, know more about newborn screening than their counterparts. The significantly lower understanding of newborn screening among women with lower education levels shows that healthcare providers should also take into consideration and address health literacy gaps. At the same time healthcare providers should not assume that women with higher education necessarily have more knowledge on newborn screening. The findings from the study also show a roughly equal distribution of when knowledge about newborn screening was obtained: before the current pregnancy, during the current pregnancy, and after delivery. Previous studies have shown that parents prefer information in the prenatal period.[22, 23] This fact, taken with the results of the study, could provide an opportunity to intervene and present relevant information regarding newborn blood spot screening early on in order to give mothers the most informed choice. Providing information during the prenatal period as opposed to postnatal allows time for mothers to develop and ask questions and gain a better understanding of a quick yet important diagnostic tool.

In light of these results, the limitations of this study should also be taken into consideration. Participants may not feel comfortable providing honest answers if they perceived their knowledge to be inadequate or their answers to be incorrect, which would affect the reliability of the survey data. Participants might also have an unclear understanding of the questions, which would lead to inaccurate answers.

With an understanding of the lack of knowledge that mothers have regarding newborn screening, there can be an educational intervention during prenatal care. In order to provide better knowledge to mothers about newborn screening and in a grander sense other tests or procedures, health care providers must be diligent in providing accurate and timely information to all patients, regardless of education level, age, or other demographic factors. In addition to advising healthcare providers about conveying newborn screening information to patients, perhaps more accurate and easily accessible material on the Internet would beneficial to patients. Key areas that should be targeted include:

purpose of NBS, screened diseases and how to interpret the results of the test. These next steps will promote informed consent among mothers in regards to newborn blood spot screening.

Conclusion

Many mothers lack general and specific knowledge about NBS and the diseases screened for. Health education that provides accurate and complete information on the newborn blood spot screening should be provided to all parents prior to the administering of any genetic testing. Key areas that should be targeted include: purpose of NBS, screened diseases and how to interpret the results of the test.

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Declarations

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Author contributions

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

Conflicts of interest

The authors declare that they have not conflict of interests.

Data availability

Data available on request from the authors.

Ethics approval

The study was approved by the Medical College of Wisconsin/Froedtert Health IRB as well as the Office of Clinical Research and Innovative Care Compliance, USA (1/06/2015).

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ORIGINAL PAPER

Addiction in university students – determining the levels of cigarette, alcohol, substance, game, and Internet addiction

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ABSTRACT

Introduction and aim. This study aims to determine the average addiction levels of university students and the effects of different demographic variables on addiction levels and types.

Material and methods. The study included 783 volunteer students in the Seydişehir campus of Necmettin Erbakan University. Data were collected face-to-face using Sociodemographic Information Form, Fagerström Test for Nicotine Dependence (FTND), Cut-off test (CAGE), Digital Game Addiction Scale (DGAS-7), and Internet Addiction Test (IAT).

Results. Of the students, 27.7% were using tobacco, 14.8% were using alcohol, 2.6% were using ecstasy, 2.3% were using cannabis, 1.4% were using inhalants, 1% were using pills. FTND, DGAS-7, and IAT mean scores were 3.80±2.55, 12.04±5.57, and 43.56±15.73, respectively, and 21.5% had risky alcohol use. Also, 2.2% were game addicts, 3.8% were internet addicts. Digital game addiction, internet addiction, and nicotine addiction were positively correlated. There was no significant relationship between nicotine and internet addictions. Individuals with risky alcohol use had higher rates of nicotine addiction.

Conclusion. Students' addiction rates were similar to the country in general. Addictions gained in the youth years continue in the following years and bring many health problems. Therefore, it is crucial to include the subject of combating addiction more in university education and increase studies on the subject.

Keywords. addiction, alcohol, cigarette, game, Internet, university student

Introduction

Addiction is a clinical presentation that starts with taking increasing amounts of a substance outside its intended use, progresses with the inability to stop using it even though it causes problems in the person's life, and lastly, reducing the substance intake causes withdrawal symptoms. In the scientific literature, it is examined under substance and behaviour addiction.[1] The Diagnostic and Statistical Manual of Mental Disorders-5 (DSM-5) lists addictive substances as alcohol, caffeine, cannabis, hallucinogens, inhalants, opioids, sedatives-hypnotics and anxiolytics, stimulants, nicotine, and other (or unknown substances)[2]. The individual's actions, including computer, television, gaming, shopping, food, internet, gambling, porn, exhibitionism, sex, being in virtual environments, using digital tools and equipment are behavioural addictions.[3]

Although preventable, tobacco smoking is one of the leading causes of mortality and morbidity. According to the World Health Organization (WHO), nearly 1 billion people worldwide use tobacco.[4,5] In Turkey, smoking prevalence among university students varies between 20% and 48%.[6,7] Alcohol/substance or technological addictions may be accompanied by decreased academic/work achievement, social and physical problems, bringing along psychiatric disorders, attention/concentration difficulties.[8]

Internet addiction constitutes the main framework of other internet-related addictions. In this context, besides the internet itself, the activities carried out over the internet are also a source of addiction.[9] Therefore, "social media addiction", "digital game addiction" and "smartphone addiction" can be considered as addictions in which the active substance is the internet[10]. Studies have shown that in parallel with the risk of internet addiction for adolescents, digital game addiction is also high and digital games are more stimulating than other games because they increase curiosity and excitement and provide instant gratification.[11-15]

Cannabis remains by far the world's most used drug an estimated 209 million people used cannabis, 61 million people used opioids, 21.5 million people used cocaine, 34 million people used amphetamines, NPS (Synthetic cannabinoids and ketamine) were consumed in most countries in 2020.[16]

At the same time, the desire of university students who are adolescents to lead an independent life and the fact that both substance and behavioural addictions affect all areas, especially in the field of health, causes them to become a priority by leading them to negative situations. Addiction has become a major threat affecting societies. It is crucial to determine the factors causing addiction, to raise awareness for determining the addiction status, to quickly produce solutions to prevent addiction, and to inform students in educational institutions about the negative consequences.

Aim
This study aims to determine the average addiction levels of university students and the effects of different demographic variables on addiction levels and types.

Research Questions
What are the a. Nicotine addiction, b. Alcohol dependence, c. Internet addiction, d. Digital game addiction levels, and e. Substance use rates of university students? What is the socio-demographic variables that affect these rates?

Material and methods
Ethical approval
Ethics committee approval was obtained before starting the study (App. No:2022/18-108-05.01.2022). Institutional permissions were obtained from the relevant University (Number: E-15812146-200-141779 / E-79170238-044-142345 / E-33205045-100-141777). Participants' verbal and written consents were obtained. The research was carried out per the principles of the Declaration of Helsinki.

Type of research
This is a cross-sectional field study.

Research population and sample
The population includes 1545 students studying at the Necmettin Erbakan University Seydişehir campus. The sample size calculated with the Raosoft program using the sample calculation formula for groups with a known population, with a 0.05 margin of error, a 95% confidence interval, was 308. The literature recommends increasing the number obtained by at least 15% for possible losses.[17] Accordingly, the number of students to be sampled was increased by 15% to 354. A total of 783 students who met the inclusion criteria and volunteered to participate were included in the study.

Data collection techniques and tools
Data were collected using "Sociodemographic Information Form", "Fagerström Test for Nicotine Dependence (FTND)", "Cut-off Test (CAGE)", "Digital Game Addiction Scale (DGAS-7)" and "Internet Addiction Test (IAT)".

Sociodemographic information form
It includes questions about the socio-demographic characteristics of individuals and whether they have used addictive substances in DSM-V in the last year.[2,18]

Fagerström Test for Nicotine Dependence (FTND)
Fagerström first proposed the Fagerström Tolerance Test for the assessment of smoking addiction in 1978. This test was reconsidered by Heatherton et al. in 1991, creating the Fagerström Test for Nicotine Dependence (FTND). FTND consists of 6 questions, each having a different score. The validity and reliability analysis of the test was performed by Uysal et al. A total score of 0-2 indicates low dependence, 3-4: low to moderate dependence, 6-7: moderate dependence, and 8-10: high dependence. The Cronbach reliability coefficient of the test was found to be moderately reliable at 0.56. Cronbach was found to be 0.66 in our study, and it can be said that the scale is reliable.[19,20]

Cut-off test CAGE (Cut down, Annoyed, Guilty, Eyeopener)
It was developed by Ewing and Rouse, and its Turkish validity and reliability study was performed by Arıkan et al. It is a short and simple test consisting of four questions. The recommended cut-off is two; that is, two or more positive answers indicate risky use.[21,22]

Digital game addiction scale (DGAS-7)
DGAS-7 was developed by Lemmens et al. to identify problematic digital gaming behaviours. It is a seven-item short form of DGAS-21 consisting of 21 items and seven sub-dimensions. It is a single-dimension, five-point Likert type scale scored between 1 and 5 (1=never, 5=always) (range: 7-35). Scoring three or more on all seven items implies that the person is a game addict. In addi-

tion, a relationship can be established between the increase in the score obtained from the scale and the level of addiction. They reported the Cronbach's alpha coefficient of the scale as 0.72.[23,24]

Internet addiction test (IAT)

It is a 20-item Likert-type scale developed by Dr. Kimberley Young (1996). Turkish validity and reliability studies were conducted. Those who score '80 and above' in the questionnaire are supposed to have significant impairments in functionality and are considered "internet addicts" (IA). Those who score between '50-79' are regarded as those frequently having problems with the internet in their daily lives and difficulty in controlling themselves and are called "risky internet users" (RIU). Those who score "49 and below" are considered "average internet users" (AIU). The reliability level of the scale was found to be 0.895 with the Cronbach's alpha coefficient.[25,26]

Data collection

Data were collected by face-to-face interview method between January 9 and February 28, 2022. Data collection took approximately 10-15 minutes for each individual. As the study was conducted during the COVID-19 pandemic, the interviews were held following the pandemic measures, wearing masks, and maintaining social distance.

Study limitations

The study group was selected from accessible volunteer students.

Statistical data analysis

Data were analysed using the SPSS (IBM, Armonk, New York, United States) for Windows 22 package program. Besides numbers, percentages, minimum and maximum values, mean and standard deviations, normally distributed measurements were compared with t-test for two independent groups, and analysis of variance for multiple groups (as further analysis, LSD was used in cases where variances were homogeneous, and Dunnet C was used in cases where they were not). In the evaluation of statistical relationships, Pearson correlation analysis was used for normally distributed measurements, and Spearman correlation analysis was used for non-normally distributed measurements. Cronbach α coefficient was used for internal validity, Kurtosis and Skewness coefficients were used for normality distribution of the data. In normal distribution analysis, Skewness coefficients for each scale were as follows: FTND (n: 219) SE: 0.16, Kurtosis coefficient SE: 0.327; DGAS-7 (N: 780) SE: 0.088, Kurtosis coefficient SE: 0.175; IAT (n: 769) SE: 0.088 Kurtosis coefficient SE: 0.176. Accordingly, Fageström Nicotine Addiction Test, Digi-

tal Game Addiction Scale, and Internet Addiction Test scores show normal distribution. Age and GPA were not normally distributed; age had a Skewness coefficient of (n: 779) SE: 0.088 and Kurtosis coefficient of SE: 0.175, GPA had a Skewness coefficient of (n: 458) SE: 0.114 and Kurtosis coefficient of SE: 0.228. Internal validity (Cronbach α) coefficients of the scales were as follows; FTND: 0.666, DGAS-7: 0.871, IAT: 0.917.

Results

Of the participants, 36.7% were associate degree (Associate degree is a 2-year education given in vocational schools of universities), 37.8% were second-year students, 67% were male, 83.7% were born in the city centre, 53.9% had a middle socioeconomic level, 72.3% did not smoke, and 85.2% did not use alcohol. As for substance use, 97.7% did not use cannabis, 99.4% did not use ecstasy, and 99.5% did not use heroin. In addition, 99.5% did not use cocaine, 99.6% did not use roofies, 99.7% did not use Rohypnol, 98.6% did not use inhalants, 99% did not use pills, 99.4% did not use amphetamines, and 99.6% did not use other substances (Table 1).

Table 1. Demographic characteristics of the participants

		n	%
Department	Nursing	196	25
	Mechanical Engineering	92	11.7
	Computer Engineering	208	26.6
	Associate degree	287	36.7
Grade	1	352	45
	2	296	37.8
	3	42	5.4
	4	93	11.9
Gender	Male	478	61
	Female	305	39
Place of birth	City	655	83.7
	Town	68	8.7
	Village	60	7.7
Socioeconomic status	Below	47	6
	Below average	238	30.4
	Average	422	53.9
	Upper average	69	8.8
	Upper	7	0.9
Smoking	Yes	217	27.7
	No	566	72.3
Drinking Alcohol	Yes	116	14.8
	No	667	85.2
Medications usage	None	775	99
	Only a few times	3	0.4
	1-3 times a month	1	0.1
	1-5 times a week	1	0.1
	Almost every day	3	0.4

Continuous Variables	n	Min.	Max.	Avg.	SD.
Age	779	17	48	20.47	2.97
Grade Point Average	458	1	98	17.52	29.37

* Multiple markings

2.7% of the participants were using electronic cigarettes. Electronic cigarette use was not an effective factor in any type of addiction (p<0.0059).

Participants scored an average of 3.80±2.55 on the Fagerström Test for Nicotine Dependence, and scores ranged from 0 to 10. According to the scale cut-off points, 35.6% of the students had a low dependence on nicotine (Table 2).

Table 2. Distribution of scale scores

Scales and Subscales	n	Min.	Max.	Avg.	SD.
Fagerström Nicotine Addiction Test	219	0	10	3.8	2.55
According to Scale Breakpoints	**n**	**%**	–	–	–
Slightly addicted	78	35.6	–	–	–
Little addicted	56	25.6	–	–	–
Moderately addicted	30	13.7	–	–	–
Highly addicted	35	16	–	–	–
Very highly addicted	20	9.1	–	–	–
Incision Test (CAGE)					
According to Scale Breakpoints	**n**	**%**			
Risky usage	26	21.5	–	–	–
No risky usage	95	78.5	–	–	–
Total	121	100	–	–	–
Digital Game Addiction Scale	780	7.00	35	12.04	5.57
According to Scale Breakpoints	**n**	**%**			
No risk	765	97.8	–	–	–
Game Addicted	17	2.2	–	–	–
Internet Addiction	769	20	101	43.56	15.73
According to Scale Breakpoints	**n**	**%**	–	–	–
Internet addicted	29	3.8	–	–	–
Risky internet users	194	25.2	–	–	–
Average internet user	546	71.0	–	–	–

The difference in Fagerström Test for Nicotine Dependence score was statistically significant according to the participants' class, gender, alcohol use/trial, alcohol use, and pill use (p<0.05) (Table 3). Further analyses to specify which groups the differences originated from determined that the mean score of the 4th-year students was lower than the 1st and 2nd-year students (p<0.05), and the mean score of men was higher than that of women (p<0.05). Those who used/tried alcohol had higher scores than those who did not (p<0.05), and those who used pills almost every day had higher scores than those who never used pills or used only 1-2 times (p<0.05). The difference in Fagerström Test for Nicotine Dependence scores according to department, place of birth, socioeconomic level, and smoking status was not statistically significant (p>0.05). The parts with missing SD in the table; Since the number of respondents was 1 person, it could not be checked (Table 3).

There was no statistically significant correlation between age and GPA and Fagerström Nicotine Dependence test scores (p>0.05) (Table 6). There was risky alcohol use in 25% of the participants.

The difference in Alcohol Abuse Disorder Rates according to department and pill use status was statistically significant (p<0.05) (Table 4). Further analyses (LSD) to determine from which groups the differences originated revealed that those who studied in the nursing department (p<0.05), those who never used pills, and those who were in the no alcohol abuse risk category obtained higher scores than the other groups. The difference in Alcohol Abuse Disorder Rates according to class, gender, place of birth, socioeconomic level, smoking, alcohol use/trial status was not statistically significant (p>0.05) (Table 4). Age and GPA scores were not statistically significant in terms of alcohol abuse status (p>0.05) (Table 5).

Participants scored an average of 12.04±5.57 points on the DGAS-7. The scores varied between 7-35 and based on the scale cut-off points, 2.2% of the participants were game addicts (Table 2). The difference in DGAS-7 scale scores by department, class, gender, socioeconomic level, alcohol use/trial, and inhalant use was statistically significant (p<0.05) (Table 3). Further analyses (LSD) to distinguish from which groups the differences originated determined that the scores of those in the nursing department were lower than those in the computer engineering department, the scores of the second-year students were higher than those in the other years. The mean score of men was higher than that of women. Those in the upper economic classes had higher scores than those in the lower, lower-middle, middle, and upper-middle classes. The mean score of those who used/tried alcohol was higher. The scores of those who never used inhalants were lower than those who used them only 1-2 times and those who used them every day. The difference in DGAS-7 Scale Scores according to the place of birth and smoking status was not statistically significant (p>0.05) (Table 3). There was no statistically significant association between age and DGAS-7 Scale score (p>0.05). There was a statistically significant, low-level negative correlation between GPA and DGAS-7 Scale score (p<0.05). As the DGAS-7 Scale score increased, the overall grade point average decreased (Table 6).

Participants obtained an average of 43.56±15.73 points from the Internet Addiction Test. The scores varied between 20-101, and based on the scale cut-off points, 3.8% of the participants were internet addicts (Table 2). The difference in Internet Addiction Test scores according to socioeconomic level, inhalant use, and other substance use was statistically significant (p<0.05) (Table 3). Further analyses (LSD) to discern from which group the differences originated determined that those in the upper economic class had higher scores than those in the lower, lower-middle, middle, and upper-middle classes. The scores of those who used inhalants only 1-2 times were higher than those who never used them and those who used them almost every day.

Table 3. Comparison of FTND, DGAS-7 and IAT scores according to demographic characteristics

		FTND n	FTND Avg.	FTND SD.	FTND Test	FTND p	DGAS-7 n	DGAS-7 Avg.	DGAS-7 SD.	DGAS-7 Test	DGAS-7 p	IAT n	IAT Avg.	IAT SD.	IAT Test	IAT p
Department	Nursing	20	3.20	3.00			196	11.20	4.73			193	43.30	12.01		
	Mechanical Engineering	35	3.94	2.03	F=2.347	0.074	92	11.85	5.73	F=3.091	0.026	88	43.09	16.49	F=0.066	0.978
	Computer engineering	57	3.19	2.68			206	12.87	5.56			204	43.77	16.21		
	Associate degree	107	4.19	2.49			286	12.07	5.98			284	43.73	17.37		
Grade	1	95	3.57	2.43			350	11.47	5.44			343	42.47	15.90		
	2	95	4.43	2.58	F=5.491	0.001	295	13.06	5.92	F=5.642	0.001	294	45.39	16.79	F=2.616	0.050
	3	5	3.60	2.88			42	10.81	4.72			42	44.62	11.11		
	4	24	2.25	2.11			93	11.49	4.79			90	41.22	12.56		
Gender	Male	177	4.04	2.58	t=2.914	0.004	476	13.22	5.79	t=8.058	0.000	468	43.24	15.95	t=-0.703	0.482
	Female	42	2.79	2.18			304	10.19	4.66			301	44.06	15.40		
Place of birth	City	189	3.79	2.59			652	11.87	5.46			645	43.14	15.67		
	Town	19	4.05	2.59	F=0.193	0.824	68	13.12	6.52	F=1.967	0.141	66	45.61	15.42	F=1.441	0.237
	Village	11	3.45	1.69			60	12.67	5.55			58	45.91	16.67		
Socioeconomic status	Below	9	4.11	2.32			47	11.68	5.28			47	42.91	18.19		
	Below average	69	3.48	2.44			237	12.16	5.44			231	44.20	16.77		
	Average	109	3.96	2.53	F=1.452	0.218	420	11.95	5.50	F=2.583	0.036	417	43.45	14.49	F=2.623	0.034
	Upper middle	28	3.50	2.90			69	11.72	5.52			67	40.75	14.92		
	Upper	4	6.25	2.06			7	18.57	11.84			7	60.14	30.53		
Smoking	Yes	217	3.79	2.55	t=-0.390	0.697	215	12.35	5.89	t=0.960	0.338	213	43.84	16.50	t=0.301	0.763
	No	2*	4.50	3.54			565	11.92	5.45			556	43.45	15.44		
Tried Alcohol	Yes	84	4.83	2.58	t=4.988	0.000	115	13.48	6.88	t=2.506	0.013	115	45.31	17.34	t=1.297	0.195
	No	135	3.16	2.31			665	11.79	5.28			654	43.25	15.43		
Using alcohol	None	104	3.21	2.31			603	11.70	5.23			592	42.99	15.21		
	Only a few times	51	3.84	2.41			85	12.51	5.78			86	44.15	16.51		
	1-3 times a month	39	4.21	2.77	F=4.791	0.001	59	14.02	6.68	F=3.693	0.005	58	46.71	18.23	F=1.344	0.252
	1-5 times a week	17	5.53	2.76			25	12.80	7.00			25	45.28	15.54		
	Almost every day	8	5.50	2.62			8	15.88	9.78			8	51.13	24.14		
Medications usage	None	214	3.78	2.53			772	12.01	5.57			761	43.55	15.66		
	Only a few times	3	2.33	2.31			3	14.00	6.00			3	45.33	35.44		
	1-3 times a month	–	–	–	F=3.284	0.039	1	11.00	.	F=1.065	0.373	1	41.00	.	F=0.700	0.592
	1-5 times a week	–	–	–			1	12.00	.			1	67.00	.		
	Almost every day	2	8.00	1.41			3	18.33	4.62			3	37.00	16.52		
Using volatile substances	None	210	3.75	2.51			769	11.96	5.52			758	43.44	15.59		
	Only a few times	6	6.00	2.97			7	15.86	6.72			7	39.57	14.32		
	1-3 times a month	–	–	–	F=2.458	0.088	1	10.00	.	F=5.388	0.001	1	57.00	.	F=5.383	0.001
	Almost every day	3	3.00	3.46			3	23.33	4.93			3	78.33	22.50		
Using Ras	None	216	3.81	2.54			777	12.02	5.56			766	43.51	15.68		
	Only a few times	1	1.00	.	F=1.336	0.263	1	8.00	.	F=1.725	0.160	1	29.00	.	-	0.036
	1-3 times a month	1	1.00	.			1	20.00	.			1	86.00	.		
	Almost every day	1	7.00	.			1	21.00	.			1	53.00	.		
Other substance use	None	216	3.81	2.54			777	12.02	5.56			766	43.51	15.68		
	Only a few times	1	1.00	.	F=0.609	0.545	1	8.00	.	F=2.583	0.076	1	29.00	.	F=3.168	0.043
	Almost every day	2	4.00	4.24			2	20.50	0.71			2	69.50	23.33		

*Two people who said no were not included as smokers because they stated that they used electronic cigarettes.

The difference in Internet Addiction Test score by department, class, gender, place of birth, smoking, alcohol use/trial status was not statistically significant (p>0.05) (Table 3). There was no statistically significant correlation between age and GPA and Internet Addiction Test Score (p>0.05) (Table 6).

Table 4. Incision test by demographic characteristics of participants: comparison of alcohol abuse disorder rates by CAGE

		Risky usage		No risky usage		Importance
		n	%	n	%	
Department	Nursing	4	66.7	2	33.3	x^2=12.260 p=**0.007**
	Mechanical Engineering	1	5.0	19	95.5	
	Computer engineering	3	12.5	21	87.5	
	Associate degree	18	25.4	53	74.6	
Grade	1	11	20.4	43	79.6	x^2=3.564 p=0.313
	2	15	26.3	42	73.7	
	3	2	100	–	–	
	4	–	–	8	100	
Sex	Male	24	23.3	79	76.7	p=0.356*
	Female	2	11.1	16	88.9	
Place of birth	City	24	23.1	80	76.9	x^2=1.689 p=0.430
	Town	2	16.7	10	83.3	
	Village	–	–	5	100.0	
Socioeconomic status	Below	2	28.6	5	71.4	x^2=2.842 p=0.585
	Below average	7	20.0	28	80.0	
	Average	11	20.0	44	80.0	
	Upper middle	6	31.6	13	68.4	
	Upper	–	–	5	100	
Smoking	Yes	21	23.9	67	76.1	x^2=1.080 p=0.299
	No	5	15.2	28	84.8	
Alcohol usage/ experience	Yes	23	19.8	93	80.2	p=0.066*
	No	3	60.0	2	40.0	
Medications usage	None	24	20.5	93	79.5	x^2=7.921 p=**0.019**
	Only a few times	–	–	2	100	
	1–3 times a month	–	–	–	–	
	1–5 times a week	–	–	–	–	
	Almost every day	2	100	–	–	

* The value of x2 is not available as Fisher's exact chi-square test is used

There was a statistically significant low-level positive correlation between Fagerström Test for Nicotine Dependence and Digital Game Addiction Scale scores (p<0.05). As the Fagerström Test for Nicotine Dependence score increased, the Digital Game Addiction Scale score also increased. There was no significant association between the Fagerström Test for Nicotine Dependence and the Internet Addiction Test (p>0.05). There was a statistically significant, moderate positive correlation between the Digital Game Addiction Scale score and the Internet Addiction Test score (p<0.05). As the Digital Game Addiction Scale score increased, the Internet Addiction Test score also increased (Table 6).

Table 5. Comparison of FNAT, DGAS-7, IAT Scores by age and GPA according to alcohol abuse disorder

	Alcohol risky usage			No risky usage			Meaningfulness
	n	Avg.	SD	n	Avg.	SD	
Fagerström Test for Nicotine Dependence	21	6.24	2.43	67	4.46	2.45	t=2.908 p=**0.005**
Digital game dependence scale	26	13.50	6.75	94	13.44	6.87	t=0.042 p=0.966
Internet dependence scale	26	46.54	13.95	94	45.16	17.92	t=0.363 p=0.717
Age	26	21.54	4.17	95	20.42	1.22	t=1.351, p=0.188
General score average	16	18.92	29.06	49	15.70	26.33	t=0.413, p=0.681

Table 6. Examining the relationship between FNAT, DGAS-7, IAT, age and GPA

		Fagerström Test for Nicotine Dependence	Digital game dependence scale	Internet dependence scale	Age	General score average
Fagerström Test for Nicotine Dependence	r	1	0.137	0.029	0.108	−0.135
	p		**0.043**	0.673	0.110	0.144
	n	219	217	215	218	118
Digital game dependence scale	r	0.137	1	0.534	0.038	−0.155
	p	**0.043**		**0.000**	0.291	**0.001**
	n	217	780	766	776	457
Internet dependence scale	r	0.029	0.534	1	−0.032	−0.061
	p	0.673	**0.000**		0.379	0.193
	n	215	766	769	766	453

Fagerström Test for Nicotine Dependence scores varied significantly by alcohol abuse disorder risk status (p<0.05). Individuals with risky alcohol use had higher Fagerström Test for Nicotine Dependence scores (Table 5).

Discussion

This research was conducted to evaluate the levels of addiction: cigarette, alcohol, substance, game and internet addiction among university students living in a borough. Universities are an important reflection of the general smoking behaviour of the young population. Many studies in the scientific literature have determined the smoking rates of university students. As there are studies in our country with findings similar to our study (22-34%), there are also studies that have found much higher rates (40-50%).[6,27-29] The smoking rate found in some of the studies conducted in other countries is; 51.4% in Syria, 24% in Italy, and 21% in the USA.[30-32] Two people who said no were included as smokers because they stated that they used electronic cigarettes. When we look at our country and the world in general, it is seen that the rate of smoking among university students is high.

The alcohol consumption rate of university students was determined as 14.8%. Study findings are similar to scientific literature.[33] A multicentre study found that among seven countries (Germany, Poland, Bul-

garia, Denmark, Lithuania, Spain, and Turkey), Turkish students had a lower prevalence of daily alcohol use compared to students studying in other countries (male 19%, female 8%).[34] These results may make us think that living in the borough and religious values also affect it.

Of the participants, 2.3% stated that they use cannabis, 1.4% use volatile substances, and 1% use pills. The rate of students' addictive substance use was found to be 13.4% in a study.[18] Studies in Germany show that alcohol and substance use is more common among university students than in Turkey.[35] Obtaining the study data from a campus in a borough constitutes one of the limitations of the study. Therefore, results cannot be generalized. However, it can be said that the access to the substance of the participants included in the study is low because it is lower than in the city centre.

The participants obtained 35.6% had a very low level of nicotine dependence, 25.6% had a low level of nicotine dependence, and 13.7% had a moderate nicotine dependence. A study conducted with 622 undergraduate students studying at a private university determined that students had an average FNTP score of 3.6, male students scored higher, and the FTND scores indicated low (29.3%) and moderate (9.7%) dependence Evli found the mean FNTP score of the students as 3.52. The present study is consistent with these findings.[30,36] It can be said that a small part of the students have never met cigarettes. The nicotine addiction rate of the last-year students was lower than those in the 1st and 2nd-years. In their study with medical school students, Şenol determined that the first three years were the riskiest years for smoking.[27]

There was risky alcohol use in 21.5% of the participants. Studies on university students found alcohol use risks to be 13% in Denmark, 7.2% in Hong Kong, 1% in Indonesia.[37-39] Many studies determined the alcohol use rate and addiction levels to be higher in males than in females.[18,33,34] Unlike these, our study found no significant correlation between gender and risky alcohol use. The rate of risky alcohol use was higher in students in the nursing department (66.7%). This finding is consistent with the literature.[38]

According to the Household Information Technologies (IT) Usage Survey, 2021 report, the internet usage rate in our country is 82.6% in the 16-74 age group. According to the Digital 2021 report, the worldwide average daily internet usage time is 6 hours 54 minutes, and in our country, it is above the world average with 7 hours and 57 minutes. The scores varied between 20-101, and based on the scale cut-off points, 3.8% of the participants were internet addicts. In a study conducted in Ordu, the average score obtained from the Internet Addiction Test was 89.87.[40] In their systematic review examining the internet addiction levels in the Gulf countries, Al-Khani et al. reported the internet

addiction rate as 33%.[41] Both the total scores and the addiction rates of the participants were lower than the mentioned studies.

No significant relationship was found in the rate of internet addiction according to the gender variable. Besides similar studies in the scientific literature, there are also studies reporting higher internet rates in men.[42,43] Internet addiction rates of those with a high-income level were higher in accordance with the literature.[42,43] Since the students live in the borough, it can be predicted that the internet speed is low.

Participant 2.2% of the students are game addicts. Aktaş and Bostancı found students' Digital Game Addiction Scale mean score as 35.86±16.97, and reported that 6.3% of the students were in the addicted group and 1.6% were in the highly addicted group.[44] The digital game addiction scores differed significantly by gender. The addiction scores of male students were higher than female students. There are similar studies in the scientific literature.[42]

The study revealed a statistically significant, low-level negative correlation between the overall grade point average and the DGAS-7 Scale score. As the DGAS-7 Scale score increased, the overall grade point average decreased. This finding is similar to the scientific literature.[45] While gaming is a healthy deal with, it can pose some risks for college students who are still in their teens.

There was a statistically significant, low-level positive correlation between Fagerström Test for Nicotine Dependence and Digital Game Addiction Scale scores, consistent with the scientific literature.[46] There was no significant association between the Fagerström Test for Nicotine Dependence and the Internet Addiction Test. Besides studies in the scientific literature that are compatible with the findings of the studies, there are also studies showing a positive relationship between nicotine addiction and internet addiction.[6,47,48] A moderate positive relationship was found between the participants' Internet addiction and digital game addiction levels. There are studies several studies have associated Internet addiction with smartphone addiction.[42,49]

In the study, nicotine addiction levels were found to be higher in those who used/tried alcohol and those who used pills. Similar to the findings of the study, the scientific literature indicates a significant relationship between the use of tobacco, alcohol, and other substances.[35,50]

Conclusion

Despite the fact that societies continue to stand against all kinds of addiction and take precautions, all addiction rates continue to threaten society and mental health. In our study, the risky use of alcohol by students in the nursing department was higher than in other departments, indicating that health workers are

also under threat. In addition, individuals with risky use of alcohol have higher rates of nicotine addiction, the increase in game addiction as nicotine addiction increases and the increase in internet addiction as digital game addiction increases suggest a connection between addictions. We are of the opinion that individual approaches should be taken with all types of addiction, taking into account the level and degree of addiction, and that protection programs should also be organized for health workers.

Among the examined addiction types, although nicotine, alcohol, and substance addictions are old concepts, internet and game addiction are relatively newly defined concepts. All the addiction types discussed are significant public health problems for young individuals both in Turkey and around the world. This study demonstrates that individuals with any addiction are also at risk for other types of addiction. Hence, it is clear that it is necessary to provide university students with sufficient knowledge and equipment about the types of addictions, the diseases they can cause, the harm they cause to society, the methods of getting rid of this habit, and to develop protective approaches.

The research was carried out per the principles of the Declaration of Helsinki.

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ORIGINAL PAPER

Compliance of temozolamide with concurrent radiotherapy as an adjuvant in patients with high grade glioma – a retrospective study

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ABSTRACT

Introduction and aim. High grade glioma is the most aggressive form of primary brain tumour with a median survival of one year. Maximal safe resection followed by temozolamide-based concurrent chemoradiation and adjuvant chemotherapy is the standard of care. To assess the compliance of temozolamide in patients of high-grade glioma who underwent concurrent chemoradiation followed by adjuvant chemotherapy

Material and methods. 30 patients of high grade glioma diagnosed and treated in our Oncology department during the period of March 2016 to March 2018 were analyzed retrospectively. Cases included in this study were patients with biopsy proven high grade glioma who underwent maximal safe surgery, temozolamide-based concurrent chemoradiation, followed by adjuvant chemotherapy with temozolamide. Data regarding age, gender, histopathology, extent of surgery, performance status, radiotherapy dose, chemotherapy cycles and treatment toxicity profiles were recorded.

Results. Treatment was generally well tolerated with most patients experiencing grade 1 and 2 toxicities, which were managed with supportive care. Grade 3 toxicities were noted as follows: anaemia (6.7%, n=2), neutropenia (16.7%, n=5) and thrombocytopenia (16.7%, n=5). Treatment with TMZ was discontinued in 6.7% (n=2) of individuals due to myelosupression. No grade 4 hematological toxicities were observed in the study group.

Conclusion. The compliance of temozolamide in high grade gliomas is high with less treatment interruptions and manageable side effect profile.

Keywords. glioblastoma multiformae, high grade gliomas, radiotherapy, temozolamide

Introduction

High grade gliomas (HGG) are the most aggressive primary brain tumours. The standard of care for newly diagnosed high-grade glioma includes maximal safe resection of the tumour followed by 6 weeks course of radiotherapy with concurrent temozolamide (TMZ), followed by adjuvant TMZ for 6months. Previous studies proved that postoperative radiotherapy compared to surgery alone provided significant survival advantage.[1,2] A phase III trial by stupp et al showed that temozolamide given concurrently with radiotherapy gives better overall survival than radiotherapy alone in glioblastoma multiforme patients.[3] The European Organisation for the Research and Treatment of Cancer (EORTC) study proved that concurrent TMZ along with radiation followed by Adjuvant TMZ had increased the median

survival & 2-year survival rate. Another study showed that patients completing 6 cycles of adjuvant TMZ had significantly better outcome.[4]

Temozolamide is an oral second-generation alkylating agent which readily crosses the blood brain barrier and has near 100% bioavailability. TMZ is the prodrug of alkylating agent 5-(3 methyltriazen-1-yl) imidazole-4-carboxamide (MTIC). After administration, TMZ spontaneously hydrolyzes to the active metabolite MTIC & subsequently to the active methylating agent, methyl hydrazine. TMZ exerts its anti-tumor effects by methylating guanine at the N7 (70% of adducts) and O6 (5% of adducts) positions and adenine at the N3 position (9% of adducts).[5,6]

The antitumour activity of the TMZ has been shown in preclinical studies.[7] Currently the recommended dosing schedule for TMZ is 75mg/m2 for concurrent chemoradiation and 200mg/m2 for 5 days every 28 days in the adjuvant setting. Many studies tried different schedules to determine the efficacy without enhancing the toxicity.[8-11] Aforementioned doses were used in this study.

Aim

The aim of the study was to analyze the compliance of the TMZ in patients getting concurrent radiation and followed by adjuvant TMZ in a tertiary care hospital.

Material and methods

Ethical approval

Ethical approval was obtained from the Institutional ethical committee, Government Stanley Medical College and hospital (ECR/131/Inst/TN/2020/RR-21).

Study design

30 patients of high-grade gliomas diagnosed and treated in our Oncology department during the period of March 2016 to March 2018 were analyzed. The cases included biopsy proven high grade glioma patients who underwent maximal safe surgery, postoperative radiotherapy and concurrent temozolamide followed by adjuvant TMZ. Adequate bone marrow reserve, normal renal parameters and hepatic parameters were ensured before the study. Data regarding age, gender, histopathology, extent of surgery, performance status, radiotherapy dose, chemotherapy cycles and toxicity profiles were recorded. Side effects were graded in severity based on CTCAE 4 guidelines.

– Patients receiving transfusions were classified as having grade 3–4 cytopenias during the transfusion period.

– This adjustment was not made for hematopoietic growth factor administration.

– Cytopenias were attributed to concomitant daily TMZ and RT administration if they occurred at any

point before thestart of adjuvant TMZ (usually four weeks after the completion of concomitant therapy).

– In patients who developed hematologic toxicity, TMZ administration was delayed, dose adjusted, or discontinued according to the guidelines Additionally, patients developing hematologic toxicity underwent a careful medication review (Table 1).

Table 1. Blood and lymphatic system disorders

Adverse events	Grade				
	1	2	3	4	5
Anemia[*]	Hemoglobin (Hgb) <LLN– 10g/dl; <LLN–6.2mmol/L; <LLN–100g/L	Hgb <10.0–8.0g/dL <6.2–4.9 mmol/L; <100–80g/L	Hgb <8.0g/dL <4.9 mmol/L; <80g/L; Transfusion indicated	Life-threatening consequences; urgent intervention indicated	Death
Bone marrow Hypocellular[**]	Mildly Hypocellular Or <=25% reduction from normal cellularity for age	Moderately Hypocellular Or >25–<50% reduction from normal cellularity for age	Severely Hypocellular Or >50–<=75% reduction from normal cellularity for age	Aplastic persistent for longer than 2 weeks	Death
Febrile Neutropenia[***]	–	–	ANC <1000/mm3 with a single temperature of >38.3°C (101°F) or a sustained temperature of ≥38°C (100.4°F) for more than one hour	Life threatening consequences; urgent intervention indicated	Death

[*] A disorder characterized by a reduction in the amount of Hemoglobin in 100 ml of blood. Signs and symptoms of anemia may include pallor of the skin and mucous membranes, shortness of breath, palpitations of the heart, soft systolic murmurs, lethargy and fatigability; [**] A disorder characterized by the inability to produce hematopoietic elements; [***] A disorder characterized by an ANC <1000/mm^3 and a single temperature of >38.3°C (101°F) or a sustained temperature of ≥38°C (100.4°F) for more than one hour

Radiotherapy and chemotherapy treatment

After maximal safe surgery, postoperative radiotherapy was given at a total dose of 6000cGy in 200cGy per fraction over a period of 6-7 weeks in 2 phases. 5000 cGy was given to the tumour bed/residual tumour along with the surrounding edema with 3cm clearance in the initial phase. Second phase of radiation was administered to tumours with 3 cm margin. The radiotherapy was delivered by two opposing lateral fields with Telecobalt machine. Patients received concurrent TMZ (75mg/m^2/day) 7 days per week one hour before radiation and 6 cycles of adjuvant TMZ (200mg/m^2/day) day 1-5 in every 28 days.

Statistical analysis

Data was entered in MS-Excel (Redmond, Washington, USA) software and analysed by using SPSS version 26 (IBM, Armonk, NY, USA). Descriptive statistics were seen by mean and standard deviation for numerical data, whereas for categorical data, frequency table was represented.

Results

Patients and treatment characteristics

The median age of the study group was 46.7 years (Range: 17–67years). Male:Female ratio=1:1. The demographic data of the patients are given in Table 2. 43.3% of the patients had poor performance status (KPS<70, n=13). 86.7 % of the patients had completed the full 60 Gy of postoperative radiotherapy (n=26). 83.3% of the patients had completed the concurrent TMZ and 66.7 % were able to complete the entire treatment protocol (including adjuvant TMZ). 33.3% of patients did not receive adjuvant chemotherapy due to disease progression, toxicity etc.

Table 2. Demographic data of the patients

Variables	Variable	No (%)
Age	≤30yrs	5 (16.7)
	31-40	5 (16.7)
	41-50	8 (26.7)
	51-60	9 (20)
	>60	3 (10)
Sex	Male	15 (50)
	Female	15 (50)
Kps	<70	13 (43.3)
	≥70	17 (56.7)
Tumor size	<4	9 (30)
	≥4	21 (70)
Histology	GBM	17 (56.7)
	Anaplastic astrocytoma	10 (33.3)
	Anaplastic oligo	3 (10)
Grade	3	13 (43.3)
	4	17 (56.7)
Surgery	Biopsy only	10 (33.3)
	Partial excision	2 (6.7)
	Subtotal resection	7 (23.3)
	Near total resection	10 (33.3)
	Radical excision	1 (3.3)
RT	<60Gy	4 (13.3)
	≥60gy	26 (86.7)
Concurrent chemo	Yes	5 (16.7)
	No	25 (83.3)
Adjuvant chemo	<60	10 (33.3)
	≥60	20 (66.7)

Toxicity assessment

All patients were evaluated for toxicities during concurrent chemoradiation and adjuvant chemotherapy with TMZ. Toxicities were assessed and graded according to CTCAE version 4.0 developed by national cancer institute. Hematological toxicities include thrombocytopenia, anaemia, neutropenia and the non hematological toxicities include nausea, vomiting, headache, skin rashes, pruritis. Patients in the combined modality group were able to complete the 6 cycles of adjuvant TMZ. Majority of the hematological & non hematological toxicities were grade 1& 2. 52% of the study group had non hematological side effects and 28% had hematological side effects. Non hematological toxicities like nausea and vomiting were managed by antiemetics and dopamine receptor antagonists. Treatment was generally well tolerated with 6.7% of patients developing grade 1&2 anaemia, 16.7% of patients with grade 1&2 neutropenia (n=5) and 16.7% of patients(n=5) with grade 1&2 thrombocytopenia during the treatment. Concurrent TMZ was discontinued in two patients (6.6%) due to grade 3 myelosuppression and one patient (3.3%) developed grade thrombocytopenia. No grade 4 hematological toxicities were observed in the study group. Hematological toxicities were managed with delay or discontinuation of temozolamide therapy that was decided by the treating physician. The toxicity profile of the study group was given in Table 3.

Table 3. Toxicity profile

Toxicities	Variable	Grade 1&2 No (%)	Grade 3 No (%)
Hematological	Anemia	2 (6.7)	
	Neutropenia	5 (16.7)	2 (6.6)
	Thrombocytopenia	5 (16.7)	1 (3.3)
Non Hematological	Nausea	15 (50)	
	Vomiting	7 (23.3)	
	Headache	4 (13.3)	
	Constipation	10 (33.3)	
	Skin rash	1 (3.3)	

Discussion

Postoperative radiotherapy with concurrent TMZ followed by adjuvant chemotherapy after safe maximal surgery is the standard of care in high grade gliomas. The feasibility and safety of TMZ along with radiotherapy is higher when compared with nitrosoureas. TMZ also has radio sensitizing properties that have proved in vivo and in vitro studies.[11,12] The standard schedule of TMZ in high grade glioma patients is 200mg/m^2 for 5 days every 28days.[13-16]

In this retrospective study only 9% of the patients had grade 3 haematological toxicity. These values are comparable with the earlier study by Stupp et al. in which haematological grade 3& 4 toxicities were noticed in 7% of the patients, mainly neutropenia and thrombocytopenia.[17]

Another study found a myelosuppression in 8.7% of their patients. Leucopenia grade 3 and 4 were noticed in

3.5% and thrombopenia grade 3 and 4 in 5.2%.[18] Many retrospective studies analyzed the temozolamide toxicities and found that the frequency of grade 3 & 4 myelosuppression at a rate of 3% to 15% and grade 3 & 4 thrombocytopenia at 0–15%.[19-21] Majority of toxicities in our study were grade 1&2 and were tolerable to our patients. Gastrointestinal disturbances such as nausea and vomiting were exceedingly common. Previous studies showed that these are known as two of the most important serious side effects which affects the quality of life of the cancer patients during chemotherapy.[22]

The main hematological side effect of our study was myelosuppression, which was reversible. Most of the patients in our study were able to complete the treatment. The awareness of toxicities and evidence-based interventions for managing toxicities are important to maintain the quality of cancer care in glioma patients. The concurrent administration of radiotherapy and TMZ was feasible and well tolerated with few hematological and non-hematological toxicities. The compliance of the adjuvant therapy was satisfactory and was comparable to the previous studies. The grade 3&4 non hematological toxicities were nil in the study group. Hematological toxicities commonly occurred during concurrent chemoradiation and non haematological toxicities were common during adjuvant chemotherapy cycles.

In summary, the results of our study suggest that concomitant chemoRT with TMZ followed by six cycles of adjuvant TMZ was safe and well tolerated, and patients were able to sustain therapy. The recently published international EORTC phase III trial has demonstrated a similar benefit. Patients received Temozolomide 75 mg/m² everyday with radiotherapy as recommended by the EORTC/NCIC trial.

