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ORIGINAL PAPER

Selegiline modulates inflammatory indicators in RAW 264.7 macrophages and LPS-aggravated CFA-induced rheumatoid arthritis in rats

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ABSTRACT

Introduction and aim. Rheumatoid arthritis (RA) causes pain, inflammation, and deformities in numerous joints. Monoamine oxidase B (MOA-B) inhibitor selegiline exhibits anti-inflammatory characteristics and has the propensity to scavenge free radicals. Therefore, the aim of this research comprises of assessing the effect of selegiline on proinflammatory cytokines in RAW 264.7 macrophages as well as its capacity to improve various arthritic parameters in rats with lipopolysaccharide (LPS) accelerated complete Freund's adjuvant (CFA) induced RA.

Material and methods. In RAW 264.7 cells (lipopolysaccharide accelerated), nitric oxide (NO), tumor necrosis factor alpha (TNF-α), interleukin 6 (IL-6), inducible nitric oxide synthase (iNOS), and prostaglandin E_2 (PGE_2) were determined after treatment with selegiline. Different arthritic parameters were analyzed after administration of selegiline in LPS accelerated CFA-induced arthritis in rats.

Results. LPS escalates NO, TNF-α, IL-6, iNOS, and PGE_2 quantities in the RAW 264.7 cells, which was minimized by selegiline at 100 µg/mL and 150 µg/mL respectively. In rats, CFA induction causes a decrease in body weight, elevation of paw volume, splenic index, and arthritic index, which are further accelerated by LPS. 20 mg/kg of selegiline managed all these arthritic parameters effectively, including TNF-α, IL-6, and a few other biochemical parameters.

Conclusion. Selegiline may be beneficial in RA extenuating joint and cartilage damage, and modulating inflammatory responses.

Keywords. CFA, cytokines, RAW 264.7, reactive oxygen species, rheumatoid arthritis, selegiline

Introduction

Rheumatoid arthritis (RA) is categorized as symmetrical polyarticular arthritis, predominantly impacting the tiny diarthrodial joints of the limbs, resulting in synovial inflammation.[1] Due to its chronicity, autoimmunity, rapid inducing inflammatory effects on multisystem, its destroys cartilage, bone, joint tissues, articular structure irreversibly resulting untimely death, impairment, and diminished quality of life within contemporary society.[2] Recent studies have provided evidence of synovial cellular infiltration and the presence of peripheral blood inflammatory cells, including monocytes, lymphocytes, and neutrophils.[3] Polymorphonuclear neutrophils and lymphocytes play crucial roles in the progression of

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synovial inflammation and subsequent joint damage.[4] Cytokines and chemokines as primary products of macrophages, together with degradative enzymes, assume a crucial function in the genesis, pathophysiology, and development of RA. Additionally, cytokines may aid in the generation of inducible nitric oxide synthase (iNOS), which in turn triggers the synthesis of local nitric oxide (NO) that results in tissue damage, inflammation, and angiogenesis. It also exhibits excessive activation and proliferation, inducing pronounced inflammation by activating immune cells, stimulating fibroblasts, and polarizing T lymphocytes.[5–7] Furthermore, critical role of macrophages in the process of synovial angiogenesis is widely recognized, which is a fundamental pathogenic mechanism in RA.[8] Correspondingly, Fibroblast-like synoviocytes, which are cells generated from mesenchymal cells, significantly involve in the autoimmunological process of RA. These cells contribute to tissue injury and sustain the intricate disease process by undergoing morphological and phenotypical changes.[9,10] Neutrophils, a crucial kind of leukocyte in inflamed joints, significantly contribute to reactive oxygen species (ROS) production during their breakdown process. This release of ROS contributes to the manifestation of endothelial dysfunction, as well as lipid and protein oxidation. Additionally, the damage caused to DNA by these ROS leads to the creation of auto-antibodies.[11] In fact, it has been found that ROS has the power to control the processes involved in immune cells' activation as well as their activity.[12] The primary objectives of therapeutic interventions for RA encompass the mitigation of joint swelling and discomfort, optimization of joint functionality, and the prevention of joint deterioration and deformity.[13] Treatment methods often involve the use of pharmacological agents in combination with weight-bearing activity, patient education regarding the disease, and periods of rest.[14] In light of current advancements in treatment approaches, it is imperative to emphasize the significance of early identification and intervention in order to mitigate the occurrence of severe impairments and the potential loss of vital physiological capacities despite the beneficial outcomes of glucocorticoids, nonsteroidal anti-inflammatory drugs (NSAIDs) and disease modifying antirheumatic drugs (DMARDs).[15,16] Furthermore, the exorbitant expenses and significant adverse effects pose numerous obstacles for DMARDs employment in the treatment of RA.[17] Selegiline is a chemical moiety having approval from the FDA for treating Parkinson's disease as an adjuvant and major depressive disorder in adults. Its primary mechanism of action involves the inhibition of monoamine oxidase (MAO) impeding the process of reuptake of monoamine neurotransmitters inside the central nervous system, leading to an increase in the quantities of physiologically active monoamines present at the synaptic cleft.[18,19] Selegiline is additionally utilized off-label for the management of attention-deficit hyperactivity disorder.[20] One of the byproducts of the MAO-catalyzed amine oxidation reaction is hydrogen peroxide, which can be further processed into reactive oxygen species to cause cytotoxicity. Thus, reducing oxidative stress may be possible through MAO activity inhibition.[21] Furthermore, the observed effects of selegiline on numerous organs cannot be simply attributed to its inhibition of MAO-B activity. Instead, these effects are believed to stem from selegiline's ability to modulate cellular oxidation pathways and mitochondrial enzymes.[21] The efficacy of selegiline in scavenging free radicals and its anti-inflammatory properties have been previously demonstrated.[22] Hence, based on the aforementioned facts, it is evident that oxidative stress is one of the major pathological pathway of RA leading to destruction of synovial joints and which is again facilitated by inflammatory cytokines, it is justifiable to propose that the utilization of selegiline could potentially yield positive outcomes while managing of RA by impeding the occurrence of oxidative harm and the activation of inflammatory cytokines caused by free radicals.

Aim

The aim of this research encompasses of evaluating the potential contribution of selegiline in the prevention of proinflammatory cytokines in the RAW 264.7 macrophages cell including iNOS mediated NO, as well as its ability to improve various arthritic parameters in complete Freund's adjuvant-induced RA rats, which is further accelerated by lipopolysaccharide (LPS).

Material and methods

The compound selegiline was acquired from Abcam (Product Code: ab120604), a reputable supplier known for providing selegiline with a purity exceeding 99%. This compound is readily soluble in water. The majority of the chemicals utilized in the cell line assay and in vivo animal experiment were obtained from Sigma, while the other chemicals were sourced from reputable suppliers. All the substances utilized in the experiment were of analytical grade.

Cell culture and treatment

The RAW 264.7 cell line, specifically a macrophage cell line, was acquired from the National Centre for Cell Sciences located in Pune, India. According to the protocol followed by Sahlan et al., RAW 264.7 macrophages cells were cultured.[23] Briefly, these cells were nurtured in Dulbecco's Modified Eagle Medium (DMEM) with 10% fetal bovine serum (FBS) and 1% solution of penicillin – streptomycin at 37°C with 5% carbon dioxide followed by subculture until they reached 70–80 % confluence in a humidified incubator. The cells were allowed to grow in fresh growth medium replaced in an interval of 2–3 days following washing. After trypsin-EDTA treatment, the

cells were isolated and plated for testing. Selegiline was separately prepared into 2 mM solution, stored at –20°C.