Limitation of this retrospective study is the small sample size (n=30). The demographic data & few prognostic variables of the current study could be correlated with previous studies. We have found that the compliance of the patients to radiotherapy and chemotherapy is good.

Conclusion
The compliance of temozolamide in high grade gliomas is high with less treatment interruptions and the toxicities are minimal and manageable with good outcomes, making it a good feasible protocol for high grade gliomas.

Declarations
Funding

Author contributions
Conceptualization, V.A.R. and S.J.; Methodology, V.A.R., S.J. and K.R.S.; Formal Analysis, K.R.S.; Investigation, X.X.; Resources, V.A.R. and S.J.; Data Curation, V.A.R. and S.J.; Writing – Original Draft Preparation, V.A.R. and S.J.; Writing – Review & Editing, V.A.R., S.J. and K.R.S.

Conflicts of interest
The authors have no conflict of interest.

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

Ethics approval
Ethical approval was obtained from the Institutional ethical committee, Government Stanley Medical College and hospital (ECR/131/Inst/TN/2020/RR-21).

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ORIGINAL PAPER

Effect of some characteristics of fathers on paternal-infant attachment in the postpartum period

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ABSTRACT

Introduction and aim. The paternal attachment can affect the academic, social and emotional development of the child in the future. The study was conducted in a descriptive-cross-sectional design to determine the impacts of some characteristics of fathers on paternal attachment.

Material and method. The study was conducted with 278 fathers who had 3-12 months old infants. The data were collected by using the "Father Information Form" and the "Postnatal Paternal-Infant Attachment Questionnaire (PPAQ)".

Results. It was found in the present that the mean PPAQ score of the fathers who were exposed to domestic violence in their childhood was significantly lower than those who were not (p=0.001). The mean PPAQ score of the fathers whose infant was born by cesarean section (p=0.017) and who had physical contact for the first 24 hours (p=0.047) was found to be significantly higher. The mean PPAQ score was significantly higher in fathers who had a physical contact duration of 7 hours or more than those with less physical contact durations (p=0.030).

Conclusion. In this study, some characteristics of fathers were found to affect father-infant attachment. It may be recommended to plan preventive interventions for risky fathers.

Keywords. attachment, father, infant

Introduction

The attachment of the parent with the infant begins during the intrauterine period, and the attachment of the infant with the parent begins after the delivery.[1,2] Postpartum attachment is an important process in the postpartum period and there is evidence that attachment difficulties are associated with the mental health of the parents and have negative outcomes on the development of the child.[3] It was found that the character of the infant affects the parenting behaviors shared between parents, especially in the 0-24-month period of the infant's life.[4] The attachment taking place in infancy (1-12 months) shows limited variability in the following periods.[5] Based on the opinion that adult attachment is the continuation of childhood attachment, it was found that attachment styles in adulthood are 72% similar to childhood attachments and affect the social lives of individuals.[6] It is not likely that the secure or insecure attachment of the infant will change later.[7]

It was found in the literature review that studies conducted within the scope of parental attachment are mostly on mother-infant attachment, and studies on father-infant attachment are limited.[8,9] Considering the impacts that each parent has on the development of the child, it is already known that it is important not to ignore the impacts of the father on the development of the child.[10]

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Although the main attachment figure is considered the mother, infants can also establish a secure attachment with their fathers as well.[11] The attachment relationship that children establish with their fathers affects their academic, social and emotional development significantly.[12] The importance of integrating the cognitive, affective, and behavioral aspects of parenting is emphasized in the attachment.[13] It is possible to argue that father-infant attachment has long-term impacts on individuals' lives at significant levels.[14] The father's experiences in his childhood and the way he established bonds with his parents also affect the father's bond with his infant.[15,16] It is speculated that fathers who have secure adult attachment styles enjoy parenting more.[17] However, childhood experiences also affect adulthood, and the child who witnesses or is abused can carry it into adulthood. Also, it is considered that children with insecure attachment relations with their parents are in danger of neglect and abuse.[18] It is already known that the physical contact and attachment of the infant with the mother is at the forefront because of the infant's need for the mother in the postnatal period, but the physical contact and attachment between the father and the infant remains in the background. If the father comes into physical contact with his infant in this period, the bond between the father and the infant is initiated.[19] Also, the physical stimulation of the infant by the father has a positive impact on toddlerhood.[20] If there is a problem in father-infant attachment and insecure attachment style happens, problems emerge in the mental, spiritual and physical development of infants.[21] Insecure attachment styles in infancy and childhood are considered as the source of psychopathologies in the infant's later life.[22] It is also known that low paternal involvement is associated with more peer aggression, and infants with a secure attachment style overcome adolescence problems more easily.[23,24] Securely attached children have more receptive and positive behaviors.[25]

Attachment forms, which are the part of characteristics of an individual, bring the individual to the forefront in cases such as separation or loss.[26] The absence of the father between the ages of 0-3 may cause the infant to be more prone to self-harm during adolescence.[27] It is speculated that individuals with an attachment figure that they can reach at any time in case of need have less fear and anxiety when compared to those who are deprived of this.[28] It has been revealed that securely attached parents are more sensitive and interested in their children, and these children are more strongly attached to their parents.[29] Due to working mothers, fathers are spending more and more time with their babies today.

Aim
Studies on parent-infant attachment mostly involve mothers. It is considered that the findings obtained in the present study, which was conducted to determine the impacts of some characteristics of fathers on paternal attachment in the postpartum period, will shed light on the development of healthy attachment styles and the planning of mental health protective practices for children.

Research questions
–   Do father's sociodemographic characteristics affect father-infant attachment?
–   Does the father's experiences with his parents during childhood affect his bonding with his baby?
–   Do baby's characteristics affect father's attachment?
–   Does the father's physical contact with the baby in the first 24 hours affect the attachment?

Material and methods
Ethical approval
The study was approved by the Human Research Ethics Committee of Zonguldak Bülent Ecevit University (approval no: 2021/106). The participants read the information text before they filled out the questionnaires, and those who approved were included in the study.

Study design
The study was conducted in a descriptive and cross-sectional design to determine the impacts of some descriptive and sociodemographic characteristics of fathers on paternal attachment in the postpartum period.

Population and sample
The data of the study were collected with the Snowball/Chain Sampling Method between May 2021 and February 2022 in Turkey. This study includes participants from all geographical regions of Turkey. A total of 285 fathers participated in the study. Seven fathers were excluded from the study because they did not accept to participate in the study. The sample of the study consisted of 278 fathers who volunteered and approved to participate in the study.

Data collection
The data of the study were collected by using an online survey between May 2021 and February 2022. The online survey was created using Google Forms. The questionnaire form was sent to the participants using online networks (e-mail, WhatsApp, Instagram, etc.). Before starting the survey, the participants read the explanations about the study (purpose, subject, criteria for participation in the study, voluntary participation in the study, etc.) and approved. The time to fill out the questionnaire varied between 5 and 10 minutes. To evaluate the suitability of the questionnaire, a preliminary application was made with 10 participants. After the necessary arrangements were made in the questionnaire form as a result of this pre-application, the application stage was started.

Father Information Form: The form consisted of 24 questions on demographic characteristics of fathers (age, occupation, educational status, etc.) and characteristics of the infant (age, gender, etc.).

Table 1. The sociodemographic characteristics of the fathers (n=278)

Sociodemographic characteristics		
Age (years) Mean±SD	31.16+4.94 (min:20-max:50)	
Marital status	**n**	**%**
Married	273	98.2
Single	5	1.8
Educational Status		
Literate	3	1.1
Primary education	30	10.8
High school	107	38.5
University and above	138	49.6
Working Status		
Working	242	87.1
Not working	36	12.9
Profession		
Employee	42	15.1
Officer	72	25.9
Private sector	126	45.3
Unemployed	11	4.0
Other	27	9.7
Health Insurance		
Yes	258	92.8
No	20	7.2
The family type lived longest in		
Elementary	162	58.3
Extended	96	34.5
Single Parent	20	7.2

The postnatal paternal-infant attachment questionnaire (PPAQ)

The Postnatal Paternal-Infant Attachment Questionnaire (PPAQ) was developed by John T. Condon to evaluate postnatal father-infant attachment.[12] The Turkish validity and reliability study of the scale that was developed by Condon (2008) was conducted by Güleç & Kavlak in 2013.[14,30] The scale consisting of 19 items; Items 1, 2, 6, 11, 13, 17 and 18 are 'patience and tolerance', items 4, 5, 8, 9, 10, 12, 15, 19 are 'pleasure in interaction' and items 3, 7, 14 they constitute the 'love and pride' dimension. The lowest score that can be obtained from the scale is 19, the highest score is 95 and a high score on the scale shows a high level of attachment. In the study of Güleç, the Cronbach Alpha value of the scale was calculated as 0.76.[30] The Cronbach Alpha value was calculated as 0.833 for this study.

Statistical analysis

The data analysis was made by using the SPSS 21.0 (Statistical Package for Social Sciences) package program. Descriptive statistics, mean, standard deviation, frequency, and percentage were used in the evaluation of the data.

The conformity of the quantitative data to the normal distribution was tested with the Kolmogorov-Simirnov Test. The t-test was used for the comparison of quantitative variables between two groups because the data showed a normal distribution. The ANOVA Test was used for the comparisons between more than two groups. The Tukey Analysis was used as a Post-Hoc Test to evaluate the differences between the groups. In the comparisons, p<0.05 was accepted as the significance level.

Table 2. The characteristics of the lives of fathers with their parents (n=278)

Father's life with his parents	n	%
Marital status of their parents		
Married	256	92.1
Divorced	22	7.9
Age when parents divorced (n=22)		
0-2 Years	11	50.0
3-6 Years	2	9.1
7-11 Years	1	4.5
12-18 Ages	4	18.2
18 years and over	4	18.2
Custody parent (n=21)		
Mom	20	95.2
Father	1	4.8
Exposure to domestic violence during childhood		
Yes	90	32.4
No	188	67.6
Type of violence exposed (n=90)		
Physical Violence	59	65.6
Verbal Violence	19	21.1
Emotional Violence	10	11.1
Other	2	2.2
People applying violence (n=90)		
Mother	21	23.3
Father	61	67.7
Other (Grandmother, etc.)	8	8
Receiving psychological help when exposed to violence (n=90)		
Yes	1	1.1
No	89	98.9

Results

The mean age of the fathers was found to be 31.16±4.94 (min:20, max:50), 98.2% were married, 49.6% were at university or higher education level, 87.1% were working in a job and 45.3% were working in the private sector in the present study. A total of 92.8% of the fathers had health insurance, and 58.3% of them lived the longest in an elementary family (Table 1).

It was found that 92.1% of the parents of the fathers who were included in the study were married, 7.9% were divorced, 50.0% were in the 0-2 age group when their parents were divorced, and 95.2% of them had their custody in their mothers. Also, 32.4% of the fathers were exposed to domestic violence in their childhood, 65.6% of those who were exposed to violence experienced phys-

ical violence, and 67.7% of the people who applied violence were fathers. A total of 98.9% of the fathers stated that they did not receive psychological help when they were exposed to violence (Table 2).

When the characteristics of the fathers were examined, it was found that the mean infant age was 6.69±3.04 (min:3-max:12) months and 51.1% were female. Among the fathers, 27.7% of whom said that they had other children, 88.8% of them had planned pregnancy, 55.4% of them were born by cesarean section, 79.9% of them had physical contact in the first 24 hours when the infant was born, and 44.6% of those who had physical contact had less than 1 hour of contact. Also, 43.2% of the fathers stated that they were with their spouses during the delivery (Table 3).

Table 3. The characteristics of the infants of the fathers (n=278)

The characteristics of the infants of the fathers		
Infant's age (months) Mean±SD	6.69±3.04	(min:3-max:12)
Number of children Mean±SD	1.76±0.73	(min:1-max:5)
Infant's gender	**N**	**%**
Girl	142	51.1
Boy	136	48.9
Other children		
Yes	77	27.7
No	201	72.3
Planned pregnancy		
Yes	247	88.8
No	31	11.2
Birth type		
Normal	124	44.6
Cesarean section	154	55.4
Physical contact in the first 24 hours when the baby was born		
Yes	222	79.9
No	56	20.1
Physical contact time (n=222)		
Less than 1 hour	99	44.6
1-3 hours	63	28.4
4-6 hours	26	11.7
7 hours or more	34	15.3
Status of being with the spouse at the time of delivery		
Yes	120	43.2
No	158	56.8

Table 4. PPAQ sub-scale and total mean scores

PPAQ sub-scale	Mean±SD	Minimum	Maximum
Patience and tolerance	23.08±2.67	14.9	28
Pleasure in interaction	27.07±5.14	8	35
Love and pride	18.52±2.2	4	20
PPAQ scores	68.17±8.55	40	81

The mean patience and tolerance sub-scale score was 23.08±2.67 32, the mean pleasure in interaction sub-scale score was 27.07±5.14, the mean love and pride

sub-scale score was 18.52±2.20, and the PPAQ mean total score was 68.17±8.55 (Table 4).

Table 5. The distribution of some characteristics of the fathers according to the PPAQ scores[a]

Characteristics of the fathers	Mean±SD	Test	Post-hoc*
Marital status			
Married	68.15±8.55	t=-0.311	
Single	69.36±9.92	p=0.756	
Educational Status			
Literate-Primary Education	64.62±11.55	f=4.481	1 and 3
High school	67.71±8.99	p=0.012	p=0.016
University and above	69.38±7.04		
Working Status			
Working	67.82±8.64	t=-1.775	
Not working	70.53±7.64	p=0.077	
Health Insurance			
Yes	68.11±8.61	t=-0.478	
No	69.08±7.88	p=0.633	
Type of the family lived longest			
Elementary	68.82±9.23	f=0.674	
Extended	67.99±8.51	p=0.511	
Single Parent	66.53±4.79		
Marital status of the parents			
Married	68.28±8.75	t=0.7	
Divorced	66.95±5.80	p=0.485	
Exposure to domestic violence in childhood			
Yes	65.66±9.31	t=-3.455	
No	69.38±7.92	p=0.001	
Baby's Gender			
Girl	67.62±8.61	t=-1.092	
Boy	68.74±8.49	p=0.276	
Other children			
Yes	65.42±9.16	t=-3.376	
No	69.22±8.09	p=0.001	
Planned pregnancy			
Yes	68.55±8.19	t=2.119	
No	65.12±10.69	p=0.035	
Birth type of the infant			
Normal	66.81±8.50	t=-2.4	
Cesarean section	69.27±8.47	p=0.017	
Physical contact in the first 24 hours when the baby was born (n=222)			
Yes	68.70±8.38	t=2.045	
No	66.09±8.98	p=0.047	
Physical contact time (n=222)			
less than 1 hour	66.86±8.32	f=3.025	1 and 4
1-3 hours	69.89±8.00	p=0.03	p=0.05
4-6 hours	69.26±8.19		
7 hours or more	70.96±8.35		
Status of being with the spouse present at the time of delivery			
Yes	69.30±8.06	t=1.922	
No	67.32±8.84	p=0.056	

[a] PPAQ – postnatal paternal-infant attachment questionnaire; t – independent samples t test; f – one way ANOVA; *Post-Hoc: Tukey test

It was found in the present study that the mean PPAQ scores of the fathers differed at statistically significant levels according to their educational status (f=4.481, p=0.012). When the origin of the difference between the groups was analyzed in the Post-Hoc Analysis, it was found that the mean PPAQ score of university graduates was higher at a significant level than that of literate/primary school graduates (p=0.016). It was also found that the mean PPAQ score of those who were exposed to domestic violence in their childhood was lower at a significant level compared to those who were not (t=-3.455, p=0.001). The mean PPAQ score of the fathers who did not have any other children was found to be higher at a significant level (t=-3.376, p=0.001). The mean PPAQ score of fathers whose infants were born by cesarean section (p=0.017) and who had physical contact for the first 24 hours (p=0.047) when their infant was born was also higher at a significant level. In addition, the mean PPAQ score of the fathers who had a physical contact duration of 7 hours or more was higher at a significant level than those with less physical contact (f=3.025, p=0.03) (Table 5).

Discussion

Attachment is a strong bond, which develops between the infant and the primary caregiver, and establishes a sense of security in the infant. The effect of the attachment pattern that is established in the early days of life in determining the future relationship styles of the person is an indisputable fact. The origin of most psychopathological conditions that emerge in the early three years of life stems from the relationship between the infant and the primary caregiver. Any disruption in this relationship can be effective in the formation of a pathological structure.[31] In this context, it is important to determine the effect of father-infant attachment along with sociodemographic and other factors. The present study was conducted to investigate the impacts of some characteristics of fathers on paternal attachment in the postpartum period.

The mean PPAQ scale score of the fathers who participated in the study was found to be 68.17±8.55. The highest score that can be obtained from PPAQ is 95. As the score increases, the level of father-infant attachment also increases. In the study conducted by Aydın Kartal & Erişen investigating the father-infant attachment, the mean total score was reported to be 71.37±10.55.[32] In their study, Yu et al. found the mean total score to be 75.68±10.01.[33] Aslan et al. found the mean father-infant attachment score to be 70.81±8.22 in their study and they accepted that the attachment was good.[34] The result of this study is similar to the results of the studies carried out.[32,33,34]

The attachment scores of the fathers who had a high level of education were found to be significantly higher in the study. In his study, Dinç reported statistically significant differences in the mean PPAQ score of the father's education level.[35] The high educational level of fathers causes them to become conscious fathers. In his study, Nkwake argued that attachment was high because the higher education level of the father increases the participation in the infant's care.[36] In the study of Kuzucu it was stated that fathers with a high level of education have higher self-confidence and are more knowledgeable about baby care.[37] The findings of the present study show parallelism with previous studies. In the study conducted by Kılan, no significant differences were reported in the mean PPAQ score of the education level of fathers.[38] The findings of our study are not consistent with Kılan's study.

Also, it was found in the present study that the mean PPAQ score of those who were exposed to domestic violence in their childhood was significantly lower than those who were not and 65.6% of those who were exposed to violence experienced physical violence, and 67.7% of the people who applied violence were fathers. It is considered that physical violence experienced by fathers in childhood affects father-infant attachment negatively. The physically abused individual carries it into adulthood.[18] In the literature, the opinion that fathers' bad experiences in childhood affect their attachment relationships negatively with their children supports this study. It was also emphasized that risk groups must be determined and the education level of fathers on this subject must be increased to develop fathers' attachment to their infants in a healthy way.

It was also found that the mean PPAQ score of the fathers who did not have any other children was significantly higher. In the study conducted by Türk Düdükçü and Aslan, it was reported that as the number of children increased, the total scale score of PPAQ decreased at significant levels.[39] The increasing number of children may cause the father's interest, attention, and energy to be divided, and the rate of participation in the infant's care and the level of father-infant attachment to decrease. Dinç found that attachment decreased as the number of children increased.[35] In the literature, it has been stated that the increase in the number of infants decreases the participation in care.[40] Uçakçı Asalıoğlu speculated in his study that as the number of children increases, the attachment of fathers is affected negatively.[19] The study data are compatible with the literature data. In the present study, 88.8% of the fathers stated that their infants were planned. Karakulak found that 92.1% of fathers wanted the baby.[41] It is stated that in unplanned pregnancies, father candidates cannot adapt to fatherhood and experience anxiety in this aspect.[14] The study conducted by Aydın Kartal and Erişen was similar in that 72.8% of fathers had planned infants.[32]

The mean PPAQ score of the fathers whose infants were born by cesarean section and who had physical con-

tact for the first 24 hours when the infant was born was significantly higher in the study. The first hours after the delivery are important in terms of initiating father-infant bonding and becoming used to the new role of the father. Studies are reporting that skin-to-skin contact affects father-infant bonding positively.[42,43] It was found in another study that skin-to-skin contact made fathers more interested in their infants.[44] In his study, Huang reported that the father-infant skin-to-skin contact after cesarean section reduces the stress and depression levels of fathers, and fathers become more interested in their infants.[45] After cesarean delivery, the meeting of the infant with the mother is later than the infants born with vaginal delivery. For this reason, father-infant physical contact occurs during the period between the end of the cesarean section and the first contact of the mother with her infant.[46] This explains the high-level father-infant attachment since fathers' first contact with their infants occurs earlier after cesarean delivery. Vaginal delivery is recommended by stating that it has positive effects such as normal and physiological vaginal delivery, rapid recovery in the mother, less risk of infection, and early breastfeeding for the newborn.[47] Although the finding of the present study is compatible with the literature data that physical contact affects attachment in the first 24 hours positively, there are differences in terms of the effect of the infant's birth type on the father's level of attachment with the infant.

The duration of the contact of the infant is as important as the first 24 hours of contact with the father. In the present study, fathers who had first physical contact with their infants for 7 hours or more had higher levels of attachment than fathers who had physical contact with their infants for less than 7 hours. The more time the father spends with his infant, the greater the attachment. Previous studies reported that postpartum physical contact strengthens attachment.[21] The literature on how long fathers must be in physical contact with their infants is not sufficient. In this respect, it is considered that the present study will contribute to the literature.

In the study, no relationship was found between the gender of the baby and the level of father-infant attachment. Düdükçü and Yılmaz also stated in their studies that the gender of the baby did not differ statistically significantly at the level of attachment.[39,48] Evcili et al. found that fathers have high levels of attachment to their baby boy.[49] The reason for the differences in the literature is thought to be the increase in the level of education, the increase in the awareness of equality between girls and boys, and the social change in cultural norms for girls.

Conclusion

The factors such as fathers' educational levels, exposure to domestic violence in childhood, the infant's birth type, the infant's physical contact status and duration of physical contact with the father in the first 24 hours, and the status of having another child were found to have a significant effect on father-infant attachment in the present study.

It was also found in the study that factors such as the father's age, marital status, employment status, occupation, region of residence, type of family he lived for the longest time, gender of the infant, and presence of the spouse during birth did not affect father-infant attachment.

To develop father-infant bonding, which has an important place in terms of community mental health, risk groups must be determined and measures must be taken in this respect. Because of the scarcity of studies conducted on father-infant attachment in the literature, it is considered that the number of studies on this subject must be increased.

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ORIGINAL PAPER

Role of healthcare professionals in drug-drug interactions and clinical interventions

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ABSTRACT

Introduction and aim. Healthcare professionals including prescribers, pharmacists and nurses must have adequate knowledge of drug-drug interactions because they can cause toxicity, loss of efficacy, and side effects. This study was aimed to assess the respective roles of healthcare professionals in preventing drug-drug interactions by clinical interventions.

Material and methods. This study was conducted at a Secondary Care Hospital of Pakistan in which total 1000 prescriptions were assessed for drug-drug interactions. Questionnaires and descriptive statistics were tools to assess the satisfaction of prescribers with pharmacists and their own prescribed medications before and after the clinical interventions. Modifications in medication therapies were done accordingly after the evaluation and acceptance of interventions.

Results. The p-value was highly significant ($p<0.05$) which showed that the collaboration between healthcare professionals is necessary to avoid drug-drug interaction by clinical interventions. Acceptance rate of interventions was 77%. Clinical interventions are a useful tool in minimizing and preventing drug-drug interactions. The compliance of prescribers with their own prescribed medication regimens increased after clinical interventions.

Conclusion. Prescribers, pharmacists and nurses have their respective roles in preventing drug-drug interactions and they must review the appropriateness of every medication order for clinical interventions.

Keywords. clinical interventions, drug-drug interactions, healthcare professionals

Introduction

Drug-drug interactions are a result of reaction between two or more than two drugs that can lead to the unanticipated side-effects, increase in the effect of a drug, decrease in action of a drug or other potential problems. It is necessary for the prescribers to have the knowledge of drug-drug interactions for the prescribed medications and pharmacists must also review the prescriptions for drug-drug interactions before the medications are dispensed to the patient.[1] Prescribing drugs to the elderly patients carries high chances of drug interactions that can lead to failure in therapy, toxicity or loss of efficacy of drug. Alterations in body physiology, diseases, poly-therapy and homeostasis modify pharmacokinetics

as well as responses of drugs as the age progresses. It is essential to periodically evaluate the drug regimens of patients and prescribers must know all the medications taken by patient along-with the herbs and diet.[2] Administration of more than one drug at a time may cause untoward adverse drug reactions due to drug-drug interactions. Poly-therapy accounts for 20-40% drug-drug interactions amongst elders in developed countries. It enhances the complexity in medication management and risk of adverse drugs reactions or reduction in the therapeutic efficacy of medicine. A drug-drug interaction may either be pharmacokinetic or pharmacodynamic.[3] Hence, it is necessary to recognize these drug-drug interactions by clinical knowledge about dif-

ferent isozymes of cytochrome P450, their inducers, inhibitors and drug transporters. Geriatric pharmacists have suggested de-prescribing in relation to poly-pharmacy to avoid potential drug-drug interactions. It has also been emphasized that the healthcare bodies must promote the preventive strategies for drug-drug interactions among prescribers and pharmacists in the form of databases, drug alerts and periodic health assessments of patients.[4] Potential cytochrome-P (CYP)-mediated drug-drug interactions have high prevalence in older adults with poly-pharmacy and clinical knowledge of pharmacist is the key component for the interventions regarding drug-drug interactions along with drug alert or drug caution software.[5] A study revealed that the efficacy of most frequently prescribed medications at an emergency department of United States was changed due to CYP2D6 drug-drug interactions.[6] Nurses also take caution of drug-drug interactions when they schedule the frequency and time of medication administration and are considered as an important healthcare professional in pharmaco-therapeutic care plan design.[7] Although nurses also have pharmacological knowledge and they serve as the last pillar between the patient and drug-drug interaction but the medication schedules designed by nurses also need a thorough review by prescribers and pharmacists.[8] Hence, the monitoring and management of drug-drug interactions is necessary at all the stages of patient care.

Pharmacists have an importance role in medication safety assurance and prevention of drug-drug interactions. A comparative study of absence and presence of critical care pharmacist during ward rounds showed that the presence of critical care pharmacists for medication review lead to a significant decrease in number of drug-drug interactions and also decreased the length of stay of patients in hospital.[9] Pharmacy information systems cannot completely eliminate the need of pharmacists to prevent potential drug-drug interactions.[10] The incorporation of only clinically relevant drug-drug interactions, color codes and removal of dual entries in clinical decision support software along with the periodic review of all these alerts by team of competent pharmacists can timely catch drug-drug interactions.[11] While prescribing any new medication or making any change in already designed therapeutic regimen, pharmacovigilance is essential which is possible mainly with significant knowledge of drug-drug interactions and secondly by electronic decision support system.[12] The feedback of prescribers is also important while incorporating drug-drug interaction data bases in software because it has an impact on compliance of drug alerts.[13]

In all the healthcare settings where multiple drugs are prescribed or administered to a patient, it is the prime responsibility of the pharmacist to monitor the drug-drug interactions and inform to the physician to avoid toxicity or any untoward effect for patient safety.[14] Pharmacokinetic and pharmacodynamic both the parameters should be evaluated when monitoring drug-drug interactions because both types of drug interactions exist and a special focus must be upon the cardiovascular prescriptions carrying concomitant respiratory medications.[15] A prescribing physician must assess the risk v/s benefit ratio for drug-drug interactions notified by pharmacist along with other biochemical, clinical, pathological and physiological parameters because potential drug-drug interactions are higher in count than the actual drug-drug interactions and risk factors play a vital role in the occurrence of clinically significant adverse effects.[16] The continuous introduction of new drug formulae and already established drug-drug interactions need periodic inclusion as well as updating in clinical decision support systems because although patient safety against drug-drug interactions must be highly focused by pharmacists and prescribers but it is not possible to memorize all existing clinically significant drug-drug interactions.[17] Drug interaction screening software cannot completely replace the need of pharmacists. Drug-drug interaction screening software combined with interventions by pharmacists proved to be the most helpful tools in significant reduction of risk for drug interactions in hospitalized patients.[18]

A study reported that out of 400 patients, potential drug-drug interactions were noticed in the prescriptions of 52.8% patients at two renowned tertiary care hospitals of a province of Pakistan.[19] At a secondary care hospital of Pakistan where this study was conducted, every 4 out of 10 patients were previously reported to have drug-drug interactions due to the lack of interventions by healthcare professionals. Software and published data for tracing drug-drug interactions was available at that hospital but the lack of collaboration between prescribers, pharmacists and nurses while prescribing, dispensing or administering the medications leads to the negligence of drug alerts which are important for consideration in patient-centric approach of therapeutic care.

Aim

The aim of this study was to evaluate the respective roles of healthcare professionals including prescribers, pharmacists and nurses in preventing drug-drug interactions by interventions.

Material and methods
Ethics approval
This study was approved by ethics committee of the Secondary Care Hospital of Karachi, Pakistan on August 2021. Healthcare professionals were informed about this study at the hospital. Ethical approval number of

this study is 2021/004 and the ethical principals were followed throughout this study.

Study design

This was an interventional study that was conducted at a Secondary Care Hospital of Pakistan for a period of one year from 2021 to 2022. In this study, the epidemiology of drug-drug interactions, the acceptance of interventions related to drug-drug interactions, their intensities and mechanisms, and the modifications done by healthcare professionals in the medication therapy along with their compliance from the intervened drug-drug interactions for patients were assessed to determine the respective roles of healthcare professionals in preventing drug-drug interactions. The study included inpatients as well as outpatients. Male and female both the genders were included in this study. From total 1000 prescriptions, 690 prescriptions were of female patients and the remaining 310 were of male patients. Verbal consents were obtained from the caretakers of inpatientsand from the ambulatory patients of outpatient clinics. The mean age of patients was 40±20 years. They belonged to the city of Karachi, Pakistan. From the inpatient domain, medication orders from gynecology and obstetrics, general medicine and general surgery were considered for this study while from outpatient domain, psychiatry, neurology, cardiology, general medicine, gastrointestinal and pulmonology clinics. These departments were thought to have most of the drug-drug interactions from IPD and OPD domains and hence the prescribers and nurses from these departments were selected to be the part of this study. All the prescriptions of patients received at pharmacy and other written or verbal medication orders were assessed for drug-drug interactions by qualified registered pharmacists. The pharmacists were trained in hospital pharmacy and had minimum one year of working experience as healthcare professional. Prescribers and nurses of the hospital were also informed by pharmacists about this study.

Data collection

Total 30 prescribers agreed to participate and provided their views for this study. Questionnaires were distributed to those prescribers carrying six questions in them to assess the level of satisfaction of prescribers with pharmacists and with their own prescribed medication regimens before and after the interventions were pointed-out for drug-drug interactions. Prescriptions of all the age groups and genders were included in this study. Total 1000 prescriptions were assessed. Pharmacists and nurses informed prescribers about these drug-drug interactions with their suggestions. The needed steps of modifications in medication therapy were then taken accordingly by prescribers, pharmacists and nurs-

esafter the evaluation and acceptance of those interventions related to drug-drug interactions. The nature of drug-drug interactions and most frequently observed mechanisms of drug-drug interactions were also noted by healthcare professionals.

Statistical analysis

Descriptive statistics were performed to draw the results of this study. Percentages of each identified drug-drug interaction in prescriptions and their acceptance rate were determined. A comparative assessment of the views of prescribers was done through the questionnaires that were distributed to them before and after the interventions of drug-drug interactions by the pharmacists. The intensities and mechanisms of identified drug-drug interactions were also assessed. The modifications that were done in the therapy by the healthcare professionals after acceptance of interventions were also recorded.

Data of this interventional study was statistically analyzed and evaluated through Statistical Package for Social Sciences (SPSS) software 22.0 version (IBM, Armonk, New York, United States). Using Z-test, the difference between the satisfaction of prescribers regarding pharmacists as healthcare professionals in preventing drug-drug interactions and with their own medication therapies before and after the identification of drug-drug interactions by pharmacists and nurseswas compared. SPSS software was used for this purpose. The level of significance was also determined and p value less than 0.05 (P<0.05) was considered highly significant.

Results

Table 1 shows that out of total 300 interventions pointed out by pharmacists in 1000 prescriptions, the accepted number of interventions by prescribers were 233. So the acceptance rate of interventions was 77%. Out of accepted 233 interventions, 38 interventions were from inpatient department while 195 interventions were from outpatient department. The most frequently observed drug-drug interaction amongst all was that of gabapentin and tramadol which was prevalent in the psychiatry and neurology clinics of outpatient department. The modification adopted by prescribers for this frequently observed drug-drug interaction was that they prescribed the lowest possible dose of gabapentin and tramadol as per need of the patient. The intermittent interval between the intake of gabapentin and tramadol was kept sufficient. The care-takers and patients were counseled by pharmacist to be vigilant about the respiratory conditions as well as sedation and to immediately report to the prescriber if either of these symptoms is observed. The most accepted intervention was to avoid the prescribed medicine carrying drug-drug interaction and to prescribe any alternate medication for it. As fer-

Table 1. Drug-drug interactions, suggested interventions and proportion of accepted intervention

S No	Drug Interaction	Department	Identified Drug Interactions		Suggested Interventions	Accepted Interventions	
			n = 300	%		n = 233	%
1	Ferrous sulfate decreases levels of levoflo-xacin by inhibition of gastrointestinal absorption.[20]	Gynecology and Obstetrics (OPD & IPD)	43	14.3	Avoid or Use Alternate Drug	38	88.3
2	Fluoxetine increases carbamazepine levels by hepatic/intestinal enzyme CYP3A4 metabolism.[21]	Psychiatry and Neurology (OPD)	58	19.3	Modify the therapy or monitor plasma levels of carbamazepine	50	86.2
3	Effects of clopidogrel are decreased by omeprazole through hepatic enzyme CYP2C19 metabolism.[22]	Cardiology and General Medi-cine (OPD)	46	15.3	Use alternative gastroprotective medication with clopidogrel	39	84.7
4	Gabapentin and tramadol have phar-macodynamic synergism and increase effects of each other.[23]	Psychiatry and Neuropathy (OPD)	81	27	Closely monitor the effects if necessa-ry to be given simultaneously	62	76.5
5	Ceftriaxone decreases prothrombin activity of heparin.[24]	General Medicine and General Surgery(IPD)	26	8.7	Use antibiotic of alternative drug class of same coverage with heparin	19	73
6	Fluconazole will increases the effect of alprazolam by hepatic/intestinal enzyme CYP3A4 metabolism.[25]	Psychiatry and General Medicine (OPD)	20	6.7	Dose of alprazolam must be minimum when given with fluconazole	13	65
7	Effects and levels of dexamethasone are potentiated by clarithromycin through P-glycoprotein transporter. [26]	Gastrointestinal and Pulmono-logy (OPD)	15	5	Avoid their concomitant admini-stration	8	53.3
8	Metronidazole increases the level or effect of simvastatin by hepatic/intestinal enzyme CYP3A4 metabolism. [27]	Cardiology and General Medi-cine (OPD)	11	3.7	Switch to rosuvastatin if possible as alternative hypolipidemic agent	4	36.3

Table 2. Prescribers' compliance before drug-drug interactions interventions by pharmacists, n (%)

S No	Views of Prescribers	Strongly disagree	Somewhat disagree	Neither agree nor disagree	Somewhat agree	Strongly agree
1	… carry knowledge about drug-drug interactions	0	5 (16.6%)	13 (43.3%)	10 (33.3%)	2 (6.6%)
2	… feel comfortable in communicating with pharmacists	0	0 (0.0%)	11 (36.6%)	8 (26.6%)	9 (30.0%)
3	…consider pharmacological knowledge of pharmacists	0	5 (16.6%)	10 (33.3%)	5 (16.6%)	10 (33.3%)
4	…consider pharmacists for clinical rounds	0	3 (10.0%)	15 (50.0%)	6 (20.0%)	6 (20.0%)
5	… pharmacists are extremely important for pharmaco-therapeutic care plan	0	10 (33.3%)	12 (40.0%)	8 (26.6%)	0 (0.0%)
6	…perception about role of pharmacists in patient care	0	0 (0.0%)	22 (73.3%)	5 (16.6%)	3 (10.0%)

rous sulfate decreases the gastrointestinal absorption of fluoroquinolone antibiotic and this drug-drug interaction implies to only oral dosage forms of both the agents hence prescriber avoided prescribing any oral medication containing ferrous during the oral antibiotic therapy of levofloxacin. If administration of ferrous was essential for the patient due to low hemoglobin levels, doctor prescribed intravenous iron therapy to such patient during levofloxacin intake period. Nurses were advised to vigilantly monitor such patients while infusing intravenous iron. Pharmacist counseled such patients to avoid intake of products containing iron till the oral levofloxacin therapy completes.

Table 2 revealed a clear change in the views of prescribers for pharmacists as healthcare professionals after the acceptance of their suggested interventions for drug-drug interactions. The p-value calculated for it using Z-test showed high level of significance (p<0.05). Medication therapies were modified by healthcare professionals through these interventions and consider-

ation of pharmacists for clinical rounds and designing of pharmaco-therapeutic plans for patient care in collaboration with pharmacists and nurses was emphasized.

67 out of total 300 interventions of drug-drug interactions were not accepted by prescribers. Some of the reasons of their non-acceptance were,

1. Drug-drug interactions were minor or non-significant
2. According to risk v/s benefit ratio assessment, risk was less than benefit of the medication
3. Monitoring parameters for medications carrying drug-drug interactions were already explained by prescriber to the patient or caretaker as there was no other therapeutic equivalent.

Some of the interventions that were not accepted by prescribers are,

1. Phenobarbital decreases the levels of carvedilol by P-glycoprotein efflux transporter
2. Rifampicin decreases the effect of loperamide by MDR1 efflux transporter

Table 3. Prescribers' compliance after drug-drug interactions interventions by pharmacists, n (%)

S No	Views of Prescribers	Strongly disagree	Somewhat disagree	Neither agree nor disagree	Somewhat agree	Strongly agree
1	… these drug-drug interactions were mostly new to me.	0	7 (23.3%)	1 (3.4%)	17 (56.6%)	5 (16.6%)
2	… made me feel more comfortable in communicating with pharmacists	0	1 (3.3%)	4 (13.3%)	13 (43.3%)	12 (40.0%)
3	… encouraged me to consider pharmacological knowledge of pharmacists	0	2 (6.6%)	2 (6.6%)	7 (23.3%)	19 (63.3%)
4	… encouraged me to consider pharmacists for clinical rounds	0	1 (3.4%)	2 (6.6%)	6 (20.0%)	21 (70.0%)
5	… pharmacists are extremely important for pharmaco-therapeutic care plan	0	3 (10.0%)	6 (20.0%)	17 (56.6%)	4 (13.3%)
6	… improved my perception about role of pharmacists in patient care	0	1 (3.3%)	4 (13.3%)	9 (30.0%)	16 (53.3%)

3. Ciprofloxacin increases half-life and serum levels of duloxetine by CYP1A2 metabolism
4. Ofloxacin and fluvoxamine both increase QT interval.[28]

Table 3 showed that the compliance of prescribers with their prescribed medication regimens increased after suggested interventions for drug-drug interactions.

Table 4 shows that 37% of the modification post intervention was to prescribe alternative medication to possibly avoid drug-drug interaction which was highest amongst all and 35% was that of monitoring the drug effects through periodic lab tests. Complete drug informationsuch as to avoid the simultaneous administration of drugs carrying chances of interaction or to monitor the anticipated effects resulting from any drug-drug interaction was also provided by healthcare professionals to the caretakers of patients. This was also practiced by nurses when they administered medications to the admitted patients.

The serum levels of patients undergoing carbamazepine therapy concomitantly with fluoxetine were repeated after every third, sixth and ninth week of therapy by prescriber and carbamazepine was prescribed in minimum possible dose to avoid its toxic serum levels due to fluoxetine. Omeprazole was replaced by sucralfate as a gastro-protective agent in the prescriptions of patients that were prescribed clopidogrel by prescriber. Patients were counseled to avoid any antacid or other gastro-protective agent of their choice and to inform the prescriber if gastric discomfort occurs. The cephalosporin antibiotic of patients of general surgery carrying heparin in their therapeutic regimen was replaced by amoxicillin infusion because of their almost similar coverage in ear, skin, upper and lower respiratory tract infections while in some cases such as bone or joint infection, clindamycin was prescribed. Other such antibiotics were prescribed as alternative therapy of cephalosporin to the general surgery patients. Bleeding and clotting time of these patients was checked by nurses daily during their hospital stay.Alprazolam was prescribed in the lowest possible dose of 0.5mg/day by the prescriber if administration of fluconazole was essential to the patient. Moreover, pharmacist avoided dispensing extended release tablet of alprazolam to such patients and also counseled them to keep sufficient interval between alprazol-

am and fluconazole. Prescriber replaced clarithromycin by azithromycin where necessary for patients taking dexamethasone. Similarly, simvastatin was replaced by rosuvastatin in patients taking metronidazole. All the mentioned modifications in the therapy occurred when pharmacist intervened about these drug-drug interactions.

Table 4. Modifications by healthcare professionals (prescribers, pharmacists and nurses) after accepting the interventions, n=233

S No	Modification	N	%
1	Alternative medicine prescribed	96	37
2	Dose adjusted	13	5.5
3	Drug information to attendants	25	10.7
4	Medication review	12	5.1
5	Lab test for monitoring	82	35
6	Dose interval adjusted	10	4.2
7	Dosage form of medicine altered	5	2.2

Figure 1 shows that 55.3% of the identified drug-drug interactions were significant in nature that needed consideration by healthcare professionals due to their intensities.
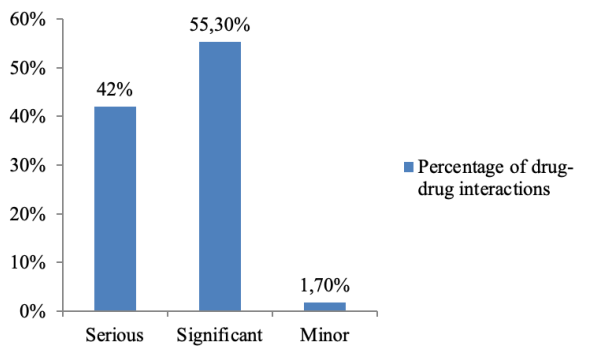

Fig. 1. Intensity and Percentage of drug-drug interactions, n=233

Figure 2 shows that 65.2% of the drug-drug interactions had pharmacokinetic mechanism while others were pharmacodynamic drug-drug interactions.

Reviewing and notifying the drug-drug interactions in the medication orders by pharmacists, vigilant monitoring while administering the medications carrying

drug-drug interactions by nurses and therapeutic modification by prescribers after acceptance of drug-drug interactions are the roles played by these healthcare professionals in preventing drug-drug interactions.
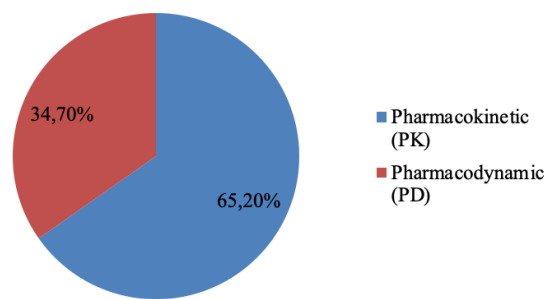

Fig. 2. Mechanism and percentage of drug-drug interactions, n=233

Discussion

Change in the effect of drug when taken together with the other drug is referred to as Drug-drug interaction. This can lead to enhanced or delayed absorption of drug, increased or decreased effect of one or more drugs or may even cause adverse effects on the body. Several efforts have been made till date by healthcare professionals including prescribers, pharmacists and nurses to prevent or possibly reduce the severity of these drug-drug interactions but their complete elimination seems to be impossible to them. It had previously been noted through various researches that the interventions by pharmacists greatly reduced the incidence of clinically relevant drug-drug interactions in a number of patients.[29] Strategic implementation of clinical decision support system at both the prescriptions and administration level can promote the acceptance of drug alerts and clinical interventions of pharmacists.[30] Even though over-the-counter non-steroidal anti-inflammatory drugs are considered safe but the adverse drug reactions from their drug-drug interactions suggest that they must be used in lower doses for short span.[31] It means that the knowledge of pharmacists for drug-drug interactions is compulsory even for the dispensing of over-the-counter medications. Nurses are also important healthcare professionals in preventing drug-drug interactions as they are responsible of safe medication administration as well as monitoring of drug effects at their respective level. An extreme pharmacovigilance is needed for narrow therapeutic index drugs such as anti-cancer therapy because medication assessment for interventions by pharmacologist and hematologist can promote safe use of oncology drugs.[32] A team of healthcare professionals including clinical pharmacist is essential to adjust, manage and monitor the medication therapy for optimized care of patients.[33] Although online tools and drug alert software are important parameters but they cannot completely obliterate the much required collaboration between physicians and pharmacists in minimizing adverse effects resulting from drug-drug interactions because risk versus benefit ratio exists for medications with close monitoring of patients.[34,35] It is also necessary for the healthcare professionals specially clinicians to search drug-drug interaction data bases and medication literatures periodically to remain updated from newly added drug-drug interactions.[36]

This study evaluated the role of healthcare professionals in minimizing drug-drug interactions through interventions and it was done by assessing the prevalence of drug-drug interactions, the acceptance of the related interventions, the intensities as well as mechanisms of the intervened drug-drug interactions, and the modifications done by healthcare professionals after complying from those interventions. The descriptive statistical analysis was done by Statistical Package for Social Sciences (SPSS) software 22.0 version through Z-test on the data of total 300 interventions and questionnaires filled by 30 prescribers at a Secondary Care hospital of Karachi during a period of one year. 22% interventions were rejected by the prescribers due to certain reasons such as, non-significant drug-drug interaction, benefit of the intervened medication was more than its risk, lack of authentic reference or therapeutic equivalent. 49% of the accepted interventions were of the Psychiatry and Neurology clinics of outpatient department. The clinical interventions proved to be a useful tool in minimizing and preventing drug-drug interactions as it sidelined the drug alert fatigue of software faced by prescribers and nurses while entering any medication order.[37] It also reduced the burden of risk versus benefit ratio evaluation by prescribers while prescribing any medication because it added a second step of double check of prescription for drug-drug interactions by pharmacists and a third step of medication assessment by nurses before the medication is administered to the patient.[38] This third step was modified for outpatients by educating them completely for any untoward drug-drug interaction while they take their medications at home.[39] It was revealed by this study that the prevention of drug-drug interactions through interventions carries respective roles of all the healthcare professionals in a healthcare set-up such as, pharmacists reviewed and notified the drug-drug interactions in the received medication orders to the prescribers, nurses vigilantly monitored the administering of medications carrying drug-drug interactions as well as their symptoms and prescribers modified the therapy after acceptance of drug-drug interactions.