Cell viability assay (MTT)

The cell viability assay for selegiline in RAW264.7 macrophages was accomplished in accordance with Wen et al. following the capability of viable cells to reduce yellow MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) into a purple formazan product.[24,25] To summarize, $5x10^3$ cells were planted into a 96 well plate following incubation at 5% CO_2 and at 37°C for 24 hours. After discarding the old medium, the cells were supplied with fresh growth medium (180 mL) and 20ml of sample containing selegiline (50, 100, 150, 200 µg/mL respectively) and again incubated for 24 hrs. Later 20 µL of MTT (5 mg/mL) was incorporated to each of the wells and the plate is placed for incubation at 5% CO_2, 37°C for 3 hours. At last, the medium was discarded and in each well 150 µL DMSO was transferred to dissolve the formed formazan. At 490 nm, the absorbance was recorded and cell viability was measured considering untreated cells as control through subsequent mathematical expressions.

$$\text{Cell viability (\%)} = \\ = (\text{absorbance of test/absorbance of control}) \times 100$$

Estimation of iNOS, TNF-α, PGE_2, and IL-6 in LPS-induced RAW 264.7 cells

To estimate different proinflammatory cytokines, 96-well plates have been seeded with $5x10^3$ numbers of RAW 264.7 macrophages followed by incubation with selegiline (10, 50, 100 and 150 µg/mL). After 1 hr LPS (1 µg/mL) is incorporated and incubated for 24 hrs. Cells without LPS or selegiline serves as negative control and Cells with LPS but without selegiline serves as positive control. After centrifugation, the culture supernatant was recovered and the liberation of iNOS, TNF-α, PGE_2, and IL-6 was estimated through ELISA kits following manufactures protocol. All the ELISA kits were obtained from Abcam (ab253219 for iNOS, ab208348 for TNF-α, ab287802 for PGE_2, and ab222503 for IL-6, Cambridge, United Kingdom) Prior to usage, all reagents were equilibrated at room temperature (18–25°C) and prepared accordingly. A volume of 50 µL of each sample, in addition to 50 µL of the antibody cocktail, was introduced into the corresponding wells. Subsequently, the plate was incubated for a duration of 1 hour at room temperature on a plate shaker operating at 400 rpm. Each well was subjected to three rounds of washing using 350 µL of Wash Buffer PT. A volume of 100 µL of tetramethylbenzidine development solution was introduced into each well, followed by an incubation period of 10 minutes in a dark environment on a plate shaker operating at 400 rpm. Following the addition of 100 µL of Stop Solution to each well, the plate was subjected to agitation on a plate shaker for a duration of 1 minute in order to facilitate thorough mixing. Subsequently, the absorbance at a wavelength of 450 nm was measured and recorded. The stock solution used for the standard was maintained at a concentration of 2000 pg/mL with an additional seven serial dilutions, same protocol was performed in order to generate the standard curve. Using the standard curve, the concentration of these cytokines was determined.[26,27]

Estimation of NO in LPS-induced RAW 264.7 cells

Griess reagent was used to quantify the NO level in terms of measuring the nitrite in the supernatant. The Griess reagent was prepared by combining equal volume of Sulfanilamide solution (1% w/v in 5% phosphoric acid) and N-(1-naphthyl) ethylenediamine dihydrochloride (NED) solution (0.1% w/v in distilled water). In short, in a 96-well microplate, 100 µL of the cell culture supernatant was taken in each well and equal volume of Griess reagent was added. A microplate reader measured absorbance at 540 nm after incubating the mixture for 15 minutes. A sodium nitrite standard curve determined the supernatants' nitrite concentration.[28]

Experimental animals

After receiving clearance from the Institutional Animal Ethics Committee (CPCSEA Registration No. 544/PO/C/02/CPCSEA), the college of veterinary science at the Assam Agriculture University in Guwahati, Assam, provided healthy young adult nulliparous and non-pregnant Albino Wistar rats of both genders (M/F). A total of 40 animals were acquired for this experimental technique, ensuring an equal distribution of males and females. The animals were between 150 and 200 grams in weight and 8 to 10 weeks old. They were kept in a well-furnished, well-ventilated polypropylene cage accompanied by diurnal cycle characterizing 12 hours of daylight and 12 hours of darkness, 22±3° C of room temperature with relative humidity of 53–60%. The animals spent five days getting used to the laboratory setting before the experiment, and they had unrestricted access to pelleted food and fresh water.

Experimental design

The experimental procedure described by Brand et al. was employed to produce arthritis in rats.[29] This involved the administration of an emulsion containing collagen type II (CII) with complete Freund's adjuvant (CFA), with minor adjustments made to the original approach. In summary, a drop-wise addition of CII was performed to combine it with CFA, whereby both substances were pre-chilled and had an equivalent concentration of 2 mg/mL. This process aimed to create a CII-CFA emulsion. On day zero, 200 µL of this combination is administrated at the base of the tail intradermally on 34 rats (17 males + 17 females).

No immunization was done in remaining 6 rats and kept for negative control. On the day 11, Animals that had displayed clinical signs after being stimulated with CII-CFA emulsion were randomly divided into four groups consisting of total 24 animals (Gp II – Gp V). These groups received the treatment of selegiline and Methotrexate form day 11 to 27. On the fourteenth day, each animal in these groups was intraperitoneally delivered 100 µL of LPS solution, which was made as 1 mg/mL in phosphate buffered saline (PBS) with 7.2 pH and a molarity of 0.02 M.[30] On the fifteenth day, each animal received an intradermal booster dose of CII–CFA emulsion, with a volume of 100 µL. Each group consists of six animals, with an equal distribution of three females and three males. Group I was designated as negative control where neither immunization nor treatment was provided. Group II was positive control where arthritis was induced with Collagen type II, CFA and LPS. In group III, treatment was done with Methotrexate 2.5 mg/kg i.p. after induction of arthritis. Group IV and group V represents the animals treated with selegiline 10 mg/kg and 20 mg/kg i.p. respectively after induction of arthritis

Evaluation of RA

The evaluation and severity of RA were conducted by measuring the volume of the paws in bilateral pairs and calculating the mean value based on three repeated measurements (n=3). The quantitative arthritis scoring in this study, as defined by De et al., involves assigning a value between 0 and 4 to several parameters based on the degree of erythema and swelling seen.[31]

Evaluated parameters

The evaluation starts with measuring the body weight of the rats before stimulation in combination with paw volume and at 7-day intervals up to 28 days following CII+CFA inoculation. The percentage change in bodyweight and paw volume were calculated. Hind paws radiograph was captured and the degree of bone damage was evaluated from 0 to 5 based on changes in bone density, joint space, and exudates as no change, slight change, slightly moderate, moderate, slightly severe and severe respectively. Every animal was subjected to sacrifice 24 hours subsequent to the final intervention, on day 28, and the plasma was extracted from the heparinized blood collected through retro-orbital plexus following centrifugation at 2000g×10 minutes at stored at –20°C before being promptly processed for biochemical analysis. The spleens were taken out from every rat, promptly weighed, and spleen to body weight ratio was followed to compute the splenic index.