88% accepted intervention of any alternative medication or to avoid the prescribed medication by prescriber highlighted the much needed collaboration

between prescribers, pharmacists and nurses to avoid drug-drug interactions for patient-care. The p-value was highly significant (p<0.05). The compliance of prescribers for pharmacists and nurses due to their clinical interventions hinted that the pharmaco-therapeutic plan for advanced clinical needs of patients requires knowledge and collaboration between healthcare professionals along with clinical decision support system and drug alerts. 55% of the drug-drug interactions observed in medication orders or prescription were of significant intensities while others were serious or negligibly minor. Out of 233 accepted interventions, 65.2% of them were pharmacokinetic drug-drug interactions while 34.7% were pharmacodynamic drug-drug interactions. 16% of the accepted interventions were from inpatient department while 84% were from outpatient department of the secondary care hospital of Karachi, Pakistan.

The implication of this study underlies the fact that it is necessary for the healthcare professionals, i.e., prescribers, pharmacists, and nurses to collaborate with each other to combat the inclining trend of drug-drug interactions at all the levels of healthcare set-ups.[40] None of these can supersede the domain of the other in this phase of advanced patient care. For example, a prescriber can diagnose and prescribe the therapy efficiently with all the knowledge and cautions for drug-drug interactions but dispensing of medications after review of their intended use, dose, frequency etc is the responsibility of a pharmacist. It is the critical first point where a drug-drug interaction could be intervened. Secondly, the medication monitoring during or after administration as well as their effects are vigilantly recorded by nurses. This is the second critical point for intervention of any drug-drug interaction so that the therapy could be immediately modified. Acceptance or rejection of the interventions is the sole authority of the prescriber in the light of available authentic clinical references or guidelines. The reported number of drug-drug interactions reduced by 30% after this study as the number of drug-drug interactions reduced to 3 out 10 patients in the clinics or departments where this study was conducted. It was because all the healthcare professionals played their respective roles there with collaborative efforts to design appropriate therapeutic care plan for patients. So, it is evident by this study that the healthcare professionals have their respective vital roles in preventing drug-drug interactions and these criteria of drug interventions should be applied at all the healthcare domains.

Conclusion

Healthcare professionals, i.e., prescribers, pharmacists and nurses have their respective vital roles in preventing drug-drug interactions and they must review the appropriateness of every medication order at their respective ends with collaboration to ensure advanced patient-care.

Declarations

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ORIGINAL PAPER

Suicidal behavior as a result of maladjustment of servicemen to the conditions of military service in Ukraine

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ABSTRACT

Introduction and aim. The purpose of the article is to analyze the peculiarities of suicidal behavior as a result of maladjustment of servicemen to the conditions of military service in Ukraine. The tasks of the article are to identify among militaries: 1) the most significant risk factors for autoregressive and suicidal behavior; 2) the psychological peculiarities of adaptive disorders that may lead to suicide; 3) protective factors against autoregressive and suicidal behavior.

Material and method. To solve the problem of our research, a set of methods was used: theoretical methods – theoretical and methodological analysis of scientific sources, their systematization, classification, generalization; empirical methods – the observation, the interview, a questionnaire, testing, the method of expert assessments. In general 420 militaries were participated in our research. The participants of the 1st stage of the study were 240 militaries in the age 18-25 years old with suicidal and auto-aggressive behavior in anamnesis and one or more attempts of suicide. These militaries were treated in the psychiatric hospital № 1 in Kyiv (Ukraine). At the 2nd stage of the study 180 militaries were participated. They were treated in the Main Military Clinical Hospital (the Center), Kyiv, Ukraine. The research was organized during May–November, 2021.

Results. We investigated that 120 militaries had various forms of post-suicidal encephalopathy, such as acute affective, non-psychotic state. The number of patients with residual psychoorganic pathology was 41 people (34.16%); after poisoning there were 37 people, the number of patients with toxic encephalopathy – 33 people (27.5%); after self-arson – 9 people, there were 4 patients (3.33%) with burn encephalopathy. We identified the following clinical variants of depressive reactions as a result of maladjustment and suicidal attempts: 1) the reaction of disadaptation in combination with neurosis-like disorders (48.9%); 2) the reaction of maladjustment including hypochondriacal inclusions (in 23.4% of cases of respondents); 3) maladaptive reactions with an anxious component were observed in 28.6% of cases.

Conclusion. It was shown that the suicidal behavior of soldiers depended on many external and internal risk factors. We showed protective factors against autoregressive and suicidal behavior.

Keywords. poisoning, post-suicidal encephalopathy, residual psychoorganic pathology, suicides

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Introduction

Recently, there has been a tendency towards increasing of aggression, auto-aggression and various forms of addiction of youth. According to the WHO, among cases of self-injury, including suicide, we can see about 20% of cases having been done by young people.[1]

Suicide is a deliberate, conscious and rapid deprivation of life by the person himself/herself.[2-4] This phenomenon is characterized by three main criteria: 1) the presence of the man's intention to the end of his/her life; 2) the awareness of his/her own actions; 3) a speed.[5,6] There are some actions which do not belong to the sphere of suicidal behavior. They are: 1) accidental or unintentional self-harm; 2) self-harm of persons who could not understand the meaning or consequences of their actions due to a high degree of dementia or severe mental disorder at the time of the act has been presenting; 3) habitual auto-traumatization. It is deliberate self-harm, which is not associated with the idea of death and suicide; 4) habitual long-term use of psychoactive substances, efficiency, inclination to take risks as personally significant ones.[7-10]

Among the various forms of suicidal behavior there are the main ones: 1) antivital experiences; 2) passive suicidal thoughts; 3) suicidal intentions; 4) suicidal wishes.[11-14] The psychological meaning of suicide is shown as: 1) a cry for help; 2) a protest, revenge, punishment of a significant other person; 3) avoidance of punishment or suffering; 4) self-punishment suicide; 5) suicide-sacrifice.[15,16] Relative socio-demographic factors of suicidal behavior are: male gender, loneliness, violation of a professional stereotype, religion (for example, Buddhism). Medical factors of suicidal risk include: 1) depression; 2) a syndrome of alcohol dependence; 3) dependence on opiates; 4) schizophrenia; 5) chronic somatic pathology; 6) physical deformities; 7) HIV-infection; 8) mental disorders; 9) oncological pathology.[17-19]

In many European countries suicide is the leading cause of death of youth, and in addition to suicide many young people commit non-lethal auto-aggressive acts. These acts are found, according to various sources, 10-100 times more often than suicides, although there is no exact information about such auto-aggressive actions. The forms of auto-aggressive activity, its manifestations and socio-psychological predictors, clinical and psychopathological disorders, combined with self-destructive behavior are changed.[20,21] And in this case the ability for socio-psychological adaptation is also changed.[22-24]

If we talk about the statistics of suicides in foreign countries, we've to note that in general each next suicide occurs in the world every 40 seconds. Almost 800000 people committed a suicide every year. At the same time, these figures do not take into account the attempts of suicide, which, according to the WHO, occur approximately 20 times more often than "successful" suicides.[1] Ukraine occupies a rather prominent place in these "black statistics". It is not among the top of ten countries with the highest rates of suicides per capita, but Ukrainian rates are almost twice higher than the world average level. It is mostly because Ukrainian men (not women) kill themselves seven times more often than women do.[25]

Nowadays only 38 of 183 countries, that are the members of the WHO, have "A National Suicide Prevention Program" (this list of countries includes Australia, Israel and the USA). As a result of this program the suicide rate is quite low in these countries. The WHO believes that the process of restricting accesses to the means of doing suicide is one of the most effective measures to prevent suicides.[1]

Scientists[26] call one of the reasons for suicide in Israel "loneliness". This quality leads to depression, anxiety and personnel crises. The authors also emphasize, that loneliness is also understood as the main factor according to psychosomatic disorders, breast cancer, cardiovascular disorders.[26] In other cases loneliness is combined with poor physical and mental health of the person.

There are a number of social, cultural, personal, medical and other prerequisites for the development of psychoemotional maladjustment with such kind of access as auto-aggressive behavior.[27,28] Experts of WHO point out that the frequency of suicide is the most important indicator of problems according to social well-being and public health of the population. Presented statistics of suicides does not correspond to the surrounding us reality at all, because many cases of suicide are not classified as such accidents, but they are seen as cases which belong to other qualifications.[29,30] Experts of WHO believe that the number of suicides having been registered is only the visible tip of so called iceberg.[1] If we look at the reality, we understand that a real data is underestimated by about 20%, and sometimes by 70% in some regions of Ukraine.[31,32] The exceptionally high level of suicides in the Ukrainian army has been repeatedly pointed out in Ukrainian researches. Thus, G.Ya. Pilyagina said that at least half of suicides in Ukraine were committed by soldiers and officers, and this can be the indicator of a severe crisis and complete degradation of moral and psychological climate of the Ukrainian army. In the Ukrainian army from 18% to 50% of all deaths are suicides. The reasons of this fact are in great difficulties of adaptation of recruits (combatants) to the harsh military conditions of life and excessive stress.[33]

The mechanisms of the formation of suicidal behavior of servicemen were highlighted in the scientific literature.[34] There is no suicidal behavior in the pre-positional phase of the person's activity.[35,36] There is a formation of low self-esteem, the person's own worthlessness. The suicidal phase begins during the formation

of suicidal tendencies and continues until the suicide is committed.[37-39] The presuicidal period is characterized by antivital experiences with the denial of the positive meaning of life.[40] Some scientists identified 5 main types of suicide motives: a protest, appealing, avoidance, self-punishment, refusal.[41-42]

After revealing the fact that suicides in the military environment are the main cause of the death of militaries, the attempts were made to resolve this problem. Some researches has found that Ukrainian military command staff, instructors, officers, medical staff and psychologists are unable to detect signs of suicidal behavior among militaries. After having done testing of their knowledge according to this topic it was shown that they could answer only 48% of the questions of proposed questionnaires about the identification of suicidal behavior.[33]

From the time of the development of the armed conflict in the south-east of Ukraine, suicides in the Ukrainian Army became implicit information, and it was reflected in the discussions. These discussions were about a great increasing of the number of suicides in the army of Ukraine. As it was noted, since the beginning of hostilities in the east of the country the Ukrainian army have lost more than 3340 people, from whom which 2394 servicemen were killed directly as a result of hostilities. It means that for every four deaths there is one non-military person having been lost. And from this amount a large number of persons are for suicides. "In the army of Ukraine, the number of which is a quarter of a million people, there are 55 cases of suicides for every 100 thousand people", the UNIAN news agency said.[43]

The media note that these statistics sharply differs for the worse side from similar data of other countries of the world. According to the information of UNIAN, there are only 9-10 suicides per 100000 servicemen in Israel, and also 25-28 cases of suicide of militaries per 100000 servicemen in the United States (in spite of this fact that soldiers of the US are also involved in various military "hot" events).[43]

Thus, the assessment of the risk of suicide among militaries of the Ukrainian army is the urgent state and scientific problem. At the same time, the scientific researches of this problem is in a great degree difficult due to the secrecy of statistical data. In our research we have done the attempt to assess the risk of suicidal behavior of 80 militaries of the Ukrainian army, who were hospitalized into a psychiatric clinic after committing incomplete suicides, using a special questionnaire that includes markers of suicide.[44]

Also the importance of our research we'll explain in such a way. The disorders of adaptation among conscripts at the present time in Ukraine often receive, as we know, a wide public response. This is due to that fact

that they are diagnosed when military man defends our Fatherland and because of this reason this study is quite actual nowadays. Disorders of adaptive reactions of young servicemen also affect a number of other important problems: ethical and legal ones, psychological and psychiatric problems, social and general medical ones. In the army the first question which is actualized, it is a question about the prevention of adaptive disorders of recruits. However, the regularities of their occurrence, the development and the course of these deviations, the signs of their earliest manifestations and the methods of their identification – all these factors are not clearly and insufficiently clarified. Adjustment disorders are often associated with suicidal behavior. When we mean military service this problem is much more greater due to the possibility of using firearms and the occurrence of extended suicides or homicides. Predicting of suicidal behavior is one of the key tasks of the medical and psychological services in the army. However, when there are some disorders of adaptation, suicidal behavior is the most difficult to predict and its diagnostic criteria still need to be developed.

Aim

The purpose of the article is to analyze the peculiarities of suicidal behavior as a result of maladjustment of servicemen to the conditions of military service in Ukraine. The tasks of our research are to identify among militaries: 1) the most significant risk factors for autoregressive and suicidal behavior; 2) the psychological peculiarities of adaptive disorders that may lead to suicide; 3) protective factors against autoregressive and suicidal behavior.

The object of our research is the suicidal behavior of servicemen during their military service in the Ukrainian army. The subject of this research is the predictors of such behavior of these militaries.

The hypothesis of our research

1. Personal qualities and traits of the character play a leading role in the formation of suicidal behavior.

2. Internal mental tension accumulates gradually, combining heterogeneous negative emotions. They are superimposed on one another, concern is replaced by anxiety, and anxiety, in turn, – by hopelessness. Internal suicidal behavior includes suicidal thoughts, representations, experiences and even intentions.

3. Despite the fact that internal factors have been formed from early childhood, the recruits are greatly influenced by the situation of the war, which has taken place in Ukraine, Donetsk and Luhansk regions, since 2014. "The war situation" means moral, physical and psychological overload, uncertainty of the military situation, instability of the political situation in Ukraine, various criteria for assessing this situation (feelings about recruits' families, about their immediate future,

attitude to self-aggression in general and suicide in particular).

4. Suicidal behavior was a complex phenomenon caused by a variety of motives directed on realizing different changes in the behavior of "significant other people" or alleviating (interrupting) severe mental and/or physical suffering of the person. The consequences of suicidal acts are experienced as severe stress not only for the military servicemen themselves, but also for their relatives and specialists who provide them with assistance.

Material and methods

Ethical approval

The ethical examination of the conducted empirical research was carried out and it was approved by the Committee on Ethics of Scientific Researches of the Public Organization "National Academy of Sciences of Higher Education of Ukraine", protocol № 12, dated from the 14th of December, 2021.

Participants

In general 420 militaries were participated in our research. There were different participants at the 1st and the 2nd stages of the study.

The participants of the 1st stage of the study were: 240 militaries in the age 18-25 years old (the average age is 20±0.5 years old) with suicidal and auto-aggressive behavior in anamnesis and one or more attempts of suicide. These militaries were treated in the psychiatric hospital № 1 in Kyiv (Ukraine). At this stage all respondents were included into one experimental group.

These militaries were sent for inpatient treatment by the military commissariats of Kyiv to resolve the issue of fitness for military service. All soldiers have being served in the army in the combat zone of Ukraine (Donetsk and Lugansk regions). All these militaries were participated in hostilities in Donetsk and Lugansk in the south-east of Ukraine. They all were included by us into experimental group, which was formed by the help of method of randomization. The 1st stage of the study was organized in May–June, 2021.

The place of organizing the 2nd stage of the experiment was the Main Military Clinical Hospital (the Center), Kyiv, Ukraine. 180 militaries were studied. At the 2nd stage of the study all respondents were distinguished into experimental and control groups. From them there are: 120 conscripts with adaptive disorders, suicidal thoughts and attempts (experimental group) and 60 conscripts without adaptive disorders (a control group). All these people agreed to participate in our research. All recruits who were the respondents in our research had been men in the age 18-22 years old. We organized this stage of the study in July–November, 2021.

In general the research was organized during May–November, 2021. We have followed the main ethical standards of providing the empirical research. Ethical principles were followed in the process of conducting the empirical research: the principle of voluntary consent; the principle of minimizing risks for participants; the principle of confidentiality; the principle of informing participants about the content of the research; the principle of mandatory documentation of the stages and the results of the research; the principle of reliability of methodical instruments of the research having been conducted; the principle of validity of research data processing.

Research methods and techniques

To solve the problem of our research, a set of methods was used, the choice and combination of which have been determined by the subject, the purpose and the objectives of this research:

- theoretical methods – theoretical and methodological analysis of scientific sources and available according to the problem of our research psychological approaches: their systematization, classification, generalization;

- empirical methods – the observation, the interview, a questionnaire, testing, which have been used for a deeper, holistic research of the structure of psychological disadaptation of adaptive disorders of servicemen.

The observation was carried out at the Main Military Clinical Hospital (the Center), Kyiv, Ukraine. The type of the observation was included one, when the researcher acted directly as a participant in the treatment process (as a psychiatrist), during which we conducted our empirical research. The purpose of our observation was to know exactly about the type of depressive reactions of combatants according to their reasons for suicides.

We used the interview method in order to identify the factors of suicidal ability of combatants. We asked the respondents the following questions:

- How do you try to react to stressful situations in your life – emotionally or rationally?

- Does your family have certain family (or collective) traditions for solving complex, conflicting problems through auto-aggression?

- Has a history of suicides been diagnosed in your family?

- Have you ever felt an unreasonable or excessive sense of guilt?

- Have you in certain cases shown a dominant willingness to «sacrifice» yourself for the sake of satisfying the interests of others?

- Do you have a tendency to perceive even the least unpredictable changes in your life as quite undesirable ones?

- Have you shown in certain cases the inability to see a life in all its diversity?

- In what cases do you experience such kind of irritable-depressive attitude to life?

- In what cases do you demonstrate emotional expressiveness instead of emotional restraint?

- Do you feel a tendency to fixate on problems and to look for a way out of a difficult situation?

- Are you often dissatisfied with yourself?

- Are you able to assess yourself as a loser?

- Is it typical for you to perceive life as a routine, monotonous and joyless process?

- Do you often feel powerless and hopeless (lack of meaning in life)?

- In what cases do you feel unable to get pleasure from any events?

- In what cases do you feel like a victim of uncontrollable circumstances?

Interviews and observations were organized by one of the authors of this article, who worked as a psychiatrist at the Main Military Clinical Hospital (the Center), Kyiv. This psychiatrist gave informed consent of combatants to participate in this research.

For the each stage of our research we will identify and specifically describe specific methods of empirical research according to the aim of this stage. For example, to assess mood and mental state of the person we used the test "Well-being, Activity, Mood".[45] Personal characteristics and peculiarities of interpersonal relations were determined using the MMPI questionnaire and Multivariate questionnaire 16-PF by the R.B. Cattell.

The adaptive resources of military servicemen were assessed by measuring stress resistance and social adaptability (Holmes and Rage method), as well as using the complex multifactor test "Adaptability". Multilevel personal questionnaire "Adaptability"[46] (165 questions) has the aim to study various aspects of adaptation. This test includes the following scales: "Neuropsychic stability" (NS), "Personal adaptive potential" (PAP), "Communicative skills" (CS), "Moral normativeness" (MN) and "Correction scale" (C). The basic scale of the test is "Personal adaptive potential". It contains information about behavioral regulation and communicative potential of a person and allows him/her to differentiate people according to the degree of his/her resistance to the effects of psychoemotional stressors. These questionnaires helped us to show protective factors against autoregressive and suicidal behavior.

For the purpose of statistical data processing of the empirical results of our research we used the procedure of the exploratory factor analysis (computer package of statistical programs "Statistica" by StatSoft: Version 12.5.192.7 (2015, Dell, Round Rock, Texas, USA).

The 1st stage of the study

The purpose of the 1st stage of our research: to identify and do comprehensive assessment of clinical and socio-psychological predictors of suicidal and auto-aggressive behavior of persons of pre-conscription and

conscription age. These predictors will help us to develop measures with the aim of reducing the risks of auto-aggressive behavior.

To achieve this goal, the following tasks were set:

- to determine the clinical structure of psychopathological disorders, taking into account the diagnostic criteria of "International Statistical Classification of Diseases and Related Health Problems. 10th Revision (ICD-10)";[47]

- to provide a comprehensive assessment of biological and social-situational factors that influence the formation of suicidal and auto-aggressive behavior of militaries in the Ukrainian army.

Material and methods of the 1st stage of the study

Clinical and diagnostic qualification of revealed mental pathology was carried out on the basis of "International Statistical Classification of Diseases and Related Health Problems. 10th Revision (ICD-10)".[47] The reliability of the results having been obtained was determined by the help of Fisher multifunctional statistical criterion (F-criterion) and by use of t-criterion of Student.

The 2nd stage of the study

At the 2nd stage of our research conscripts were examined. The purpose of this stage of the study is to identify among militaries the psychological peculiarities of adaptive disorders that may lead to suicide; to show protective factors against autoregressive and suicidal behavior.

Material and methods of the 2nd stage of the study

The methods of experimental psychological research were selected according to its tasks. So, to assess mood and mental state of the person we used the test "Well-being, Activity, Mood".[45] Personal characteristics and peculiarities of interpersonal relations were determined using the MMPI questionnaire and Multivariate questionnaire 16-PF by the R.B. Cattell. With the help of these tests we identified among militaries the psychological peculiarities of adaptive disorders that may lead to suicide.

The adaptive resources of military servicemen were assessed by measuring stress resistance and social adaptability (Holmes and Rage method), as well as using the complex multifactor test "Adaptability".[46] Multilevel personal questionnaire "Adaptability" (165 questions) has the aim to study various aspects of adaptation. This test includes the following scales: "Neuropsychic stability" (NS), "Personal adaptive potential" (PAP), "Communicative skills" (CS), "Moral normativeness" (MN) and "Correction scale" (C). The basic scale of the test is "Personal adaptive potential". It contains information about behavioral regulation and communicative potential of a person and allows him/her to differentiate people according to the degree of his/her resistance to the effects

of psychoemotional stressors. These questionnaires helped us to show protective factors against autoregressive and suicidal behavior.

Results

1ˢᵗ stage of the study

The results of our research showed the following phenomenological picture of mental disorders: 20.6% of respondents with autodestructive patterns of the behavior were suffered from emotionally unstable personal disorders; 11.3% had organic affective disorders; 6.3% of people had mild mental retardation; 20.1 of recruits of all identified nosology suffered from anxiety-phobic disorders; neurasthenia, also as asthenic, infantile and schizoid personality disorders. For the period of inpatient psychiatric diagnostics 33.7% of men were considered as mentally healthy persons.

These studies reliably demonstrate the role of numerous factors in the genesis and the development of suicidal and auto-aggressive behavior of young recruits. For each group of disorders the contribution of risk factors is different, mathematical processing of the data made it possible to identify the level of the significance of these factors:

- the development of organic disorders reliably depends (0.7385, p<0.001) on perinatal pathology, the presence of pathologically burdened heredity, also the presence of craniocerebral trauma;

- for the development of personal disorders the main were social indicators with high values of such factors as upbringing in disharmonious families, conflicts at school, deviations from social norms of the behavior (being brought to the police, convictions, addictive behavior, alcohol consumption). The last ones are significantly significant (0.7730, p<0.001);

- for the development of mental retardation statistically significant (0.7534, p<0.001) there is the presence of perinatal pathology, developmental delay of the person.

The results having been obtained, determined the need to strengthen measures of primary prevention directed on preventing the action of adverse social factors that in a great degree contributed to the development of mental illnesses. These social factors can also be the basis for the formation of risk groups and the implementation of preventive measures in these groups (we mean the implementation of measures of secondary prevention). Timely identification of risk factors will reduce the incidence of suicidal and auto-aggressive behavior in people of military age.

2ⁿᵈ stage of the study

Characteristics of mental problems in the study group
Biological risk factors for suicidal behavior
The results of our research of suicides with various forms of post-suicidal encephalopathy were such, as:

among the young age who have hung in an acute affective, non-psychotic state (120 people). The number of patients with residual psychoorganic pathology was 41 people (34.16%); after poisoning there were 37 people, the number of patients with toxic encephalopathy – 33 people (27.5%); after self-arson – 9 people, there were 4 patients (3.33%) with burn encephalopathy. The obtained indicators speak in favor of greater pathogenicity, when a psychoorganic syndrome occurs a clear post-hypoxic factor (which usually occurs during strangulation); much less toxic (in case of poisoning); the burn factor, having been mixed in its structure, is also distinguished by a high degree of pathogenicity – due to the hypoxic component that occurs during self-arson, and toxic one – due to septicotoxemia, which subsequently is joined in a clinical period.

Clinically, the psychoorganic syndrome of studied patients was characterized predominantly by similar psychosymptomatics: general adynamia, less often hyperactivity, torpid thinking, impaired memory function (in mild cases – short-term, in more severe cases – long-term, often with amnesia of the fact of suicidal actions), intellectual functions (different levels), in violation of criticism to their condition, situation; apathy, sometimes complacency (up to euphoria), inadequacy of behavior; with toxic and especially burn form – more pronounced asthenia, depreciation. Dominant in the clinic of psychoorganic syndrome, in all forms of encephalopathy, is an intellectual-mnestic defect of varying severity form (from mild memory loss up to deep dementia). The time of formation of the psychoorganic (intellectual) defect is corresponded to the nature (asphyxia, toxic, toxic-posthypoxic) and the severity of the pathogenic process and ranged from 3-7 days in posthypoxic form of encephalopathy; 1-2 weeks – with toxic; 1-1.5 months – in a case of burn form.

From a neurological point of view for patients with posthypoxic and toxic encephalopathy cerebral symptoms are initially predominated, often with vegetative-vascular dystonic disorders, usually persisting persistently by the type of cerebral-vascular disorders. For patients with burn encephalopathy focal symptoms were more often observed: lesions of the oculomotor, facial, hypoglossal nerves, anisoreflexia according to the hemitype, pathological hand and foot reflexes, phenomena of oral automatism, etc.; the indicated symptomatology was generally persistent. Somatically patients with post-suicidal encephalopathy, who were initially in a serious condition (with impaired vital functions up to coma), were compensated at different time periods: with post-hypoxic encephalopathy – within 2-7 days; toxic one – 2-4 weeks; burn encephalopathy – 1-3 months (the duration was different due to joining septicotoxemia), with pronounced asthenization in the last two forms (especially burn one). The interesting fact is that

one of the frequent discrepancy between the severity of the somatic state and the increased background of the mood of patients with encephalopathy (mainly in moderate and especially severe degrees), which is explained by the violation of a critical assessment in connection with the existing intellectual defect.

In a paraclinical study the results were such as: with Ro-graphy of the skull in a greater degree of patients with encephalopathy, hypertesio-hydrocephalic manifestations are found (in the form of increased digital impressions, expansion of the pachyon fossae, sometimes thinning of the walls of the sella turcica), characterizing in a certain way by the phenomena of venous stasis, such as hypoxia of the brain; in posthypoxic encephalopathy, Ro-logical confirmation of this fact was noted for 67.42% of patients; with toxic one – for 54.12%; with burn encephalopathy – for 60.12% of patients). Neurophthalmologically: pathological changes in different forms of encephalopathy have a specific picture; with post-hypoxic – the main pathological changes observed primarily are leveled out in dynamics, after 2-3 weeks the phenomenon of clogged venous blood flow is preserved; with toxic form: retinal edema, spasm of the third order arteries, hemorrhages, peripapillary edema of the retina and optic nipples; for burn encephalopathy: decalorization of the nipples of the optic nerves, a decrease in the number of vessels in the vascular zone of the optic nerves. Common for different forms were the phenomena of venous stasis and optic-asthenia state. In the study of cerebrospinal fluid the majority of the studied patients revealed the phenomena of hypertension, increasing the actual aggravation of the severity of the psychoorganic process (with a functional degree – there is no; with mild degree – 10-12% of patients; with medium form – 40-43% of patients; with the severe form – 69-75% of patients).

By the experimental study, the most of the patients with encephalopathy, pronounced pathological changes in the form of slow-wave activity, the presence of sharp waves, alpha rhythm disorders, pathological paraximal activity, etc., are found to some extent inherent for patients with different forms of encephalopathy.

The patients were selected as they arrived from the military unit to the hospital. The diagnosis of "adjustment disorder" was established according to the following criteria:

- the presence of stressful events and situations in the circumstances of military service during the period of adaptation of young servicemen to it (also we've paid our attention onto suicidal behavior);

- the presence of the state of subjective distress of a serviceman before the disease, such as actualized mental states, as stress, frustration, crisis experiences, feelings of loneliness and humiliation, deprivation of support, hopelessness, despair, etc.;

- the presence of emotional disorders in the clinical picture of the disease in combination with a low level of productivity in social functioning or with its complete impossibility;

- the presence of comorbid adaptive disorders, such as suicidal ideas, intentions, fantasies and incomplete suicidal actions;

- the presence of relevant anamnesis data and data on premorbid traits of the person, which could, on the one hand, explain the origin of adaptive disorders, and, on the other one, exclude mental disorders of a different nature.

Clinical-psychopathological, clinical-dynamic, experimental-psychological and statistical research methods were used in this part of our research, as well as the analysis of the condition of patients in the process of their individual and group psychotherapy. Service characteristics from the unit where the service was held were studied for each patient.

With the help of a clinical and psychological approach and psychotherapeutic interviewing in the process of our research and psychotherapy, the system of views of the person and his life positions, directions of his behavior and lifestyle, strategies for organizing adaptive behavior were clarified.

Psychological risk factors for suicidal behavior
On the 2nd stage of the experiment the study of pathogenic situational factors and premorbid traits of the person contributing to adaptive disorders showed that the immediate causes of adaptive disorders of conscripts are, in general, not some extreme distressing circumstances, but everyday difficulties at the process of ordinary military service.

On the first place (64.9%) among the causal factors of adjustment disorders of conscripts, according to their own assessment, were the usual difficulties of military service. The patients complained about the inability to comply with the strenuous daily routine. These difficulties, recruits emphasized, were largely determined by the fact that they had their military service in the army in "hot spots" (in our research the events had taken their place in Donetsk and Luhansk regions, Ukraine). They were greatly affected by the deaths of their friends, commanders, and severe injuries, which in most cases had led to the disability of recruits.

The second place in the etiological structure was occupied by worries about a change in the sense of military men's own status (26.2%). Among them 18.7% of patients suggested that the commanders had communicated with them, often humiliating the dignity of their subordinates. Other 7.5% of recruits felt humiliated by the very need to obey and follow orders.

As for the third place in terms of frequency (8.9% of recruits) is the experience of separation from family and

friends, separation from home. This feeling is combined with longing, memories and resentment towards "fate".

And, at last, for the fourth factor – so-called hazing (we mean attitudes of officers to recruits with psychological harassment, demands to perform some kind of the activity, etc.). This reason, as the most significant one, wasn't noted at all by recruits, who had their military service on the territories of Donetsk and Luhansk regions, Ukraine (Fig. 1).
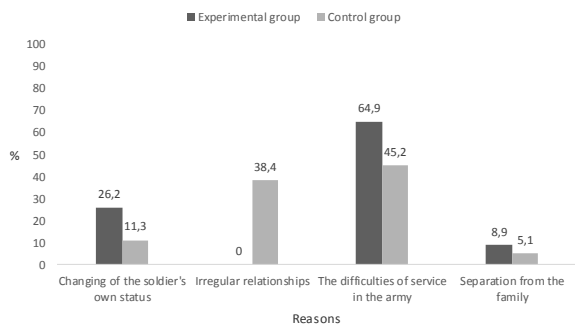

Fig. 1. Reasons of adaptive disorders of military servicemen

For control group the results differ from the same ones, which are diagnosed for respondents of control group. So, on the first place there is the same criteria – the great difficulties of military service (45.2%). But on the second place there is criteria "hazing", which means negative attitude of officers towards recruits (it is 38.4%). The latter we explain in the same way. The experimental group was not diagnosed with "hazing attitudes" because the situation of war did not allow for successful combat operations combined with injustice, humiliation and pressure from the side of officers on young recruits. In real circumstances officers allow themselves to belittle recruits, often requiring certain activities for several times (cleaning toilets, cleaning the floor in the barracks, etc.), to redo this work even at night, despite fatigue or malaise.

The third place among the respondents of the control group is the indicator "change in the sense of military men's own status", but it is not significant (11.3%). The last place took the criteria "the separation from family and friends", but it is also insignificant in its value (5.1%). The results are shown by Fig. 1.

Further research of this issue showed that the circumstances of the initial period of military service are closely intertwined with the factors of pre-army life and with the personal characteristics of sick servicemen (Table 1).

Firstly, most of them (77.5% in experimental and 79.3% in control group) had their upbringing in low-income families, in which family situations in the most cases were aggravated by conflicting interpersonal relationships. Thus, 73.6% patients of experimental and 69.1% in control group had had frequent quarrels between parents in their families for many years. 49.6% persons of experimental and 52.3% in control group were divorced and lived separately. 21.7% of respondents of experimental and 20.6% of soldiers of control one showed the loss of one of their parents because of various reasons. 58.6% patients of experimental and 59.7% – in control one had their upbringing in an incomplete family (more often without a father), either due to divorces even before the birth of a future serviceman, or due to the death of one of the parents (also more often this parent is the father).

It should be noted that the data of the respondents of the experimental and control groups are almost the same, there is no statistically significant difference in the results by t-criteria of Student by 1% level of confidence. However, difficulties in adapting to military service in the army were diagnosed, first of all, according to the respondents of experimental groups, which indicate that these difficulties are influenced primarily by the factor of hostilities in regions of Donetsk and Luhansk, and not the factors presented in Table. 1.

Despite all these factors (which in a great degree aggravate the life) had being studied by the patients, nevertheless, before the military service in the army were

Table 1. The factors of pre-army life that in a great degree aggravate adaptation to military service (highly probable signs are highlighted)*

Significant factors of pre-army life	Frequency and confidence limits of the likelihood of pre-army life factors that burden adaptation and service in the army					
	Experimental group			Control group		
	%	min	max	%	min	max
Single-parent family factor	58.6	0.5211	0.6417	59.7	0.5563	0.6824
Loss of one parent	21.7	0.2301	0.2516	20.6	0.1834	0.2177
Loss of a deeply loved person (a sister, a brother)	17.9	0.1892	0.2212	21.3	0.2451	0.2631
Conflicts between parents over the years	73.6	0.6931	0.7912	69.1	0.7105	0.7628
Material difficulties in the parental family	77.5	0.7830	0.8276	79.3	0.8004	0.8223
One (or two) parents have used alcohol (or drugs, other psychoactive substances)	29.4	0.2709	0.3144	25.6	0.2418	0.2932
Divorce of parents	49.6	0.4863	0.5428	52.3	0.5412	0.5894

* min – minimum meaning of the factor by factor analysis procedure; max – maximum meaning of the factor by factor analysis procedure

mainly socially adapted.

Clinical and psychopathological study of individuals in both groups confirmed that the military medical commission had all reasons to recognize them fit for military service. None of them, according to the data of our research, had any explicit accentuations of the character, or specific (psychopathic) personal disorders, or dependence on psychoactive substances, or adjustment disorders, or other mental disorders. At the same time, a psychobiographical analysis of the development of the person of sick servicemen revealed a common feature for them, such as increased personal vulnerability, low stress resistance, a low barrier to be tolerant for difficulties, which had been clearly identified long before conscription into the army.

Assessing the state of adaptation among conscripts, it was important to study also the characteristics of their person and interpersonal relationships. Using the test MMPI, it was shown that there are significant differences in these groups according to the following scales: Correction (K), Overcontrol (Hs-1), Pessimism (D-2), Emotional lability (Hy-3), Impulsivity (Pd-4), Feminine qualities (Mf-5) and Rigidity (Pa-6). At the same time, in the group of servicemen with adaptation disorders to a greater extent than for healthy individuals, there were presented traits of the person which determined easy neuropsychic decompensation (including decompensation according to a psychosomatic type of the person) and social maladjustment. These features include a tendency towards neurotic ways of responding to life's difficulties (low frustration tolerance combined with a tendency to "withdraw into the illness"); emotional instability and tendency to affective fluctuations; sensitivity; lack of plasticity and a tendency to fix themselves on their own shortcomings, problems or symptoms of their disease; great dependence and passivity in the sphere of social contacts (Fig. 2).
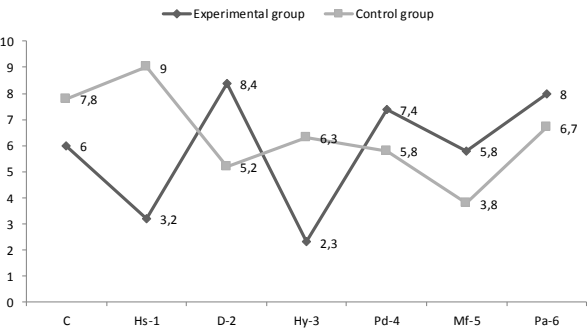
Fig. 2. Average profiles of the person in the experimental and control groups (according to MMPI: The scales of the test MMPI: C – correction; Hs-1 – Overcontrol; D-2 – pessimism; Hy-3 – emotional lability; Pd-4 – impulsivity; Mf-5 – feminine qualities; Pa-6 – rigidity)

The analysis of the results using Multivariate questionnaire by R.B. Cattell showed that patients with disorders in adaptation in the emotional-volitional sphere have instable mood, weak volitional control over impulses and wishes, deviations in emotional sensitivity, in emotional response, regression of will with behavior predominantly based on prevailing stereotypes and automatisms (Fig. 3). At the same time, the personality of patients differs according to the factors having been analyzed (we mean low tolerance in relations to frustration, a tendency to mood lability and emotional instability, fatigue and nervousness, as well as increased sensitivity, impressionability, sentimentality, anxiety, vulnerability and depression).
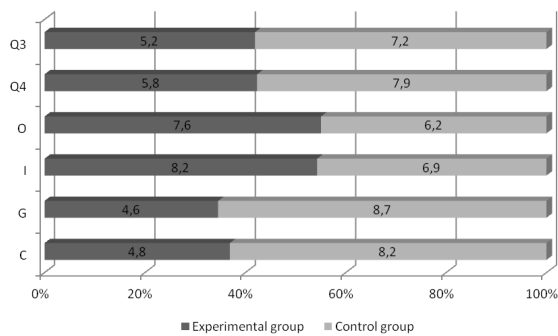
Fig. 3. Average data per person in the studied groups of recruits according to the factors characterizing the emotional and volitional sphere (by the test 16-PF of R. Cattell: Factors in Multivariate questionnaire of R.B. Cattell: C – emotional instability; G – undisciplined; I – tough-minded; O – self-reliance; Q4 – being relaxed; Q3 – non-conforming, rule-consciousness)

The control group of recruits, according to data obtained by Multivariate questionnaire by R.B. Cattell, differed from other patients because of greater emotional stability, self-control, constancy of interests, emotional maturity.

Our observation of the respondents in the experimental group showed that among the reasons for suicides 30-45% of servicemen were directly related to the difficulties of their adaptation to the conditions of military service. Based on our experiment, we identified the following clinical variants of depressive reactions as a result of maladjustment:

1) the reaction of disadaptation in combination with neurosis-like disorders (48.9%). In this group of respondents suicidal attempts were noted in 53.5% of cases examined (self-tapping screws, self-hanging);

2) the reaction of maladjustment including hypochondriacal inclusions (in 23.4% of cases of respondents). Hypochondriacal experiences were accompanied by thoughts of their own failure, helplessness, hopelessness. All this accompanies the formation of suicidal behavior (10.5% of soldiers have done the attempts of

self-hanging, and in 18.6% of cases there is a form of self-tapping screws). Suicidal attempts were in the most cases (in 80.94% of cases) impulsive;

3) maladaptive reactions with an anxious component were observed in 28.6% of cases. These respondents had anxious and suspicious traits of character. These soldiers are characterized by hazing, unauthorized departures from the military unit and suicidal thoughts.

In the occurrence of prolonged depressive reactions with impaired adaptation, prolonged stressful situations were of significant importance. Suicidal behavior in this category of the respondents was observed in 32.4% of cases. Sometimes we noted endoform symptoms.

As a result of our research it was found that the formation of borderline mental disorders in the conditions of military service occurs under the influence of a complex of biological, individual-psychological, environmental factors, having been interacted in different proportions and in varying degrees with the dependence of the duration of the stressor action, which subsequently determines the quality and the level of social functioning and the suicidality of a military man. The first period of the military service is the most suicidal. The formation of short-term depressive reactions was noted almost from the first days of being in the unit and it was

manifested itself in the form of pseudo-cognitive disorders, which gave a great rise of decreasing of self-esteem of young soldiers. But later on decreased mood, sleep and appetite disorders were observed. In 47.5% of cases servicemen with short-term depressive reactions were noted as such people who had had auto-agressive behavior, in 25.6% of cases this mood was limited by suicidal fantasies and predictable statements.

All data having been obtained in our research were processed by us by the procedure of factor analysis. Into the first, basic factor (59.35% of the variance), which has a clearly defined social orientation and was called by us "Problems inherent in family upbringing", with the largest factor weight were included the following characteristics: "lack of parental love and care, friendship and support from parents lack parental love and care, friendship and support from the side of parents" (0.7134), "shame for the behavior and lifestyle of parents" (0.6512), "presence of a psychological complex of inferiority, shame, failure" (0.6302).

The second factor (40.65% of the variance), which, in contrast to the first one, has a purely personal orientation and was called by us "Personal qualities and characteristics". The second factor contains indicators with a low factor load (<0.06), that's why we wouldn't analyze these results in our research.
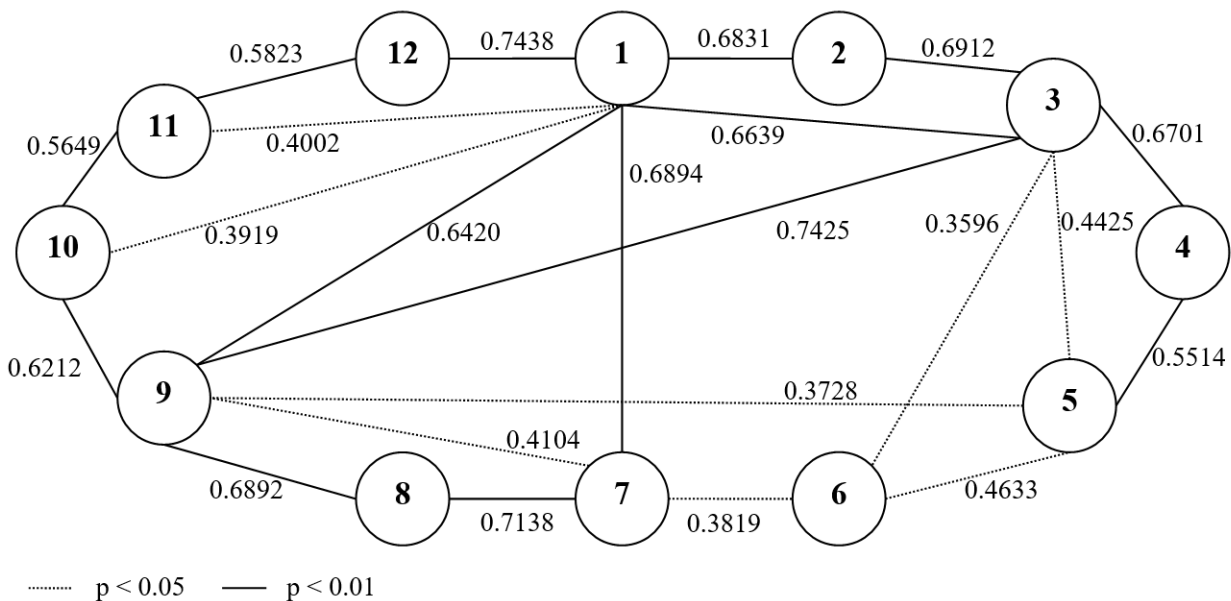
Fig. 4. Correlations between characteristics of adaptive disorders of military servicemen (1 – lack of parental love and care, friendship and support from parents lack parental love and care, friendship and support from the side of parents; 2 – shame for the behavior and lifestyle of parents; 3 – presence of a psychological complex of inferiority, shame, failure; 4 – envy towards more successful peers; 5 – fear of more successful peers and negativism towards them; 6 – in a great degree increased need for protection, support and patronage from more "strong" and "successful" people; 7 – passive obedience to "the stronger" and "more successful" people up to complete dependence on them; 8 – feeling of lack of self-sufficiency and self-doubt; 9 – insufficiently developed ability for psychological mobilization; 10 – increased need for protection, support and patronage; 11 – decreased tolerance to frustrations; 12 – shyness, timidity and indecision in new situations and situations with strangers).

The data having been obtained by us, had been processed by the procedure of factor analysis, were also processed by the procedure of correlation analysis of empirical data (Fig. 4). The highest number of positive correlations was obtained by the following indicators:

– "lack of parental love and care, friendship and support from the side of parents" has positive correlations with: "shyness, timidity and indecision in new situations and situations with strangers" (r=0.7438, p<0.01), "passive obedience to "the stronger" and "more successful" people up to complete dependence on them" (r=0.6894, p<0.01), "shame for the behavior and lifestyle of parents" (r=0.6831, p<0.01), "presence of a psychological complex of inferiority, shame, failure" (r=0.6639, p<0.01), "insufficiently developed ability for psychological mobilization" (r=0.6420, p<0.01), "decreased tolerance to frustrations" (r=0.4002, p<0.05), "increased need for protection, support and patronage" (r=0.3919, p<0.05);

– we diagnosed such significant positive correlations "insufficiently developed ability for psychological mobilization» with: "presence of a psychological complex of inferiority, shame, failure» (r=0.7425, p<0.01), "feeling of lack of self-sufficiency and self-doubt" (r=0.6892, p<0.01), "lack of parental love and care, friendship and support from the side of parents" (r=0.6420, p<0.01), "increased need for protection, support and patronage" (r=0.6212, p<0.01), "passive obedience to "the stronger" and "more successful" people up to complete dependence on them" (r=0.4104, p<0.05), "fear of more successful peers and negativism towards them" (r=0.3728, p<0.05).

So, in our research we proved, that mental disorders and personality abnormalities were high risk factors for suicide. Most of those people who commit suicide have never seen a psychiatrist before. Therefore, early diagnosis of mental disorders and assessment of suicidal risk in primary health care is an important prerequisite for reducing the risk of suicide. The success of such a risk assessment depends both on the completeness of the doctor's objective registration of predictors of suicidal risk in the patient's behavior, and on careful observation of his/her own feelings in the process of communication. The clinical interview should be based on empathic, non-moral acceptance of the patient and his/her emotions, regardless of the severity of the psychopathology and the nature of a mental disorder.

In our research it was shown that the suicidal behavior of soldiers depends on many external and internal risk factors. The leading factors in the formation of suicidal behavior are internal factors (neuropsychic states, the presence of latent mental illnesses, a tendency to depression, etc.). All these factors are formed in the soldiers' childhood. As we showed in our research the main internal factors are: "feeling of lack of self-sufficiency and self-doubt", "insufficiently developed ability

for psychological mobilization", "increased need for protection, support and patronage", "decreased tolerance to frustrations" and others.

To a large extent, these factors, according to our research, are caused by children's experiences, namely external risk factors, such as: "lack of parental love and care, friendship and support from parents lack parental love and care, friendship and support from the side of parents", "shame for the behavior and lifestyle of parents", "presence of a psychological complex of inferiority, shame, failure", "envy towards more successful peers", "fear of more successful peers and negativism towards them", "in a great degree increased need for protection, support and patronage from more "strong" and "successful" people", "passive obedience to "the stronger" and "more successful" people up to complete dependence on them".

Based on the empirical data having been obtained in our research, their factor and correlation analysis, the psychological peculiarities of adaptive disorders of servicemen, which in 30-45% of cases lead to suicide attempts were proposed. These psychological peculiarities are:

– a high level of adaptive disorders of soldiers is due to socially oriented characteristics, among which some of them occupy a dominant place. They are: "lack of parental love and care, friendship and support from the side of parents", "shame for the behavior and lifestyle of parents", "presence of a psychological complex of inferiority, shame, failure", "fear of more successful peers and negativism towards them";

– military servicemen, prone to maladaptation during their involvement into military conflicts, usually have certain personal problems that were inherent in their childhood. Among such problems some of them take a dominant place: "insufficiently developed ability for psychological mobilization", "increased need for protection, support and patronage" and "decreased tolerance to frustrations".

In our research we proved all the hypothesis. We showed, that personal qualities and traits of the character played a leading role in the formation of suicidal behavior of the individual. We showed, that internal mental tension accumulated gradually, combining heterogeneous negative emotions. They were superimposed on one another, concern was replaced by anxiety, and anxiety, in turn, – by hopelessness. We proved, that internal suicidal behavior of combatants included suicidal thoughts, representations, experiences and even intentions.

In our research we showed, that despite the fact that internal factors had been formed from early childhood, the recruits were greatly influenced by the situation of the war, which had been taken place in Ukraine, Donetsk and Luhansk regions, since 2014. We became

sure, that "the war situation" meant moral, physical and psychological overload, uncertainty of the military situation, instability of the political situation in Ukraine, various criteria for assessing that situation (feelings about recruits' families, about their immediate future, attitude to self-aggression in general and suicide in particular).

In such a way we've to conclude that suicidal behavior was a complex phenomenon caused by a variety of motives directed on realizing different changes in the behavior of "significant other people" or alleviating (interrupting) severe mental and/or physical suffering of the person. The consequences of suicidal acts are experienced as severe stress not only for the military servicemen themselves, but also for their relatives and specialists who provide them with assistance.

So, we showed protective factors against autoregressive and suicidal behavior. To assess the severity of suicidal intentions and predictive reality of suicidal behavior it is necessary to remember that about the majority of suicides, before a real suicidal act, soldiers directly or indirectly tell, say about their suicidal intentions, declare fluctuations between the desire to live and to die. Recrutes with suicidal tendencies often give very clear instructions on what they are going to do. Many suicides occur during a period of improvement into the context of depression, when the soldier has enough energy and will to turn desperate thoughts into decisive action. Suicidal thoughts, as a rule, are not constant, they tend to be returned when a conflict or psychologically difficult situations became worse and worse, which is quite common during military operations. Direct conversation of a psychologist with a soldier about his problems can help to relieve the emotional state of a recruit.

Discussion

The results of our study indicate that personal qualities and traits of the character play a leading role in the formation of suicidal behavior. Other scientists also tell about a number of predisposing psychological and psychiatric factors of suicidal behavior. These factors are: increased tension of the person's needs, a desire for emotional intimacy, a low ability to form psychological defense mechanisms, inability to ease frustration, impulsiveness, explosiveness and emotional instability, increased suggestibility, uncompromisingness and lack of life experience; guilt and low self-esteem; hyporeactive emotional background in the process of conflicts, difficulty in restructuring value orientations.[48,49]

Suicide is often associated with a crisis of identity.[50] Such a psychological crisis can be arisen suddenly (under the influence of strong state of affect).[51-53] But more often internal mental tension accumulates gradually, combining heterogeneous negative emotions. They are superimposed on one another, concern is replaced by

anxiety, and anxiety, in turn, – by hopelessness. A person loses faith in himself/herself, because of the ability to overcome adverse circumstances. In such a way the internal conflicts of "self-rejection", "self-denial" are arisen, and a feeling of "loss of the meaning of life" appears.[54-55] In our research we also proved that the leading factors in the formation of suicidal behavior were internal factors (neuropsychic states, the presence of latent mental illnesses, a tendency to depression, etc.). All these factors are formed in the soldiers' childhood. As we showed in our research the main internal factors were: "feeling of lack of self-sufficiency and self-doubt", "insufficiently developed ability for psychological mobilization», "increased need for protection, support and patronage", "decreased tolerance to frustrations" and others.

So, internal suicidal behavior includes suicidal thoughts, representations, experiences and even intentions. Passive suicidal thoughts are characterized by the ideas, fantasies about the person's death, but not about taking by him/her the person's own life as a spontaneous activity. As a bright example of this we've to propose such statements of militaries with one attempt of suicide: "It would be nice for me to die…", "I'd like to fall asleep and not wake up"; "If something happens to me and I die…" These results are related to current scientific knowledge.[56,57] In these researches the authors proved, that people with hysterical personality disorder were the most suicidal. For these respondents scientists counted more than 35% of all completed suicides. The highest frequency of repeated suicide attempts with a tendency to demonstratively-blackmailing forms also correlates with hysterical personality disorder. In addition to suicidal behavior, the following types of deviant behavior are very often observed in accordance with these individuals: dependence on alcohol and other psychoactive substances, sexual promiscuity and perversion, collective types of deviant behavior (sectarianism and participation in informal groups).[58-60]

In our empirical research we also proved that despite the fact that internal factors have been formed from early childhood, the recruits are greatly influenced by the situation of the war, which has taken place in Ukraine, Donetsk and Luhansk regions, since 2014. "The war situation" means moral, physical and psychological overload, uncertainty of the military situation, instability of the political situation in Ukraine, various criteria for assessing this situation (feelings about recruits' families, about their immediate future, attitude to self-aggression in general and suicide in particular). During the military conflict in Donetsk and Luhansk regions the soldiers often are influenced by senseless actions of soldiers based on orders of commanders, social misunderstanding of these situations (which, in turn, facilitates low level of social maturity, use of psychoac-

tive substances, hetero- and autoaggression). Post-traumatic stress disorders, anxiety and depressive disorders and neurotic states, sometimes – even psychopathic disorders, which also have taken a place, actualize children's experiences, which take a more severe form than in childhood, facilitates the acquisition of psychopathic traits of soldiers. As a rule the latter leads to suicide.

Scientists proved that suicidal ideation was an active form of manifestation of suicidality, that is a tendency to suicide, the depth of which increases because of the degree of the development of a plan for suicide implementation.[61] The current researches state that, as a rule, the person realizes the ways of suicide, a time and a place of committing suicide actions. In our research we came to the opinion that in a great degree suicidal intentions involve the addition of a decision and a volitional component to the idea, prompting a direct transition to external behavior of the person.[62-64]

So, the structure of suicidal experiences is based on the person's attitude towards two polar opposite values: one's own Life and Death. The attitude towards the person's own life in presuicide is often expressed in four main forms: 1) by a great feeling of indifference; 2) by a feeling of regret about personal existence of a man; 3) the experience of the life's burdensomeness, intolerant attitude to everything that is around people; 4) by the experience of being disgusted with the person's life. The attitude towards death appears in its forms: 1) in a form of fear because of death, which is although reduced by its intensity; 2) by a feeling of indifference; 3) by a feeling of inner consent to die; 4) showing a great desire to die.[65] In our research we also came to the conclusion that some psychotraumatic factors had a suicidogenic effect: personal; interpersonal; family or production situations, accompanied by underestimation of others; refusal or obstacle to the achievement of purely selfish goals; dissatisfaction with the claims of the person to his/her exclusive role; situations of uncertainty; some combination of all of the above factors. The period from the movement when suicidal thoughts appear and when the attempts are implemented is traditionally called presuicidal (presuicide). It always lasts for several minutes ("acute presuicide") or some months (so called "chronic presuicide"). The presuicidal period is a key to point out the problem of so-called "impulsive" suicides. All deaths without the idea of the person about his/her own death should be classified as accidents, but not suicides. External forms of suicidal behavior always include suicidal attempts and completed suicides.[66]

In current researches of scientists it is said that when it was about repeated suicidal attempts, there was a tendency to move from true suicidal behavior to demonstratively blackmailing with rent-seeking attitudes.[67-68] Attempts of a manipulative type predominate, they have the aim of providing psychological control over others

by a psychopathic personality.[69-71] There is also a transformation of personal meanings with the dominance of some hysterical reactions, such as a call for help, a protest, blackmail. There is also a change in the methods of suicide from more severe to some lighter (self-poisoning, self-cutting) and less lethal forms. With each subsequent attempt suicidal behavior is consolidated and becomes a style of the behavior in resolving conflict situations and defending one's interests. This style of behavior is formed after the situation is resolved in a favorable direction for the suicidal person after the first suicidal attempt.[72]

According to the empirical results of our study (we mean militaries) we also distinguish the following types of suicidal behavior: 1) a really conscious behavior (when the person does conscious actions, the purpose of which is to commit the act of suicide); 2) emotionally-affective type (suicidal actions are done due to a strong effect of some psycho-traumatic accident or event); 3) demonstratively-hysterical (conscious manipulation of a military with life-threatening actions with the purpose to change the conflict situation in a favorable direction).

In the researches of scientists it was shown, that people with emotionally unstable personality disorders were among the most suicidal ones. Among all individuals with personality disorders who have committed suicide there are about 75% ones who are emotionally unstable.[56-59]

Repeated suicidal attempts tend to defiantly blackmail forms. They take on the character of manipulative-type attempts and discharge-type attempts. Attempts of the discharge type are impulsive by their mechanism of growth. During a suicidal act the emotional stress is discharged with a simultaneous narrowing of the paradigm of the person's consciousness. A control over the person's behavior is weakening. Therefore, a person's behavioral response in the form of a suicidal attempt often looks inadequate to this or that situation. Each subsequent attempt "sensitizes" the person, creates a psychological readiness for repeated suicidal attempts, which are carried out in more risky, often fatal ways.[70]

The results of our research showed, that a really conscious suicide of a military was developed in such a way. The predispositional phase of suicide is characterized by exceptional emotional intensity for a suicidal person. During this period the militaries' attention is fixed on insurmountable difficulties of the service, on different thoughts about the lack of potential opportunities to solve the problems that have been arisen. When a military is in a conflict or critical situation, personally significant needs are frustrating, and a military in the most cases begins to write home anxious, shocked, panicked letters. In such situations militaries ask their wives, parents, rela-

tives to organize leave from military service. Militaries often address to commanders with extremely persistent requests for hospitalization, and sometimes for practical psychological help. In such a way a military seeks a great support. This phase can be characterized as the phase of psychological social maladaptation. The life is perceived only in its retrospective, without a bright, encouraging future. The military feels inner emptiness and meaninglessness of his/her existence. The loss of the meaning of life is a central link in the suicidal behavior of a military.[61]

In modern studies it is also emphasized that different symptoms of depersonalization greatly influence the commission of suicides.[51] In current researches there are shown personal characteristics that potentiate suicidal behavior: a pronounced tendency to act quickly, impulsively, without taking into account the probabilistic consequences; a pronounced tendency to conflict behavior, especially in cases where impulsive actions are rebuffed or criticized; a tendency to outbursts of anger or aggression, culminating in "behavioral outbursts" that the individual is not able to control; propensity to self-reassessment; unstable, changeable mood.[70]

Having been on the predispositional suicide phase, different symptoms of depersonalization appear. They are: the indefinite feeling of internal change, alienation, oppressive or depressive mood, the experience of some kind of "unnaturalness" of the environment, its incomprehensibility and hostility. Some militaries, after one or more failed attempts of suicide, feel internal panic, internal catastrophe, tell about a premonition of some kind of the disaster. Militaries also feel a hypertrophied desire for self-analysis, self-observation, there is a heightened feeling of reflection.[61]

Modern researches deal with such point of view that in a case of militaries suicidal risk is due not only to personal, but also to endogenous factors, such as "phases of oppression" with a gloomy-dreary mood, maliciously aggressive discharges, conflicts, senestopathic sensations.[73]

In our research we also proved, that suicidal thoughts of militaries appeared, and later the person thought about the method of suicide, "trying it on him/her". The suicidal period, the beginning of which is associated by us with the appearance of suicidal thoughts, lasts until the attempt of the person to finish his/her own life. The very decision to commit suicide, even as a result of "thinking over", testifies about great experiences of extraordinary thoughts how to commit suicide.

Often in the very suicidal phase, before committing a suicidal attempt, a military displayed some behavioral features due to an affective narrowing of the person's consciousness. At the same time, there is a fragmentary perception of the environment, a decrease of the reaction to some external stimuli, emotional retardation and inadequate actions and statements, which precede suicide. At the same time, other forms of manifestation by

militaries of behavioral forms of suicide activity were also noted by us: prudence, decisiveness, composure (so called ominous or mortal calmness), and great aggressiveness. Sometimes the adoption of a suicidal decision is accompanied by fussiness, motor excitement, causeless gaiety. However, in all cases of committing a suicide, the unnatural behavior is immediately evident.

Study limitations

There were some limitations in our research. Firstly, the research was organized only in one Hospital (it is the Main Military Clinical Hospital (the Center), Kyiv, Ukraine). Secondly, in our research in experimental group there were participated only combatants with adaptive disorders, suicidal thoughts and attempts. Thirdly, into a control group we included 60 conscripts without adaptive disorders. Fourthly, in our research there were participated men in the age 18–22 years old. Fifthly, there were only men, not women, who participated in our research (because we organized this stage of the study in July – November, 2021, and in this period of time there were no women in the age 18–22 years old with suicidal thoughts and (or) attempts at the Main Military Clinical Hospital (the Center), Kyiv, Ukraine). And, the last one, sixthly, the terms of our research were limited by July–November, 2021.

Conclusion

We proved, that suicidal behavior was a complex phenomenon caused by a variety of motives directed on realizing different changes in the behavior of "significant other people" or alleviating (interrupting) severe mental and/or physical suffering of the person. The consequences of suicidal acts are experienced as severe stress not only for the military servicemen themselves, but also for their relatives and specialists who provide them with assistance.

Summing up, we should point out the expediency of the main provisions that are of practical importance in the activity of psychiatrists with suicidal militaries. All suicides who have committed severe suicidal attempts (hanging, complex poisoning, self-arson), according to their somatic compensation, must be examined by a psychiatrist without fail, using the developed classification of post-suicidal encephalopathies and certain experimental methods of psychophysiological research, in order to objectively identify the severity of the psychoorganic process, which in turn determines the treatment tactics of the doctor. Experimental psychophysiological examination of suicidal patients with residual post-suicidal encephalopathies should be carried out before and after the course of treatment in order to determine the objective results of treatment, the nature of rehabilitation, issues of medical and labor psychiatric examination and medical and social prognosis of patients, done by the doctors. The obtained statistical data on the dy-

namics of severe suicidal attempts in an acute affective, non-psychotic state (mainly among the militaries in the age 18-25 years old), followed by a residual psychoorganic defect, having been traced since 2014 in connection with military operations in the East of Ukraine, reflect the general trends of suicidogenesis among the male population of Ukraine, which contributes to the objective assessment and the possibility of solving this problem by appropriate medical and social preventive measures. According to our data, the motives of protest and conscription prevailed among soldiers. Therefore, the most important preventive factor is the early diagnosis of presuicidal changes in the behavior of young soldiers in the unit, which also indicate the need to use a model of psychological support for military servicemen. It will be done in further our articles.

Declarations

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Author contributions
Conceptualization, N.M. and Y.K.; Methodology, Y.P.; Software, Eduard I.; Validation, Y.K., W.Z. and Ernest I.; Formal Analysis, Eduard I.; Investigation, Ernest I.; Resources, A.Y.; Data Curation, Y.K., Eduard I. and Ernest I.; Writing – Original Draft Preparation, N.M.; Writing – Review & Editing, Y.P.; Visualization, A.Y.; Supervision, W.Z.; Project Administration, N.M.; Funding Acquisition, N.M.

Conflicts of interest
The authors declare no conflict of interest.

Data availability
The empirical results of our research of use of suicidal behavior of combatants in the conditions of their military service on the territory of Ukraine were presented in the repository "*Social Science Research Network (SSRN)*".[74]

Ethics approval
The ethical examination of the conducted empirical research was carried out and it was approved by the Committee on Ethics of Scientific Researches of the Public Organization "National Academy of Sciences of Higher Education of Ukraine", protocol № 12, dated from the 14th of December, 2021.

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ORIGINAL PAPER

Comparison between traditional and disposable bed baths in Intensive Care Unit

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ABSTRACT

Introduction and aim. This study aimed to compare the difference in the number of microorganisms (microbial counts) between traditional and disposable bed baths.

Material and methods. This study is quasi-experimental with two groups. The sample consisted of 30 respondents from the traditional bed bath and the disposable bed bath groups. Cultures of the groin were obtained to compare the number of microorganisms before and after bathing.

Results. Bathing with disposable bed baths has proven to be more effective in reducing the number of microorganisms in both the control group and the intervention group on day I and day II with p=0.014 and p=0.033.

Conclusion. Disposable bed baths are more effective in reducing the number of microorganisms on the skin than traditional bed baths.

Keywords. disposable bed baths, number of microorganisms, traditional bed baths

Introduction

Nosocomial infections are associated with increased length of hospital stay, mortality rates and costs. The increased risks of mortality and disability in patients treated in the Intensive Care Unit (ICU) are not only caused by chronic diseases but also secondary causes, namely nosocomial infections.[1,2] Each year, about 1.75 to 3.5 million patients are admitted to hospitals in the United States, and about 5% to 10% of whom, suffer from nosocomial infections.[3] Prevention of nosocomial infections can be done by bathing patients regularly to improve body hygiene and skin integrity and prevent disease.[4]

Previous studies have shown a very high contamination rate of basin baths from 62% to 98%.[5]

Patients in the ICU who are sedated and on a mechanical ventilator are too weak to be able to perform personal hygiene measures on their own.[6] Nurses perform personal hygiene measures by using a basin of warm water, soap and washcloths.[7] The use of soap causes an increase in the pH of the skin, stripping the skin of moisture – thus causing the skin to become dry.[8]

Personal hygiene measures aim to keep patients clean, refresh them and make them feel comfortable. Also, it can help reduce body odor, stimulate circula-

tion, eliminate sweat and reduce the potential for infection. Traditional basin bath has long been the standard in bathing patients in bed, but bed hygiene measures are considered potential workloads on nurses because they are really tiring.[9] The bathing method using disposable bed baths (DBB) eliminates the use of traditional basin baths which have been identified as a significant potential source of waterborne and basin-borne infections.

Aim

Aimed to compare the difference in the number of microorganisms (microbial counts) between traditional and disposable bed baths.

Material and methods

Ethical approval

Ethics committee approval was obtained before starting the study (Universitas Hasanuddin with number 718/H4.B.4.5.31/PP36-KOMETIK/2018).

Study design

This study used a quasi-experimental with a two-group design. Respondents were divided into 2 groups, group I was bathed using the traditional method, while group 2 was bathed using the DBB method, each group was bathed once a day for 1 day. On day 2, cross-over was performed, the first group (traditional method) was bathed using the DBB method and the second group (DBB group) was bathed using the traditional method, each group was bathed once a day for 1 day.

Respondents in this study consisted of 30 patients, namely 15 in the control group and 15 in the intervention group. This study was conducted in the ICU room at RS Universitas Hasanuddin, Makassar from October 22 to November 22 2018; bacterial culture examination was carried out in the Microbiology Laboratory at RS Universitas Hasanuddin, Makassar. Inclusion criteria were respondents who do not have skin problems, agree to participate in the study, have never been bathed in bed with DBB regularly. Meanwhile, exclusion criteria were respondents who were only hospitalized for one day in the ICU, suffered from cervical fractures and burns, infants and had diarrhea – and referred to other hospitals thus had not followed all stages of the study.

The procedure for bathing with the traditional and DBB methods is relatively the same, what makes it different is only the tools and materials used. The traditional method uses a basin, water, soap, lotion and towels to dry, while the DBB method only uses a disposable wash glove. Research assistants involved in this study were trained on procedures and how to bathe using traditional and DBB methods. Cultures of the Skin were obtained in moist areas, namely the groin.[10,11] Swabs of the skin were performed before the patient was bathed and

5-10 minutes after the patient was bathed. Swabs were performed using sterile cotton swabs which were then rubbed on the groin in a circle, then swabs that have been rubbed were put in sterile bottles to be examined in the microbiology laboratory.

Data were analyzed using SPSS 21.0 (IBM, Armonk, New York, United States). Univariate data were data on participants' initial characteristics and bivariate data were to compare the number of microorganisms. Data analysis was done using Paired t-test if the data were normally distributed – and the alternative Mann-Whitney test if the data were not normally distributed.

Results

Table 1 shows characteristics of respondents based on age, gender, type of antibiotic and medical diagnosis.

Table 1. Distribution of Respondents in the ICU at RS Universitas Hasanuddin, Makassar (n=30)

		Control (n = 15)	Intervention (n = 15)	p
Age	Mean (SD)	53.40 (±17.204)	48.60 (±19.508)	0.52
	(Min-Max)	12-80	12-87	
Gender	Males	6 (20%)	6 (20%)	1.000
	Females	9 (30%)	9 (30%)	
Types of Antibiotics	Ceftriaxone	8 (26.7%)	9 (30%)	0.908
	Ceftazidime	1 (3.3%)	1 (3.3%)	
	Cefotaxime	2 (6.7%)	1 (3.3%)	
	Meropenem	1 (3.3%)	0 (0%)	
	Ciprofloxacin	0 (0%)	1 (3.3%)	
	Levofloxacin	0 (0%)	1 (3.3%)	
	No Antibiotics	3 (10%)	2 (6.7%)	
Medical diagnosis	Surgery	6 (20%)	8 (26.7%)	0.526
	Non Surgical	9 (30%)	7 (23.3%)	

Table 2. Microorganisms distribution of cultured swabs in the groin (n=30)

		Control (n = 15)	Intervention (n = 15)
Types of Microorganisms Day 1 Bathing			
Resident	Staphylococcus aureus	5 (33.3%)	3 (20%)
	Staphylococcus epidermidis	6 (40%)	3 (20%)
	Staphylococcus hemolyticus	0 (0%)	1 (6.7%)
Transient	Eserichia coli	1 (6.7%)	2 (13.3%)
	Alkaligenes faecalis	1 (6.7%)	1 (6.7%)
	K. pneumonia	1 (6.7%)	0 (0%)
	A. calcoaceticus	1 (6.7%)	0 (0%)
	Pseudomonas aerogenosa	1 (6.7%)	1 (6.7%)
	Gram Positive Basil	0 (0%)	4 (26.7%)
Types of Microorganisms Day 2 Bathing			
Resident	S. aureus	6 (40%)	7 (46.7%)
	S. epidermidis	6 (40%)	2 (13.3%)
	S. hemolyticus	0 (0%)	1 (6.7%)
Transient	E. coli	1 (6.7%)	0 (0%)
	K. pneumonia	0 (0%)	1 (6.7%)
	A. calcoaceticus	1 (6.7%)	0 (0%)
	P. aerogenosa	1 (6.7%)	1 (6.7%)
	Gram Positive Basil	0 (0%)	3 (20%)

In the control patients, the average age of the patients was 53.4, while in the intervention group, the average age of the patients was 48.60. The average gender in the control group and the intervention group were

6 females (20%) and 9 males (30%) respectively. The most widely used antibiotic in the control group was ceftriaxone, with as many as 8 people (26.7%), while in the intervention group, the most widely used antibiotic was also ceftriaxone, with as many as 9 people (30%). Medical diagnoses in the control group were more on non-surgical patients, namely 9 people. Furthermore, Table 2 shows the distribution of microorganisms which was more dominant on gram-positive bacteria, namely *Staphylococcus aureus* which percentage on day 1 in the control group was 33.3% while in the intervention group was 20%. Meanwhile, *Klebsiella pneumoniae, Acinotobacter calcoaceticus* were less on day 2, both in the control group and the intervention group.

Table 3 shows that on day 1, the control group showed an increase in the number of microorganisms (microbial counts) after bathing with the traditional method with a p-value = 0.221, meaning that there was no difference in the increase in the number of microorganisms before and after bathing with the traditional way. On day 2, the control group patients were bathed in the DBB method and there was a decrease in the number of microorganisms after the bath with a p=0.152. Meanwhile, Table 4 shows that on day 1, the intervention group showed a decrease in the number of microorganisms after bathing with the DBB method with a p=0.007. On day 2, the intervention group patients were bathed in the traditional method and there was an increase in the number of microorganisms after the bath with a p=0.035.

Table 5 shows the average decrease in the number of microorganisms in the control and intervention groups on day 1 with a p=0.014; there was a significant difference between the number of microorganisms after the bath, with the number of microorganisms decreasing greater in the group bathed with the DBB method. Analysis using the Mann-Whitney test on day 2, the traditional and DBB groups obtained a p=0.033, statistically there was a greater decrease in the number of microorganisms in the group that was bathed with the DBB method. Clinically there was a significant difference between the number of microorganisms after bathing with the traditional method and the DBB method with the median value in the intervention group (traditional bed bath day 2) was -110 x 106 while in the control group (DBB day 2) was 1.1 x 106 .

Discussion

Changes in the number of microorganisms (germs) before and after bathing with traditional methods

In this study, it was found several types of microorganisms, there were gram-negative microorganisms and gram-positive bacteria. Gram-negative microorganisms appear more in humid areas than in dry areas.[12,13] Microorganisms found on the skin are classified into 2, namely resident microorganisms and transient microorganisms. Normal flora causes disease when it reaches internal organs through trauma or surgical equipment, for example *S. epidermidis*. The type and number of microorganisms still varied from individual to individual and

Table 3. Measurement of the number of colonization of microorganisms in the control group (n=15)

Day I				Day 2			
Pre Traditional (n = 15) Median (cfu/ml) (Min – Max)	Post Traditional (n = 15) Median (cfu/ml) (Min – Max)	Mean Difference (cfu/ml) (SD)	p	Pre DBB (n = 15) Median (cfu/ml) (Min – Max)	Post DBB (n = 15) Median (cfu/ml) (Min – Max)	Mean Difference (cfu/ml) (SD)	p
20 x 106 (32 x 105 – 59 x 108)	330 x 106 (5 x 105 – 62 x 108)	-210 x 106 (17 x 108)	0.221*	120 x 106 (11 x 103 – 74 x 108)	20 x 106 (17 x 103 – 64 x 108)	160 x 106 (45 x 107)	0.152*

*Wilcoxon test

Table 4. Measurement of the number of colonization of microorganisms in the intervention group (n=15)

Day I				Day 2			
Pre DBB (n = 15) Mean (SD)	Post DBB (n = 15) Mean (SD)	Mean Difference (SD)	p	Pre Traditional (n = 15) Median (Min – Max)	Post Traditional (n = 15) Median (Min – Max)	Mean Difference (SD)	p
1200 x 106 (19 x 108)	820 x 106 (17 x 108)	440 x 106 (54 x 107)	0.007**	160 x 106 (5 x 105 – 13 x 108)	230 x 106 (2 x 106 – 36 x 108)	-330 x 106 (68 x 107)	0.035*

*Wilcoxon Test **Paired Test

Table 5. Differences in the mean number of microorganisms in the control and intervention groups' patients (n=30)

Day I				Day 2			
Control (Traditional Bed Baths) Median (cfu/ml) (Min - Max)	Intervention (DBB) Median (cfu/ml) (Min-Max)	Mean Difference (SD)	p-value	Control (DBB) Median (cfu/ml) (Min – Max)	Intervention (Traditional Bed Baths) Median (cfu/ml) (Min – Max)	Mean Difference (SD)	p-value
-30 x 106 (-36 x 108- 43 x 108)	490 x 106 (-31 x 107 – 18 x 108)	110 x 106 (13 x 108)	0.014*	1.1 x 106 (-59 x 107- 10 x 108)	-110 x 106 (-25 x 108 – 25 x 107)	-84 x 106 (62 x 107)	0.033*

*Mann Whitney test

differed between body regions. Most microorganisms remained harmless.[13] Transient microorganisms consist of non-pathogenic microorganisms that have the potential to become pathogenic. Under conditions of altered balance, transient flora can cause disease. Microorganisms present on the skin become transient such as *S. aureus* concentrated in the nostrils, some Gram-negative bacteria such as *E. coli*.[14]

Traditional basin baths can reduce the number of microorganisms on the patient's skin by scrubbing carefully, using products/soaps containing anti-septic ingredients. Study conducted by has proven that anti-septic soap is effective in reducing microorganisms. Another study conducted by Abbas has also shown that antiseptic soap with a concentration of 50 mg/ml is effective for reducing microorganisms on the skin.[15-17]

In this study, the number of microorganisms after bathing with the traditional method increased, although the increase in the number of microorganisms was not statistically significant, but clinically from 15 patients in the control group who were bathed on day I with the traditional method, 8 patients experienced an increase in the number of microorganisms and only 5 patients experienced a decrease in the number of microorganisms. Meanwhile, the other 2 patients experienced stagnation in the number of microorganisms. Likewise, from 15 patients in the intervention group on day 2 after cross-over, who were bathed using the traditional method, 5 patients experienced an increase in the number of microorganisms and 8 patients experienced a decrease in the number of microorganisms. Meanwhile, the other 2 patients also experienced stagnation in the number of microorganisms with a p=0.035; there was an increase in the number of microorgaisms (germs) before and after bathing with the traditional method.

This study contradict the study conducted by Larson where from 33 patients who underwent culture swabs before and after bathing, there was a decrease in microorganisms cultured in the groin area, from 5.09 to 4.85. Traditional bathing using a bath basin has many weaknesses where according to studies, the bath basin used has proven to be a reservoir of pathogenic microorganisms.[10]

However, this study is supported by the study conducted by Marchaim that the bath basin used for patient care in the ICU, medical surgical ward, and general patient ward has a role as a reservoir of pathogenic microorganisms in various hospitals. 686 basins from various hospitals were tested, as many as 62.2% were contaminated with one or more pathogenic microorganisms.[5] This finding is consistent with study conducted by Johnson, that the bath basin is prone to be contaminated with pathogenic microorganisms up to 98%.[3] Besides that, traditional bathing using a bath basin can cause a potential workload on nurses because the work is tiring – it can cause back pain; musculoskeletal disorders due to strenuous, repetitive activities. Also, preparing water can cause fatigue and impact on the workload of nurses.[20]

Changes in the number of microorganisms (germs) before and after bathing with the DBB method

In this study, from 15 patients in the intervention group, swabs of the skin were performed to measure the number of microorganisms before and after bathing, it was found that there was a decrease in the number of microorganisms with a p=0.007, meaning that there was a difference in the number of microorganisms before and after bathing with the DBB method. The analysis carried out on the control and intervention groups (traditional bathing and DBB) on day I showed that the number of microorganisms decreased in the DBB method was much higher/ greater than in the traditional method with p=0.014; likewise, after the cross-over was carried out on day 2, it showed that the number of microorganisms decreased in the DBB method was much higher/ greater than in the traditional method with p=0.033. This study is consistent with study conducted by Larson, where from 33 patients who were bathed with the DBB method compared to the traditional method, there was a decrease in the number of microorganisms in the groin area from 5.05 to 4.79 with p=0.78.[10]

Bathing with the DBB method is quite practical because it only uses disposable washcloths that contain disinfectant. The product used in this study is the "Docare" brand washcloth bath. One of the ingredients of the wash glove used in bathing with the DBB method is Polyhexamethylene Biguanide (PHMB). PHMB is a disinfectant and preservative used for skin disinfection and cleaning contact lenses; it has very low toxicity to organisms such as human cells. PHMB is not cytotoxic. PHMB kills direct intracellular interactions of MRSA with pathogens in keratinocytes and host cells.[21,22] PHMB is effective in killing Gram-positive (*S aureus*), Gram-negative (*E. coli* and *Salmonella enterica* serovar Typhimurium) and acid-fast (*Mycobacterium smegmatis*) bacteria.[23]

Study limitations

This study period was short, namely only 1 day of intervention and 1 day of cross-over were carried out. Besides, this study only measured the types of microorganisms as well as the number of microorganisms in the control group and the intervention group; there is no specific data on the number of microorganisms in each type of microorganism so that it cannot be known the decrease in the number of microorganisms in each type of microorganism after bathing intervention was given.

Conclusion

The results of this study can be a recommendation for the use of DBB in the ICU because the number of mi-

croorganisms increases after bathing with the tradition-
al method while it decreases after bathing with the DBB
method. Disposable bed baths are more effective at re-
ducing the number of microorganisms on the skin than
traditional bed baths.

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H.A.; Data Curation, A.M.I.; Writing – Original Draft
Preparation, M.Y. and H.H.I.; Writing – Review & Edit-
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Conflicts of interest
The authors have no conflict of interest.

Data availability
The datasets used and/or analyzed during the current
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sonable request.

Ethics approval
The study was approved by the Universitas Hasanuddin
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ORIGINAL PAPER

Impact of face mask use during the non-stress test in pregnancy

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ABSTRACT

Introduction and aim. Face masks used to protect against the COVID 19 pandemic have become a daily routine. The aim of this study was to examine the possible effects of mask use on non-stress test (NST) results during pregnancy.
Material and methods. A total of 951 pregnant women were included in the study. They were divided into two groups as those who wear masks and those who do not. These pregnant women were also divided into subgroups as preterm and term periods.
Results. The mean age of the pregnant women was 31.2±4.9 and their gestational weeks were between 34+0 and 40+6. There was no significant difference between 34 and 37 gestational weeks pregnants in terms of FHR, reactivity, non-reactivity, deceleration, FHR category distribution and number of fetal movements ($p>0.05$). The variability was significantly higher in those who did not wear a mask ($p<0.05$). In pregnancies >37 gestational weeks there was no significant difference in terms of FHR, reactivity, non-reactivity, variability, deceleration and FHR category distribution ($p>0.05$). The number of fetal movements was significantly ($p<0.05$) lower in the mask-wearing group.
Conclusion. Mask use should be considered in NSTs where variability is reduced or fetal movements are low. Thus, misinterpretation of the NST can be avoided.
Keywords. COVID-19, face mask, fetal heart rate, non-stress test

Introduction

Antepartum fetal surveillance aims to reduce mortality and morbidity during pregnancy. There are several methods used for this purpose. These are maternal perception of fetal movements, non-stress test (NST), contraction stress test, umbilical artery doppler velocimetry, biophysical profile and modified biophysical profile.[1]

NST is routinely recommended to assess fetal well-being during the third trimester.[2] It is a non-invasive assessment method. Fetal heart rate (FHR), fetal movements, presence of uterine contraction and deceleration, number of accelerations and reactivity/non-reactivity are evaluated. If a possible risk is detected during the evaluation, the decision to perform other fetal well-being tests or urgent intervention can be discussed.

COVID-19 was identified in December 2019 in China and was declared a pandemic by the World Health Organization (WHO) on March 2020.[3] Although it generally progresses with clinical findings such as cough, fever, loss of taste, loss of smell, shortness of breath, headache and sore throat, it can also cause serious complications. According to data obtained to date, being infected with COVID-19 during pregnancy may increase the likelihood of hospitalization, admission to the intensive care unit and need for life support.

Main transmission routes of COVID-19 include droplet, contact transmission and airborne transmis-

sion. The usage of a disposable medical or surgical mask that covers the mouth and nose is routinely recommended to prevent viral transmission. Wearing a mask, which is the most well-known method of protection from COVID-19 during the pandemic, continues in hospital visits as well as daily routine. In a study, it was determined that the usage of surgical masks in term pregnancies significantly reduced oxygen saturation.[4]

Aim

Based on this finding, we planned to investigate whether wearing a mask during NST for assessment of fetal well-being would affect NST results.

Material and methods

Ethical approval

Ethical approval was obtained from Maltepe University Ethics Committee (No: 2021/900/83) and the study was carried out in accordance with the principles of the Declaration of Helsinki.

Study design

The study was carried out in Maltepe University Hospital, outpatient clinic of obstetrics between April 2019 and May 2021. A total of 951 pregnant women who met the criteria were included in the study. Masks have been routinely used since April 2020 to protect against COVID infection. Based on this date, two groups as wearing masks (group B) and not wearing masks (group A) were created.

Those with a singleton pregnancy and >34 gestational weeks were included in the study. Exclusion criteria were multiple pregnancy, <34 weeks of gestation, active labor, maternal disease (hypertension, diabetes mellitus, kidney disease, heart disease) and obstetric risk (preeclampsia, intrauterine growth retardation, chromosomal or structural abnormality).

The age, body mass index and gestational week were checked from patient files. FHR, number of accelerations, presence of deceleration and fetal movements detected in NST were retrospectively analyzed and recorded from the archive. All NST recordings were made by a single device. Those whose NST records could not be fully analyzed, those with a duration of less than 20 minutes and those with missing data in their files were not included in the study.

NST interpretation

By following the fetal heart rate tracing in NST; basal FHR, variability, accelerations, and decelerations can be measured. FHR is the average beats per minute (bpm) over a 10-minute interval. The normal value is between 110-160 bpm. Basal FHR <110 bpm is called bradycardia and >160 bpm is called tachycardia. Significant and sudden increases in FHR are called accelerations. It is

defined as an increase of ≥15 bpm lasting at least 15 seconds and maximum 2 minutes at ≥32 weeks of gestation. Absence of accelerations may be associated with fetal metabolic acidemia and hypoxic injury.[5-7]

Early decelerations are defined as FHR decreases with normal variability accompanying uterine contractions. They are not associated with hypoxia and acidosis. Prolonged decelerations are decreases in FHR of at least 15 beats lasting the shortest 2 minutes and the longest 10 minutes. The absence of variability or the presence of minimal variability and absence of accelerations requires urgent evaluation for hypoxic risk. Variable decelerations are sudden drops in FHR. The shape and size of the deceleration are not related to uterine contractions. They account for most decelerations during labor and reflect the baroreceptor-mediated fetal autonomic response to transient mechanical compression of the umbilical cord. Late decelerations are symmetrical decreases and outputs in heart rate together with uterine contractions. These decelerations reflect the chemoreceptor-mediated response to fetal hypoxemia.[8,9]

Variability occurs depending on the integration of the sympathetic and parasympathetic systems. A normal (moderate) variability is defined as the amplitude in the range of 5-25 bpm. It shows that oxygenation of the central nervous system is normal, hypoxic damage and metabolic acidemia are absent.[10] Amplitude >25 bpm is called saltatory pattern and <5 is called minimal variability.

One of the most important indicator in the evaluation of fetal well-being is the reactivity of NST. Reactive (negative) NST is a normal result showing that there are accelerations that occur at least 2 times within a maximum of 20 minutes. Nonreactive (positive) NST is the absence of two or more accelerations of at least 15 beats lasting, at least 15 seconds within 20 minutes. While 50% of NSTs are non-reactive under 28 weeks of gestation, 15% of NSTs between 28-32 weeks are non-reactive.[1] NST of a normal preterm fetus is usually non-reactive rather than reactive.

FHR patterns were classified in 3 categories in the workshop held by the National Institute of Child Health and Human Development, the American College of Obstetricians and Gynecologists and the Society for Maternal-Fetal Medicine in 2008 (Table 1).[6] Category I FHR monitors predict normal fetal acid-base status at the time of observation. They are routinely followed and no special action is required.[7] Category III monitoring is associated with abnormal fetal acid-base status. In these cases, a prompt clinical evaluation should be made. To quickly resolve the abnormal FHR pattern, maternal oxygen support, change in maternal position, cessation of labor stimulation, treatment of maternal hypotension, and treatment of tachysystole can be planned. If a positive response is not achieved despite the precau-

tions taken, delivery can be planned. Category II FHR follow-ups are uncertain and do not predict abnormal fetal acid-base status.

Table 1. Three-tier fetal heart rate interpretation system[6]

CATEGORY I

Category I fetal heart rate (FHR) tracings include all of the following:
- Baseline rate: 110–160 beats per minute (bpm)
- Baseline FHR variability: moderate
- Late or variable decelerations: absent
- Early decelerations: present or absent
- Accelerations: present or absent

CATEGORY II

Category II FHR tracings include all FHR tracings not categorized as Category I or Category III. Category II tracings may represent an appreciable fraction of those encountered in clinical care. Examples of Category II FHR tracings include any of the following:

Baseline rate
- Bradycardia not accompanied by absent baseline variability
- Tachycardia

Baseline FHR variability
- Minimal baseline variability
- Absent baseline variability not accompanied by recurrent decelerations
- Marked baseline variability

Accelerations
- Absence of induced accelerations after fetal stimulation

Periodic or episodic decelerations
- Recurrent variable decelerations accompanied by minimal or moderate baseline variability
- Prolonged deceleration ≥2 minutes but <10 minutes
- Recurrent late decelerations with moderate baseline variability
- Variable decelerations with other characteristics, such as slow return to baseline, "overshoots," or "shoulders"

CATEGORY III

Category III FHR tracings include either:
- Absent baseline FHR variability and any of the following:
  - Recurrent late decelerations
  - Recurrent variable decelerations
  - Bradycardia
- Sinusoidal pattern

Statistical analysis

In the descriptive statistics of the data mean, standard deviation, median minimum, maximum, frequency and ratio values were used. The distribution of variables was measured with the Kolmogorov Smirnov test. Mann-Whitney U test was used in the analysis of quantitative independent data. Chi-Square test was used in the analysis of qualitative independent data and the Fischer test was used when the Chi-Square test conditions were not met. SPSS 28.0 program (IBM, Armonk, New York, United States) was used in the analysis.

Results

In our study, pregnant women were divided into two groups because it was planned to examine the effects of mask use on maternal and fetal oxygenation. Pregnant women whose NST data were analyzed in the pre-pandemic period were named group A and pregnant women whose NST data were analyzed during the pandemic were named group B. In addition, because the rate of non-reactive NST was found to be higher in the

preterm period than in the term period, the pregnant women were divided also into subgroups according to their weeks.

Those between 34-37 gestational weeks were classified as A1 and B1 groups and those with >37 gestational weeks were classified as A2 and B2 groups. The mean age of the pregnant women was 31.2±4.9 and their gestational week was between 34+0 and 40+6 (Table 2).

Table 2. Demographic characteristics of all participants

		Min-Max		Median	Mean±SD/n-%
Age		19	– 44	31	31.2 ± 4.9
Gestational Week		34	– 40.6	37.1	36.8 ± 1.6
FHR		100	– 170	130	129.6 ± 10.1
Non-reactivity					162 17%
Reactivity					789 83%
Variability	I				64 6.7%
	II				719 75.6%
	III				168 17.7%
FHR Category	I				766 80.5%
	II				154 16.2%
	III				31 3.3%
Deceleration	No				905 95.2%
	Yes				46 4.8%
Fetal Movements		0	– 36	9	11.5 ± 8.6

There was no significant difference between the A1 and B1 groups, in which only pregnancies below 37 weeks were compared in terms of gestational weeks, FHR, reactivity, non-reactivity, deceleration, FHR category distribution and fetal movement number (p>0.05) (Table 3). The variability in the A1 group was significantly higher than in the B1 group (p<0.05).