Statistical analysis

The findings are displayed in accordance with mean ± standard error of the mean (SEM). Tukey HSD test, was employed subsequent to the completion of a one-way analysis of variance (ANOVA) in order to determine the statistical significance (p value) in SPSS (IBM, Armonk, NY, USA). The significance limits were established to be *$p<0.05$ and **$p<0.01$, indicating statistical significance and strong statistical significance, respectively.

Results

Cell viability assay

Selegiline was tested in vitro to examine how long previously cultivated RAW 264.7 cells could survive in 50, 100, 150 and 200 µg/mL of concentration respectively. While calculating the cell viability (%) after recording the absorbance at 490 nm, it was found to be 98.37±0.65, 98.32±0.60, 97.97±1.06, 97.88±0.75, 96.56±1 respectively for the aforementioned concentrations while the p value was appeared to be 0.133 ($p>0.05$) that represents of being no significantly different among the groups. Hence, 50 µg/mL to 200 µg/mL of selegiline exhibited no cytotoxic effects on RAW 264.7 cells, as evidenced by the absence of cell viability suppression (Fig. 1) nor increase any cell viability compared to the negative control. The cell viability of 200 µg/mL selegiline exhibited the lowest but non-significant results. Therefore, for subsequent investigations on anti-inflammatory markers in RAW264.7 cells, concentrations up to 150 µg/mL are deemed appropriate.

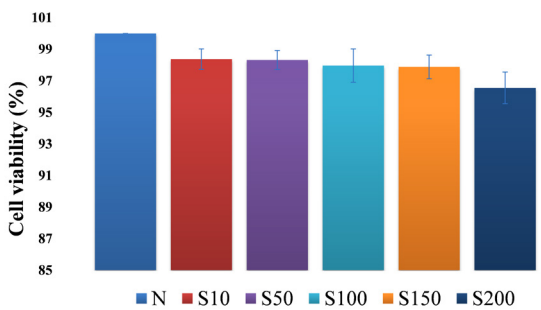

Fig. 1. Determination of cell viability through MTT assay, "N" represents RAW264.7 cells without treatment, S10, S50, S100, S150 and S200 represents RAW264.7 cells intervened with selegiline 10, 50, 100, 150, 200 µg/mL

ELISA for TNF-α, IL-6 estimations

The involvement of inflammatory cytokines in the pathophysiology and progression of RA has been well-established.[32] The quantification of iNOS, TNF-α, PGE_2, NO, and IL-6 release was conducted using ELISA kits while the standard curve provides the concentration of these cytokines. After incubation with selegiline in the concentration of 10, 50, 100, 150 µg/mL, LPS was incorporated to the RAW264.7 cell and again incubated for 24 hours. Fig. 2A and 2B represents highly significant ($p<0.01$) elevation in the value of TNF-α and IL-6 in RAW264.7 cells after incubation with LPS. 100 µg/mL

and 150 µg/mL of selegiline quite capable of decrease in the production of TNF-α in contrast to LPS provoked cells ($p<0.05$). However, 10 and 50 µg/mL of selegiline did not show a notable drop in TNF-α production (Fig. 2A). On the other hand, in the context of assessing the inhibitory potential of selegiline on IL-6 in LPS-induced RAW264.7 macrophages, 10 µg/mL of selegiline did not demonstrate any effectiveness ($p=0.103$). Notably, dose-dependent reduction in IL-6 production was executed by selegiline at concentrations of 50, 100, and 150 µg/mL in LPS-induced cells, with statistical significance ($p<0.01$) (Fig. 2B).

ELISA for iNOS, PGE_2 estimation

In order to provide more clarification of selegiline curbing inflammation, PGE_2 release was assessed in LPS provoked RAW 264.7 cells. The findings are presented in Fig. 2C, where it is observed that treatment with 1 µg/mL LPS greatly enhanced the level of prostaglandin E_2 (PGE_2) ($p<0.01$). Conversely, the concentration of only 150 µg/mL of selegiline resulted in reduction in PGE_2 production in the respective cell line ($p<0.05$), while the other incorporated concentrations didn't have any significant effect in case of PGE_2 reduction. Fig. 2D denotes the effect of selegiline mitigating the production of iNOS after treating with LPS in RAW264.7 cells. iNOS may be made to continue producing excessive NO after being stimulated by the immune system or microbes, which in turn release more inflammatory cytokines.[33] Following treatment with LPS, the level of iNOS is increased significantly in RAW264.7 cells ($p<0.01$). This study revealed that the level of iNOS in LPS induced cell did not exhibit a significant drop following treatment with a concentration of 10 µg/mL of selegiline (Fig. 2D). But interestingly, 100 and 150 µg/mL of selegiline significantly diminished iNOS production in LPS induced RAW264.7 cells ($p<0.01$) whereas 50 µg/mL made it little significant ($p<0.05$).