Table 3. Comparison of NST features <37 gestational week

		A1		B1		p
		Mean±SD/n-%	Median	Mean±SD/n-%	Median	
Gestational Week		35.5 ± 1.0	35.5	35.5 ± 0.8	35.6	0.858 [m]
FHR		129.5 ± 10.2	130	130 ± 10.9	130	0.535 [m]
Non-reactivity		41 17.7%		41 17.1%		0.852 [x^2]
Reactivity		190 82.3%		199 82.9%		
Variability	I	8 3.5%		15 6.3%		0.027 [x^2]
	II	175 75.8%		195 81.3%		
	III	48 20.8%		30 12.5%		
FHR Category	I	179 77.5%		197 82.1%		0.482 [x^2]
	II	43 18.6%		35 14.6%		
	III	9 3.9%		8 3.3%		
Deceleration	No	219 94.8%		227 94.6%		0.914 [x^2]
	Yes	12 5.2%		13 5.4%		
Fetal Movements		11.4 ± 7.4	9	10.9 ± 8.2	9	0.351 [m]

[m] Mann-Whitney U test/[x^2] Ki-kare test

Furthermore, those with pregnancies >37 weeks of gestation were also analyzed as A2 and B2 groups. There

was no significant difference (p>0.05) in terms of gestational weeks, FHR, reactivity, non-reactivity, variability, deceleration and FHR category distribution. Solely, the number of fetal movements in the B2 group was significantly (p<0.05) lower than the A2 group (Table 4).

Table 4. Comparison of NST features >37 gestational week

		A2		B2			p
		Mean±SD/n-%	Median	Mean±SD/n-%	Median		
Gestational Week		38.2 ± 1	38.1	38 ± 0.8	38		0.259 [m]
FHR		128.6 ± 9.1	130	130.1 ± 10.2	130		0.134 [m]
Non-reactivity		34	15.4%	46	17.8%		0.475 [x^2]
Reactivity		187	84.6%	213	82.2%		
Variability	I	21	9.5%	20	7.8%		0.609 [x^2]
	II	162	73.3%	187	72.5%		
	III	38	17.2%	52	20.2%		
FHR Category	I	185	83.7%	205	79.1%		0.279 [x^2]
	II	32	14.5%	44	17.0%		
	III	4	1.8%	10	3.9%		
Deceleration	No	215	97.3%	244	94.6%		0.1 [x^2]
	Yes	6	2.7%	15	5.8%		
Fetal Movements		13 ± 9.1	10	11 ± 9.1	8		0.011 [m]

Discussion

During pregnancy, maternal and fetal metabolic activities increase. To compensate these increases, significant changes are observed in the respiratory system and cardiovascular system. Adaptive changes are observed in static lung volumes, gas exchange and ventilation. Besides, cardiovascular changes such as increased plasma volume and cardiac output and decreased vascular resistance are also observed.[11] In addition to these possible changes observed during pregnancy, we planned our research considering that the use of masks can also change respiratory physiology. In our study, we examined the possible effect of the mask use on NST results. As a primary outcome, we evaluated the difference in terms of FHR, reactivity, non-reactivity, variability, deceleration rate, FHR category distribution and fetal movements.

The first usage of mask in the literature was described by Mikulicz in 1897. He suggested the usage of a mouth bandage made of gauze in operations and took the first step regarding surgical masks.[12] The possible physiological effects of mask use over time were investigated. While some studies did not show a possible harm of using masks, some studies showed a disruptive effect on vital signs. A study by Zhang et al showed that wearing a surgical mask in healthy young people had adverse effects on cardiopulmonary function during exercise.[13] In a similar study, Shaw K et al. showed that wearing a face mask during exercise had no significant effect on healthy young people in terms of percutaneous oxygen saturation (SpO_2), exercise maximum load, tissue oxygenation index, exercise hearth rate and rating of perceived exertion.[14]

On the other hand, it was found that as long as the surgeons used a mask, their saturation was lower even if they were within the normal range.[15] In a study conducted with 50 university students, it was determined that the usage of masks caused an increase in heart rate and a decrease in blood oxygen saturation.[16] Lässing et al. showed that the heart rate and cardiac output were higher while wearing a surgical mask but there was no change in the values of blood pressure and blood lactate level during the exercise.[17]

Since pregnancy has different dynamics, maternal and fetal effects of mask use during pregnancy have been investigated for a long time. The physiological changes detected were variable similar to the general population. In a study conducted with pregnant healthcare workers using N95 masks, it was shown that the exhaled oxygen concentration increased by 3.2% and the exhaled carbon dioxide increased by 8.9%. These values are indicative of increased forced expired CO_2 concentration and decreased forced expired O_2 concentration. In contrast, there was no change in maternal and fetal heart rates, fingertip capillary lactate levels and oxygen saturation, and the degree of perceived exertion.[18] In a case-controlled study of 48 patients using masks there were no differences between the pregnant and non-pregnant in heart rate, respiratory rate, transcutaneous carbon dioxide level and oxygen saturation. Likewise, there was no significant effect on FHR.[19] A systematic review examined the physiological effects of N95 face mask use by pregnant women. It was determined that short-term usage of N95 filtered face mask did not have a negative effect on maternal heart rate, respiratory rate, blood oxygen saturation and FHR.[20] On the other hand, Roberge et al. found an increase in subcutaneous CO_2 levels over time during exercise in pregnant women using N95 FFR face masks.[21] In another study supporting this result, it was determined that the usage of surgical masks in term pregnancies significantly reduced oxygen saturation.[4]

In our study, while examining the pregnant population, we paid attention to the distinction of preterm fetus, which may constitute a handicap. We know that NST of most preterm fetuses is often non-reactive.[22] In order to avoid possible misinterpretation that may arise from this, we compared 34-37 weeks of pregnancy and >37 weeks of pregnancy as two groups. No negative effects of mask use on FHR, reactivity rate, non-reactivity rate, deceleration rate and FHR category distribution were found in the comparison of both groups (p>0.05).

As negative effects of mask usage, we found a tendency to decrease in variability in the preterm period and a decrease in the number of fetal movements in the term period (p <0.05). Fetal movements are one of the oldest method used to demonstrate fetal well-being. Although fetal movement count is still being used, studies have not found a proven effectiveness of fetal move-

ment count in predicting fetal well-being.[23] Location of the placenta, amniotic fluid volume, fetal presentation, maternal smoking, fetal sex, primiparity, obesity, and acute exercise have been associated with decreased fetal movements.[24] Similarly, there are many factors that can affect variability. Possible variables include gestational week, maternal daily exercise amount, daily rhythm, fetal respiratory movements, fetal gross movements, fetal behavioral conditions, smoking, fetal gender and ethnic differences.[25] Since we did not investigate these parameters, we think that the decrease in fetal movement numbers or differences in variability cannot be associated with the usage of masks alone.

It has been reported that the usage of masks during physical activities in people with known lung disease will cause physiological changes, even if minimal. From this point of view, it can be concluded that attention should be paid to possible decrease in saturation in prolonged NST scans in pregnant women with known lung disease.[26]

The negative side effects caused by masks become more evident over time. Since it can affect many parameters such as temperature increase, humidity, facial irritation, itching, headache, acne, vocal fatigue, perceived voice problems, increased stress, impaired motor function and cognition, a decrease in the use of masks is observed in the society from time to time.[20,27,28] We think that the most important limitation of our study is that pregnant women may have removed the mask even for a short time due to these possible side effects during NST. Other weaknesses of our study are that we did not question the type of masks patients used and how long they had been wearing them. We state this as another limitation, since CO_2 uptake in the dead space increases due to long-term use of masks and each mask has a different filter mechanism.

Conclusion
It seems that the usage of masks will take place in our lives for a while due to the COVID-19 pandemic. No significant effect of mask usage on FHR, reactivity, non-reactivity, deceleration rate and FHR category distribution was observed. On the contrary, we determined that the usage of masks may cause a decrease in baby movements and a decrease in variability. We think that possible misinterpretations will be avoided when NSTs with decreased baby movements and decreased variability are evaluated in the light of this information. Prospective studies with large samples are needed for more comprehensive results on this subject.

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Author contributions

Conflicts of interest

Data availability

Ethics approval

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ORIGINAL PAPER

From ground zero of the pandemic – nurses' stories via Photovoice

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ABSTRACT

Introduction and aim. With the ongoing COVID-19 pandemic, healthcare workers continue to work selflessly and intensively despite many occupational hazards. Although various studies have been carried out to evaluate the experiences of nurses who play an important role in the fight against the pandemic and the effects thereof, qualitative studies carried out using the photovoice technique are very limited. The aim of this study was to evaluate the experiences of nurses working during the COVID-19 pandemic and show the impact that COVID-19 has had on nurses' work and daily life using creative photographic data.

Material and methods. This qualitative study employed a participatory action research design, and photovoice technique was used. Research data were collected from nurses working in COVID-19 clinics of various hospitals between May and September 2021. Data analysis was performed via a phenomenological interpretation method.

Results. Five themes emerged from the data: (1) burnout, (2) anxiety, (3) social isolation, (4) emphasis on professional value, and (5) the value of life. Each theme was presented with representative photographic and written narratives provided by the participants.

Conclusion. The findings of this study suggest that nurses experience problems such as burnout, anxiety, and social isolation. All the participants associated these problems with the negative effects of the COVID-19 pandemic on work and daily life. On the other hand, the participants emphasized professional value and the value of life despite all the negativities and risks.

Keywords. COVID-19, pandemic, Photovoice, nursing care, qualitative research

Introduction

The first COVID-19 outbreak associated with exposure to a seafood market was detected in the Wuhan province of China in December 2019.[1] On January 30, 2020, the World Health Organization (WHO) declared the outbreak as an Internationally Important Public Health Emergency after a new type of coronavirus was detected. On March 11, 2020, COVID-19 was declared as a pandemic.[2] In Turkey, the first case was seen on the same day, and the first COVID-19 related death occurred 6 days later. By February 2022, the daily number of new cases exceeded 100,000 and the number of deaths exceeded 200.[3] While the COVID-19 pandemic continues to affect the public health, social, and economic sectors, the overarching objective of the Strategic Preparedness and Response Plan for COVID-19 – preventing the transmission of SARS-CoV-2 and the occurrence of related diseases and deaths – has been confirmed.[4] Healthcare workers, who played a key role in the fight against the COVID-19 pandemic, faced the highest risk to occupational disease exposure including injury and death. Occupational infections due to COVID-19, skin problems caused by long-term use of personal protective equipment, toxicity due to frequent use of disinfectants, and chronic fatigue are some of the problems experienced by healthcare workers. In addition, the pandemic also has a negative effect on the mental health of healthcare workers.[5]

Of the COVID-19-related deaths in Turkey, only 6,643 healthcare workers died. The estimated number of deaths, which is quite high for healthcare workers, may increase if the working conditions and workload are not improved and protective measures are not implemented.[6] On the other hand, the International Council of Nurses (ICN) issued a statement similar to that of the WHO stating that the number of deaths of nurses reported during the pandemic was contradictory and underreported. Despite the potentially fatal risks, nurses continued to work more intensively than ever during the pandemic, attracting the attention of the community with their success.[7] In a meta-analysis study, the prevalence of emotional exhaustion was 34.1%, depersonalization was 12.6 % among nurses during the COVID 19 pandemic.[8]

The clinical picture and symptoms of the disease led to increased hospitalizations and nursing care demand. Nursing care, which is often carried out in infection services and intensive care units, contributes significantly to the achievement of many goals from saving the patient's life to the success of treatment, preventing complications, and maintaining holistic, uninterrupted daily life activities.[9-10] The professional performance displayed by nurses during the pandemic was sometimes described as heroic. Furthermore, the literature emphasized that such a performance is already due to the nature of the nursing profession wherein patient advocacy and high-level care are continued even in the most difficult times. Societies once again remembered the critical role of nurses in the healthcare system as they went through an unprecedented pandemic.[11-12]

We can now examine in more detail the experiences of nurses in their fields of work during the COVID-19 pandemic through scientific research. Changing working conditions and increased risks also affect other areas of life. These effects show that nurses may have difficulty maintaining their mental and physical health.[13-15] We believe that nursing care experiences shaped during the pandemic and archived via scientific publications will contribute to the planning of future research and delivery of care services in the field. In the present study, we incorporated the power of qualitative research design to generate in-depth information on the subject as well as the richness of photographic expression in our data collection methods.

Aim

In this context, the aim of this study was of the experiences of nurses on active duty during the COVID-19 pandemic and reveal the impact of COVID-19 on nurses' work and daily life using creative photographic data.

Material and methods

Ethical approval

Ethical approval for the study was obtained from Necmettin Erbakan University Ethics Committee for Scientific Research in Health Sciences (decision no. 2021-6 date: 2/2021). The participants were properly informed and written consent was obtained. Nicknames were used instead of full names to ensure privacy. It was ensured that the privacy of third parties (patients, relatives, and other healthcare workers) was protected in the photographs.

Study design

This qualitative study was designed as a participatory action research and photovoice technique was used.[16] Photovoice is a technique that engages participants through photographic data generation to create a positive change and increases people's interest and responsiveness to the research question/subject by utilizing the impressive power of photographs.[17] Photovoice establishes a bridge between researchers and participants and enables stronger data to be produced regarding the meaning of the information. In addition, it makes participants freer in the data generation process, making it easier to focus on them and authenticity rather than the researcher's priorities.[18]

Research population and sample

The research population consists of nurses working in various hospitals in Turkey during the COVID-19 pandemic. Sample size calculation is not recommended in qualitative studies.[19] However, it is stated that 5–25 participants are enough for purposive sampling.[20] It is reported that qualitative data analysis will be difficult if there are many participants, stressing the importance of achieving data saturation.[21,22] Within this framework, the present study included 14 participants based on the inclusion and exclusion criteria. It was thought that data saturation was reached when the same answers started to be received from the participants. In this process, the data collection process has been terminated. Inclusion criteria were as follows: (a) Working as a nurse in COVID-19 clinics/units during the pandemic, (b) being interested in expressing one's experiences and thoughts with photographs, and (c) giving informed consent for voluntary participation in the study.

Nurses from six different provinces working in university hospitals and a private hospital throughout Turkey were included in the study via the snowball sampling technique. At the time of the research, various restrictions were imposed in Turkey due to the COVID-19, especially curfews covering certain hours. Therefore, sampling and the application phase were carried out online.

Procedure

The procedural steps of the study were defined by the researchers based on an extensive literature review.[23-24] The researchers gave information about the research to the nurses they could contact and asked them to share this

information with other nurses they knew and provide the contact information of those interested in participating with their permission. A digital presentation was prepared by a researcher about the purpose of the research, its scope, what the application will be like, and the rules of photography. With this presentation, an introductory meeting was held online for the nurses who provided their contact information. For those who could not attend, the meeting was repeated on a different date. Immediately after the meetings, the presentation file was shared with the participants and a voluntary sample group was formed.

Participants were told they had one month to take photographs with their own cameras. The photographs taken individually were evaluated together with the participants, and they were asked to create titles and written narratives for the agreed photographs. In-depth individual interviews were conducted with each participant based on photographic narratives. Interviews were conducted online by a researcher having experience in conducting qualitative research and semi-structured interviews, whereas the other researcher participated in interviews as a rapporteur for making observations and preparing field notes. For each participant, two group meetings lasting at least 30 and a maximum of 55 minutes and at least one individual interview were held. The interviews are concluded after the answers are repeated or the preliminary evaluations of the photographs with photographic and written statements are completed. Afterward, a session was held by all the researchers and the photographs to be included in the study were selected. The steps from reaching the participants to data collection were carried out between May and September 2021.

Data collection

Research data were collected mainly via the photovoice technique. The tools used for this purpose included:

<u>Participant information form (PIF)</u>: The form consists of ten questions on the general characteristics of the participants.

<u>Photograph- and written narrative-related instructions</u>: These include considerations to be made regarding photography and storage. During the photography period, an e-mail address was shared with the participants for additional information requests and possible questions and the necessary support was provided. An e-mail account was created to collect the photographs taken.

<u>Semi-structured interview form (SSIF)</u>: This form consists of two parts – the first is a six-question section standardized for photovoice technique and briefly called SHOWED[25] and the other part consists of three open-ended questions created by the researchers of this study. The standardized questions for photovoice technique are: (a) "what do you see here?", (b) "what is really happening here?", (c) "how does this relate to our lives?", (d) "why does this concern, situation, or strength ex-

ists?", (e) "how can we become empowered through our new understanding?", and (f) "what can we do?".

Other questions present on the interview form are as follows: (a) "what have you been through during this period?" (b) "how has this affected your profession?" and (c) "how has the pandemic affected the lives of nurses?". Two external specialists and two nurses were consulted for the evaluating SSIF.

The PIF was an online survey. Semi-structured interviews were recorded with participant permission and transcribed within 48 h after the interview to minimize data loss. The conformity of the transcripts with the interviews was checked by a separate researcher other than the rapporteur. The photographs and narratives included in the research were converted into a digital file. At the end of the data collection process, 35 photographs, written narratives for 14 photographs, data collected using PIF, and 50 pages of interview records and field notes were included in the data analysis process. The photographs that strongly represented the themes were determined by joint decision of the researchers. Photos and other data are stored encrypted on the first researcher's computer.

Data analysis

Colaizzi's seven-step phenomenological interpretation method was used in the evaluation of qualitative data, and the application steps were shaped accordingly (Table 1).[26] The qualitative data was first evaluated individually by each researcher, then jointly in the presence of all the researchers, and finalized with consensus. Miles–Huberman conformity analysis was performed for SSIF and the themes determined by encoders to ensure reliability.[27] The conformity coefficient for SSIF and the themes determined by the encoders were calculated to be 1 and 0.97, respectively. Induction was used in the grouping of themes and deduction was used to finalize the themes. In case of differences of opinion between the researchers, a joint decision was made after taking the opinion of a faculty member specializing in qualitative research. Data analysis was carried out according to Colaizzi's phenomenological interpretation method (Data recording, Extracting significant statements*, Formulating meaning*, Organizing formulated meanings into clusters of themes*, Exhaustively describing the investigated phenomenon*, Understandable expression of the investigated phenomenon, Verification of the basic structure**; *Miles–Huberman model was used for determining, making sense of, and grouping the statements.**The participants were contacted, and the data was verified in case of hesitation).

Consistency and verifiability were provided for reliability and credibility, and transferability were provided for validity.[28] In order to ensure verifiability, opinions were taken from two external experts, and expert auditing technique was used. In order to ensure consistency,

standardized questions for photovoice were used, and expert opinions were obtained for the other three questions in SSIF. Verification/member checking was done when all interpretations were member checked. In addition, an interview template was created and adhered to in order to standardize all the interviews. To ensure credibility, the interviews were held in the appropriate time frame. The moderator summarized the answers given by the interviewee during the interviews and confirmed with participant feedback.

Table 1. Social demographic data of the participants

	Variables	Average	Standard deviation
Professional experience (months)		7.71	7.043
		Number (n)	**Percent (%)**
Worked provinces	Konya	9	64.2
	Kırıkkale	1	7.14
	Kocaeli	1	7.14
	Bursa	1	7.14
	İstanbul	1	7.14
	Ankara	1	7.14
Employed Institutions	Special	1	7.14
	Public — University Hospital	2	14.28
	Ministry of Health Training and Research Hospital	4	28.56
	City Hospital	2	14.28
	Public Hospital	5	35.71
Gender	Female	13	87
	Male	1	13
Educational Status	Licence	11	78.6
	Degree	2	14.3
	Doctorate	1	7.1
Marital status	Married	7	50
	Single	7	50
Childbearing status	childless	9	64.3
	1 child	2	14.3
	2 children	2	14.3
	3 children	1	7.1
Her/His Covid status	Infected	4	28.6
	Non-infectious	10	71.4
Covid status of family members	Infected	8	57.1
	Spouse	2	14.3
	Brother	1	7.1
	More than one person in the family (mother, father, sibling, spouse)	5	35.7

All data collected during the research were protected using encrypted files and an antivirus program. This research was reported in accordance with the Standards for Reporting Qualitative Research checklist.[29]

Results

The majority of the 14 nurses included in the study were women (87%). Their mean age was 29.5 ± 6.5 years, and their mean work experience was 7.7 ± 7 years. Further-

more, 78.6% of the nurses received a bachelor's degree, 50% were married, 64.3% of the nurses did not have any children, 28.6% of the nurses had COVID-19 infection themselves, and 57.1% reported that at least one of their family members had COVID-19 (Table 1).

Themes

On performing qualitative analysis of the collected data, five main themes and a total of 16 sub-themes were created. Due to the large number of sub-themes, only the main themes are presented in this section with their representative photographic and written narratives to simplify the presentation of the findings (Table 2, Fig. 1, Fig. 2).

Table 2. Distribution of themes and sub-themes

Theme	n	Sub- theme	n
Burnout	30	Burnout	6
		Despair	12
		Hope/Despair	8
		Depersonalization	2
		Unmet expectations	2
Anxiety	16	Anxiety of infection	8
		Anxiety about inexperience	5
		Anxiety associated with family and children	3
Social Isolation	13	Social isolation (Contingency included)	13
Professional degree emphasis	18	Awareness	12
		A sense of self-actualization	3
		The importance of communication with the patient	2
		Increase in team awareness	1
Value of life	7	--	7

Theme 1: Burnout

According to the analysis, there was a strong emphasis among participants on "burnout" caused by the pandemic. In addition to data highlighting burnout directly, opinions reflecting desperation and despair were also shared. Factors such as hours of strenuous routines, patient losses, treatment practices and nursing care not yielding the desired result, inability to care for relatives with COVID-19 infection, and inability to support their child's daily routines were among the reasons for the feeling of helplessness experienced by the nurses.

Participant 1. *"... I've never had a situation where treatment and medication were so inadequate. We were just struggling without knowing what to do. Time was not important. No one was asking about the time. Patients were going blue and dying..."*

Participant 2. *"When I was going to the shift, I was saying goodbye to my children, saying "I might not be able to come home if I get sick tomorrow, don't worry" and leaving. They were crying and sending me on shift…"*

Participant 3. *"When the condition of our patients did not improve and our interventions were inadequate, I was falling to the aside with feelings of exhaustion and helplessness."*

Theme 2: Anxiety

The theme of "anxiety" put forward in this research was associated with several factors. For example, one participant with limited experience in the profession experienced anxiety due to the inexperience.

Participant 4. *"I had 1.5 months of work experience...They put me in the COVID-19 ward; I was so nervous. I was inexperienced and that was difficult for me; I asked experienced nurses for help, but then I was increasing their workload."*

Analysis of the interviews showed that all participants felt anxious about transmitting COVID-19 to their relatives and loved ones.

Participant 5. *"...I found out in September that I was COVID-19 positive. When my first CT scan showed that I had lung involvement, I was terrified and I cried. The only thing I could think of at the time was my family. My father has hypertension, and I was worried about transmitting COVID-19 to him."*

Theme 3: Social isolation

It was found that the exhausting routines expressed in the first theme and the anxiety about transmitting COVID-19 to others expressed in the second theme pushed nurses into social isolation. According to the data, social isolation affected the families of the nurses and also their social lives. One participant described social isolation in her own verses along with the photo in Figure 1-c.

Participant 6.

Who would have thought we'd look at life behind masks
Who would have thought smiles would be hidden

Who would have thought life would end up on the other side of the bars
Wait, life, we are fighting, and we're going to win...
Participant 7. *"Covid limited our freedom, we were left alone… On the other hand, the sense of being together and acting together strengthened in our team."*

Theme 4: Emphasis on professional value

Despite the negative feelings they experienced and the difficult working conditions, the participants stated that they played a very important role in providing valuable service, treatment, and care to patients during the pandemic.

Participant 8. *"It was a common opinion that nurses just prepared injections and only did what the doctor said, but now the patients see us the most, we touch them the most. We are valued more now."*

The participants frequently stated that the value given to nurses by the community and patients increased. One nurse said: "*...some people came to us and said 'you have worked so hard, you were always on duty when we were at home,' and some people coming to get vaccinated brought us little presents and thanked us for our efforts. In fact, the community in general understood how hard nurses work.*" (**Participant 9.**)

Theme 5: The value of life

The participants stated that they were affected by severely ill or dying patients, and that their clinical experiences during the pandemic strongly reflected the value and fragility of life. Participants also stated that similar thoughts were shared by the patients receiving care

Fig. 1. Photographic narratives of the participants representing (a) burnout, (b) anxiety and (c) social isolation
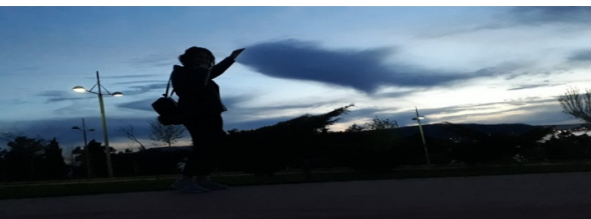

Fig. 2. Photographic and written narrative representing the theme of the value of life

(Figure 2). The COVID-19 pandemic has been an unprecedented experience so far. One nurse associated the inability of holding traditional ceremonies for relatives who have died due to COVID-19 with the value of life. **Participant 10.** *"... A lot of people experienced this disease and lost a relative. Losing someone close and no one coming to the funeral showed the importance of simple things and life."*

Participant 11. *"Even those with mild disease started to look at life differently. They're afraid of death. Everything, even money, is losing importance. My wife and I went through this. My wife's been through a lot. Both her lungs were half shut down. Nothing else was important to us at that moment."*

Discussion

The findings obtained in this study were discussed within the framework of five main themes covering the feelings and experiences of nurses providing care to infected patients in various clinics during the COVID-19 pandemic. These themes are burnout, anxiety, social isolation, emphasis on professional value, and the value of life.

Burnout is a psychological syndrome that involves emotional exhaustion, desensitization, and a feeling of decreased personal achievement, especially in relation to working with clients or patients.[30] Intensive care unit nurses participating in this study reported that they often experienced a feeling of burnout during the pandemic. Burnout can have dangerous consequences for nurses, patients, and health institutions, such as poor quality of care and service. Excessive workload, financial and human resources, and psychological factors such as the presence of social support in the workplace are important in explaining burnout.[31] It has been reported that work environments affect the psychological health of medical personnel.[32] In particular, working in areas, such as intensive care units, where working conditions are more difficult and require high level of attention greatly increases the workload of nurses. Working in very difficult conditions during the pandemic, having inadequate social support, and experiencing disruptions in familial dynamics can lead to the depletion of nurses' emotional resources and the manifestation of burnout syndrome.[7,33] The COVID-19 pandemic has increased the stress experienced by nurses[34], and the increased patient load due to the pandemic further triggered burnout among nurses.[34] If the problem of burnout among nurses is not addressed in such a period, it may lead to various negative psychological effects in the future.[35]

Anxiety is defined as a feeling of worry, nervousness, or unease about something with an uncertain outcome, accompanied by various physiological symptoms that can occur at any time in the lives of individuals.[36] Due to the COVID-19 pandemic, healthcare workers experience significant levels of anxiety and stress, and stress is even considered as an independent health related risk factor.[37] Among healthcare workers, nurses exhibit the most anxiety-related symptoms.[38] It was reported that 85.7% of nurses showed moderate-to-severe anxiety[39] and 20.7% showed severe anxiety.[40] When emotions such as stress, fear, and anxiety experienced during the pandemic cannot be controlled, they can lead to undesirable problems such as anger, loneliness, despair, depression, and post-traumatic stress disorder.[40,41] The United Nations urged governments to focus on initiating mental health services for their citizens, especially healthcare workers, during the pandemic.[42]

Since COVID-19 is a respiratory disease, various isolation measures have been introduced to prevent its spread. As a result of these measures, schools were closed, intercity travel and movement were restricted, and curfews were imposed. In addition to all these measures, nurses who provided care for patients diagnosed with COVID-19 were concerned about infecting family members and further isolated themselves. Social isolation is without doubt the most effective measure against COVID-19, but it can negatively affect the mental health of individuals.[43,44] Social isolation can trigger various psychological problems such as panic disorder, anxiety, and depression.[45,46] During the pandemic, hospitals have become an intensive and stressful workplace where nurses spend more time. Nurses tried to overcome the problem of social isolation with the support of their colleagues.[47]

The pandemic has led to a situation where many individuals are infected at the same time and treatment is carried out in hospital conditions. This period highlighted the importance of the concept of "care," which is the main purpose of the nursing profession. ICN declared the theme of 2020 as "Nurses: A Voice to Lead – Nursing the World to Health".[48] In the fight against COVID-19, nurses have worked with dedication on the front line, and nursing care plays a key role in ending this pandemic.[49] Therefore, institutions should carry out activities to increase the professional motivation of nurses. Nurses felt loneliness, fear, and restlessness and experienced instability and emotional exhaustion at the beginning of the pandemic. However, mutual social support provided by team members, financial incentives offered by institutions, positive feedback from patients and their relatives, public praise, and support increased the motivation of nurses.[50]

Although nurses worked with superhuman strength and dedication during the COVID-19 pandemic, this challenging period has led to changes in their lives as it has in the lives of other individuals. The complex feelings and desperation felt between life and death have caused nurses to focus on the value of life. During this period, nurses lost not only the patients they cared for but also their colleagues. Many nurses and their relatives got in-

fected with COVID-19. In Turkey, compared with the general population, the incidence of COVID-19 was 10 times higher among healthcare workers.[51] This painful experience has also resulted in some positive changes. These include thinking about the meaning of one's own life; questioning life in general; and associating events, people, and experiences with positive meanings.[52]

Study limitations
Not every experience can be investigated by the photovoice method. Creating photographic data for certain situations and experiences may not be legally and ethically possible. This is the main limitation of the present research.

Conclusion
This research conducted during the COVID-19 pandemic revealed the signs of burnout and anxiety experienced by nurses and showed that nurses experienced social isolation. The photographic narratives of the participants formed strong representations about their experiences during the pandemic process. Participants had the opportunity to express themselves freely and share common outputs in the sessions. Photo narratives selected for a stronger and more widespread impact were published on the official website of the researchers' institution. These feelings and experiences were reflected by the photographic and written narratives. These problems need to be addressed quickly and effectively as the feeling of burnout, uncontrollable intense anxiety, and social isolation will have further negative consequences among nurses as well as their relatives, patients, and the healthcare system. However, despite all the negativities and risks, nurses participating in the present study emphasized the value of life and nursing profession, which is undoubtedly admirable. Governments, managers, and nongovernmental organizations and societies should be aware of the great dedication shown by nurses during the COVID-19 pandemic and continue to appreciate and honor the positive impact of nursing care throughout the pandemic.

Declarations

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REVIEW PAPER

A potential usage of probiotics in prevention and treatment of neutropenic enterocolitis

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ABSTRACT

Introduction and aim. Neutropenic enterocolitis is a severe disease that affects patients with immunodeficiency and is often related with the chemotherapy of the hematologic malignancies. Its pathophysiology is not fully understood. Neutropenic colitis may lead to sepsis, gastrointestinal bleeding and even perforations requiring surgical management. The therapy consists of antibiotic therapy, transfusions, hematopoietic growth factor treatment, usage of fluids and electrolytes, bowel rest and even surgical operations. The aim of this review is to consider the potential usage of probiotics in the prevention and treatment of neutropenic enterocolitis.

Material and methods. References for that article were found through PubMed and Google Scholar, using terms: "neutropenic enterocolitis" and "probiotics", or "gut microbiota" and "neutropenic enterocolitis". The research was limited to abstracts and available full-text articles.

Analysis of the literature. The most possible mechanism of neutropenic enterocolitis development appoints bacterial invasion with co-existing immunodeficiency. The probiotics appeal as beneficial agents in both prevention and treatment of neutropenic enterocolitis in according to their impact on gut immune barrier improvement. However older societies' guidelines were cautious, the most modern ones appoint probiotics as a promising agent in neutropenic enterocolitis, what corresponds with results from current randomized clinical trials.

Conclusion. As neutropenic enterocolitis is a severe disease we need to look for better or alternative therapies of that state. The probiotics seems to have beneficial effects in terms of prevention and treatment of neutropenic enterocolitis due to their impact on gut immune barrier. Benefits of such therapy are reflected in current societies' guidelines which consider probiotics as a promising agent in neutropenic enterocolitis.

Keywords. oncology, neutropenic enterocolitis, probiotics

Introduction

Neutropenic enterocolitis (NE) is a severe disease that affects patients with immunodeficiency and is often related with the chemotherapy.[1,2] However usually NE is associated with hematological malignances, it may also occur in according to solid tumors therapy.[3] Usually it affects terminal ileum and right colon.[4]

Its pathophysiology is not fully understood. Damaged intestinal mucosa combined with neutropenia (neutrophil count < 500/μL increases the risk) and immunodeficiency leads to interstitial edema, engorged vessel, vascular congestions and bacterial, fungal or viral invasion (predominatingly *Pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus cereus*, *Klebsiella* spp., *Enterococci, Clostridium* spp., and *Candida* spp.).[5–7] That mucosal damage may result from the usage of chemotherapeutics with gastrointestinal toxicity.[5] Medications that predispose to NE are taxane drugs, cyclophosphamide, vincristine, methotrexate, cytarabine, daunorubicin, fludarabine, doxorubicin, idarubicin, 6-thioguanine, deoxycoformycin, 5-fluorouracil, epirubicin, docetaxel, cyclosporine, arabinoside, gemcitabine, leuvocorin.[8–10]

As NE is a rare syndrome and has a nonspecific presentation it may resemble other diseases.[5] That diagnosis may be indicated by concomitance of symptoms such as neutropenia, abdominal pain, diarrhea, nausea and vomiting, fever and thickening of bowel wall seen in radiological examination.[5–7]

NE may lead to sepsis (predominantly *C. septicum*, *Citrobacter freundii*, *Stomatococcus mucilaginosus*, and *Stenotrophomonas maltophilia*), gastrointestinal bleeding and even perforations requiring surgery.[6,11] Estimated mortality varies from 23% in intensive care unit patients to 42.2% of those who needed surgical treatment.[6,12]

There are still discussion about optimal therapy for NE. Currently it consists of antibiotic therapy, transfusions, hematopoietic growth factor treatment and surgical operations.[6]

On the other hand, recent studies focused on gut microbiota condition and its meaning for human health resulted with significant usage of probiotics in diseases like *Helicobacter pylori* infection[13] or inflammatory bowel disease.[14–16]

Aim

The aim of this review is to consider the potential usage of probiotics in the prevention and treatment of NE.

Material and methods

References for that article were found through PubMed and Google Scholar, using terms: "neutropenic enterocolitis" and "probiotics", or "gut microbiota" and "neutropenic enterocolitis". The research was limited to abstracts and available full-text articles.

Analysis of the literature

However the pathophysiology of NE remains unclear, the most possible mechanism of its developments is local bacterial invasion of intestine walls, unprotected due to neutropenia and dysbiotic microbiota (both caused by aggressive chemotherapy).[6] Oncological chemotherapeutics have impact on drug pharmacokinetics, what may result up with gastrointestinal toxicity, making a gate for bacterial invasion.[17] Disorders in gut microbiota appeals as common among hematological patients, even as a result of stem cells transplant.[18]

In patients with NE level of dysbiosis corelates with higher degree of disease, on the one hand prolonging the treatment time, but on the other hand – without a significant increase of mortality rate.[19] This dysbiosis may also be iatrogenic – the antibiotic therapy used before the oncological treatment increases the risk of NE development, what may suggest that microbiota impairments are more dangerous for the patient than neutropenia indeed.[20] The important factor increasing risk of NE is gut mucositis.[6] In according to mucositis, the beneficial effect of probiotic intake also is suggested.[21,22]

The usage of probiotics (particularly *Lactobacillus rhamnosus GG*) significantly reduced the prevalence and severity of gastrointestinal side effects of chemotherapy in patients with acute lymphoblastic leukemia in a prospective cohort study.[23]

The first randomized clinical trial on the usage of probiotics in chemotherapy-related diarrhea was published in 2007. The study was concerned on the impact of *Lactobacillus rhamnosus* GG supplementation in patients with 5-fluorouracyl therapy. The patients with probiotic had significantly lower prevalence of severe diarrhea (22 vs 37%, p=0.027), reported less abdominal discomfort and needed chemotherapy doses reduction less frequently.[24] The other research group appointed similar results using *Lactobacillus acidophilus* LA-5 plus *Bifidobacterium animalis* subsp. *lactis* BB-12 in patients with cervical cancer – the research group reported significantly lower occurrence of acute diarrhea in compare to control group (53.8 and 82.1%, p < 0.05).[25] The other group appointed that probiotics intake results up with decreased level of pro-inflammatory cytokines (TNF-α, IL-6, IL-10, IL-12, IL-17A, IL-17C and IL-22) in patients after surgical treatment of colorectal cancer, with significantly lower prevalence of acute diarrhea.[26,27] The intake of *Enterococcus faecium* M-74 resulted with lower risk of severe neutropenia (crucial condition for NE) in patients with leukemia treated by chemotherapy.[28] In according to other post-chemotherapy complications, other randomized clinical trial appointed significantly lower occurrence of fever among pediatric patients ongoing chemotherapy.[29]

The probiotics usage in NE is a dynamic topic of scientific research. Nevertheless, its intake in patients

isn't currently recommended according to current guidelines of numerous scientific associations including European Society of Medical Oncology[30] or German Society of Hematology and Medical Oncology[31]. The recent recommendations of European Society of Pediatric Gastroenterology, Hepatology and Nutrition appoint usefulness of probiotics in treatment and prevention of NE, but on the other hand they underline the need of further research on the particular strains.[32]

Conclusion

The probiotics appeal as beneficial agents in both prevention and treatment of NE in according to their impact on gut immune barrier improvement. However older societies' guidelines were cautious, the most modern ones appoint probiotics as a promising agent in NE.

Declarations

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Author contributions

Conceptualization, J.G. and P.H.; Methodology, J.G.; Software, Ł.D.; Validation, P.H., J.G. and Ł.D.; Formal Analysis, J.G.; Investigation, J.G., P.H. and Ł.D.; Resources, J.G., P.H. and Ł.D.; Data Curation, J.G and P.H..; Writing – Original Draft Preparation, J.G., P.H. and Ł.D.; Writing – Review & Editing, P.H., J.G.; Visualization, J.G.; Supervision, J.G..; Project Administration, J.G., P.H. and Ł.D.

Conflicts of interest

Authors have no conflicts of interest to declare.

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REVIEW PAPER

Transcranial magnetic stimulation as a new therapeutical approach in autism spectrum disorders and attention deficit hyperactivity disorder – a review

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ABSTRACT

Introduction and aim. Autism spectrum disorders (ASD) and attention deficit hyperactivity disorder (ADHD) neurobiology reveals a complex picture of altered excitation-inhibition balance, aberrant neuronal and neurotransmitter activity, and network disorganization that could be addressed through repetitive transcranial magnetic stimulation (TMS). In this paper, we provide a narrative review of the most recent literature on the use of TMS to treat patients with ASD and ADHD.

Material and methods. Literature search from 2018 up to November 2022 has been conducted on PubMed database. Keywords reflected diagnoses and treatment modalities of interest.

Analysis of the literature. Eleven clinical trials regarding the use of TMS as a therapeutic tool in ASD, and seven studies (of which 3 are case reports) for ADHD have been reported. The dorsolateral prefrontal cortex (DLPFC) is the most frequent area stimulated. Heterogeneity in stimulation parameters, patient age, and outcome measures limited the interpretation of findings.

Conclusion. TMS as a therapeutic tool for neurodevelopmental disorders is still in its infancy. To define the real efficacy of TMS, future studies must be randomized, sham-controlled, and double-blind, and should include a larger sample with adequate inclusion/exclusion criteria, and longitudinal follow-up.

Keywords. ADHD, ASD, autism, neuromodulation, neurodevelopmental disorders, TMS

Introduction

Autism spectrum disorder (ASD) and attention deficit hyperactivity disorder (ADHD) are complex, heterogeneous, neurodevelopmental disorders caused by a strict interplay of genetic vulnerability and environmental factors that typically onset in childhood. Heterogeneity in etiology, phenotype, comorbidities, and outcomes are common hallmarks among these diseases. Treatment options for these neurodevelopmental disorders are limited, mainly focusing on early behavioral interventions. While for ASD there are no specific pharmacological treatments to address the core symptoms, psychostim-

ulants are considered the most effective therapy for patients with ADHD, with various side effects and the potential for abuse.[1-3]

In this scenario, transcranial magnetic stimulation (TMS) could emerge as a therapeutic option to treat ASD and ADHD in children and adolescents. TMS involves magnetic stimulation of the brain to cause long-term changes in excitability and neurochemical activity, healing the key neurobiological alterations known to be involved in neurodevelopment disorders.[1]

ASD refers to a group of clinical conditions that share the symptomatic core dyad of impaired social communication and interaction, as well as restricted, repetitive patterns of behavior and interests. ADHD is characterized by pervasive symptoms of age-inappropriate inattention, and/or hyperactivity/impulsivity, which interferes with daily functioning and development.[1] In both cases, symptoms persist across the lifespan, with a higher prevalence among males.[1]

Treatment options for these neurodevelopmental disorders are limited, mainly focusing on early behavioral interventions. While for ASD there are no specific pharmacological treatments to address the core symptoms, psychostimulants are considered the most effective therapy for patients with ADHD[3]. Unfortunately, various side effects and the potential for abuse with no reduction in symptom severity in long-term use can restrict its administration.[4]

In this scenario, alternative therapeutic strategies have been explored. Noninvasive brain stimulation, and in particular TMS could emerge as a therapeutic option for the treatment of ASD and ADHD in children and adolescents.[5]

TMS is based on the scientific principle of electromagnetic induction, consisting of the rapid pulse of electrical current in a copper wire coil, which in turn induces a rapidly fluctuating magnetic field. The magnetic field passes through the skull and generates an electric field, able to depolarize and fire brain networks safely and painlessly.[6]

Three TMS paradigms have been extensively used: single-pulse, paired pulses, and repetitive pulses. Among these, repeated TMS (rTMS) pulses protocols show the greatest therapeutic potential and longest-lasting effects.[6,7] By convention, low-frequency rTMS (<5 Hz) plays an inhibitory effect on the underlying cortex, while high-frequency stimulation (>5Hz) typically induces cortical facilitation.[2,6] Besides these classic rTMS procedures, there are other TMS protocols with the potential to modulate cortical activities for therapeutic purposes, such as theta-burst stimulation (TBS).[6,8,9] Continuous theta-burst stimulation (cTBS) shows an inhibitory effect on the cortex, while intermittent theta-burst stimulation (iTBS) is excitatory.

Changes in synaptic strength determine the long-lasting effects of TMS on the brain, which also result in specific structural modifications of dendritic spines and sprouting.[10]

In animal models, rTMS promoted complex neurobiochemical effects such as early genes stimulation, neurotransmitters release modulation, and expression of glutamate AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid)/NMDA (N-methyl-D-aspartate) receptors.[11] These molecular effects influence the electrophysiological properties of neurons, leading to synaptic plasticity-related phenomena, including long-term potentiation (LTP) and long-term depression (LTD).[11-13] When TMS is applied repetitively, a number of AMPA receptors (that open quickly and briefly) are recruited with secondary induction of NMDA transmission. NMDA receptors activation increases calcium influx and, therefore, calcium-sensitive signaling pathways. Accordingly, long-term changes in both the presynaptic and postsynaptic neurons lead to increased synaptic strength.[11,14] LTP is enhanced, in part, by retrograde signals that further release glutamate and BDNF. This in turn, activates the BDNF-TrkB pathway, which prompts the NMDA-dependent after-effects on synaptic plasticity.[15] In an *in vitro* model has been demonstrated that rTMS may increase the steady-state current in the presynaptic compartment independently from NMDA postsynaptic transmission. In this way, it is likely that a longer duration of rTMS may strengthen these presynaptic steady-state currents, thus prolonging the TMS-induced sequelae.[11,16]

Since rTMS produces a non-invasive form of brain cells activation, it has been considered for the treatment of several neuropsychiatric conditions, where behavioral disability is associated to altered cortical excitability and plasticity.[2]

In the pediatric population, rTMS has been demonstrated to be safe and tolerable.[17] Krishnan et al. reviewed data from 48 studies involving over 513 children and adolescents (aged 2.5-17.8 years old) and found that TMS side effects were generally mild and transient, such as headache, scalp discomfort, twitching, mood changes, fatigue, tinnitus. Seizures are the most serious side effect, and there have been very few cases in adolescents receiving TMS.[18] Overall, the risk of seizure is considered to be less than 0.01% across all patients and all paradigms.[2]

Aim

Here we provide an overview of the most recent literature on the use of TMS as treatment in patients with ASD and ADHD. We also briefly discuss the biological mechanisms of these disorders and how TMS may modulate them. Then we summarize the current evidence associated with safety, tolerability, and efficacy of rTMS in ASD and ADHD populations.

Material and methods

Although this article is not intended to be a systematic review, to identify all relevant articles a comprehensive search of the PubMed database has been conducted up to November 2022. Search terms reflected the diagnoses of interest (ASD, Autism, Autism Spectrum Disorders, Asperger, ADHD, Attention Deficit Hyperactivity Disorder, Neurodevelopmental disorders) and the interventions of interest (Transcranial magnetic stimulation, TMS, rTMS, TBS, neuromodulation). Only full articles published in English and in peer-review journals were considered.

Analysis of the literature

Neurobiology of ASD and ADHD: the rationale behind TMS treatment

The neurobiology of ASD and ADHD reveals a complex picture of altered excitation-inhibition (E/I) balance, aberrant neuronal activity, neurotransmission, and disorganization of brain networks.[19]

ASD

One of the most recent theories on the etiopathogenesis of ASD suggests that it may depend on the structural disarray of the neocortex's vertical morpho-functional units, otherwise known as mini-columns.[20] Mini-columns have a clearly defined hierarchical structure, with combinations of GABAergic interneurons that control inputs and outputs of pyramidal cells at their periphery, and radially oriented arrays of pyramidal projection neurons in their core.[21]

Minicolumns were both more numerous and smaller than usual in autistic patients' cerebral cortex, and there was less peripheral neuropil compartment. Because this compartment contains the unmyelinated projections of certain interneurons, researchers hypothesize that inhibition is impaired in autism.[20]

In ASD, cortical dysplasia which underlies the hypothesis of an inhibitory deficit appears in overabundance within the prefrontal lobes.[22] Immunocytochemical studies have localized this inhibitory deficit to a subset of interneurons containing the calcium-binding protein parvalbumin (PV). The loss of PV interneurons has been proposed to determine gamma oscillation abnormalities, which are thought to be a neurophysiological biomarker of ASD. Gamma oscillations (typically defined as 30 to 120 Hz with a low amplitude of 10-20V) are generated locally as a result of reciprocal interactions between excitatory pyramidal cells and the rhythmic perisomatic inhibition of PV interneurons.[23] In autism, uninhibited gamma activity suggests that none of the circuits of the brain comes to dominance as many of them are active simultaneously, with the consequent inability to focus attention on relevant, social stimuli. Thus, some researchers point that altered-gamma oscillations could explain the "weak central coherence" theory and its associated deficits in ASD patients.[23-25]

ADHD

One of the most influential theories for the neural basis of ADHD has focused on deficits in key domains of executive functions (EF).[26] The most consistent deficits seem to involve the "cool" EF such as motor response inhibition, working memory, sustained attention, response variability, and cognitive switching.[26-28] However, less severe impairments have also been observed in the so-called "hot" EF, including motivation control and reward-related decision making.[28,29]

ADHD patients appear to report multisystemic deficits affecting the front-striate-parietal-cerebellar system, which controls different cognitive functions.[28,29] In neuroimaging studies, during motor inhibitory control and attentional tasks, the right ventrolateral and dorsolateral prefrontal cortices (VLPFC and DLPFC) show consistently lower activation in people with ADHD. This makes the PFC a potential target for rTMS treatment.[30,31] In addition, during "hot" EF tasks (like decision-making tasks involving rewards or tasks requiring temporal discounting), children with ADHD have also demonstrated underactivation in the ventromedial prefrontal cortex (vmPFC), orbitofrontal cortex (OFC), and striate-limbic region.[28]

ADHD patients display also abnormally increased activation in areas of the default mode network, which consists of intercorrelated activation of the ventromedial frontal cortex, posterior cingulate, precuneus, inferior parietal, and temporal regions and is supposed to reflect task-irrelevant thoughts.[30,32] It has been proposed that poor performance on attention-demanding higher-level cognitive tasks in ADHD is caused by a combination of decreased activation of task-relevant regions and decreased deactivation of the default mode network, which reflects more mind-wandering.[29,33]

A large number of ADHD studies have also revealed regional volumetric changes, an abnormal trajectory of brain development, and abnormal functional connectivity.[9] Structural and functional differences in the ADHD brain are accompanied by defects of the catecholaminergic neurotransmitters, dopamine, and norepinephrine, which are believed to be critical in the pathophysiology of ADHD.[9] Low dopamine levels in the prefrontal cortex are linked to increased hyperactivity and irritability.

Interestingly, it has been shown that TMS might produce a similar effect on the dopamine system as D-amphetamine.[34] rTMS over prefrontal regions in animals and humans would alter neurotransmitter systems, including changes in serotonin, striatal dopamine release, and metabolite levels, as well as in striatal glutamate release and concentration.[28] Due to this and the

TMS's known safety profile, it can be considered a secure and efficient therapy option for ADHD.

TMS and ASD

Several trials indicate that specific rTMS protocols target certain regions of the cortex leading to improvement in behavioral deficits.[35]

These results have been also confirmed by recent meta-analyses showing that TMS may improve social abilities, repetitive and stereotyped behaviors, as well as errors in executive function tasks.[36]

In the next section, we will discuss articles on this topic published from 2018 to 2022 (Table 1A and 1B).

Clinical effects of TMS in ASD

A 3-week open-label trial (aged 9-17 years) delivered fifteen sessions of iTBS at 100% motor threshold on the right DLPFC in a population of 10 male children.[37] Improvements were reported in parent report scores on the repetitive behaviors. The iTBS treatments were well tolerated with no serious adverse effects.

In 2018, Kang et al. explored the efficacy of low-frequency rTMS on brain activity and behavior of 32 autistic children with intellectual disabilities (Intelligence Quotient, IQ < 70.[38] The autistic children were divided randomly into an experimental group and a control group. Participants received 18 times rTMS treatment with Fig8 coil, two times per week (9 weeks). The coil was placed over the left DLPFC for the first six times, then over the right DLPFC for the next six times, and finally over the bilateral DLPFC for the remaining six times. Low-frequency rTMS at 1 Hz with 90% MT was used. The Autism Behavior Checklist (ABC) scores revealed positive effects of rTMS on behavior. Furthermore, they recorded electroencephalographic (EEG) data before and after TMS treatment, highlighting an improvement in neurophysiological markers of cognitive function and brain connectivity. Particularly, an augmented peak alpha frequency (PAF) in the frontal, left temporal, right temporal, and occipital regions and alpha coherence between the central and the right temporal regions were reported.

More recently, using a similar stimulation protocol, the same research group published new findings on children with low-functioning autism.[39] Thirty-two children were divided equally into 2 groups: 16 children received a real rTMS treatment, while the other 16 children sham stimulation. Data EEG and ABC scores were collected before and after rTMS. To characterize the deterministic features of cortical activity, three recurrence quantification analysis (QRA) measures were extracted from EEG signals: recursive rate (RR), deterministic (DET), and mean diagonal length (L). Significant differences in RR and DET were observed between the experimental group and the sham group, highlighting a positive

outcome for brain activity. They also found an improvement in the ABC score post-rTMS only for the experimental group.

High-frequency rTMS has been used on the left inferior parietal lobule in 11 low-functioning ASD children to improve autism core symptoms.[40] Patients received two rTMS treatment courses six weeks apart. Each treatment course consisted of 5-second trains at 20 Hz, with 10-minute inter-train intervals, on each consecutive weekday for three weeks. Subjects were evaluated five times: before and after the first and second rTMS courses, and six weeks after the second rTMS treatment course. Participants showed a significant and long-lasting reduction in language and social-related symptoms measured by ATEC (Autism Treatment Evaluation Checklist). Furthermore, caregivers referred to some improvements in imitation and cognition.

In 2020 Gwynette et al. conducted an open-label, single-arm study to evaluate the safety and effects of rTMS on depression and autism symptoms in individuals with both major depressive disorder (MDD) and ASD.[41] Ten participants aged 23–29 years with ASD and MDD (without any medication changes in the last month, with IQ >60) were involved in the study. They underwent 25 sessions of rTMS applied to the left DLPFC. Overall, rTMS was well tolerated with just minor adverse effects. The Hamilton Rating Scale for Depression (HDRS) significantly improved, and 40% of participants achieved depression remission after rTMS treatment. There was no change in self-reported autism questionnaires following treatment, while parents' clinical scales of core symptoms of autism suggested an improvement in the ritualistic, sameness, and restricted behaviors that lasted over the next 3 months.

The Aimes' group published a pilot, double-blind, randomized controlled trial on the use of rTMS as treatment for EF deficits in ASD.[42] Thirty-eight autistic patients (age 16-35 years old) with IQ > 70, were equally randomized into two groups: the active group (n=18) and the sham group (n=20). Participants received TMS stimulation bilaterally on DLPFC for each session. Clinical and cognitive assessments were completed before and 4 weeks later the rTMS cycle. No evidence for the efficacy of active versus sham rTMS on EF was found. However, they found preliminary evidence of EF improvement following active vs. sham rTMS in participants with ASD with more severe adaptive functioning deficits. Adverse events experienced across groups were mild or moderate.

From the same lab, Moxon-Emre et al. conducted a randomized double-blind sham-controlled trial designed to investigate the impact of excitatory rTMS on glutamatergic (Glx) and γ-aminobutyric acid (GABA) metabolite brain levels, in ASD patients with EF defi-

Table 1A. Summary of studies on ADS, outcome measures, and results, including use of medication and adverse effects

Study	TMS modality/ Study design	N of patients on TMS	N of controls	Age (Years)	Target	Number of sessions	Frequency	Hz	MT(%)	Pulses/Sessions	Inter-Train Interval	Duration/session (minutes)
Abujadi et, al, 2018	iTBS //case-study (open-label)	10 (males)	/	Age range: 9-17	R DLPFC	15	Daily (5 weekday, over 3 weeks)	50 (x 3 pulse bursts at 5 Hz)	100	900 PPS (300 burst)	8 s On/ 2 s Off	5min
Kang et al., 2018	rTMS // case-study (randomized, sham controlled study)	16 (13 males: 3 females)	Sham group 16 (13 males: 3 females)	Active group=7.8 ± 2.1; Sham group=7.2 ± 1.6	L DLPFC & R DLPFC	18 (6 L DLPFC, 6 R DLPFCS, 6 bilaterally)	Twice every week, for 9 weeks	1	90	180PPSx18 (180 for each session)	20 s	Not reported
Kang et al., 2022	rTMS // case-study (randomized, sham controlled study)	16 (13 males: 3 females)	Sham group 16 (13 males: 3 females)	Active group=7.8 ± 2.1; Sham group=7.2 ± 1.6	L DLPFC & R DLPFC	18 (6 L DLPFC, 6 R DLPFCS, 6 bilaterally)	Twice every week, for 9 weeks	1	90	180PPSx18 (180PPS for each session)	20 s	Not reported
Yang et al., 2019	rTMS // case series	11 (7males: 4females)	/	7.09 ± 2.88	L inferior parietal lobule	30 (15 sessions for each course)	Daily (5 weekdays over 6 weeks)	20	50	Not reported	10 min	Not reported
Gwynette et al., 2020	rTMS / case-study (open label)	10 (9males: 1 female)	/	25.5	L DLPFC	25	Daily	10	100-120	3.000 PPS	5 s On/ 10 s Off	Not reported
Ameis et al., 2020	rTMS/case-study (double blind, randomized, sham controlled study)	20 (14 males: 6 females); 2 withdrew	Sham group 20 (14 males:6 females)	22.58 ± 4.5	L DLPFC & R DLPFC	20	Daily (5 weekdays over 4 weeks)	20	90	1500 PPS= 750 pulses/ hemisphere	30sec	Not reported
Moxon-Emre et AI, 2021	rTMS/ case-study (Double blind, sham-controlled study)	16 (16 males: 2 females)	Sham group 12 patients (8 males: 4 females)	23.3 ± 4.69	L DLPFC	20	Daily (5 weekday for 4 weeks)	20	90	1500 PPS	30sec	30-45 min
Casanova et al., 2021	rTMS / case-control study	19 (14 males:5 females)	19 healthy controls, who did not receive TMS	14.2 ± 3.61	L DLPFC & R DLPFC	18 (6 left DLPFC, 6 r DLPFCS, 6 bilaterally)	Weekly	1	90	180 PPS (9x20)	20-30s	Not reported
Darwish et al., 2021	rTMS/ randomized control study	15 (11males: 4 females)	15 (9 males: 6females)	Active group (5.13±1.89) Sham group (5.92±2.33) Total range (3-10years)	Broca's area on L IFC	4	Weekly	1	70	1800 PPS per day session	20 s	40 min
Ni et al., 2021	iTBS/ a randomized, single-blind parallel sham-controlled trial, followed by additional 4-week open-label	75 divided into Active group 40 (35males: 5 females)	35 (30 males: 5 females)	Active group (13.0±2.8) Sham group (12.5±2.9) Total range (8-17years)	Bilateral pSTS	16	Twice per week (2 days/week for 8 weeks)	50	80	2400 PPS/session.	10 s	Not reported
Ni et al., 2021	iTBS/ randomized, single-blinded, sham-controlled crossover trial	13 (11males: 2 females)	12	22.7±1.4	Bilateral pSTS	10	Daily (5weekday for 2 weeks)	50	80	2400 PPS/ session.	10 s	Not reported

Table 1B. Summary of studies on ADS, outcome measures, and results, including use of medication and adverse effects*

Study	Clinical	Cognitive	Effects	Adverse Effects	Medication
Abujadi et al., 2018	ASD	IQ>50	Yale-Brown Obsessive-Compulsive Scale improvement, reduced perseverative errors on the Wisconsin Card Sorting Test and lower total time for the Stroop test were highlighted.	None	Not reported
Kang et al., 2018	LF ASD	IQ<70	Significant increases in the Peak Alpha Frequency at the frontal region, the left temporal region, the right temporal region and the occipital region and a significant increase of alpha coherence between the central region and the right temporal region. Improvements or repetitive behaviours	Not reported	Not reported
Kang et al., 2022	LF ASD	IQ<70	Significant differences in RR and DET were observed between the experimental group and the control group, highlighting a positive outcome for brain activity. They found also, an improvement in the Autism Behavior Checklist (ABC) score post-rTMS only for the experimental	Not reported	Not reported
Yang et al., 2019	LF ASD	IQ<70	Participants showed a significant reduction in language- and social-related symptoms measured by Autism Treatment Evaluation Checklist (ATEC). Moreover, some possible improvements in imitation and cognition were reported by caregivers	1 participant showed more Irritability during the first 3 days of each treatment course; 1 patient reported emotional dysregulation and restless after the second course, which recovered in 5 days; 1 patient showed Hyperactivity and irritability during the first 5 days of the first course	None
Gwynette et al., 2020	ASD + MDD	IQ>60	The Hamilton rating scale for depression (HAM-D17) improved, and 40% of participants achieved remission. Clinical scales of core symptoms of autism also suggested improvement, though no change was observed by the participants themselves	1 participant had anticipatory anxiety; 1 reported increased irritability; 1 participant had transient muscle spasms. Other adverse events included mild scalp dicomfort, mild headache, and fatigue, were reported within the first week of treatment. 1 participant had a seizure due to a programming error	Not reported wich medication
Ameis et al. 2020	ASD	IQ>70	No evidence for efficacy of active versus sham rTMS on EF performance was found. However, we found promising preliminary evidence of EF performance improvement following active versus sham rTMS in participants with ASD with more severe adaptive functioning deficits	Adverse events experienced across groups included: headache, pain, nausea, nose bleed, congestion, laceration. The rate of adverse events in the active group was 1.37 times the rate in the sham group	26/40 partecipants used psychotropic medications. Active group= 16/20; Sham group= 10/20
Moxon-Emre et al., 2021	ASD	IQ>70 (Active group=112+19.5; Sham group=112±16.1)	Normalization of local Glx levels in adults with ASD.	Not reported	Psychotropic medication=12/16 partecipants of Active group; 5/12 partecipants of Sham group
Casanova et al., 2021	ASD	IQ>80	A significant reduction in the time period to reach peak amplitude and an increase in the decay phase (settling time) og gamma oscillations.	Not reported	None
Darwish et al., 2021	ASD	Not reported	There was a statistically significant clinical improvement in the active group comparing baseline Childhood Autism Rating at Scale (CARS) assessment. No significant difference between inter-groups. There was significant difference in improvement between the two groups according to eye contact and in response to examiner. Significant difference between active and sham groups in improvement of the active expressive language. No difference in passive vocabulary.	Not reported	Not reported
Ni et al., 2021	ASD	IQ>70	No therapeutic efficacy on the clinical symptoms and cognitive performance of social impairment	Local pain during iTBS intervention (10% in the Active at both phases and 29% in the Sham group at Phase 2) as well as headache, dizziness, tinnitus, and anxiety	24/75 patients on Methylphenidate; 2/75 on Atomoxetine; 10/75 on Antipsychotics
Ni et al., 2021	ASD	IQ= 102.9 ±17.4	Positive effects on parent-rate autistic symptoms	Not reported	1/13 was os sertraline; 1/13 on Fluoxetine; 1/13 on Methylphenidate; 1/13 on Alprazolam

* LF ASD – low function autism; DLPFC – dorsolateral prefrontal cortex; pSTS – posterior superior temporal sulcus; IFC – inferior frontal cortex; L – left. R – right; iTBS – intermittent theta-burst stimulation; rTMS – repetitive transcranial magnetic stimulation; MT – Motor threshold

cits.[43] Twenty-eight participants aged 16 to 33, underwent two magnetic resonance spectroscopy (MRS) scans of the left DLPFC, before and after randomization to receive active or sham rTMS in the same area. Baseline MRS data was available for 19 typically developing controls, matched for age and sex. Metabolite levels for Glx and GABA+ were compared between ASD and control groups at baseline and post-TMS treatment. Absolute Glx level was greater in the active rTMS group compared to the sham group, on the contrary GABA+ did not differ between groups. There was a significant difference between the rTMS and sham groups in terms of participants' use of psychotropic drugs and comorbidities, particularly depression. These findings highlight excitatory rTMS's ability to modulate local Glx levels and improve depression symptoms in young adults with ASD.

In 2021, the Casanova group conducted a control-trial study involving 19 ASD patients and 19 healthy controls, matched for age and sex.[44] Both groups received rTMS weekly (2 sessions/week for 9 weeks in total). The initial six rTMS sessions were administered over the left DLPC, followed by 6 sessions targeting the right DLPC, and an additional 6 treatments were done bilaterally (over the left and right DLPFC). Gamma frequency oscillations were also analyzed in response to a visual classification task (Kanizsa figures). Besides the normalization of time to peak amplitude and ringing decay of autistic subjects after TMS therapy (considered an index of E/I normalization), and a reduction of total error percentage at the visual task, the ABC and RBS-R (The Repetitive Behavior Scale-Revised) parental behavioral checklists rating changes showed statistically significant improvements, especially in repetitive and restricted behaviors.[44]

In a randomized control study, Darwish et al. evaluated the impact on language of rTMS on Broca's area in a sample of 30 autistic children aged between 3 and 10 years.[45] Patients were randomly divided into active (n=15) and sham (n=15) groups. The rTMS was administered weekly for 4 weeks over the left inferior frontal cortex (IFC), at 1.0 Hz and 70% of MT. CARS (Childhood *Autism* Rating Scale) showed significant improvement in the active group compared to baseline evaluation, though no significant difference between the two groups was highlighted. Moreover, there was a significant difference in the improvement of eye contact and active expressive language.

More recently, Ni et al. assessed the efficacy of iTBS over the bilateral posterior superior temporal sulcus (pSTS) in ASD, in a 4-week randomized, single-blind parallel sham-controlled trial, followed by a 4-week open-label intervention.[46] Seventy-eight children and adolescents (aged 8-17 years) were randomly assigned to one of two groups: active or sham. During the first

4 weeks, the active group received two-session/week of iTBS, whereas the control group received the same number of sham stimulation. After unblinding, both groups received eight-session of real stimulation over the additional 4 weeks. The within-group analysis revealed that 8 weeks of iTBS achieved greater efficacy than 4-week interventions. Participants with higher intelligence and better social cognitive abilities, as well as less severe attention-deficit hyperactivity disorder at baseline, were more likely to respond. However, the clinical efficacy of iTBS of pSTS was insignificant.

In a single-blinded, randomized, crossover, and sham-controlled pilot study, the same research group investigated the effects of 5-day multiple sessions of iTBS over the bilateral pSTS in 13 adults with ASD.[47] Each TBS train was comprised of a burst of 3 pulses at 50 Hz, given 1 every 200 ms for 10 times. The TBS train was given every 10 s for 20 times to have 600 pulses in total for each iTBS course. In this study, two iTBS courses, which were separated by a 5-min break, were first applied to the left and then to the right pSTS. The stimulus intensity of iTBS over the pSTS was 80 % of MT. For the sham control, iTBS sessions were given to the inion. The results revealed significantly immediate effects of multi-session iTBS over the bilateral pSTS on parent-rate autistic symptoms, but also that baseline social impairment and cognitive performance impacted iTBS efficacy.

The prefrontal cortex (PFC), especially DLPFC, is the main stimulation target region in ASD patients. Although most of these studies have reported positive effects of rTMS there is high heterogeneity and variability associated with patient characteristics, study designs, and stimulation parameters. Furthermore, it is still difficult to establish the real impact of variables such as age, sex, severity of the disorder, medications on TMS outcomes.

TMS stimulation in ADHD

There is a limited number of clinical trials on the use of TMS in ADHD population. A recent review reported 6 studies conducted principally in adults (four out of six) over 45 years, highlighting relatively little evidence for rTMS efficacy on ADHD symptoms or cognition.[28]

Here we report the latest trials published, including case reports as well (Table 2A and 2B).

Clinical effects of TMS in ADHD

In 2008 Niederhofern described a case of a 42-year-old female with ADHD resistant to methylphenidate, stopped 2 months before the trial.[48] A 5-day rTMS over the motor area with B65 coil had been administered at a frequency of 1 Hz, for 1200 pulses per day. Sham stimulation was also administered four months after the active rTMS. Conner's rating scale (CSRS) for adults

Table 2A. Summary of studies on ADHD, outcome measures, and results, including use of medication and adverse effects

Study	TMS modality/ Study design	N of patients	N of controls	Age (Years)	Target	Number of sessions	Frequency	Hz	MT(%)	Pulses/Sessions	Inter-Train Interval	Duration/session (minutes)
Niederhofer et al., 2008	rTMS//case-report	1 (female)	/	42	Motor Area	5	Daily	1	Not reported	1200 PPS/die	Not reported	60 min
Niederhofer et al., 2012	rTMS//case-report	1(female)	/	42	Additional Motor Area	21	Daily	1	Not reported	1200 PPS/die	Not reported	60 min
Ustohal et al., 2012	rTMS//case-report, sham controlled-study	1 (male)	patient received also sham treatment	36	L + R DLPFC	10 (5 for each emisphere)	Not reported	10	120%	1500 PPSfor session	10sec	Not reported
Shahar et al., 2015	Deep rTMS//Double-blind radomized control study	5	5 (sham group)	Not reported (Adults)	Right PFC	15	Not reported	Not reported (High frequency)	Not reported	Not reported	Not reported	Not reported
Harmelech et al. 2018	rTMS// Blinded sham-controlled trial	34 (divided in Active group and sham group)	Not reported	Not reported (Adults)	Bilateral DLPFC	15	Daily (5 weekdays over 3 Weeks)	Not reported (High frequency)	Not reported	Not reported	Not reported	Not reported
Cao et al, 2019	rTMS// Double-Blind sham-controlled study	22= rTMS group; 21 = rTM+ ATX group	the ATX group (n = 16) and the placebo group (n = 16); + Control group (18 healthy children)	<18	Right DLPFC	30	Daily (5 weekdays over 6 weeks)	10	100%	2400 PPS for session	26sec	30 min
Cardullo et al., 2021	rTMS// retrospective, open- label, no sham control study	22 (21 males: 1 female)= (Cocaine Abuse+ ADHD and 208 (203 males: 5 females)= (Cocaine Abuse)	/ (everyone received TMS)	22-59. ADHD +Coc A(37.91± 8.71); Coc A (37.67± 7.05)	Left DLPFC	30	Twice-daily rTMS sessions for the first 5 consecutive days, followed by twice-daily rTMS sessions once a week over 11 weeks	15	100 % (MT)	2400 PPS(60x40)	60 impulses per stimulation train, inter-train interval 15 s, and 40 total trains, for a session duration of 13 min	13 min

Table 2B. Summary of studies on ADHD, outcome measures, and results, including use of medication and adverse effects

Study	Clinical	Cognitive	Effects	Adverse Effects	Medication
Niederhofer et al., 2008	ADHD resistant to methylphenidate	Not reported	Self-reported improvement (Hyperactive symptoms domain)	None	Methylphenidate interrupted 2 months before TMS
Niederhofer et al., 2012	ADHD combined type with MPH (20 mg)	Not reported	After 3 week reduction in dosage of MPH to 10 mg + Self-reported improvement (Hyperactivity domain)	None	Methylphenidate
Ustohal et al., 2012	ADHD and Depression resistant to atomoxetine	Not reported	Improvement in attention after L DLPFC stimulation	After R DLPFC stimulation, patient reported dysphoria, hypobulia, tension and also inattention	Not reported (previously treated treated with venlafaxine, milnacipran,mirtazapine and tianeptine)
Shahar et al., 2015	ADHD	Not reported	Attention improvement	Not reported	Not reported
Paz et al. 2018	ADHD	Not reported	No difference between Active and Sham group	None	None
Harmelech et al., 2018	ADHD	Not reported	Significant improvements were observed in CAARS inattention sub-scale and in the attention and execu- tive function scores on the Mindstreams battery in the real stimulation groups mainly in the right DLPFC group	Not reported	Not reported
Cao et al., 2019	ADHD	IQ ≥80	Improvement in all ADHD core symptoms on the SNAP-IV scale. Downregulation of miRNA-let-7d expression level only at post- rTMS or at post-ATX treatment	1 patient reported headache.	None
Cardullo et al., 2021	ADHD + Cocaine Abuse	Not reported	Improvements on cocaine use, craving, and other negative affect symptoms. No differences were observed between groups	None	9/22 of ADHD patients were on Atomoxetine

showed an improvement in the hyperactive symptoms only after TMS stimulation with a duration of 4 weeks.[48] Lately, Niederhofer administered rTMS (with the same stimulation paradigm) for 21 days over the right motor area in a patient on methylphenidate.[49] A clinical improvement in hyperactivity was seen after the first 5 days of stimulation, with a 3 weeks long-lasting.[49] Both studies did not show any difference in the inattention domain.

A 36-year-old man with ADHD and depression, resistant to atomoxetine received 5 sessions of 10 Hz rTMS on the left and right DLPFC at 120% MT. The patient showed improvement in attention at d2Attention test when treated on the left DLPFC, and reported an improvement even after sham stimulation. Neurostimulation on the right DLPFC showed adverse effects of dysphoria, inability to respond emotionally, hypobulia, tension, and impaired attention.[50]

 Shahar et al. conducted a double-blind, randomized, control study on 20 adults with ADHD, in which 15 sessions of high-frequency rTMS using either deep, Fig8, or sham coils over the right PFC were administered.[51] Conner's Adult ADHD Rating Scales (CAARS) established improvements in the attention measures and the Stop Signal Reaction Time (SSRT) test showed a better response inhibition.[51]

Thirty-four adults with ADHD were involved in a blinded sham-controlled trial, in which they received H-coil rTMS over bilateral DLPFC after cognitive training, for 15 sessions spread over 3 weeks.[52] Improvements were seen in the CAARS inattention subscale and the attention and executive function scores of the Mindstreams cognitive assessment battery for the group with right DLPFC stimulation, with increased activation of that area during a working memory task, as measured via fMRI.[52]

More recently, Cao analyzed the impact of rTMS on serologic-microRNA-let-7d (miRNA-let-7d) and miRNA-107, as diagnostic and therapeutic biomarkers of ADHD.[53] Seventy-five ADHD patients under 18 years of age, were randomly divided to receive 6 weeks of either rTMS (using a Fig 8 coil, 5 sessions per week, of 30-minute sessions of 60 cycles of 4 s of 10 Hz stimulation followed by 26 s intertrain interval at 100% MT, totaling to 2400 pulses per session of the right DLPFC), sham rTMS, atomoxetine, or placebo. Improvement in all ADHD core symptoms on the SNAP-IV (the Swanson, Nolan, and Pelham Rating scale) was observed. On the contrary, sham rTMS or placebo failed to produce any improvements. Compared with pre-rTMS or pre-ATX treatment in ADHD patients, the serum miRNA-let-7d expression level was downregulated only at post- rTMS or at post-ATX treatment. This suggested that serum miRNA-let-7d may serve as a potential biomarker for clinical diagnosis and therapeutic assessment

of ADHD.[53]

In 2021, Cardullo et colleagues conducted an interesting study on 22 adults with ADHD and CocUD (Cocaine Use Disorder) in comorbidity, compared to 208 CocUD-only subjects, which received a high-frequency (15Hz) rTMS treatment over the left-DLPFC (intensity 100% of the motor threshold, 60 impulses per stimulation train, inter-strain interval 15s, and 40 total trains, for a 13 minutes session)(54). Twice-daily rTMS sessions for the first 5 consecutive days of treatment, followed by twice-daily rTMS sessions once a week over 11 weeks were administrated. The time interval between the two sessions each day was 45–60 min. ADHD/CocUD patients, of whom 19/22 were pharmacologically treated with atomoxetine, received an rTMS treatment in addition to conventional psychosocial intervention. Significant reduction of inattentive and hyperactive symptoms, in addition to decreased cocaine use, craving, and other negative affect symptoms were reported. No differences were observed between groups.[54]

Even for ADHD population, the majority of studies focused on the stimulation of the DLPFC, with some investigating the effect on different areas, including and the motor areas. In general, it could be assumed that stimulation with high-frequency over the right DLPFC and low frequency in the left DLPFC improve ADHD symptoms.

Clinical effect of TMS
Although evidence of TMS potentiality in the treatment of ASD and ADHD, there are still critical challenges that may limit its use in clinical practice.[55]

In most existing studies, lack of blindness and randomization, and self-report evaluation are critical design issues that may significantly impact observed outcomes.[8,35,55]

No adequate amount of data exists on the TMS clinical efficacy depending on patients' age, gender, or cognitive impairment.[36] There are also ongoing concerns about safety, tolerability, and ethical questions. Over half of the existing studies did not report side effects, and the others often did not use standardized questionnaires to evaluate them. In addition, many studies enrolled patients on psychotropic drugs (such as stabilizers, antiepileptics, psychostimulants, antidepressants, and neuroleptics), which may induce long-term changes in the synaptic and excitatory balance, and consequentially may affect rTMS outcomes.[55]

Finally, another crucial weakness is the lack of longitudinal follow-up. This prevents critical questions regarding possible predictors of outcome (e.g., genetic profiling), duration of benefits, and utility of booster session.[23,56] As suggested by Oberman et al., the "one size fits all" approach may not be ideal for this application.[8,35]

Conclusion

Given the phenotypic, endophenotypic, and etiological heterogeneity of ASD and ADHD, it is not surprising to note a parallel heterogenicity in the results of TMS trials. With the spread of the concept of "personalized medicine", a more targeted approach, based on preliminary, individual measures of cortical plasticity and excitability, functional state of target networks, in combination with other behavioral or pharmacological interventions is an urgent need.

Declarations

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Conflicts of interest
The authors declare no competing interests.

Data availability
No datasets were generated or analyzed during the current study.

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REVIEW PAPER

Endophytes – untapped resources and pharmacological prospects against coronaviruses

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ABSTRACT

Introduction and aim. Viral infections stand to be among the most devastating diseases globally. Though significant efforts have been made in research and drug development against viral infections, the search for safe, affordable and effective vaccines against the current ravaging severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is still on. This is because already approved vaccines still need improvement. This review draws the attention of researchers on the potentials of bioactive substances from endophytes against the novel coronaviruses.

Material and methods. This assessment was made using references of articles published in English peer reviewed journals indexed in PubMed and Google Scholars databases up to June, 2022. The following key words were used; 'coronaviruses', 'Endophytes', 'Endophytes and viral infections, 'Endophytes and COVID-19, 'SARS-CoV'.

Analysis of the literature. *In-silico, in-vitro and in-vivo* studies revealed that natural compounds from endophytes showed antiviral activities against various human coronavirus, including HCoV 229E and a norovirus surrogate, the feline coronavirus FCV F9, COVID-19, Coronavirus 2 (SARS-CoV-2), SARSCoV-2 Mpro, among others.

Conclusion. This finding calls for researchers to also focus on endophytes, as part of drugs development in the bid to finding possible solution in combating the devastating COVID-19, an emerging situation.

Keywords. coronaviruses, endophytes, endophytic fungi, SARS-CoV, natural products

Introduction

Endophytes could be termed as microbes, mainly fungi or bacteria that reside within cells of plant tissues.[1] They have no negative impact on the host plant. They are ubiquitous in all plant species.[2] Besides their beneficial role on host plants through provision of resistance against stress, they also generate molecules of interest against divers diseases including cancer, fungal infections, viral infections, to mention a few.[3,4]

Transmission of endophytes occurs through seed in the entire plant's life cycle. However, their high nutrient demand may cause them to become parasitic and cause disease to host plants.[5] Studies have revealed the existence of endophytes in every parts of medicinal plant including resins, scales, flowers, roots, barks, stem, canals, leaves and meristems.[6] They can easily be fermented and cultured.[7] They possess the ability to generate natural and potential bioactive compounds.[6]

Several studies have demonstrated endophytes and their specific metabolites to facilitate tumor death.[8] Also an endophytic fungus isolated from the stem of *Tripterygium wilfordii* Hook.f. produced a novel cyclopeptide antibiotic which has related chemical properties as echinomycin.[7,8]

Endophytes have attracted remarkable attention as a result of their capacity to generate innovative bioactives with various biological activities.[1] The uniqueness of endophytes is attributed to their potentials against resistant human and plant pathogens.[9]

In the bid to curb the ravaging SARS-CoV, the discovery and development of more safe, potent and cost-effective antiviral drugs as well as vaccines is very necessary. Although studies have been carried out on the relevance of animal products, medicinal plants, and marine resources against SARS-CoV, no assessment have been done on the potential of natural anti-viral bioactive compounds from endophytes against SARS-CoV.[1,10]

Aim

This review would help accelerate the discovery and development of alternative remedies from endophytes against the current prevailing pandemic, SARS-CoV, COVID-19.

Material and methods

References of articles published in English peer reviewed journals indexed in PubMed and Google Scholars databases up to June, 2022 were assessed electronically using some key words viz; 'coronaviruses', 'Endophytes', 'Endophytes and viral infections, 'Endophytes and COVID-19, 'SARS-CoV'.

Analysis of the literature

Coronaviruses (COVS)

Coronaviruses (from the latin word, *Coronaviridae*) are group of RNA-containing viruses which are sub-divided into two families; *Coronavirinae* and *Torovirinae*.[11] Alpha, beta, gamma, and delta coronaviruses are four genera in the *Coronavirinae* subfamily. Among the human coronaviruses (HCoV-HKU1, SARS-CoV-2, HCoV-NL63, SARS-CoV, HCoV-OC43, HCoV-229E, and MERS-CoV), SARS-CoV-2 appears to be responsible for COVID-19 pandemic.[11,12] They are viruses with non-segmented, single-stranded and positive-sense RNA genome.[11]

Coronavirus pandemic-19 (COVID- 19) is a deadly disease discovered on 31st December 2019, identified as a novel coronavirus 9th January 2020, and declared a public health emergency of international concern on 30th January, 2020.[13,14] It is caused by severe acute respiratory syndrome (SARS-CoV-2), a single-stranded RNA (ssRNA) virus. Thus, the International Committee on taxonomy of viruses categorizes it as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).[15] Recently, as at May 2022, the World Health Organization report revealed at least 500 million confirmed cases of COVID-19, with more than 6 million deaths worldwide.[16] It is a betacoronavirus that causes several diseases.[15] Out-break of two pathogenic virus, the Severe Acute Respiratory Syndrome (SARS-CoV, or SARS) and the Middle East Respiratory Syndrome (MERS-CoV, or MERS) preceded the Coronavirus pandemic-19 (SARS-CoV-2). The origin of SARS was from Wuhan City, Hubei Province, Southern China in 2002, while MERS has its origin in Jeddah region of Saudi Arabia after infecting a patient in 2012.[12,17,18]

At the end of 2019, a new coronavirus, previously known as SARS-Co-2 was recognized as the cause of pneumonia in Wuhan, a city in the Hubei Province, China.[19] From there, it spread throughout China and other parts of the world. SARS-CoV-2 was labeled by the World Health Organization (WHO) as Coronavirus disease 2019 (2019-nCoV) on 11th March 2020 due to its pandemic nature.[12,20]

Trend in endophytes research

Existence of fungal endophytes inside medicinal plants has shifted the attention of new drugs discovery from medicinal plants to fungi endophytes.[21] Being substitutes for plants secondary metabolites, several bioactive natural products with antiviral, antifungal, antibacterial, antioxidant and cytotoxic properties have been discovered from endophytes of plants, marine and animal origin.[21]

This gap is currently attracting attention of microbiologists, pharmacologists, natural product chemists, biochemists, botanists, biologists and industrialists. This is also attested by the increase in number of endophyte research publications in recent times. Currently, advanced chemical, biotechnological and computational molecular biology methods are being used for significant production of bioactive compounds from endophytes.[22]

Although natural products from medicinal plants[23], animals[24] and marine resources[14,25] have been explored to have potentials against the ravaging SARS-CoV-2, the place of endophytes is still emerging. In this evaluation, potentials of endophytes against the novel COVID-19 virus were addressed through literature survey.

Anti-viral activities of endophytes

Activities against influenza virus

Endophytic fungal extract from mangrove plant, *Aegiceras corniculatum* displayed potent activity against influenza A viral (H1N1). Compounds isolated from the extract include isoindolones (emerimidines A and B, emeriphenolicins A and D) and other compounds, including aspernidines A and B, austin, austinol, dehydroaustin, and acetoxydehydroaustin. Cytopathic inhibition assay showed activity of eight compounds (Table 1) against influenza A virus (H1N1).[26]

Table 1. Anti-influenza activity of endophytes

Endophytic fungi	Host plant/source	Bioactive compounds	Reference
Emericella sp. (HK-ZJ)	*Aegiceras corniculatum*	Emerimidine A and B and Emeriphenolicins A and D	26
Nigrospora sp YE3033	*Aconitum carmichaeli*	6-O-demethyl-4-dehydroxy-altersolanol A, azaphilones, 8,11-didehydrochermesinone B, and (7S)-7-hydroxy-3,7-dime-thyl-isochromene-6,8-dione	27
Phoma sp.	*Aconitum vilmorinianum*	14-nordrimane sesquiterpenoid	28
Nigrospora sp. YE3033	*Aconitum carmichaeli*	hydroanthraquinone derivative, 6-O-demethyl-4-dehydroxy-altersolanol A (1), and two new azaphilones, 8,11-dide-hydrochermesinone B (6) and (7S)-7-hydroxy-3,7-dimethyl-isochromene-6,8-dione (8)	29
Phoma multirostrata XJ-2-1	*Phoma multirostrata* XJ-2-1	Ergocytochalasin A (1)	30

Similarly, the hydroanthraquinone derivatives, 6-O-demethyl-4-dehydroxyaltersolanol A, azaphilones, 8,11-didehydrochermesinone B, and (7S)-7-hydroxy-3,7-dimethyl-isochromene-6,8-dione isolated from the culture extract of *Nigrospora* sp. YE3033 (from *Aconitum carmichaeli*) showed strong antiviral activity against the influenza viral strain A/Puerto Rico/8/34 (H1N1).[27]

A novel rare 14-nordrimane sesquiterpenoid isolated from the endophyte, *Phoma* sp., of the roots of *Aconitum vilmorinianum* displayed antiviral activity by inhibiting the growth of influenza A virus (A/Puerto Rico/8/34, H1N1).[28]

Studies were done on endophytic fungus (Nigrospora sp. YE3033) derived from *Aconitum carmichaeli* (A. carmichaeli) that resulted in the discovery of three new compounds (hydroanthraquinone derivative, 6-O-demethyl-4-dehydroxyaltersolanol A (1), and two new azaphilones, 8,11-didehydrochermesinone B (6) and (7S)-7-hydroxy-3,7-dimethyl-isochromene-6,8-dione (8) as well as five already known analogues/existing compounds (2-5 and 7). Anti-influenza viral strain (A/Puerto Rico/8/34 (H1N1) with IC_{50} values of 2.59, 8.35, 7.82, and 0.80 μg/mL, respectively. Compound 7 showed low cytotoxicity. Authors remarked that such compounds are potentials in the development of anti-influenza A virus agent.[29]

Isolation study was carried out on endophytic fungus (*Phoma multirostrata* XJ-2-1) which led to the unique discovery of Ergocytochalasin A (1), an unprecedented merocytochalasan designed through Diels-Alder cycloaddition of a cytochalasin with an ergosterol. Besides the cytotoxicity activity of compound 1 against six cancer cell lines with IC_{50} values between 6.92 to 26.63 μM, *in vitro* immunosuppressive activity against ConA-induced T cell and LPS-induced B cell proliferation, and its antiviral activity against Human dengue virus type 3 (DV3), influenza A virus (H1N1) and respiratory syncytial virus (RSV), was experimented.[30]

Activities of endophytes against other viruses

Among several endophytic fungal strains (*Scopulariopsis fusca, Fusarium equiseti* and *Geotrichum candidum*) from brown alga, *Padina pavonica*, located in the red sea, *F. equiseti* demonstrated the highest antiviral activity against hepatitis C virus (HCV) NS3-NS4A protease, with an IC_{50} value of 27.0 μg/mL. Two diketopiperazines, (cyclo-L-AlaL-Leu and cyclo L-Tyr-L-Pro) and two nucleosides (cordycepin and Ara-A) were discovered following structural characterization (Table 2).[31]

Brefeldin A, a compound from endophytes associated with *Penicillium* sp. FKI-7127 was reported to exhibit potent antiviral properties.[32]

Antiviral activities against herpes simplex virus (HSV) were also reported in fungal extracts of *Phialophora* sp. (No.96-1-8-1), *Nigrospora sphaerica* (No.83-1-1-2), and *Alternaria alternata* (No.58-8-4-1). Two novel heptaketides, (+)-(2S,3S,4aS)-altenuene (1a) and (−)-(2S,3S,4aR)-isoaltenuene, alongside six already existing compounds, (−)-(2R,3R,4aR)-altenuene, (+)-(2R,3R,4aS)-isoaltenuene, 50-methoxy-6-methyl-biphenyl-3,4,30-triol, alternariol (4), alternariol-9-methyl ether, and 4-hydroxyalternariol-9-methyl ether were identified (Table 2).[33]

Fermentation products from the endophytic fungus *Aspergillus versicolor* yielded two compounds which displayed significant activity against tobacco mosaic virus with inhibition rates of 46.4% and 35.4%, which were more potent than ningnanmycin (30.8%), the positive control.[34]

In a related study, they also isolated four new Oryzaeins and five already available orayzaeins from the endophytic fungus, *Aspergillus oryzae*. Compounds 1 and 2 expressing isocoumarins characteristics possessed an unusual 2-oxopropyl group and a rare 3-hydroxypropyl group revealed activity against tobacco mosaic virus.[35]

In another study by Selin and co-workers, endophytic fungi from medicinal plants of Egyptian origin, exhibited substantial antiviral activity against two virus-

Table 2. Endophytes activity against other viruses

Endophytic fungi	Host plant/source	Bioactive compounds	Activity	Reference
Fusarium equiseti	*Padina pavonica*	Two diketopiperazines, (cyclo-L-AlaL-Leu and cyclo L-Tyr-L-Pro) and two nucleosides (cordycepin and Ara-A)	hepatitis C virus (HCV) NS3--NS4A protease	[31]
Penicillium sp. FKI-7127	*Penicillium* sp. FKI-7127	Brefeldin A	antiviral properties	[32]
Phialophora sp. (No.96-1-8-1), *Nigrospora sphaerica* (No.83-1-1-2), and *Alternaria alternata* (No.58-8-4-1)	*Nigrospora sphaerica* (No.83-1-1-2)	(+)-(2S,3S,4aS)-altenuene (1a) and (−)-(2S,3S,4aR)-isoaltenuene, alongside six already existing compounds, (−)-(2R,3R,4aR)--altenuene, (+)-(2R,3R,4aS)-isoaltenuene, 50-methoxy-6-methyl-biphenyl-3,4,30-triol, alternariol (4), alternariol-9-methyl ether, and 4-hydroxyalternariol-9-methyl ether	herpes simplex virus (HSV)	[33]
Aspergillus versicolor	*Aspergillus versicolor*	unusual 2-oxopropyl group and a rare 3-hydroxypropyl group	tobacco mosaic virus	[34]
Aspergillus oryzae	*Aspergillus versicolor*	Oryzaeins A-D (1-4), four new isocoumarin derivatives, along with five known ones (5-9) [unusual 2-oxopropyl group and a rare 3-hydroxypropyl group]	tobacco mosaic virus	[35]
Pleospora tarda strain	*Ephedra aphylla*	Alternariol and alternariol-(9)-methyl	vesicular stomatitis viruses (VSV) and herpes simplex (HSV-2)	[36]
Phomopsis sp. CGMCC No. 5416	*Achyranthes bidentata*	Three unidentified chromanones	HIV-1	[37]

Table 3. Activities of endophytes against coronavirus

Endophytic fungi	Host plant/source	Bioactive compounds	Activity	Reference
Curvularia papendorfii	*Vernonia amygdalina*	Not detected	HCoV 229E and a norovirus surrogate, the feline coronavirus FCV F9	[9]
Aspergillus terreus	Soybeans	Two dereplicated metabolites, aspergillide B1 and 3α-Hydroxy-3, 5-dihydromonacolin L	anti-COVID-19	[40]
Aspergillus versicolor	Sea crab (*Chiromantes haematocheir*)	four novel indolyl diketopiperazines, aspamides A−E (1−4) and two novel diketopiperazines, aspamides F−G (5−6), in addition to 11 existing diketopiperazines and intermediates	Coronavirus 3-chymoretpsin-like protease (Mpro) of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)	[14]
Penicillium citrinum TOPEF34(Pc)	Phoenix dactylifera (date palm tree roof)	Benzodiazepine alkaloid analogue cydopentin A and B, dehydro-cyclopeptin and cyclopeniol	Significant SARS-COV2 Mpro inhibitory effect compared to GC376 as positive control	[44]
Cladosporium sp. 7951	*Paris polyphylla var. yunnanesis.*	Eight new aspulvinone analogues, aspulvins A−H (1−8) and aspulvinones D, M, O, and R (9−12).	All isolates displayed various degrees of inhibitory activity against SARS-CoV-2 Mpro at 10 μM	[47]

es, vesicular stomatitis viruses (VSV) and herpes simplex (HSV-2). Alternariol and alternariol-(9)-methyl were two compounds isolated from endophyte *Pleospora tarda* that showed antiviral property.[36]

From the stems of *Achyranthes bidentata* the fungal strain *Phomopsis* sp., CGMCC No. 5416 were extracted which resulted to three unidentified chromanones, that revealed promising antiviral activities against HIV-1.[37]

Alternaria tenuissima QUE1Se, a fungal endophyte of *Quercus emoryi* was found to exhibit strong anti-HIV activity.[38]

Studies on potentials of endophytes against coronavirus

According to Afra and co-workers, the endophytic fungus (*Curvularia papendorfii*) isolated from *Vernonia amygdalina,* produced antiviral activity against human coronavirus HCoV 229E and a norovirus surrogate, the feline coronavirus FCV F9 (Table 3).[9]

In-silico studies play a significant role in preliminary assessment of potential drugs, which can further be subjected to *in-vitro* and *in-vivo* studies.[39] In-silico assessment of the metabolites produced by the endophytic fungus, *Aspergillus terreus* associated with soybeans against COVID-19 revealed two dereplicated metabolites, aspergillide B1 and 3α-Hydroxy-3, 5-dihydromonacolin L to be potent anti-COVID-19 drug candidates in the molecular docking study.[40] The authors submitted that the potential of Aspergillide B1 and 3α-Hydroxy-3, 5-dihydromonacolin L could be developed as phytopharmaceuticals for the management of COVID-19.