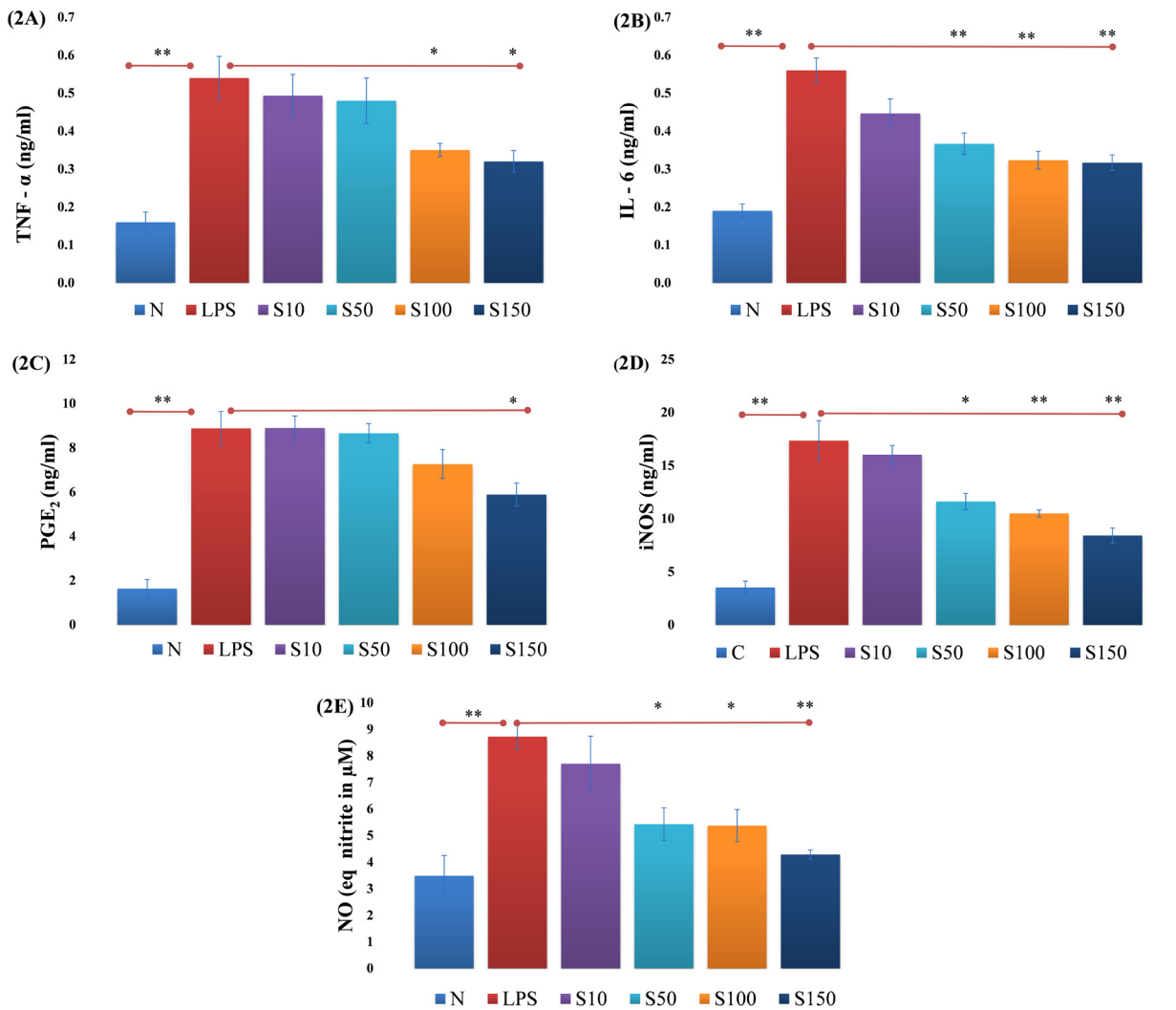

Fig. 2A. Determination of the level of TNF-α, **2B.** Determination of the level of IL- 6, **2C.** Determination of the level of PGE_2, **2D.** Determination of the level of iNOS, **2E.** Determination of the level of NO, "N" depicts RAW264.7 cells without treatment, "LPS" depicts RAW264.7 cell treated with Lipopolysaccharide 1 µg/mL. S10, S50, S100 and S150 depicts RAW264.7 cells intervened with 10, 50, 100 and 150 µg/mL of selegiline along with lipopolysaccharide 1 µg/mL

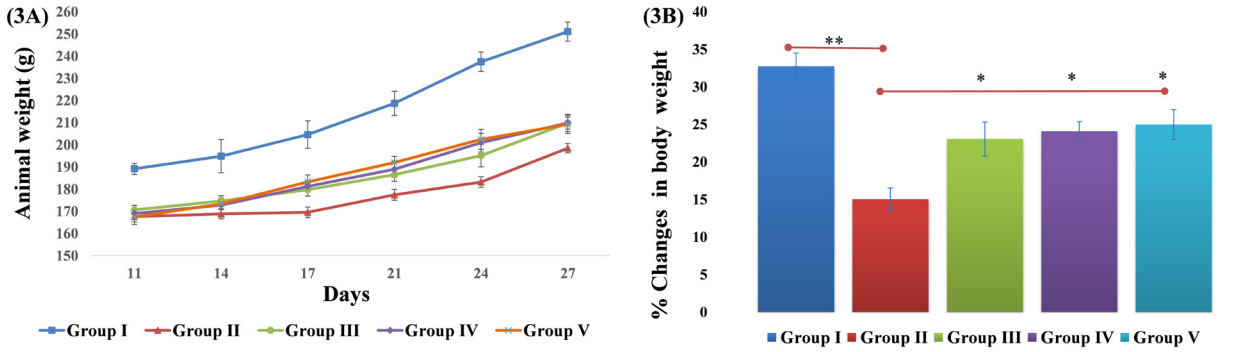

Fig. 3A. Changes in the body weight of the rats, **3B.** Percentage changes in the body weight of rat, group I is negative control, group II is positive control, group III, IV and V denotes animals treated with methotrexate 2.5 mg/kg, selegiline 10 and 20 mg/kg respectively after inducing RA

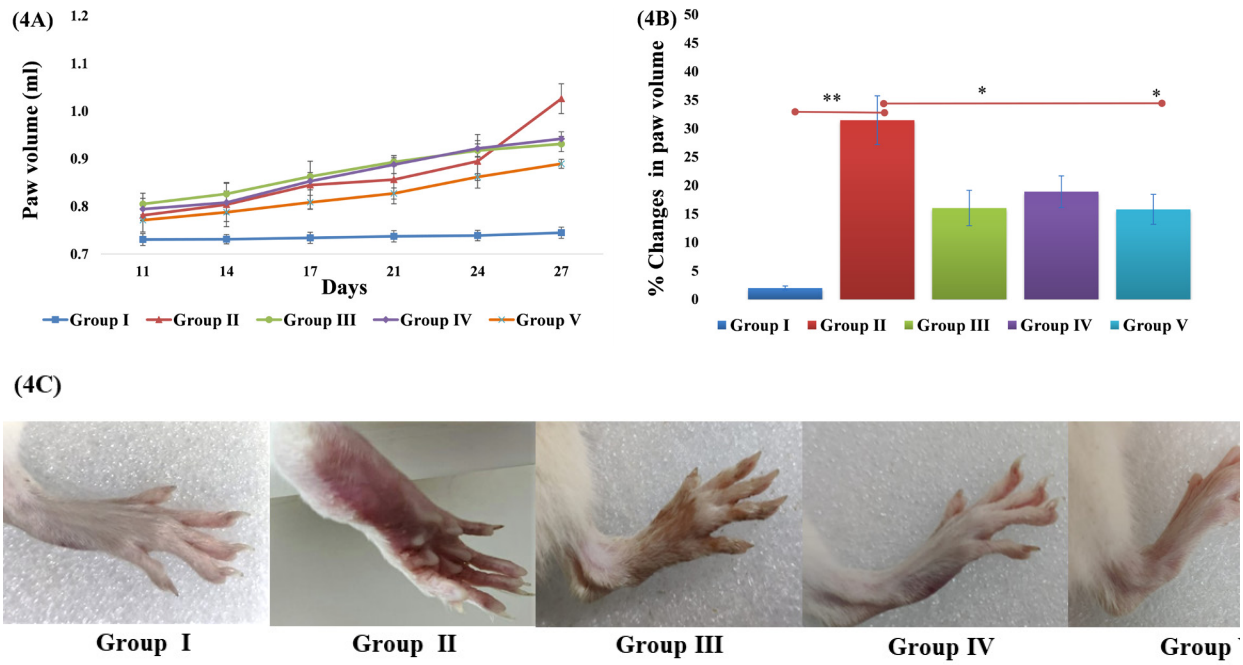

Fig. 4A. Changes in the paw volume of rats, **4B.** Percentage changes in the paw volume of rats. **4C.** Macroscopical study. Group I is negative control, group II is positive control, group III, IV and V denotes animals treated with Methotrexate 2.5 mg/kg, selegiline 10 and 20 mg/kg respectively after inducing RA

Determination of NO level

Increase in the level of NO in synovial fluid as well as in serum is has been determined to be linked with the pathogenesis of RA.[34] In our present study increase in the NO level was highly significant in the LPS (1 µg/mL) stimulated cells ($p<0.01$). The findings of this investigation indicate that the concentration of NO in LPS-induced cells did not demonstrate a statistically significant decrease after being treated with a dose of 10 µg/mL of selegiline, as seen in Figure 5. However, it is noteworthy that the efficacy in reducing NO generation in LPS provoked RAW264.7 cells was shown to be statistically significant for 50 and 100 µg/mL of selegiline ($p<0.05$), and highly significant at a dose of 150 µg/mL ($p<0.01$).

Effect of selegiline on body weight and paw volume of the animals

To evaluate the consequence of selegiline on RA, LPS provoked CFA induced arthritis in rat model was implemented. CFA rats exhibit the highest degree of similarity to human RA in terms of etiology, immunology, and genetical features.[35] Rats body weight were constantly monitored following incorporation of Collagen type II and CFA emulsion and the paw volume was recorded using digital plethysmometer at the interval of 3 days. The reduced in the body weight and elevation of paw volume was observed in animals following RA pathological mechanism (Fig. 3A and 4A). Observing the degree of erythema and swelling, division of rats were carried out and intervention was employed. In Figure 3B, our findings indicate that 10 and 20 µg/mL

of selegiline, as well as methotrexate, had significant efficacy in promoting weight gain in rats with chemically induced arthritis. This was determined by assessing the percentage change in body weight after 28 days ($p<0.05$). On the contrary, only 20 µg/mL of selegiline was capable to reduce the paw swelling in arthritic induced rats ($p<0.05$), while measuring at the end of 27 days as represented in Fig. 4B and 4C. Selegiline thereby effectively improved the delayed weight gain of rats brought on by LPS stimulated immunization, as well as reduced erythema and oedema.

Arthritic index by radiographic technique (X ray)

All the rats were proceeded for radiographic analysis with a 55kVp exposure for 6.4 mAs at day 28. As shown in the Figure 5A and 5B the degree of bone damage (arthritic index) was calculated from 0 to 5 based on changes in bone density, joint space, and exudates. A prominent bony erosion and swelling of soft tissue was observed in positive control group leading to wrecking of bones and stenosis of joint spaces. Interestingly, selegiline 20 µg/mL (Group V), also standard methotrexate (Group III) was significantly effective ($p<0.05$) in reducing arthritic index in immunized rats manifested by slowing down cartilage destruction and synovial hyperplasia. Unfortunately, selegiline 10 µg/mL failed to produce any noticeable effects.

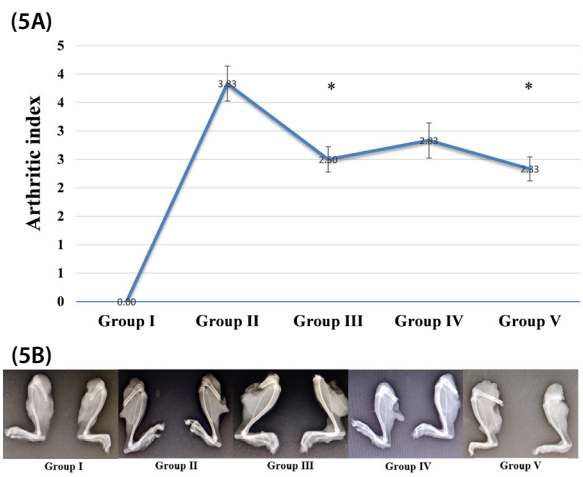

Fig. 5A. Arthritic index of the rats, **5B.** Radiographical study, group I is negative control, group II is positive control, group III, IV and V denotes animals treated with methotrexate 2.5 mg/kg, selegiline and 20 mg/kg respectively after inducing RA

Splenic index of the animals

After sacrificing the rats at day 28, spleens were taken out and spleen to body weight ratio was followed to compute the splenic index.[36] Fig. 6 depicts the splenic index for the rats of all the groups. The splenic index in group I (negative control) is reported as 0.31±0.02, but the arthritic rats without intervention (group II) had a significantly higher splenic index of 0.46±0.02. It is fascinating to mention that selegiline at the concentration of 20 µg/mL is highly efficient to reduce the splenic index up to 0.37±0.01 ($p<0.05$). Selegiline at a dose of 10 µg/mL was found to have a splenic index of 0.42±0.03, without denoting any statistical significance.

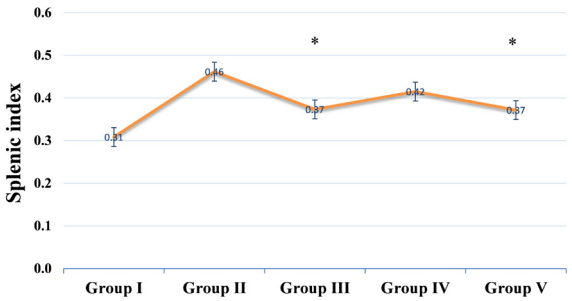

Fig. 6. Splenic index of the rats, group I is negative control, group II is positive control, group III, IV and V denotes animals treated with methotrexate 2.5 mg/kg, selegiline and 20 mg/kg respectively after inducing RA

Table 1. Evaluated biochemical parameters in experimental rats[a]

Parameter	Unit	Group I	Group II	Group III	Group IV	Group V
Erythocytes	($\times 10^6$/µL)	9.4±0.89	7.1±1.12	8.9±1.25	7.89±2.1	7.9±1.67
Leukocytes	($\times 10^3$/µL)	12±1.86	15.1±2.45	12.89±1.89	14.53±2.45	14.6±2.21
Haemoglobin	g/dl	11.3±1.99	9.3±2.3	10.1±3.42	9.8±2.35	9.7±1.94
Neutrophils	($\times 10^3$/µL)	2.4±0.45	3.02±0.99	2.85±0.77	2.99±0.88	2.19±1.01*
Lymphocyte	($\times 10^3$/µL)	8.52±2.1	10.65±1.49	9.26±2.01*	10.10±1.87	9.15±2.33*
Eosinophils	($\times 10^3$/µL)	0.6±0.75	0.83±0.86	0.73±0.66	0.83±0.55	0.82±0.42
Monocytes	($\times 10^3$/µL)	0.36±0.22	0.5±0.31	0.46±0.42	0.49±0.39	0.46±0.44
Basophils	($\times 10^3$/µL)	0.12±0.05	0.15±0.07	0.15±0.08	0.15±0.07	0.14±0.08
AST	U/L	82.9±6.46	101.75±5.5	94.33±4.9	98.59±5.51	98.99±4.38
ALT	U/L	49.8±3.48	67.56±4.6	50.19±2.8	60.23±4.12	58.55±3.78
ALP	U/L	210.3±5.49	250.65±4.1	230.86±3.88	235.35±3.87	221.35±4.04
TNF-α	pg/mL	195.87±3.56	358.66±2.45	285.38±2.79	323.48±2.15	295.64±3.15*
IL-6	pg/mL	249.56±2.95	389.47±3.45	315.43±3.78	335.35±3.58*	305.54±2.49*

[a] Animals were sacrificed on day 28 and plasma was extracted from heparinized blood collected from each animals, plasma was stored at –20°C and proceeded for biochemical analysis, group I is negative control, group II is positive control, group III, IV and V denotes animals treated with methotrexate 2.5 mg/kg, selegiline 10 and 20mg/kg respectively after inducing RA, the data are shown as mean ± SEM and one way ANOVA followed by Tukey HSD test was performed, *$p<0.05$ represents statistical significance while comparing with positive control

Biochemical analysis

The impact of selegiline on the hematological parameters of rats with induced arthritis was also examined. CII+CFA group of rats demonstrated white blood cell elevation, red blood cell and hemoglobin diminution

with regard to the normal group. Although, rats that were treated with standard methotrexate and selegiline exhibited a rise in red blood cell count, hemoglobin levels, and a decrease in white blood cell count, as compared to the rats in the arthritic condition, none of the values are statistically significant. However, selegiline 20 μg/mL (group V) decrease neutrophil and lymphocytes count significantly ($p<0.05$) in arthritis induced rats. Both methotrexate and selegiline decrease AST, ALT and ALP level non significantly in the blood of arthritis induced rats. The TNF-α and IL-6 were appeared to be much higher in Arthritic untreated group. Interestingly, selegiline 20 μg/mL (group V) can significantly decrease these cytokines levels ($p<0.05$). To a surprise 10 μg/mL (group IV) of selegiline can also decrease the IL-6 to a significant level whereas the effect of selegiline 20 μg/mL is higher than the standard methotrexate. All the results are represented in Table 1.