Aspergillus versicolor, an endophyte connected with the sea crab (*Chiromantes haematocheir*) was subjected to isolation study, which led to the discovery of four novel indolyl diketopiperazines, aspamides A–E (1–4) and two novel diketopiperazines, aspamides F–G (5–6), in addition to 11 existing diketopiperazines and intermediates. Computer-aided study on isolates showed activity against coronavirus 3-chymoretpsin-like protease (Mpro) of severe acute respiratory syndrome Coronavirus 2 (SARS-CoV-2). Authors submitted that screened molecules could be helpful against corona virus disease-19 (COVID-19).[14] Endophytic fungi could be considered as treasures of unique bioactive metabolites.[41]

Out of 16 alkaloids that previously displayed antimicrobial potential on angiotensin-converting enzyme 2 using molecular modeling and *in silico* studies, *Aspergillus Fumigatoside* E showed best fitting within active sites of angiotensin-converting enzyme 2 (ACE2) which

is an entry receptor in which SARS-COV was transmitted followed by Aspergicin.[42]

A computational study using molecular docking and molecular dynamic simulation revealed pyrrocidine A and dankasterone B, secondary metabolites of endophytic fungi *Acremonium zeae*, as potent inhibitors of viral RdRp. The authors suggested it as a promising and efficient anti-coronavirus drug which can be explored.[43]

The extract of *Penicillium citrinum*, TDPEF34, showed potential inhibition and was further analyzed to identify potential Mpro inhibitors. Following bio-guided isolation, a series of benzodiazepine alkaloids cyclopenins with good-to-moderate activity against SARS-CoV-2 Mpro were identified. The authors posited that their findings could be utilized for further *in vitro* and *in vivo* investigations to produce anti-SARS-CoV-2 drug candidates as well as critical structural information that could be used in future design of potent Mpro inhibitors.[44]

Fonsecin, a naphthopyrone pigment from *Aspergillus fonsecaeus* mutant has shown high binding affinity for SARS-COV-2 PLpro by interacting with the Tyr 268 amino acid residue of enzyme cavity based on *in silico* molecular docking and molecular dynamic studies.[43,45]

The genome of *Penicillium thymicola* contains a polyketide synthase and a nonribosomal peptide synthetase hybrid gene cluster, which upon expression leads to the synthesis of Pyranonigrin A. Pyranonigrin A. is a secondary fungus metabolite with strong inhibitory capability against the SARS-CoV-2 Mpro.[43,46]

Conclusion

As part of drugs discovery and development in the fight against the devastating Corona Virus Disease-19, the search for antiviral metabolites from endophytes is becoming an area of exploration by researchers. The above studies revealed that endophytes are vital sources of natural bioactive compounds which have potential in immunomodulations for the prevention and treatment of CoVs. While addressing the preclinical roles of endophytes against the ravaging virus, cognizance of their clinical trials as well as safety (toxicity profile) should be put into consideration. Further studies still need to be done on their mechanism of actions.

Declarations

Author contributions
Conceptualization, E.O.E.; Methodology, E.O.E., C.I., C.C.E. and M.M.; Writing – Original Draft Preparation, E.O.E.; Writing – Review & Editing, C.I., G.C.O., C.C.E., M.M. and E.O.E.; Visualization, E.O.E.

Data availability
Data are available from the corresponding author upon request.

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REVIEW PAPER

Taking medicine in the right way – most important but most neglected

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ABSTRACT

Introduction and aim. To cure any disease, proper use of medicine or taking medicine in the correct order is required. Even patients from developed countries struggle to maintain their drug compliance. There is an odd parallel between underdeveloped, emerging nations and the so-called developed world in the West when it comes to improper medicine use. The understanding and perception of the disease is the most important factor influencing whether patients stick to their treatment plan.
Material and methods. Prioritized databases for public health topics included PubMed, ALTAVISTA, Embase, Scopus, Web of Science, and the Cochrane Central Registers. Along with other online sources, journals from Elsevier, Springer, Willey Online Library, and Wolters Kluwer were thoroughly searched.
Analysis of the literature. There is a thorough discussion of medication non-adherence issues and a few adherence-improving methods.
Conclusion. Adherence to treatment guidelines is essential in the treatment of any disease.
Keywords. medication non-adherence, patient non-compliance, avoidable medical costs

The list of abbreviations:

ADR – adverse drug reactions, **BP** – blood pressure, **NSAIDs** – non-steroidal anti-inflammatory drugs, **CDC** – centers for disease control and prevention, **WHO** – World Health Organization

Introduction

To cure any disease, proper use of medicine or taking medicine in the correct order is required. According to the WHO, noncompliance with treatment regimens causes major problems in patients, particularly those with chronic illnesses. "Right administration" depends on at least 5 right factors – right patient, right drug, right time, right dose and right route.[1] "Medicines simply will not work if you don't take it right" – This simple fact is not understood by most people around the world, and as a result, more than half of chronic disease patients in the developed world do not take their medicine correctly, according to WHO.[2] Patients suffering from chronic diseases may have a particularly difficult time adhering because their medications must frequently be taken for an extended period of time, sometimes for the rest of their lives. Patients may struggle to stick to treatment regimens for a variety of reasons, and the centers for disease control and prevention (CDC) estimates that medication non-adherence accounts for 30 to 50% of chronic disease treatment failures. Poor adherence can lead to treatment failure, worsening symptoms, and health deterioration.[3]

Aim

To demonstrate that the most important issues of treatment guidelines compliance are despised in the majority of cases around the world, and to discuss a few reasons for this.

$300 BILLION ANNUAL MEDICAL COSTS
25,000 PREMATURE DEATHS YEARLY
10% HOSPITALIZATIONS IN USA

ACCOUNTS FOR 30 TO 50% OF CHRONIC DISEASE TREATMENT FAILURES

$125 BILLION IN ANNUAL MEDICAL COSTS 2,00,000 DEATHS YEARLY IN EUROPE

250,000 HOSPITALIZATIONS IN AUSTRALIA 1.1 MILLION HOSPITAL DAYS IN FRANCE

30% OF PATIENTS STOP TAKING THEIR MEDICINE 25% DO NOT FILL THEIR PRESCRIPTION IN CANADA

75% OF ELDERLY PEOPLE ARE UNABLE TO ADHERE TO LONG-TERM TREATMENT REGIMENS

POOR BP CONTROL WERE TWICE AS LIKELY IN HYPERTENSIVE PATIENTS WITH DIABETES IN WAR-TORN PALESTINE

MEDICATION NON-ADHERENCE

ONLY HALF OF PATIENTS IN DEVELOPED COUNTRIES ADHERE TO TREATMENT GUIDELINES FOR CHRONIC DISEASES

PHARMACEUTICAL COMPANIES WORLDWIDE LOST $637 BILLION WITH $250 BILLION LOST IN THE US ALONE

PHOBIA WAS THE CAUSE OF INSULIN REFUSAL AMONG 60% DIABETIC PATIENTS IN SOUTH IRAN
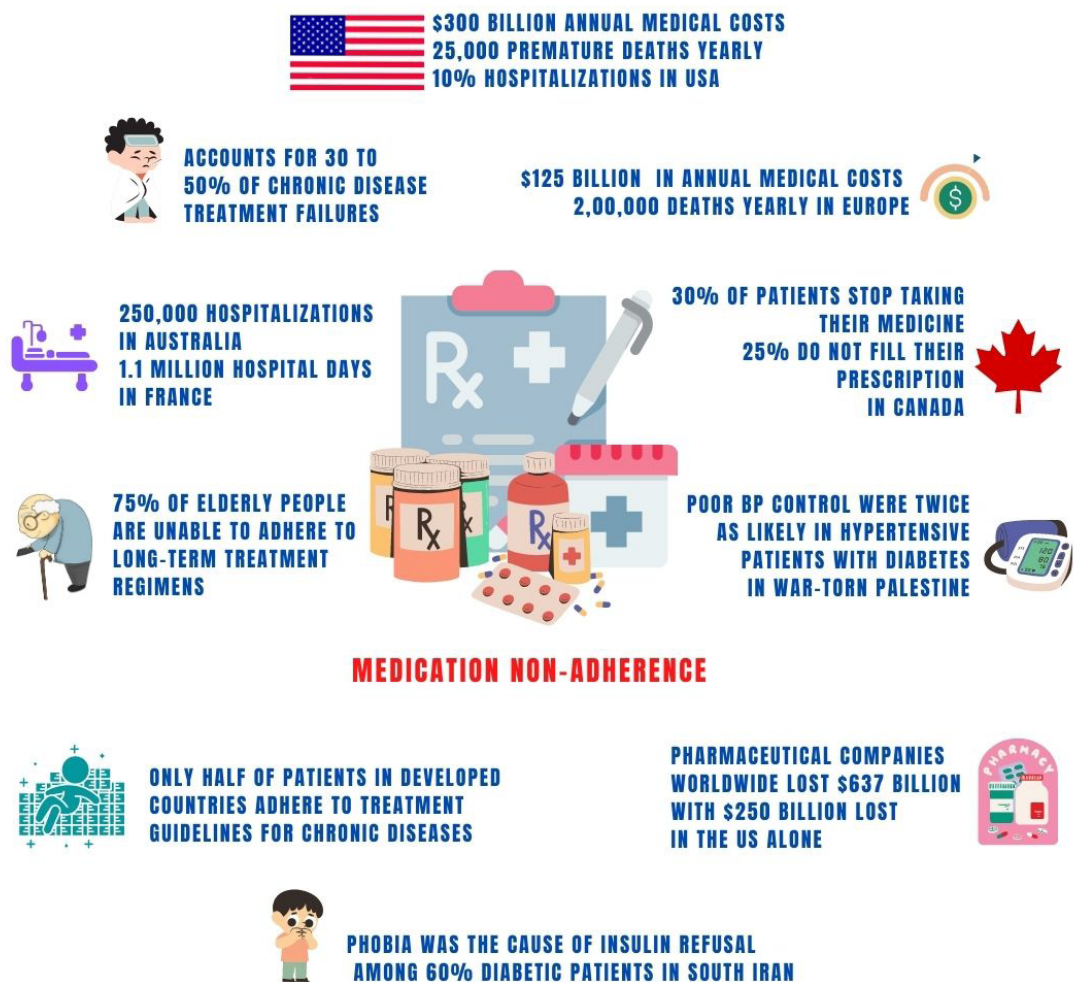
Fig. 1. Medication non-adherence and possible outcomes

Material and methods

Prioritized databases for public health topics included PubMed, ALTAVISTA, Embase, Scopus, Web of Science, and the Cochrane Central Registers. Along with other online sources, journals from Elsevier, Springer, Willey Online Library, and Wolters Kluwer were thoroughly searched.

Analysis of the literature

A literature review demonstrates that, even in highly privileged populations from developing countries, mediation treatment protocols are not consistently followed. The primary causes that are not excusable include negligence, poor health literacy, and forgetfulness, the cost of medications, poly pharmacy, and patient perception of the repercussions of non-adherence, among many other factors. The main findings of the current study are that despite millions of hours of similarly conducted research conducted all over the world, the problem of non-adherence still exists. This is because the research is only published in journals and not conducted on the general public, who are victims of recklessness with regard to their own health, whether intentionally or unintentionally. The solution to changing their health-seeking behavior is thorough patient education.

Non-adherence in the so-called developed countries

In the United Kingdom, up to 50% of medicines are not taken as prescribed, and 60% of NHS patients were unable receive the appropriate treatment within 18 weeks.[4-6] In patients with chronic diseases, noncompliance with medications leads to poorer health outcomes, higher healthcare costs, increased hospitalizations, and even higher mortality rates.[7] Medication non-adherence alone accounts for at least 10% of hospitalizations in the United States, 250,000 hospitalizations in Australia, and 1.1 million hospital days in France (Figure 1); causes $300 billion in annual medical costs in the United States and $125 billion in the European Union; and causes more than 1,25,000 premature deaths in the United States and 2,00,000 deaths in the European Union.[8,10-12] Furthermore, two-thirds of medication-related hospitalizations in Australia are potentially avoidable.[9] Ac-

cording to a recent Canadian study, 30% of patients stop taking their medicine before it is recommended, and 25% do not fill their prescription or take less than prescribed.[13] Medication non-adherence attributed to $679-$898 more preventable spending among patients who had at least one preventable encounter.[14] However, pharmaceutical companies worldwide lost $637 billion in potential sales due to non-adherence, with $250 billion lost in the United States alone last year (Figure 1).[15]

Misuse of antibiotics
More than half of all antibiotics sold in the world are sold without a prescription, and the CDC reports that 30-50% of antibiotics prescribed in hospitals are inappropriate or unnecessary.[16,17] According to a recent Lancet study funded by the Bill and Melinda Gates Foundation and the Wellcome Trust, nearly 5 million deaths worldwide in 2019 were caused by bacterial resistance, which is expected to double by 2050[18]. In South Asia, nearly 70% of hospitalized patients received one or more antibiotics, whereas 100% of ICU patients received antibiotics.[19,20] However, 70% to 80% of COVID-19 patients received various antibiotics for COVID-19 treatment.[21-23] The antibiotics most commonly prescribed were azithromycin, ceftriaxone, amoxicillin, metronidazole, and amoxicillin-clavulanic acid.[24] In addition, it has been reported that about 90% of patients with COVID-19 are being unnecessarily treated with antibiotics and close to 100% of these prescriptions were empiric.[25]

Abuse of non-steroidal anti-inflammatory drugs in patients with COVID-19, Dengue, and Chikungunya
Non-steroidal anti-inflammatory drugs (NSAIDs) are responsible for at least 650,000 hospitalizations, 165,000 deaths, and 30% of adverse drug reactions (ADR)-related hospital admissions worldwide each year.[26,27] Overuse of this class of drugs can result in kidney damage, and their side effects can be three to four times more severe in patients with kidney disease.[28] Many studies have found that these drugs are widely abused in Dengue, Chikungunya, and COVID-19 patients. It is even more important to keep the body hydrated than to reduce the fever with pain relievers, especially in Dengue or COVID-19 patients. Excessive use of Paracetamol syrup or suppositories in children can cause gastric irritation, which can lead to vomiting and hospitalization. With a few exceptions, most hospitalizations or ICU admissions among those patients could be avoided simply by halting dehydration at residence with saline and fruit juice or simply by drinking more water.[29]

A new era of uncontrolled use of prescription only and recreational drugs
Sleep disturbances are reported by approximately 40% of COVID-19 patients. Benzodiazepines increase the risk of delirium in COVID-19 patients, depress the system in patients with compromised breathing functions, and are contraindicated with some anti-viral medications.[30,31] Surprisingly, benzodiazepine dispensing increased dramatically in Canada between 2020 and 2021, while abuse of similar drugs more than doubled in Italy.[32] According to the American Journal of Public Health, approximately 300 metric tons of morphine-type analgesics are used worldwide each year, with less than 1% distributed to low- and middle-income countries.[33] As a result, the developed world retains their misuse and associated side effects. Prior to the US midterm elections, an announcement from authorities on "simple possession of cannabis" to thousands of convicted citizens exploded recreational drug abuse in both the US and the EU.[34,35]

Negative attitude towards COVID-19 vaccine
A cross-sectional study of 259 school leaders in Hong Kong carried out during the COVID-19 pandemic between April 2021 and February 2022 shows that more than 50% of participants had limited health literacy, which was strongly associated with a negative attitude towards vaccination, confusion about COVID-19-related information and secondary symptoms.[36] Earlier, a US-based study in 2020 concluded that two-thirds of the Americans will not get the COVID-19 vaccine when it is first available, while 25% report that they do not have any intention to get vaccinated at any time.[37] In India, vaccine hesitancy was high in Tamil Nadu, more than 40% and willingness for vaccine uptake was found to be close to 90% in Kerala.[38,39] Another vaccine hesitancy survey by University College London, UK finds mistrust among 16% respondents, and 23% were confused.[40]

Medical cost and low-health-literacy: the two major barriers of adherence among diabetes patients
A strange similarity can be found in under-developed, developing countries and the so-called developed world in the West or the Middle-East when it comes to not taking medicine properly. According to a WHO report, only half of patients in developed countries adhere to treatment guidelines for chronic diseases, which is much less in developing countries.[41] Several studies among diabetic patients in South Asian countries have shown that nearly half of patients do not adhere to their prescribed medication and are at risk of acute and long-term complications, resulting in increased hospitalization rates and medical costs.[42,43] "Medical costs are barriers to adherence to proper clinical guidelines for chronic diseases in poor countries" – although discussed in many forums but forgetfulness, confusion about the duration required for medication use and mistrust about the overall efficacy of medication are among the reasons for non-adherence to diabetes management

protocols in Middle Eastern countries.[44] Health literacy and medication adherence are strongly associated (Table 1). Poor glycemic control due to low-health-literacy among diabetes patients reported to both South-East Asian and Middle Eastern countries.[45-51]

Table 1. Several identified reasons for non-adherence to treatment guidelines for chronic diseases[7,71-73]

Status	Factors
Patient's socio-economic status	Low health literacy, lack of family or social support network, unstable living or homelessness, financial insecurity.
Treatment-related	Complexity and duration of treatment procedures, frequent changes in medication regimen, lack of immediate results, real or perceived unpleasant side effects, interference with lifestyle.
Health system-related	High treatment costs, limited health system for patient education and follow-up, doctor-patient relationship, patient trust in health care, long waits, lack of patient information materials.
Patient-related	Visual-hearing and cognitive impairment, mobility and dexterity, psychological and behavioral factors, perceived risk of disease susceptibility, superstitions and stigmatization by disease, etc.

Humanitarian crisis: poor blood pressure control among cardiac patients

A recent study by the American Heart Association revealed that patients with high blood pressure (BP) do not follow treatment guidelines because of: (a) suboptimal dosing or prescribing the wrong medication (b) lack of insurance or lack of health care access and (c) patient failure to comply prescribed medication or other lifestyle guidelines.[52] Among hypertensive patients, less than 50% have persistent control over BP, even though more patients have received treatment over time. Furthermore, inadequate BP control was reported among those with elevated total cholesterol, LDL, and uric acid levels in both high, low and middle income countries.[53] Humanitarian crisis is associated with increased short-term and long-term cardiac morbidity and mortality and increases in BP.[54] For example, hypertensive patients with diabetes mellitus were twice as likely to exhibit poor BP control, found in war-torn Palestine.[55] Also, a US-based survey on re-settled Rohingya refugees from Myanmar shows a higher trend of chronic diseases like diabetes, hypertension and obesity.[56]

Superstitions: an elephant in the room

Epilepsy and schizophrenia still seen in most countries of the world as an evil spirit – although two-thirds of patients can become seizure-free with adequate treatment, poor adherence to proper guidelines is a major problem for effective recovery.[57,58] In a study conducted in India, 60% of the patients believed in luck and superstition with regard to illnesses.[59] Superstitions also reported in close to 40% men and 70% women in Northern Germany.[60] In Africa, 70% of people turn to indigenous treatments such as charms and witchery to treat their illness.[61] Surprisingly, more than 40% of Americans be-

lieve in spiritual treatments and researchers found that 73% of addiction treatment programs in the USA include a spirituality-based element.[62,63] Phobia was the cause of insulin refusal among 60% diabetic patients, despite physician recommendations – found in a study conducted in South Iran.[64]

Table 2. Interventions to improve treatment guideline adherence

Interventions	Details
Psychological adaptation training – ABC taxonomy	The first stage, initiation, is measured as a time-to-event variable and refers to the interval between prescription and the patient taking the first dose of a prescribed medication. The second phase, implementation, is a continuous measurement of the difference between the amount of medication prescribed and actually taken. It covers the time from the first dose until the last one is taken. The third stage, known as discontinuation, denotes the end of therapy, when the next dose is skipped and no additional doses are given after that. The term "persistence," which is frequently used, refers to a time-to-event variable that measures how long a patient spends in the implementation phase.[74]
Behavioral interventions	Four steps are involved in the modeling of behavior: attention, retention, reproduction, and motivation. Telephone follow-up and home visits, particularly in associations with educational components, seem to have a positive impact, providing planning and support, and integrated pre and post discharge interventions.[75]
Patient education	Health promoters typically have credibility to conduct patient education programs due to their expert knowledge and appropriate training. However, knowledge by itself does not guarantee success as a health educator. The following three guidelines must be followed in patient education programs: In order to change patients' health-related behaviors, it is important to address the following factors: (a) establishing a relationship between patients and healthcare providers; (b) delivering and evaluating the education program's goals to patients; and (c) paying attention to low self-esteem and non-verbal patients.[76]
Integrated care interventions	An interdisciplinary approach relies on health professionals from different disciplines, along with the patient, working collaboratively as a team. The physician, pharmacist, or nurse invites the patient to take part in the program, but in practice, the physician is often the best person to invite the patient to participate in the program because of the established patient-provider relationship.[77]
Self-management Interventions	The medication self-management intervention consists of two weekly phone calls and three in-person education sessions spread out over six weeks. To identify the factors that affect adherence, as well as how and why these factors contribute to poor adherence, a thorough assessment of adherence problems will first be conducted. Depending on each patient's condition and potential adherence issues, medication-related knowledge and skills will be offered. For a better understanding of patients' cognitive factors influencing adherence behavior, motivational interviewing techniques will be used.[78]
Risk-communication interventions	Patients and healthcare professionals exchange information about risks in both directions. The key to reducing the risks of drug-related car accidents is verbal communication of information and the use of straightforward documents. Providing patients with accurate information can improve their sense of self-efficacy and satisfaction, which can lead to behavioral changes and risk reduction.[79,80]

Pediatric and geriatric complications to non-adherence

Due to multiple physical complications and additional medication burden, three-quarters of geriatric persons worldwide are unable to adhere to appropriate long-term treatment regimens (Figure 1).[65] Patients over the age of 65 who take at least five medications are at an increased risk of mild cognitive impairment, memory

loss, falls, frailty, impairment, and death, while ADRs are estimated to account for 5% to 28% of acute geriatric medical admissions.[66,67] For children, common non-adherences are related to family routines, child-raising issues, and to social issues such as poverty. Long-term disease conditions like asthma, cystic fibrosis, HIV, diabetes, inflammatory bowel disease and juvenile arthritis – are attributable to around 60% of non-adherence among children.[68-70]

Tools to improve medication and treatment guideline adherence

There is evidence that the number of chronic diseases and drugs increases non-adherence. Chronic disease management necessitates ongoing psychological adaptation through behavioral, educational, integrated care, self-management, and risk-communication interventions, which may result in significant changes in therapeutic indications. In addition, several newer technologies that may improve medication and treatment guideline adherence have been incorporated (Table 2).

Conclusion

Finally, it can be stated that patients' knowledge and interpretation of the disease are the primary factors influencing their adherence to the treatment regimen. Health-care providers should explore more effective health-education methods for identifying patients' attitudes toward disease, medicine trust, psychological stressors, and increasing adherence to medication.

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The author declares that he has no competing interests.

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CASUISTIC PAPER

Case of multisystem inflammatory syndrome in adults

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ABSTRACT

Introduction and aim. Multisystem inflammatory syndrome in adults (MIS-A) is a rare severe illness which is related to prior SARS-CoV2 infection in individuals>=21 years old. The condition was described few months after recognition of similar entity in children known as MIS-C (United Kingdom, April, 2020). The diagnosis of MIS-A is based on clinical symptoms andevidence of inflammation in laboratory markers. It is characterized by extrapulmonary organ dysfunction (cardiovascular, gastrointestinal), general symptoms such as fever, malaise, rash and deviations in blood tests (elevated level of ferritin, procalcitonin, CRP, IL-6, D-Dimer) with a previous or current SARS-CoV-2 infection. The purpose of this study is to present the syndrome on the basis of a clinical case example, to show the course of the disease, its symptoms and the result of applied treatment.

Description of the case. The following case describes the clinical history, diagnostic process and applied treatment of 37-year old female patient who was admitted urgently to the hospital with a suspicion of sepsis originating from pharynx. The final diagnosis – MIS-A was settled after performing a broad panel of tests. Clinical picture was non- characteristic. The patient was successfully treated with steroids.

Conclusion. MIS-A is a rare clinical entity linked with SARS-CoV-2 infection. The symptoms manifest from multiple organ systems and the diagnostic process may be challenging. The illness can be successfully treated with steroids.

Keywords. fever, hypotension, MIS-A, lymphadenopathy, SARS-CoV-2, sepsis-like clinical picture, somatoform delirium,

Introduction

COVID-19, even an asymptomatic form of the illness, is associated with the risk of developing a multisystem inflammatory syndrome- entity that can lead to severe complications, such as cardiac dysfunction, which was reported in most cases of MIS-A, shock, multiple organ failure and may trigger a rapid clinical deterioration. The pathogenesis of this inflammatory condition is not yet fully explained.[1]

Main symptoms include high fever, which can reach up to 40°C, polymorphic rash, conjunctivitis, tachycardia, hypotension, nausea, vomiting and diarrhea, with a relative absence of respiratory disease.[2,3]

Aim

The purpose of this study is to present the syndrome on the basis of a clinical case example, to show the course of the disease, its symptoms and the result of applied treatment.

Description of the case

The following case describes the clinical history, performed diagnostics and outcome of 37-year old female patient with a prior SARS-CoV-2 infection who presented sepsis-like picture on admission. The case fulfilled the Brighton Collaboration Case Definition for MIS-A. The diagnostic process was complicated, as it required the exclusion of other diseases and performance of many laboratory tests and imaging. Successful treatment involved

the use of steroids. 37-year old female patient was urgently admitted to the department of internal diseases to the University Hospital with a suspicion of sepsis. She was reporting high fever up to 40°C and shivers within the last four days, bilateral conjunctivitis, sore throat that impaired the regular intake of food and fluids. Three weeks earlier she was diagnosed on COVID- 19, which manifested with fever, general weakness, musculoskeletal pain. After 10 days from the onset of symptoms, cervical lymphadenopathy appeared. The patient was then consulted by a family doctor on-line. As a result she took azithromycin for three days. Her medical history was only noteworthy for asthma and Lyme disease (2006).Upon admission, the patient presented current body temperature 39.8°C, hypotension (90/60 mmHg) and tachycardia (110 bpm).Physical examination revealed increased neck circumference due to painful packages of enlarged cervical lymph nodes, reddish rash on the neck and legs, fungal lesions of the oral cavity, enlarged tonsils, minor crepitation in the basal parts of the lungs and slight tenderness in the epigastrium. Nasopharyngeal swab test for SARS-CoV2 upon admission was negative, nevertheless PCR test and the IgG antibody level test performed were positive. Due to her severe condition the patient receive dempiric therapy with i.v.antibiotics (vancomycin, meropenem) and antifungal(fluconazole). The blood and urine culture were negative. The laboratory test revealed elevated count of white blood cells $16.5x10^3/mm^3$, neutrophils $13x10^3/mm^3$ as well as significant increase of procalcitonin 1.63ng/ml (<0.5), CRP 389 mg/l (0-5.0), ferritin 1640,75 ng/ml (4.63-204), NTpro-BNP 7112pg/ml (<125) , D-Dimer 5771 ug/l (0-500) and Troponin I 53,6 ng/l (<15).The APTT was slightly prolonged to 40.5 s.X-ray of the chest revealed enlarged heart, pulmonary congestion, pleural adhesions and small amount of fluid in the left pleural cavity with absence of radiographic signs of pneumonia. Echocardiography exposed generalized hypokinesis of the left ventricular myocardium (EF 55%) and a slight amount of fluid in pericardium. Heart valves were without vegetations. Performed thoracic angio-CT excluded the suspicion of pulmonary embolism. The CT of head and neck exposed enlarged cervical lymph nodes. The abdominal ultrasound did not reveal abnormalities. The treatment with antibiotics was continued, the patient received antipyretic, analgesics, i.v.hydration. During hospitalization, the patient was reporting muscle ache (without elevation of creatinine kinase), diarrhea and on the third day after admission she presented an episode of somatoform delirium. After the consultation of infectious diseases specialist, the diagnostics was upgraded to include cytomegaly, mononucleosis, toxoplasmosis, syphilis, HIV infection, tuberculosis, active borreliosis- none was positive. The test for *Streptococcus pneumoniae* excluded the infection. The throat swab revealed numerous colonies of *Candida albicans*. The tests

for systemic connective tissue diseases and monoclonal gammapathy were also negative. After exclusion of the infectious disease and autoimmune disease suspected from the beginning MIS-A was confirmed and dexamethasone treatment was started – initially with iv. therapy (8 mg per day). On the third day after clinical improvement – incl. cervical lymph node size reduction, body temperature normalization, the oral treatment with dexamethasone (4 mg per day) was continued. Further hospitalization resulted in a clear improvement of the patient's general condition and a systematic decrease in inflammatory parameters. After 12 days, the patient was discharged home.

Discussion

MIS-A is thought to be underdiagnosed as it is reported in much lower frequency than MIS-C.[2] The pathophysiology of this condition is also poorly understood for the time being and the diagnostics process is relatively long and troublesome as it involves the exclusion of alternate diseases. First worldwide reports of this illness date back to autumn 2020 which makes the entity relatively new. We currently have scientific reports concerning post SARS-CoV2 vaccine multisystem inflammatory syndrome known as MIS-V.[4-6] Moreover, non-specific clinical picture can be the cause of prolonged diagnosis.

In the presented case:
- high fever (up to 40°C) 3 days before hospitalization
- rash and conjunctivitis
- cardiac illness- myocarditis, pericarditis and impaired function of right ventricle
- hypotension
- abdominal pain
- diarrhea
- elevated level of the CRP, ferritin, IL-6, procalcitonin, NT pro BNP, troponin

The patient was within the age range for MIS-A with prior SARS- CoV2 infection. This case fulfilled the Brighton Collaboration Case Definition for MIS-A.[1,3]

Conclusion

Settling the recognition of MIS-A can be challenging because health professionals are only beginning to gain experience with this unit. Its effective treatment requires the use of steroids. MIS-A is a rare disease. The diagnostic procedure must exclude other diseases in which an excessive immune response is activated. A history of preceding SARS-CoV-2 infection is important. The BCCD guidelines are helpful in establishing the diagnosis. However, medical management requires performing a full differential diagnosis and introducing adequate treatment. In the presented case, the diagnostic procedure, taking into account MIS-A, made it possible to establish the correct diagnosis. Treatment with steroids, in a short period of time, led to the patient's recovery.

Declarations

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Author contributions

Conceptualization, H.D. and B.D.; Methodology B.D.; Validation, H.D. and B.D; Formal Analysis, H.D.; Investigation, H.D. and B.D; Resources, H.D. and B.D.; Data Curation, H.D.; Writing – Original Draft Preparation, H.D.; Writing – Review & Editing, H.D. and B.D.; Visualization, H.D.; Supervision, H.D.; Project Administration, H.D.

Conflicts of interest

The authors have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this manuscript.

Data availability

The data have not been made public, but are kept with the authors, if necessary.

Ethics approval

Written informed consent for publication was obtained from the patient. We complied with the policy of the journal on ethical consent.

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CASUISTIC PAPER

Postpartum takotsubo cardiomyopathy complicated with severe pulmonary edema and cardiogenic shock – a case report

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ABSTRACT

Introduction and aim. Takotsubo cardiomyopathy (TCM) is a rare but life-threatening illness that can affect middle aged, young and pregnant women. It is a cardiac emergency and can mimic many other life threatening conditions like myocardial infarction, acute myocarditis, peripartum cardiomyopathy or dilated cardiomyopathy and is diagnosed by exclusion.

Description of the case. Here we report a rare case of takotsubo cardiomyopathy in a young 28 year old female immediately post vaginal delivery of her normal full term twin pregnancy, who was otherwise a healthy female. She went on to develop complication of left ventricular heart failure and cardiogenic shock, which were successfully managed by conservative treatment leading to a full recovery.

Conclusion. Takotsubo or stress cardiomyopathy is a rare entity and often a missed diagnosis, which if caught on time and treated leads to great prognosis. Our patient made a full recovery and is living a healthy life.

Keywords. cardiogenic shock, left ventricular dysfunction, postpartum, stress cardiomyopathy, takotsubo, twins

Introduction

Takotsubo cardiomyopathy (TCM) also known as stress induced cardiomyopathy that is characterized by acute onset left ventricular dysfunction associated with variable wall motion abnormalities with absence of any significant coronary artery disease.[1] Cardiac function often spontaneously recovers in most patients within days or weeks, if the severity of the disease does not cause death.[2] The common precipitating factors of TCM are emotional, physical psychological stressors with a greater incidence in patients with pre-existing psychiatric conditions.[3] Most of cases of TCM associated with pregnancy are reported during the peripartum period and were not related to the mode of delivery making peripartum cardiomyopathy an important differential which is difficult to distinguish.[2,4]

Aim

In this article, we present a case of a 28-year-old female who presented with normal antenatal period at 38 weeks 2 days of gestation, with twin normal vaginal delivery. She subsequently developed TCM, immediately post-delivery, which was complicated with severe pulmonary edema and cardiogenic shock, which was successfully managed conservatively. We further review the current literature on stress-induced cardiomyopathy in pregnancy.

Description of the case

A 28 year old Indian female with P1L2 on postpartum day 1 of a full term (38 weeks 2 days), normal vaginal delivery on 27/12/2021 presented to our hospital 5-6 hours after delivery of twins at a local nursing home with chief complaints of severe progressive sub sternal pressure like throbbing chest pain since 2-3 hours(2

hours after normal vaginal delivery).The pain was 10/10 on VAS (visual analog scale), non-radiating associated with diaphoresis and shortness of breath. History of feeling "fluttery" with lightheadedness and palpitations was present since 2 hours. The pain was not relieved by I.V.(intravenous) analgesics and I.V. antacids. On presenting to the emergency department, blurring of vision and severe throbbing headache present since 15-20 minutes. No history of PPH (post-partum hemorrhage) or such episodes previously.

The pregnancy was a booked one with normal antepartum period all tests within normal limits except a slight deranged SGOT/SGPT(100/121) during last few months of pregnancy. It was a spontaneously conceived pregnancy with an uneventful ante and peripartum period. The twins were one boy (2.6 kg) and one girl (2.8 kg). Both of them cried immediately post-delivery and were normal.

No history of GDM (gestational diabetes mellitus), preeclampsia, eclampsia during the pregnancy. Family history was only significant for twin pregnancies, otherwise there was no significant history of any cardiac illness or sudden deaths/cardiac arrests/heart failures. No family history of any chronic illness. Past history not significant and no history of any illness or medications. She is a non-vegetarian with normal bowel/bladder habits and no addictions, works as a social worker and does not experience much stress in her life but the twin pregnancy and delivery was a "ONE TIME BIG LIFE CHANGING EVENT" for her.

On examination patient was conscious but confused, diaphoretic. Vitals suggestive of accelerated hypertension with a high blood pressure of 220/120 mmHg, sinus tachycardia at 145 beats/min, Respiratory rate -22/min, SpO_2 94% on room air and afebrile.

In view of high BP, Intravenous infusion of Nitroglycerine (at an initial dose of 10 mcg/min) started immediately, emergency non contrast CT (computed tomography scan) head done and reported normal, basic investigations sent and shifted to medical intensive care unit (ICU). Patient was anxious, in acute distress, dehydrated, peripheries cold, clammy with no apparent rashes. Neck was soft and non-tender with no apparent masses or jugular distension or adenopathy. Thyroid was normal in size, symmetrical and no bruits. Cardiovascular examination unremarkable with sinus tachycardia, normal rhythm with no murmurs or gallops or rubs. Lung auscultation suggestive of bilateral basal crept, rest within normal limit. Abdomen was soft, tender normoactive bowel sounds, no significant organomegaly or masses. Uterus was well contracted and palpated in suprapubic region. A neurological examination showed grossly intact cranial nerves (light touch, pinprick, position & vibration sense), normal sensation, strength was full bilaterally, normal reflexes,

unable to walk but normal coordination. Pronator drift of outstretched arms absent, with normal muscle bulk and tone. Bilateral pitting pedal edema present but no skin discolorations, clubbing, deformities or cyanosis. The capillary filling time was normal. Speech was fluent, with appropriate comprehension and retention.

Initial investigations showed a baseline hemoglobin of 11.2gm/dL, TLC: 9,600/mm³, platelets 246 000/mm³ ECG on admission as in figure 1 and a differential of ACS (acute coronary syndrome) post-partum was considered. Inj. Tramadol (50 mg IV thrice a day) and O_2 Therapy with nasal prong initiated. Troponin I was 0.40 ng/ml (Normal-0 -0.2), BNP:1240 pg/ml (Normal:- <100 ng/ml). In view of acute LVF (left ventricular failure), decongestant therapy with Inj. Furosemide infusion at the rate of 5 mg/hr) was initiated. ACS Protocol activated, anti-platelet, high dose statins and anticoagulants S/C (subcutaneously) started after gynecological reference for ruling out PPH or retained tissue/placenta on ultrasound (T Aspirin 375mg stat, T. Atorvaststin 40 mg once a day, Inj. Enoxaparin (0.4 ml/40mg, S/C once a day).

All investigations sent in view of the differential diagnosis which were all normal. Also CECT abdomen and chest were reported normal. S.FDP (fibrin degradation product), APLA Profile (lupus anticoagulant), ANA Profile, Thyroid Profile, Thyroid Antibodies, Lipid Profile, Urine dipstick protein-negative. Routine investigations were normal except transaminitis as SGOT/SGPT (112/114 mg/dl) elevated. An urgent bedside 2D echo done revealed apical LV hypokinesia with LVEF (left ventricular ejection fraction) of 20%, LVDD (diastolic dysfunction) grade I with no mitral regurgitation/mitral stenosis/aortic stenosis/aortic regurgitation/clots/vegetation or PE (pulmonary embolism). A cardiac angiography was planned and performed for the patient with differentials of coronary artery disease, but reported normal. A D-DIMER and CTPS (CT pulmonary angiography) was done which was normal and ruled out pulmonary embolism (Fig. 2).

On day 2 of admission patient developed sudden onset generalized tonic clonic seizure for 2-3 minutes, after which patient's blood pressure and pulse become non recordable. Cardiopulmonary resuscitation was initiated for cardiac arrest, Inj. Adrenaline (1ml IV stat) and inotropes (Inj. Noradrenaline at the rate of 0.4 mcg/kg/minute infusion) along with IV fluids NS (normal saline) bolus of 500ml. Patient was in cardiogenic shock and post CPR Trop I increased 0.51 ng/ml. BNP increased to 2560 pg/ml, chest X-ray revealed mild bilateral pleural effusion, bilateral alveolar edema and kerley B lines suggestive of pulmonary edema, D- Dimer increased to 2500 ng/ml).

Inj nitroglycerin and Inj. Furosemide stopped, inotropic support (Inj. Noradrenaline and Inj. Vasopressin
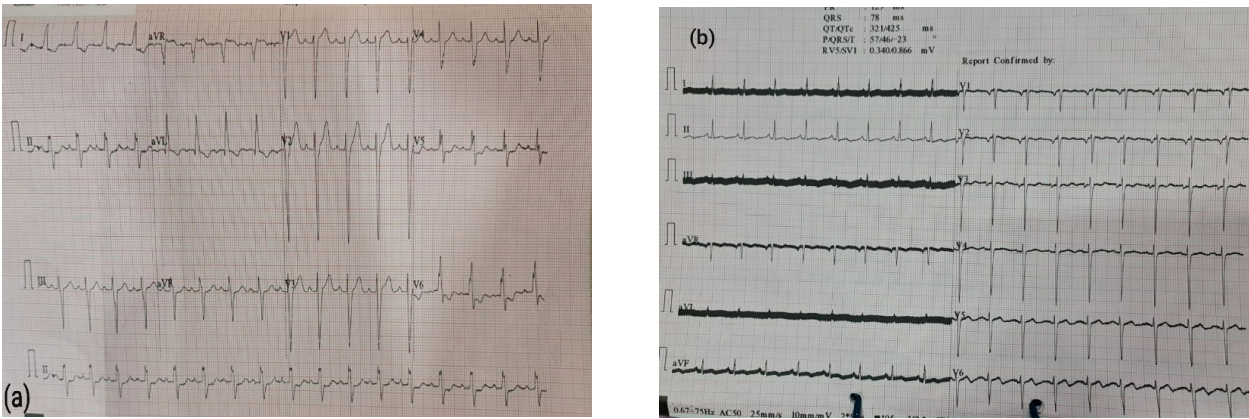
Fig. 1. (a) ECG on day 1 showing marked ST elevation changes which evolve to (b) on day 5
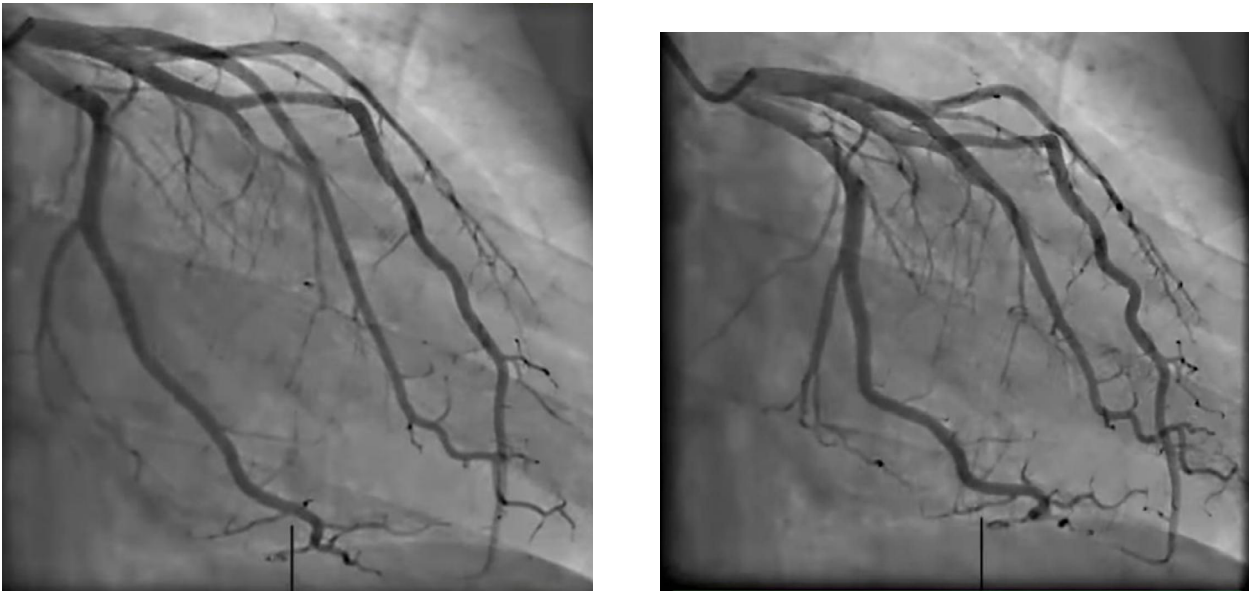

Fig. 2. Cardiac angiography which was normal in our 28yr old female patient

at the rate of 0.3 units/hr) along with IV fluids at the rate of 60ml/hr started immediately. Noninvasive ventilatory support given. Neurologist advised MRI Brain with contrast done and was normal. Further on cardiac magnetic resonance was planned. A repeat bed side transthoracic echocardiogram done which showed LVEF of 18% with a hypokinetic apical and mild distal walls and hyperdynamic basal walls of LVEF consistent with apical ballooning syndrome. Cardiac MRI done and reported as Tako-Tsubo cardiomyopathy (Fig. 3).

For the next 4 days close monitoring of vitals and routine investigations done while inotropic support, low dose Inj. Furosemide and other supportive treatment continued. Patient responded well to treatment, vitals stabilized, oxygen and inotropes tapered. On 01.01.2022 a repeat TTE (trans-esophageal echocardiography) was done which showed LV hypokinesia and LVEF improved to 43%. On day 7 patient developed one episode of high grade fever with abdominal pains and cramps.