Discussion

Numerous proinflammatory cytokines and immunomodulatory cells take part in RA, despite the fact that the exact cause is still being investigated and therefore significant number of patients with RA do not respond adequately or experience limited effectiveness after the treatment with DMARDs.[37,38] A lot of researches are going on in search of new potential drug candidate either as a regular or supportive therapy. A lot of evidence suggests the potential involvement of ROS in the elevation of oxidative stress contributing to the pathogenesis of RA.[39] Selegiline is mainly a synthetic compound primarily employed for the treatment of Parkinsons disease through the inhibition of mono amine oxidase enzyme.[21] Selegiline effectiveness in mitigating oxidative damage caused by free radicals has been studies previously and discovered to be efficient. This may be attributed to the inclusion of a propargylamine moiety that possesses an acetylene group. This functional group facilitates the donation of proton ions, enabling the scavenging of free radicals. Additionally, selegiline has been found to exhibit in vitro antiarthritic action.[22,40] In the present study, the selegiline was evaluated for antirheumatic effect both in RAW 264.7 macrophages cell line and LPS accelerated CFA induced RA in rats model. Significantly, selegiline was found to be efficient with intriguing potential in reducing proinflammatory cytokines in RAW 264.7 macrophages as well as improving different conditions such as body weight, arthritic index, splenic index, paw volume, joint and cartilage deformation in arthritic rats. Macrophages fulfill crucial duties in the initiation and progression of inflammatory processes in respond to pathogenic assaults, such as infections, and perform immune regulatory activities.[41] The RAW 264.7 cells exhibit characteristics similar to macrophages. The RAW 264.7 cells demonstrate an upregulation in the generation of NO and an enhancement in phagocytic activity by inducing iNOS activity while stimulated with LPS.[42] Therefore, the suppression of these excessive synthesis of cytokines by blocking iNOS may present a remarkable remedy while treating disorders characterized by underlying inflammation. Our findings revealed a notable augmentation of iNOS, TNF-α, PGE_2, NO, and IL-6 in LPS provoked RAW 264.7 cell line. Among all these cytokines, it was observed that selegiline 150 μg/mL is highly significant in attenuating iNOS, NO and IL-6 generation in LPS provoked RAW264.7 cell line. By promoting the migration of lymphocytes and macrophages and the synthesis of inflammatory mediators like TNF-α, IL-6, IL-1, and IL-10, the CFA has been shown to increase pervasive inflammation. Despite the several proinflammatory mediators, IL-6 is considered to have the most significant impact on arthritis generated in rats by CFA.[43] These mediators subsequently results in harm to the tissue and articular cartilages, ultimately giving rise to the characteristic signs and symptoms associated with RA.[44,45] Further these release were boosted by incorporation of LPS in experimental rats.[46] While evaluating the biochemical parameters in the blood of CFA induced arthritic rats, selegiline 20 μg/mL illustrate significantly lower in both TNF-α and IL-6. The overexpression of TNF-α, pertaining to cytokines surplus including IL-6, is currently well established to cause synovial inflammation and joint degeneration in RA patients.[47] Although, IL-6 typically controls the acute phase response, both TNF-α and IL-6 are enormously essential regulators of inflammation throughout the progression of RA.[48] Therefore, evaluating the level of TNF-α and IL-6 became enormously vital in the serum of CFA induced arthritis rats including the intervention groups. In addition, it was found that rats treated with CFA had much larger paws, lighter bodies, gradually deteriorating joints and cartilage, and higher arthritic and splenic indexes. Numerous researchers propose that the immunological response in RA inflammation may be initiated potentially through inflammatory regulator release, endothelial dysfunction, involving iNOS activation and an augmented generation of myeloperoxidase. Spleen is one of the major organs for the regulation of immune response producing more immune cell and filtering cell that are dead and hence enlargement of spleen is a common condition associated with chronic inflammatory disorder.[49] The observed elevation in the spleen index in rats induced with CFA may be attributed to the process of immune cell filtration, which includes the removal of deceased red and white blood cells. Our study represents a commendable improvement in paw swelling and splenic index in day 27 than day 11 indicating a remarkable effect of selegiline in the disease progression of RA. This result can

be attributed to the significant reduction in neutrophil and lymphocyte, the major inflammatory cells in the blood of selegiline treated CFA induced arthritic rats. Although selegiline were able to change certain other biochemical markers in a favourable way, but the results were not found to be statistically significant. At elevated doses, selegiline has the capability to hinder the activity of MAO-A and enhance the functioning of catecholaminergic neurons by means unrelated to MAO-B inhibition. These mechanisms include the stability of mitochondrial membrane potential, as well as the exertion of anti-apoptotic and antioxidant actions.[50] There exists a body of research indicating a potential involvement of MAO-A in specific inflammatory conditions such as rheumatoid arthritis; however, the underlying mechanisms remain inadequately comprehended. The potential effects of this substance include the modulation of cytokine production and immunological responses, as well as the generation of ROS through the byproduct of monoamine metabolism developing joint inflammation.[51] Collectively, all these findings hold the possibility of selegiline to counteract the inflammation and deformation of joints, bones and cartilages in CFA induced rats by inhibiting IL6, iNOS, limiting the excess production of major inflammatory cells and exaggeration of NO facilitated oxidative stress and also by suppressing peripheral MAO-A enzyme. This research demonstrates possible promise in investigating the therapeutic effectiveness of selegiline for the treatment of RA. Nevertheless, it is important to acknowledge that the study has several limitations. This study is of a preclinical nature, with a primary emphasis on conducting in vitro studies using cell lines and in vivo investigations utilizing animal models. It is important to note that the findings from these experiments may not necessarily reflect human reactions with complete accuracy. Furthermore, a restricted quantity of experimental animals is employed. The study fails to examine the potential adverse effects or safety considerations associated with high-dose administration of selegiline.

Conclusion

The research presented in this article provides inside into the potential therapeutic efficacy of selegiline in the context of RA. This study explored its antirheumatic effects both in vitro, using RAW 264.7 macrophages, and in vivo, employing CFA induced RA in rats. The results demonstrated that, in LPS provoked RAW 264.7 macrophages, selegiline effectively reduced proinflammatory cytokines, iNOS, NO, and IL-6, for instance. In the CFA-induced arthritic rat model, selegiline treatment leads to improvements in various disease parameters, such as body weight, arthritic index, splenic index, paw volume, and joint and cartilage destruction. Notably, in the serum of CFA-induced arthritic rats, selegiline significantly lowered TNF-α and IL-6 quantity. The outcomes reveal that selegiline highlights potential as a prospective therapy for RA by mitigating inflammation, preventing joint and cartilage damage, and modulating immune responses. In light of the identified limitations of this study, it is imperative to conduct more research and clinical trials in order to substantiate the findings and explore selegiline's potential as a viable treatment option for patients suffering from this debilitating autoimmune disease.

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Author contributions

Conceptualization, A.B.H. and P.C.; Methodology, A.B.H. and P.C.; Software, P.C.; Validation, A.B.H. and P.C; Formal Analysis, A.B.H. and P.C.; Investigation, P.C.; Resources, A.B.H. and P.C.; Data Curation, P.C.; Writing – Original Draft Preparation, P.C.; Writing – Review & Editing, P.C.; Supervision, A.B.H.