Patient had leukocytosis (TLC: 40,000/mm³), blood and urine cultures were sent, fever profile done, se-

rum procalcitonin done and a high vaginal swab culture (HVSC) taken. Her antibiotics were upgraded to Inj. Piperacillin and Tazobactum (4.5gm IV, 6 hourly). Serum procalcitonin was high 50.19 ng/ml, diagnosis of septic shock made and isotope support and fluid resuscitation reinitiated. HVSC showed growth of Klebsiella which was sensitive to antibiotics and her TLC started decreasing with no more fever spikes and rest of fever profile was normal. Thereafter in consultation with cardiologist inotropic support was continued. BNP was still high on day 7 with pitting pedal edema and Chest X-ray suggestive of acute LVF for which decongestion titrated up (T. Eplerenon 25mg once a day added to Inj. Furosemide) along with beta blocker (T. Metoprolol 12.5 mg BD) along with inotropes. Further on day 9 patient developed sudden severe anemia wherein her hemoglobin dropped to 5.6 gm/dl from 9.6 gm/dl on 01.01.2022. A full anemia workup to rule out hemorrhage was done (urine, stool routine, occult blood). Hemolysis ruled out by negative direct and indirect coombs test and normal total bilirubin). Gynecologist rereviewed USG to rule
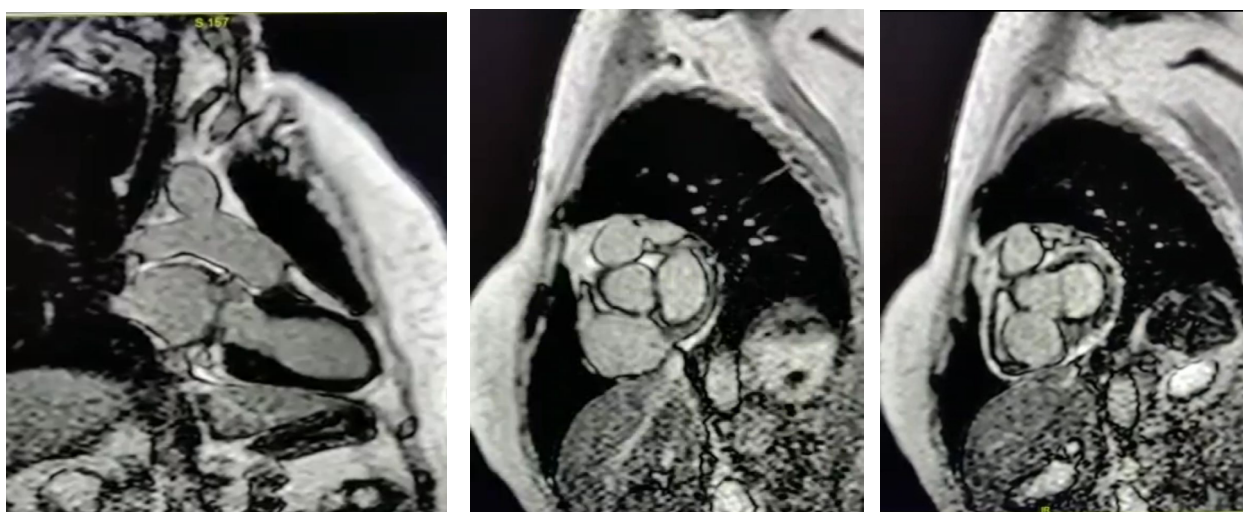
Fig. 3. Vertical and horizontal long axis in systolic and diastolic cine MR images from 28-year-old woman with Takotsubo cardiomyopathy. MRI was performed on second day of onset of symptoms. Apical akinesis produces apical ballooning, resembling Japanese takotsubo

out any retained tissue or atonic uterus and only spotting PV (per vaginal) was present. She was transfused 2 unit PRBC over 2 days and her hemoglobin increased to 8gm/dl. Gastroenterologist reference taken to rule out any gastrointestinal bleed, endoscopy and colonoscopy performed and reported normal. On day 10, hemoglobin and other reports normalized, serial BNP showed consistent decrease and she responded well to treatment. Vitals were stable off inotropes and oxygen. Patient was asymptomatic and chest X-ray cleared. Keeping everything in view, diagnosis of stress cardiomyopathy made. Our patient was discharged on day 14 on Beta blocker (T. metoprolol 25mg BD (twice a day)), T. furesomide/spironolactone) BD, anti-epileptic (T. LEVETIRACETAM 500 mg BD), antibiotics T. cefuroxime 500mg BD and T. metronidazole ER 600 mg BD and supportive treatment. On follow up, 15 days later she was asymptomatic and denied any episodes of chest pain, palpitations or shortness of breath or pedal edema. She was breast feeding both her babies along with top feed. Repeat chest X ray was clear and echocardiogram showed LVEF of 45%. An angiotensin converting enzyme inhibitor (ACEI Ramipril 2.5 mg OD) initiated on follow up. On 1 month follow up, Cardiac MR chest was repeated with 2D echo which revealed no residual cardiac anomaly and LVEF of 55%.

Discussion

TCM is a rare but life-threatening illness that can affects middle aged, young and pregnant women. It is considered a cardiac emergency and can mimic many other life threatening conditions like myocardial infarction, acute myocarditis, peripartum cardiomyopathy or dilated cardiomyopathy and is diagnosed by exclusion. TCM usually presents with acute severe retrosternal chest pain,

palpitations and diaphoresis.[2] Affected patients may progress to develop symptoms of left heart failure like paroxysmal nocturnal dyspnea, orthopnea and dyspnea caused due to fluid overload.[1]

TCM may be precipitated by physical and/or psychosocial stressors in individuals with premorbid psychiatric illnesses at higher risk.[3] Our patient had a history of general anxiety illness which may have predisposed her to TCM. In addition to this it is possible that an acute elevation of blood pressure from the baseline post-delivery triggered TCM (Fig. 4).
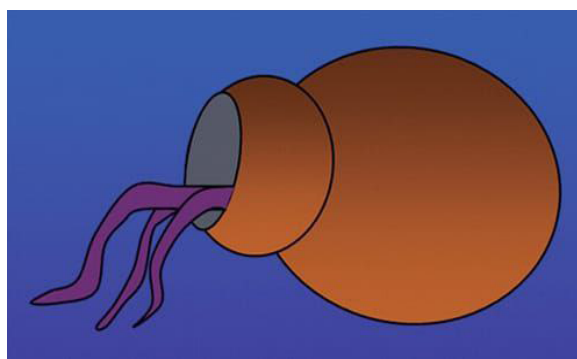

Fig. 4. Schematic drawing shows takotsubo, a pot which is used in Japan to catch octopi. It is round at bottom with a narrow neck to keep the octopus from escaping

Diagnostic evaluation of TCM is based on an electrocardiogram (ECG), positive cardiac biomarkers, echocardiography, left heart angiography and cardiac MRI. The ECG abnormalities most commonly reported include ST segment elevation in the anterior and/or precordial leads with reciprocal ST segment depression.[5] Rarely QT interval prolongation, abnormal Q waves, T wave inversion or non-specific abnormalities may be

noted.[5] Majority of cases have elevated cardiac troponin levels along with normal to mildly elevated creatinine kinase. The natriuretic peptides (brain natriuretic peptide (BNP) and pro-BNP) may be elevated in most of the cases indicative of ventricular strain.[5]

Transthoracic echocardiography very often shows a large area of regional wall motion akinesia of the LV, mostly extending beyond the territory of a single coronary artery.[6] Apical ballooning of the LV with normal basal contractility is noted in a few cases. The LV ejection fraction is invariably reduced (ranging from 20 to 49%).[7] Mitral regurgitation with or without a systolic anterior motion of the anterior leaflet may also be present in some cases.[1]

The diagnosis of TCM can be based on the Mayo clinic diagnostic criteria.[1] The criteria includes presence of left venticular transient akinesis, hypokinesis or dyskinesis with or without apical involvement; the regional ventricular wall motion abnormality typically extends beyond a single epicardial vascular perfusion territory helping rule out myocardial infarction. Absence of obstructive coronary disease or angiographic evidence of acute plaque rupture supports the diagnosis of TCM further. ECG abnormalities in TCM include ST-segment elevation with or without T-wave inversion. Cardiac troponin levels are mild to moderately increased. According to criteria myocarditis and phaechromocytoma should be excluded.[1] Our patient had transient ECG abnormalities, increased cardiac markers and no angiographic evidence of acute plaque rupture or vessel occlusion fulfilling the Mayo criteria for TCM. Hence a diagnosis of stress cardiomyopathy was made.

Few TCM cases tend to develop Left Ventricular Outflow Tract (LVOT) obstruction caused by increased contractility of base of the heart.[7] This is easily diagnosed by use of echocardiography and initiation of timely management results in good prognosis.[8] The detection of LVOT obstruction is important as the patients usually present with hypotension and the use of ionotropic agents may increase the intraventricular pressure gradient, inducing cardiogenic shock, which was very well excluded in our case by both trans-thoracic and trans-oesophageal echocardiography.[7]

TCM cases recover to normal cardiac function within 4 to 8 weeks of onset.[2] Our patient had resolution of heart failure symptoms with full recovery by 12 weeks post-delivery, confirmed by repeat echocardiography and CMR (cardiac magnetic resonance). Care of these patients should be directed at managing symptoms and medical cardiac optimization with diuretics, angiotensin-converting enzyme inhibitors or beta blockers. Our case was adequately treated conservatively and symptomatically which lead to good prognosis.

Once the physical or emotional stress resolves, resulting in rapid resolution of symptoms and left ventricular ballooning and dysfunction, though some patients develop life threatening acute complications such as acute heart failure and cardiogenic shock, like in our case requiring coronary cardiac unit admission. Some cases may require invasive techniques such as intra-aortic balloon pump or cardiopulmonary support.[8]

Recurrence of TCM in rarely occurs in premenopausal women like our case.[9] But she was subjected to a close weekly follow up upon discharge, preventing the patient to deteriorate and develop any major adverse cardiac or cerebrovascular events.[1]

Conclusion

TCM is a rare cardiac condition, even rarer especially in pregnancy, with multiple life threatening conditions like postpartum cardiomyopathy, thromboembolism, myocardial infarction etc. Management in pregnancy and post pregnancy has challenges crowded by safety concerns of the drugs used for managing the condition. Longitudinal studies are much needed for evaluating the reproductive history of women with TCM and further assess its association with preeclampsia and other condition, developing criteria and identifying pregnant women at risk of developing life threatening TCM. We strongly advocate for a multidisciplinary approach in order to optimize outcomes for the mother and child.

Declarations

Author contributions
Conceptualization, R.A.B and R.R.; Methodology, R.R.; Software, R.A.B.; Validation, R.A.B. and R.R.; Formal Analysis, R.A.B.; Investigation, R.A.B.; Resources, R.R.; Data Curation, R.A.B. and R.R.; Writing – Original Draft Preparation, R.A.B.; Writing – Review & Editing, R.R.; Visualization, R.A.B. and R.R.; Supervision, R.R.; Project Administration, R.A.B.

Conflicts of interest
The authors have no conflict of interest.

Data availability
The data may be made available to interested persons at the request of the corresponding author via e-mail.

Ethics approval
All subjects gave informed consent to the inclusion prior to participating in the study.

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CASUISTIC PAPER

Application of kinesitherapy within the process of rehabilitation of patients with Charcot-Marie-Tooth nerval amyotrophia

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ABSTRACT

Introduction and aim. Researching inherited polyneuropathy is vastly topical in the course of the contemporary practice of physical therapy and ergotherapy. The article unveils the results of the application of kinesitherapy in the process of rehabilitation of patients with Charcot-Marie-Tooth nerval amyotrophia. Inherited Charcot-Marie-Tooth neuropathy is a genetical disease, which is manifested with the slow reduction of the size of muscles of limbs and weakening of distal locations, is the most widespread clinical form of inherited polyneuropathies, which affect people regardless of generational and gender-based; mostly young and workable people become the objects suffering from its impact.

Description of the case. Due to the relatively low frequency of the multiplication of the disease within the population (according to the data from clinical statistics, the prevalence of all types of Charcot-Marie-Tooth amyotrophia per 100 thousand people is approximately 36 cases) four patients with Charcot-Marie-Tooth nerval amyotrophia aged in the area from 14 to 20 years took part in the research. In the course of the research, we applied the method of electroneuromyography, which provided the opportunity of detecting the rate of impulse impact via afferent and efferent ways, the duration of M-response and the number of movable entities within lower limbs.

Conclusion. As a result of classes being held and carried out according to the experimental kinesitherapy study program, there was the detection of positive tendencies of changing the psychophysical state of patients, diagnosed with "Charcot-Marie-Tooth nerval amyotrophia".

Keywords. Charcot-Marie-Tooth, kinesitherapy, nerval amyotrophia

Introduction

Charcot-Marie-Tooth nerval amyotrophia is one of the most widespread inherited neurological diseases in the world, which is diagnosed in 36 persons per 100000 population. In Ukraine inherited amyotrophia cases include 15000 persons, which corresponds to the correlation of 1 case per 2.5 thousand persons. Inherited Charcot-Marie-Tooth neuropathy is the most widespread clinical form among inherited polyneuropathies. This genetic disease affects all races and nationalities regardless of generational and gender-based differences, but the most frequent objects of affection are young and workable people in their 20s and 30s. Progressive course of the disease along with possible swift development of complications and absence of effective treatment of patients with Charcot-Marie-Tooth amyotrophia leads to the declination of life quality and premature corporal incapability. The disease does not affect the fertile capabilities and life duration of the objects of affection, which provides for a relatively large amount of people affected by Charcot-Marie-Tooth amyotrophia within the population.[1]

A group of developed countries bear the functioning associations, organizations and foundations concerning the provisions of medical and social aid to those who suffer from Charcot-Marie-Tooth amyotrophia and to their families. These organizations carry out active research activity seeking new methods of premature diagnosing and treatment of the disease, great emphasis is set on the measures of social and medical rehabilitation of individuals affected by Charcot-Marie-Tooth amyotrophia. Unfortunately, such organizations are nowhere to be found in Ukraine, although several scientific studies of clinical, genetic and epidemiological courses of different forms of Charcot-Marie-Tooth amyotrophia take place.[1-3]

The effective methods of physical therapy for people with the consequences of Charcot-Marie-Tooth nerval amyotrophia are prescribed individually. The amount of variations of means and methods of physical therapy is shaped by the stage of the progression of the disease, individual peculiarities of a patient and the availability of other adjoint diseases about them. Elaborated by the group of scientists and scientifically-backed kinesitherapy study program is aimed at patients with Charcot-Marie-Tooth nerval amyotrophia and leads to the improvement of indications of functioning of the neuromuscular system of patients with Charcot-Marie-Tooth nerval amyotrophia and, also, positively impacts on the psychoemotional state of the patients.[2-4]

Charcot-Marie-Tooth nerval amyotrophia is one of the most widespread inherited neurological disorders in the world, which is diagnosed in 36 persons per 100000 population. In Ukraine inherited amyotrophia cases include 15000 persons, which corresponds to the correlation of 1 case per 2.5 thousand persons.[1-4]

Inherited Charcot-Marie-Tooth neuropathy is the most widespread clinical form among inherited polyneuropathies.[2] This genetic disease affects all races and nationalities regardless of generational and gender-based differences, but the most frequent objects of affection are young and workable people in their 20s and 30s. Progressive course of the disease along with possible swift development of complications and absence of effective treatment of patients with Charcot-Marie-Tooth amyotrophia leads to the declination of life quality and premature corporal incapability.[4,5] The disease does not affect the fertile capabilities and life duration of the objects of affection, which provides for a relatively large amount of people affected by Charcot-Marie-Tooth amyotrophia within the population.

A group of developed countries bear the functioning associations, organizations and foundations concerning the provisions of medical and social aid to those who suffer from Charcot-Marie-Tooth amyotrophia and to their families. These organizations carry out active research activity seeking new methods of premature diagnosing and treatment of the disease, great emphasis is set on the measures of social and medical rehabilitation of individuals affected by Charcot-Marie-Tooth amyotrophia.[6] Unfortunately, such organizations are nowhere to be found in Ukraine, although several scientific studies of clinical, genetic and epidemiological courses of different forms of Charcot-Marie-Tooth amyotrophia take place.

The effective methods of physical therapy for people with the consequences of Charcot-Marie-Tooth nerval amyotrophia are prescribed individually.[2,7,8] The amount of variations of means and methods of physical therapy is shaped by the stage of the progression of the disease, individual peculiarities of a patient and the availability of other adjoint diseases about them.

A scientifically justified kinesitherapy study program for patients diagnosed with Charcot-Marie-Tooth nerval amyotrophia will provide for the improvement of indications of the functionality of the neural and muscular system of patients, affected by Charcot-Marie-Tooth nerval amyotrophia and, additionally, will positively impact their psychological state.

Aim

The aim of this study was to elaborate and scientifically justify the program with kinesitherapy classes for patients with Charcot-Marie-Tooth nerval amyotrophia and monitor its effectiveness.

Analysis of the literature

Analysis and unification of the literature concerning the issues about physical therapy of patients with functional deviations related to the course of Charcot-Marie-Tooth nerval amyotrophia provided for the methodological

system of the research. For the examinations of functions of the neural and muscular system in limbs, there was the application of electroneuromyography, which provided the possibility of indicating the speed of execution of the impulse by afferent and efferent methods, along with the duration of M-response and the number of mobile entities in lower limbs. Additionally, there was the application of pedagogical inspection with the objective of detecting specifications of patients' behavior and its emotional components, particularly for the understanding of the type of reaction to success and misadventures. As a secondary method of pedagogical inspection, there was the application of conversations, which were held with patients and their parents to provide for the formation of motivation of attending kinesitherapy classes. For the detection of agitation and depression levels, there were some psychodiagnostic methods, were used as well. Processing of empirical data was executed via the method of comparison of patients' physical states before and after the execution of the experimental research. Experimental research was executed for 6 months (from 10.01.2021 to 03.16.2022) in the main workshop building specialising in adaptive and physical rehabilitation "Zdorovi Rukhy". Preparation for the research provided for the execution of the comparative analysis of tendencies of indications, the motion amplitude, electroneuromyography and psychodiagnostic of the patients aged from 14 to 20 years old, affected by Charcot-Marie-Tooth nerval amyotrophia, in the process of kinesitherapy classes. For 4 patients aged from 14 to 20 years old, affected by Charcot-Marie-Tooth nerval amyotrophia, there was elaboration of the program of physical rehabilitation in the course of kinesitherapy classes. The research was held in three stages.

The first stages assured the realization of theoretical analysis of the scientific literal sources for the references, dedicated to the topic of the research, there was the examination of anamnesis and epicrisis of the patients, which became the basis of the formation of clinical characteristics and specified physical therapy program, which included the provision of kinesitherapy classes for patients, diagnosed with Charcot-Marie-Tooth nerval amyotrophia (10.01.2016 – 10.16.2021).

The second stage included the approbation and verification of the effectiveness of the physical rehabilitation program for the patients affected by Charcot-Marie-Tooth nerval amyotrophia (10.16.2021 – 03.16.2022). Bearing the objective of estimating the effectiveness of the experimental methods of kinesitherapy, there was the forming experiment, which provided for continued execution of the comparative analysis of tendencies of indication of motion amplitude, electromyography and psychodiagnostic of the patients aged from 14 to 20 years old, affected by Charcot-Marie-Tooth nerval

amyotrophia, in the course of kinesitherapy classes. The third stage of the research is the concluding one.

Description of the case
Ethics approval
This study was approved by Institute Ethics Committee, National University Yuri Kondratyuk Poltava Polytechnic, Poltava, Ukraine (Ref: NUYKPP/IEC/2022/123). We adhered to the principles of ethics thereafter throughout the study.

Participants
The course of the research, there was the elaboration of a theoretically justified and experimentally verified physical therapy program, which provided for the organization of kinesitherapy classes for the patients aged from 14 to 20 years old, affected by Charcot-Marie-Tooth nerval amyotrophia.

Procedure/Test protocol/Skill test trial/Measure/Instruments
According to the experimental program, the rehabilitation course includes 3cycles for 2 months of classes (6 months in total), with the gradual strengthening of the load charge of the physical exercises. Each class consisted of 3 stages:

1. The correct type of breathing;
2. The warm-up;
3. Kinesitherapy class;

Let us characterize each one of these stages in detail. In the course of the execution of the exercises, it's vital to remember, that inhaling must be done at the moment of muscular release, but exhaling is done at the moment of muscles being engaged when the maximum of effort is applied. Breathing must be deep (with the diaphragm being engaged), but comfortable at the same time, as one's belly must inflate in the course of inhaling. It may seem uncomfortable, but only with this way of breathing the organism is able to obtain the maximum of oxygen without losing much energy, similarly to surface-levelled frequent breathing. What also matters is what organ the patient uses – engaging their oral cavity or their nose. It is recommended to inhale using the nose of the patient, as it leads to moisturizing and purification of air from dust and microorganisms and exhaling is recommended to do using the patient's mouth, as it leads to quicker ejection of the air and organism spends much less of energy resources. Additionally, the maximum of energy is accumulated during the process of exhaling, the energy which is indispensable for the effective execution of exercises. Moreover, in the course of exhaling the air, the diaphragm and abs are engaged which provides additional endurance and helps to bear the loading without the patient's organism being harmed. At the moment

of exhaling muscles of the body are engaged in an unbalanced way, and this a person cannot apply all their energy for the execution of the exercises and loading always seems more unbearable.

The execution of second stage is initiated with the warm-up. The warm-up consists of these main exercises: "Dorsal relaxation on one's laps", "Stretching steps", "Retraction of the belly (abs)" and "Hip-bone lifting". Let us describe the indicated exercises in more detail. The first one is "Dorsal relaxation on one's laps". Executing these exercises, the initial position is pronated, with patients standing on their knees. Firstly, while exhaling, the patient steadily stretches out their back upward, then returns to the initial position, inhales and in the process of exhaling it's necessary to steadily bend their back downward (Fig. 1). The patient returns to the initial position afterwards. In the course of the execution of this exercise, the warm-up provides for 20 repetitions of previously described instruction.
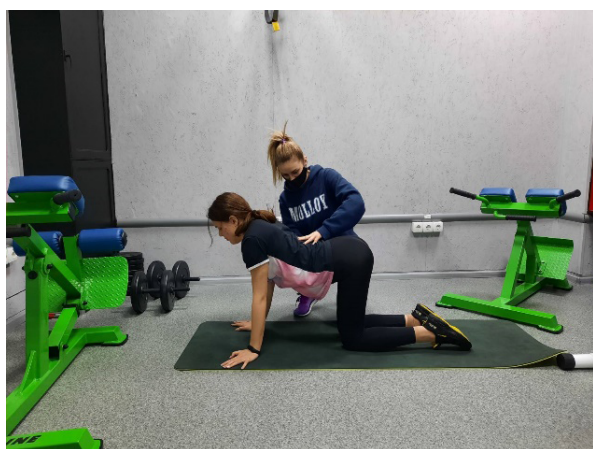

Fig. 1. Execution of the exercise "Dorsal relaxation on one's laps"

"Extending steps". In the course of execution of this exercise, the initial position is pronated with patients standing on their knees. Patients execute exercises for stretching muscles: steadily leaning on the left leg and stepping back with their right one with their

left arm stretching forward. While stretching patients inhale, up to the complete relaxation of their muscles and, afterwards, return to the initial position. Afterwards, patients inhale, lean on their right leg, step back with their left legs and stretch forward with their right ones (Fig. 2).

Fig. 2. Execution of the exercise "Dorsal relaxation on one's laps"

"Retraction of the belly (abs)". In the process of execution of this exercise, the initial position is patients laid down on their backs with their legs bent in their knee cups, with their heels on the floor, and their hands behind their heads. Patients press their chin to their chest, bend their torso while exhaling, try to lift their shoulder blades over the floor and make a motion with their elbows towards their laps (Fig. 3).

The amount of repetitions in the course of the exercise is unlimited, up to the burning sensation in the abdominal muscles.
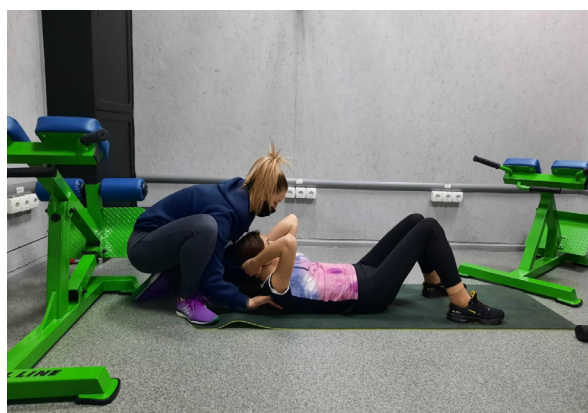

Fig. 3. Execution of the exercise "Retraction of the belly (abs)"

"Hipbone lifting". In the process of the execution of this exercise, the initial position is patients laid on their backs, with their legs bent in their knee cups, their heels on the floor, and arms along their body. While inhaling patients steadily lift their hipbone as high over the floor as possible and returns to the initial position (Fig. 4). In

the course of the execution of this exercise, the warm-up requires from 20 to 30 repetitions.

It is worth noticing, that in the course of the execution of the warm-up, as well as the next exercises, it is necessary to track patients' wellbeing, and sensations and, most importantly, respect the principle of individual approach (the variety of means and methods of physical rehabilitation is caused by the stage of the progression of the disease, individual peculiarities of the patients, and the availability of adjoint diseases).

Fig. 4. Execution of the exercise "Hipbone lifting"

Individual physical therapy program, which provided for the organization of kinesitherapy classes, consisted of a set of exercises, which are executed on the trainer "MTB-2". The exercises are "Lat Pull Down", "Abs workout", "Enhancement of quadriceps of the hips", "Enhancement of biceps of the hips", "Pulling off the knee cups towards to the abdomen", and "Pulling with a straight leg", "Triad", "Pull-over", "Rotation", "Abs enhancement + 5". Let us characterize the set of exercises, executed on the trainer MTB-2, in more detail. The exercise "Lat Pull Down". The exercise is executed with the aim of strengthening of dorsal muscles. The scheme of execution (Fig. 5).

1. The instructor sets the appropriate weight, with the preference towards the minimum one.

2. The patient sits down on the seat and adjusts the armpad roller in order for the hips to be tightly fixated and the legs tightly stood on the floor.

3. The instructor's hands are in the grip of the trainer. The patient gives it a wide grip with their hands situated slightly wider than the level of shoulder position.

4. The instructor slightly bends the torso backwards approximately to 25 degrees, which is the initial position.

5. The instructor slowly helps to pull the grip to the level of the chest, and then the patient lowers their elbows downwards to 45 degrees, with the contraction of the widest dorsal muscles. The pause must be done at the lowest point.

6. The patient returns to the initial position and maximally stretches the widest dorsal muscle at the highest point of the amplitude.

7. The amount of repetitions is from 12 to 15 times.

Fig. 5. Execution of the exercise "Lat Pull Down"

The exercise "Abs enhancement". The "Abs enhancement" exercise is executed for strengthening abdominal muscles and relaxation of the lower back. The exercise is executed on the trainer "MTB-2" with the cuffs on the legs. The scheme of the execution (**Fig. 6**) is the following: The patient lays down and grasps the fixated grips with their wrists.

Fig. 6. Execution of "Abs enhancement" exercise

The instructor attaches MTB cuffs with an additional weight of 30 kilograms to the legs of the patient. The patient's back must be able to freely attach back to the necessary surface. The instructor helps to gradually pull their knees to the ribcage at the moment of exhaling, extracting the abdominal muscles. The amount of repetitions is from 15 to 20 times.

The exercise "Enhancement of quadriceps of the hips". This exercise is used for the restoration of strength and length of muscles of the hips. The scheme of execution (Fig. 7). The patient lays down on their back with their head aimed towards the trainer. The instructor attaches one leg to the cuff from the higher block of 10 kilograms of MTB trainer. Under the pressure of the weight, one of the legs is bent in the knee, without detaching the leg from the floor, and in this phase, the patient inhales. At the moment of exhaling, the patient completely relaxes their leg in the area of the kneecap. The previously mentioned algorithm is repeated from 15 to 20 times for each leg.
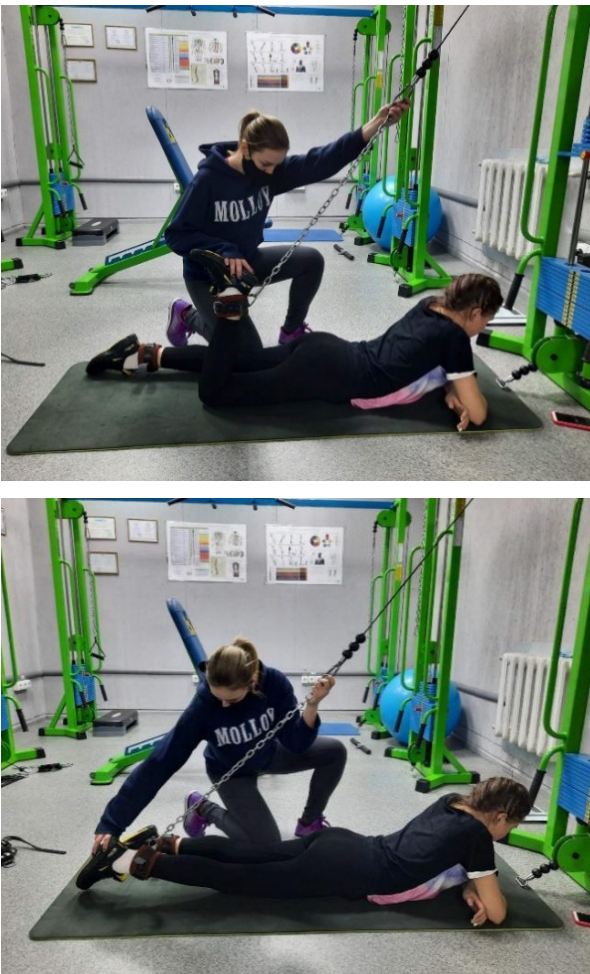

Fig. 7. Execution of "Abs enhancement" exercise

The exercise "Enhancement of biceps of the hips". The exercise is executed for the restoration of the strength of hip muscles, as well as the restoration of the length of these muscles. The scheme of execution (Fig. 8) is the following: The patient is laid on their back, with their head towards the trainer. The instructor attaches one leg to the cuff from the opposite MTB from the lower block with a weight of 2 kilograms. In the position of complete extension of the leg in a kneecap, the patient inhales. The patient performs the pulling by their legs by bending it in the kneecap after every exhaling. The amount of repetitions is from 12 to 15 times for each leg.
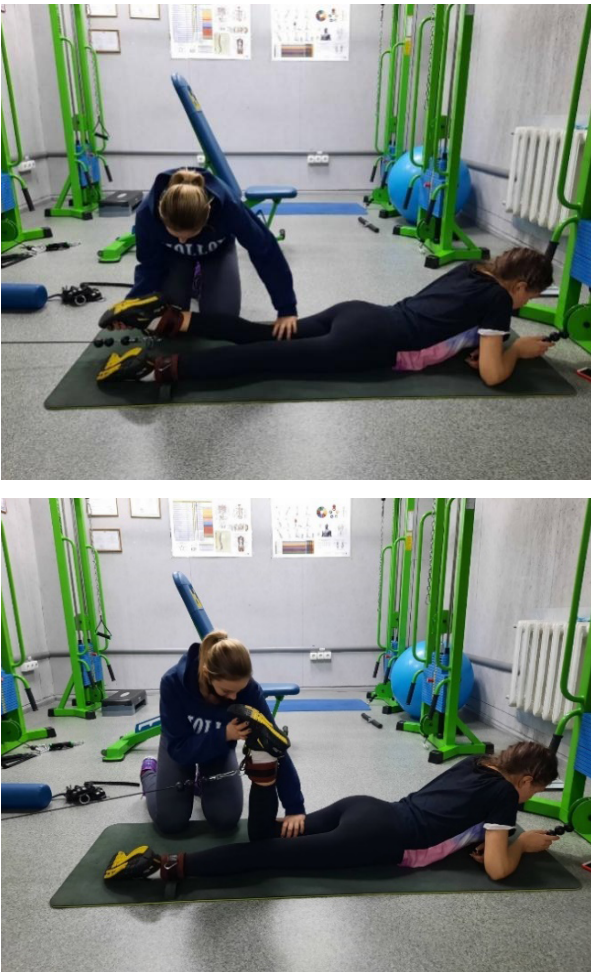

Fig. 8. Execution of the exercise "Enhancement of biceps of the hips"

The exercise "Pulling off the kneecaps towards the abdomen". The exercise is executed for the rehabilitating work with the joints of the legs in a decompressed mode, with the engaged area of the groin, muscle of illium and the lower back, muscular group of the hips, the muscles of the lower back, and the muscles of the rear side of the hips. In the process of stretching out of the leg backwards, there is the extension of the muscles of the frontal side of the surface of the hip. The scheme of execution (Fig. 9) is the following: There is the execution from the higher MTB block in the position of patients standing on their knees and hands. The objec-

tive of the patient is to maximally pull one of the knees to the abdomen with additional weight (20 kilograms). The number of repetitions for each leg must be from 12 to 15 times.

Fig. 9. Execution of the exercise "Pulling off the kneecaps towards the abdomen"

Exercise "Pulling with a straight leg". The application of this exercise is devoted to the restoration of hip muscles, as well as the restoration of their length. The execution of the exercise assures the restoration of blood provision to femoral and lumbar areas, while the restoration of blood provision assures the reduction of swelling and pain relief. The scheme of execution (Fig. 10) is the following: the patient is laid down on their back with their heads towards the MTB stand. The instructor attaches the cuff weighing 15 kilograms to the upper block with the patient's leg. The patient raises the lower limb with the weight, completely stretching out the leg in the area of the knee joint (as wide as it is physiologically possible). While exhaling the patient lowers the leg to the floor level, holding it straight (without bending). The amount of repetitions is from 15 to 20 for each leg.

Fig. 10. Execution of the exercise "Pulling with a straight leg"

"Triad" exercise. This exercise is applied for pain relieving in the arms and in the area of the shoulder joint (with the deep muscles of the shoulder joint being engaged). The scheme of execution (Fig. 11) is the following: the exercise is executed in the laid position. The patient holds the grip from the upper block of MTB attached to the cord in his hands. The weight of the block is 5 kilograms. While exhaling the patient pulls the hand towards their chin in the way to have the rearmost side of their palm pulled to the cheek and their arm being bent in the area of the elbow joint. The amount of repetitions for each hand is 15 times.

"Pull-over" exercise. This exercise is applied for the enhancement of blood circulation in the muscles of the upper part of the torso. It enhances the extension of shoulder joints, along with breath quality and cardio functioning. In the course of the execution of the exercise, abdominal muscles are normally engaged. The scheme of the execution (Fig. 12) is the following: the initial position is with the patient's back towards the trainer, sitting on the versatile bench with the angled tilting. The wide grasp is with the patient's hands of the short grip attached with the cord to the upper block (with a weight of 10 kilograms). Straight-hands-pulling from the rear side of the head is executed to the level

of the chest with exhaling. The amount of repetitions is from 12 to 15 times.

Fig. 11. Execution of "Triad" exercise

Fig. 12. The execution of the "Pull-over" exercise

"Rotation" exercise. This exercise is applied for the enhancement of decompression of the shoulder joint, with the rotators of the shoulder (subscapular, infraspinous, supraspinal, and teres minor muscles) being engaged. The scheme of execution (Fig. 13) is the following: the exercise is executed in a standing position with the patient facing toward the MTB trainer, holding for the additional handles. The arm grip is on the knot attached to the cord from the opposite upper block with a weight of 7.5 kilograms. The patient performs the rotation (the maximal turning) of the shoulder joint inwards.

Fig. 13. Execution of "Rotation" exercise

"Abs enhancement + 5" exercise. This exercise is applied for the strengthening of abdominal muscles and the relaxation of the lower back, as well as for the restoration of blood provision in the muscles of the upper part of the torso. The exercise improves the process of breathing and cardio functioning. In general, this exercise is the combined system of "Abs enhancement" and "Pull-over" exercises. The scheme of execution is in Fig. 14.
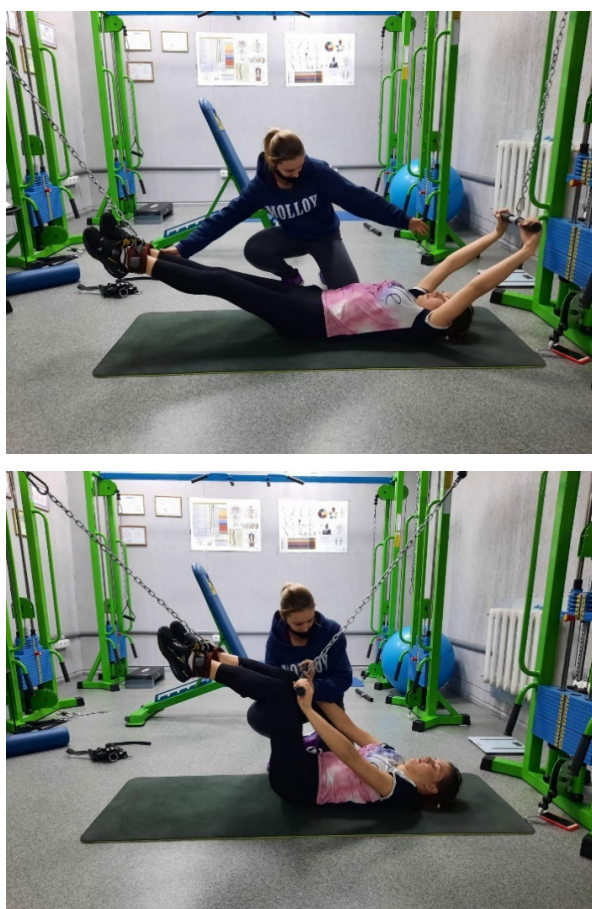
Fig. 14. Execution of "Rotation" exercise

Discussion

The effectiveness of our elaborated program of kinesitherapy classes was verified via the method of electromyographia. The criteria for the estimation of the effectiveness of the experimental program were the velocity of the affection of the impulse in afferent and efferent ways, the duration of M-response, the number of mobile entities in lower limbs, as well as the levels of agitation and depression of the patients.

As a result of our execution of the classes, elaborated according to our experimental program, there were several positive changes in the psychophysical state of the patients aged from 14 to 20 with Charcot-Marie-Tooth nerval amyotrophia disease, as was justified by the positive tendency of monitored indications. There is the highlight of the genuineness of the changes in the indications of the velocity of impulse impacting in lower limbs (average estimate): 37.1 m/s before the experiment and 60.4 m/s after the experiment on the right, with 36.8 m/s before the experiment and 59.4 m/s after the experiment on the left. The average estimate of the duration of M-response was changed from 28.8 m/s at the beginning of the experiment to 19.3 m/s after the experiment in the lower right limb, as well as from 29.1 m/s at the beginning of the experiment to 20 m/s after the experiment in the lower left limb. The average in-

dications of the number of mobile entities in the lower limbs on the right as well as on the left at the beginning of the experiment were 117.9 and 115.9 respectively, while at the end of the experiment the indications were changed to 275.5 on the right and 269.8 on the left.[3]

There is the detection of several positive shiftings of the functional state of the neuromotor system, which admits the delay of pathological motion stereotypes (it is justified by the neurophysiological impact of the exercises, whose mechanism can be explained as the amplification of the influx of neural impulses via the connected canals and the activation of the upper areas of motion analyzer).

As criteria for the estimation of the effectiveness of the experimental program, there was the application of psychodiagnostic methodology (for detection of the levels of agitation and depression) and pedagogical methods (conversations, surveys, and pedagogical supervision). The comparative analysis of depression levels of the patients before and after the execution of our classes, elaborated according to the experimental program indicated that the qualified level of agitation levels was changed, which means that the subdepressive state was changed to a "depressionless" state. In the course of personal conversation within 2 or 3 weeks, patients acquired positive emotions, the amelioration of their mood, there was the highlighted appearance of a sense of relief and pain reduction.

The results concerning the improvement of the psychophysiological state of patients, acquired after the research, correlate with the results of domestic and foreign specialists, which are occupied with the issues concerning physical therapy, and ergotherapy related to Charcot-Marie-Tooth.

We theoretically justified and elaborated the physical therapy program, which provided for the execution of kinesitherapy classes for patients aged from 14 to 20 years old, diagnosed with Charcot-Marie-Tooth nerval amyotrophia. The physical therapy course was composed of 3 cycles of 2 months of classes (6 months in total) and its physical loading gradually was getting complicated. Every class consisted of 3 stages, which are correct breathing, warm-up, and kinesitherapy classes.[1,2,4,8,15]

As a result of the execution of the classes, elaborated according to the experimental program, there is the detection of physical changes in the psychophysical state of patients aged from 14 to 20 years old, diagnosed with Charcot-Marie-Tooth nerval amyotrophia, which is confirmed by the positive tendency of examined indications.[3]

Positive changes in the functional state of the neuromotor system, which provide the possibility of delaying pathological motion stereotypes, it is explained by the neurophysiological impact of the exercises, whose

mechanism is about the amplification of the influx of neural impulses via the canals and the activation of the upper parts of motion analyzer. The application of the elaborated kinesitherapy program assured the restoration of neural connections of the patients, as well as the formation of compensatory mechanisms and provided for the correction of the psychoemotional sphere.

Conclusion

The research on the impact of the application of kinesitherapy in the sphere of rehabilitation of patients with Charcot-Marie-Tooth nerval amyotrophia, provides the possibility for us of making the following general conclusions:

1. Charcot-Marie-Tooth disease is the inherited disease of nerves, which is caused by the mutation, which admits several defects in the proteins of neurons. In the course of the progression of Charcot-Marie-Tooth disease, there is the progression of atrophy of muscular tissues, gradually progressing in the area of knuckles, and there is the deformation of the feet and wrists. Over time the disease progresses, bringing the affection of the muscles of other parts of the body, while, at the same time, only the symptomatic methods of treatment are applied. Methods of physical therapy for people with the consequences of nerval amyotrophia are composed individually for every patient.

2. We theoretically justify and elaborate a physical therapy program, which provided for the execution of kinesitherapy classes for patients aged from 14 to 20 years old, diagnosed with Charcot-Marie-Tooth nerval amyotrophia. The rehabilitation course consists of 3 cycles for 2 months of classes (6 months in total), and physical loadings were getting more complicated over time. Every class consisted of 3 stages: the correct breathing, the warm-up, and the kinesitherapy classes.

3. As a result of the execution of the classes, elaborated according to our experimental program, there are positive changes in the psychophysical state of the patients aged 14 to 20 years old, diagnosed with Charcot-Marie-Tooth nerval amyotrophia, which is justified by the positive tendency of inspected indications: 37.1 m/s before the experiment and 60.4 m/s after the experiment on the right, as well as, 36.8 m/s before the experiment and 59.4 m/s after the experiment on the left. The average estimate of the duration of M-response was changed from 28.8 m/s before the experiment to 19.3 m/s after the experiment in the lower right limb, as well as 29.1 m/s before the experiment and 20.0 m/s after the experiment in the lower left limb. The indications of the number of mobile entities in the lower limbs on the right and on the left at the beginning of the experiment were 117.9 and 115.9 respectively, while at the end of the experiment were changed to 275.5 on the right and 269.8 on the left. Positive changes in the functional state of

the neuromotor system provide for delaying pathological motion stereotypes can be explained by the neurophysiological impact of the exercises, whose mechanism is about the amplification of the influx of neural impulses via canals and activation of upper parts of the motion analyzer. It favoured the restoration of neural connections, and formation of compensatory mechanisms and caused the correction of the psychoemotional sphere of the patients.

4. The comparative analysis of depression levels of the patients before and after the execution of the classes, according to our elaborated experimental program displayed the qualified change of agitation level of the patients – the subdepressive state was changed to a "depressionless" state. On the course of personal communication, over 2 weeks of kinesitherapy classes, patients acquired positive emotions and the improvement of their mood, they started highlighting the demonstration of the sense of relief and pain killing.

Therefore, our elaborated experimental physical therapy program, providing the execution of kinesitherapy classes for patients aged from 14 to 20 years old, diagnosed with Charcot-Marie-Tooth nerval amyotrophia demonstrated its full validity. The prospects of further research can be formed in a way of creation of a more advanced type of physical therapy for patients with nerval amyotrophia via kinesitherapy treatment methods.

Our research does not contradict the opinion of the leading domestic and foreign researchers, concerning the urgent necessity for the elaboration of new theoretical concepts of physical therapy, and ergotherapy for patients, diagnosed with Charcot-Marie-Tooth nerval amyotrophia.

Declarations

Conflicts of interest
The authors declare no competing interests and no conflict of interests.

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 Institute Ethics Committee, National University Yuri Kondratyuk Poltava Polytechnic, Poltava, Ukraine (Ref: NUYKPP/IEC/2022/123). We adhered to the principles of ethics thereafter throughout the study.

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Lee JC, Seo HG, Lee WH, Kim HC, Han TR, Oh BM. Computer-assisted detection of swallowing difficulty. *Comput Methods Programs Biomed*. 2016;134(2):72-78. doi: 10.1016/j.cmpb.2016.07.010

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— ***Article from a journal, number of authors* more than 6**

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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

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— **Websites**

Author AA (if indicated). Webpage title. Name of Website. URL. Published or Updated date. Accessed date.

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— **Book**

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Doane GH, Varcoe C. *Family Nursing as Relational Inquiry: Developing Health– Promoting Practice*. Philadelphia, PA: Lippincott Williams & Wilkins; 2005:25-28.

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— 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.

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