Conflicts of interest

The authors assert that they have no conflicts of interest.

Data availability

The authors can provide the data upon request.

Ethics approval

The Institutional Animal Ethics Committee accepted the study's protocol, and it was carried out in conformity with CCSEA regulations (CPCSEA Registration No. 544/PO/C/02/CPCSEA).

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ORIGINAL PAPER

Traditional teaching method versus simulation-based teaching method in the prevention of medication errors among nursing students

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ABSTRACT

Introduction and aim. The effective and safe administration of medication is the responsibility of every health care provider involved in patient care. One of the biggest problems with nursing education is the gap between theory and practice, which pave ways for medication errors to occur at any level of the medication administration process. This can be rectified by using an efficient teaching strategy that harmoniously blends nursing theory and practical skills. the aim of the study was to evaluate the effectiveness of the traditional teaching method versus simulation-based teaching method on level of knowledge, attitude, and practice on prevention of medication errors among nursing students in selected colleges, Puducherry.

Material and methods. The research approach and design used for the study were quantitative approach and quasi-experimental pre and post-test control group design respectively. The study settings were selected 4 nursing colleges in Puducherry. The sample size was 100 (50 in experimental group I and 50 in experimental group II) which was selected by using simple random and stratified sampling technique. pre-test was done to assess the level of knowledge, attitude and practice on prevention of medication error for both experimental groups utilizing the self-administered knowledge, attitude questionnaire and checklist. The experimental group I received traditional teaching method and experimental group II received simulation-based teaching method. Post-test was done after one week using the same tool.

Results. The study results revealed that out of 100 nursing students, majority 35 (70%) and 38 (76%) of the nursing students were in the age group of 20–21 years, 40 (80%) and 39 (78%) of them were female, 50 (100%) and 41 (82%) of them were staying as day scholars, 40 (80%) and 41 (82%) had one attempt to clear the pharmacology subject in the experimental group I and II respectively. The level of knowledge, attitude, practice showed a statistically significance difference at $p<0.05$ between the pre and post-test within the experimental group I and II respectively. The effective mean scores of knowledge, attitude and practice showed a statistically significance difference at $p<0.05$ between the experimental group I and II respectively, revealed that the simulation-based teaching method was more effective over the traditional teaching method. There was a significant positive correlation at $p<0.05$ exist between the level of knowledge and attitude, knowledge and practice in the experimental group I. The association between the post-test level of attitude and the gender variable showed a statistically significant at $p<0.05$ in the experimental group II.

Conclusion. The study concluded that the simulation-based teaching method was effective which can be utilized as a means to educate the nursing students during their academic performance.

Keywords. nursing students, prevention of medication error, simulation-based teaching method, traditional teaching method

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Introduction

Patient safety is the primary objective of high-quality healthcare. It is an important component for both patients and healthcare professionals. Every person in the world will use medications to prevent and cure sickness at some point in their lives. Patients must receive the appropriate medication during the course of their medication therapy at the proper dosage, concentration, and timing.[1] However, medication can be fatal if it is inappropriately stored, prescribed, dispensed, administered and monitored inadequately. Globally, the cost associated with medication errors has been estimated at US$ 42 billion annually or almost 1% of total global health expenditure.[2]

According to the National Patient Safety in the UK and the Institute of Medicine in the US, medication errors cause a substantial mortality rate in each year. According to the Institute of Medicine (USA) 44,000 to 98,000 deaths, occur in each year because of medical errors. Among them, 7,000 deaths are linked to medication errors. In India, 5.2 million medication errors occur in each year.[3]

The healthcare systems are highly complex because it requires cooperation and collaboration between numerous organisations, different professions, and technical assistance. The complexity of the health care system may allow for the possibility of errors and increase the consequences of such medication errors. Any stage in the medication administration process, including the selection of medicine, drug preparation, dispensing, and administration, is prone to medication errors. Similarly, patients who are the ultimate consumers of medications also make mistakes at any point in their treatment. Thirty pharmacovigilance centres were recently founded in India and are supported by the World Bank. These centres have been monitoring cases of adverse medication reactions.[4]

According to the Food and Drug Administration (FDA), a medication error is defined as any preventable event that may cause or lead to inappropriate medication use or patient harm while the medication is in the control of the healthcare professional, patient, or consumer. Medication errors are one of the most common patient safety hazards in many countries. They are typically caused by a lack of collaboration among health care professionals.[5]

Medication administration errors may be made by the patient or the healthcare professional. Communication is a big part of the medication administration issue. Patients frequently aren't aware that mistakes can occur and don't actively participate in understanding what is being said to them. Medication errors are commonly recognised when the patient develops clinical manifestations after the medication has been taken and alerting the healthcare professionals. The basic approaches for identifying and evaluating medication errors are spontaneous reporting, medication chart examination, analysis of medical prescriptions, and direct observation.[6] Health professionals do not purposefully make medication errors. They receive training to provide "error-free" medical care. When mistakes are discovered, however, there is a tendency to "blame" the professionals responsible for the error. Sometimes a person receives a formal punishment from their professional bodies, which may include fines, licence suspensions, or even licence revocation. Who was responsible for a medication error is less important than what, how and why it happened. Instead of focusing on the healthcare worker who made the error, the first stage in any investigation into medication errors should be a review of the drug use and delivery pathways within a healthcare system.[7]

Intravenous administration of medicine is associated with the highest medication error frequencies. Intravenous medication errors have more serious consequences for patients than any other administration route because the bioavailability of intravenous-administered medications is high. The therapeutic dose range for intravenous drugs is generally low, and the effects of medication are difficult to reverse. Many intravenous medication administrations are high-alert medications. If administered incorrectly, it also carries a higher risk of seriously harming patients. Therefore, efforts must be made to educate the health care professionals, especially nurses who play a crucial role in the administration of medication.[8] The lack of coordination between theory and practices is one of the main issues with nursing education. The actual application of academic information is challenging for nursing students at least in India. The gap between theory and practices makes learning more difficult, and a student's professional integration suffers from a lack of comprehension of nursing terms and concepts. This is accomplished by having a thorough understanding of healthcare science, where nursing theory and practical abilities are harmoniously merged. A teaching strategy that goes in this direction is simulation-based teaching.[9]

Adult learners can gain skills, competences, knowledge, or behaviours by putting themselves in situations that are similar to those encountered in real life. The purpose of the simulation teaching methods is to address issues that occur in real-life settings and thoroughly examine them. According to Fink, "Simulation is the controlled representation of reality". The learner acts in a simulation, the simulation reacts, and the learner learns from the feedback. Simulation is the recreation of the most important parts of a real-life event. Simulation is not a technology but rather an instructional technique. It is a technique that can help in creating a successful and enjoyable experience. It engages learners by putting them in the real-life struggle. In recent decades, the simulation technique has been used successfully in education.[10]

Simulation is a teaching and training method for nursing students that attempts to make them properly

understand and enhance. It also improves the knowledge, skills, and attitudes in all nursing care. This approach reduces the likelihood that students will carry out their duties improperly. It also allows them to interact with and experience a real medical environment.[11] Simulators can be divided into low-fidelity, medium-fidelity, and high-fidelity based on how closely they resemble reality. Simulators with low fidelity are used to educate beginners on the fundamentals of technical skills. Simulators with moderate fidelity are used to introduce and further understand a spectrum of increasingly complicated competencies. High-fidelity simulators have the ability to speak, breathe, blink, and react to physical and pharmacological interventions either automatically or manually.[12]

The National Reference Simulation Centre (NRSC) was founded in October 2018 by a Tetra Partite collaboration between the Indian Nursing Council (INC), Jhpiego, Laerdal, and SGT University Delhi with the goal of implementing inter-professional education through simulation-based education. It was designed to complement the clinical components of all four years of the B.Sc. Nursing programme, including Nursing Foundations, Medical Surgical Nursing I and II, Child Health Nursing, OBG Nursing, and Community Health Nursing. Nursing professional bodies around the world have embraced simulation (National League for Nursing, Nursing & Midwifery Council).[13]

Recently the use of simulation in nursing education has increased because of growing awareness of simulation's availability and utility in nursing education. Now a days simulators are becoming more affordable. The awareness of the importance of patient safety and the scientific data supporting the use of simulation in skill development among health care professionals are increasing.[14]

Aim

To evaluate the effectiveness of the traditional teaching method versus simulation-based teaching method on level of knowledge, attitude, and practice on prevention of medication errors among nursing students in selected colleges, Puducherry.

Material and methods

Research approach and design

A quantitative research approach was adopted for this research study. A quasi-experimental, non-randomized pre-test and post-test control group design was adopted for this research study.

Variables

- Independent variable: traditional teaching method and simulation-based teaching method.
- Dependent variable: level of knowledge, attitude and practice on prevention of medication errors

Study setting

The city of Puducherry is located in the Union Territory of Puducherry. There are around 14 stand-alone institutions and 76 colleges which are affiliated to Pondicherry University which is a central university. There are around 10 colleges of nursing of which 8 are affiliated to Pondicherry University, which are considered for this study. The total student strength of final-year B.Sc. nursing students ranged from 36 to 60 in each college which worked to 262.

Population

All nursing students studying in the colleges of nursing in Puducherry.

Sample

Nursing students studying in selected colleges of nursing in Puducherry who fulfilled the inclusion criteria and were available during the period of study.

Sample size calculation

Calculated sample size to compare two means with a given power

$n = [Z(1-(\alpha/2)) + Z(1-ß))2] * (2\sigma2) (\div d2)$

Where n is the required sample size: $Z(1-(\alpha/2)) + Z(1-ß))$ were values from the standard normal distribution that account for the chance of type I error and type II error; α was the standard deviation and d was the effect size which was the difference between the means.

Were:

Alpha value = α = 1.96% CI

80% power value = ß = 0.84

Standard deviation = 2.669 (2.7)

Effect size (difference between the means) = 3

n = ((1.96+0.84) 2 x 2(2.7) 2) / 1.5 2

n = 114.3 / 2.25 = 50 for each group.

Total sample size was 50 (experimental group I) + 50 (experimental group II) = 100

Sample size

The total sample size was 100. 50 in experimental group I and 50 in experimental group II.

Sampling technique

The simple random sampling technique was adopted for college selection, and the stratified sampling technique was adopted for sample selection.

Sampling criteria

Inclusion Criteria

Students who were:

- both male and female,
- willing to participate in the study,
- available during data collection period.

Exclusion Criteria
Students who were:
- having arrear in pharmacology subject,
- had previous experience with simulation,
- sick and on leave.

Ethical consideration
Ethical clearance was obtained from the IEC. The permission from the individual college authorities and an informed consent from each of the participants were obtained.

Development and description of the tool
The tool was prepared and organized into 4 sections:

Section – A:	Demographic variables of the nursing students
Section – B: Part I:	Structured questionnaire of knowledge on prevention of medication errors (multiple choice questionnaire)
Part II:	Structured questionnaire of attitude towards prevention of medication errors (Likert scale)
Part III:	Checklist of practice on prevention of medication errors
Section – C:	The Creighton Competency Evaluation Instrument (used for simulation scenario evaluation for debriefing section but the data was not taken for statistical analysis)

Description of intervention
Traditional teaching method and simulation-based teaching method was the intervention.

Traditional teaching method
The traditional teaching method included a 45-minutes session of lecture method of teaching followed by a 15-minutes demonstration of intravenous medication administration procedure. In the lecture method, the lesson plan included that the student will be able to acquire in-depth knowledge regarding the prevention of medication errors, to value the importance of medication error prevention, and be able to perform safe medication administration. The subtopics were:
- meaning of medication error – 2 minutes,
- definition of medication error – 2 minutes,
- difference between medication errors and adverse drug reaction – 3 minutes,
- causes of medication errors – 10 minutes,
- types of medication errors – 5 minutes,
- strategies for prevention of medication errors – 15minutes,
- effects of medication errors on patient – 5 minutes.

The audio-visual aids used were blackboard, power point presentation, chart, pamphlet, and handout. At the end of the class, the topics were summarised and concluded. In the demonstration, the objectives were clearly explained to the student. The demonstration environment was oriented to the student before the procedure. Students were taught how to deliver IV medications in a safe manner while following all drug administration guidelines, reducing the risk of medication errors during the demonstration.

Simulation-based teaching method
Before the simulation-based teaching method, an introduction to simulation was given to the students with the use of a power point presentation for 5 minutes. The simulation-based teaching method was for 60-minutes, the simulation session included pre briefing: 15-minutes, running scenario: 20-minutes, and debriefing: 25-minutes (the detailed of the simulation session of 60 minutes was provided in appendix). The scenario topic was preventing intravenous medication errors. The learning objectives were to:
- administer medication through Intravenous safely without errors,
- recognize the rationale of every step of the procedure being performed,
- demonstrate therapeutic communications in care of the patient.

The simulation environment was oriented towards the students. Available equipment, articles, and how to handle the hybrid simulators were explained to the students. The simulation scenario and all the simulation information were explained to all the students. Each student separately participated in a simulation scenario and had the role of a student nurse followed by debriefing session that took place in groups. Facilitators and cofacilitators analysed the simulation act.

Validity
Six experts from the field of nursing professionals who had received training on simulation-based teaching and biostatistician scrutinized the tool.

Reliability
The reliability of the instrument was established by the split-half method. The reliability value for knowledge was found to be 'r'= 0.76, attitude 'r' = 0.79 and practice 'r' = 0.80, which indicated that the instrument was highly reliable.

Data collection procedure
The researcher got the required permission from the institute authorities to conduct the research study. Permission was obtained from the selected study settings priorly. The data was collected for a period of one month. Each subject was explained about the purpose of the study and written consent was obtained prior to the study. The researcher introduced herself to the participants and a rapport was established with the subjects. The researcher assured the subjects that all their responses would be kept confidential. Four Colleges were selected using simple random sampling technique and out of the four selected colleges two colleges were selected for experimental

group I and two other colleges for experimental group II using simple random sampling. 25 subjects were selected from each of the four colleges using stratified sampling technique for selecting boys and girls proportionately. Two colleges selected for experimental group I was given traditional teaching whereas experimental group II was given simulation-based teaching on the prevention of medication errors. During the pre and post-test, while teaching the prevention of medication errors all the student's mobile phone were collected and kept in separate box away from the students. During the skill evaluation and after the scenario act separate room were arranged for the students who completed the given task.

A pre-test was conducted on day 1st. The students were gathered in classroom without any external disturbances and a self-administered knowledge and attitude questionnaire were distributed and asked to answer the questions. The time allotted to fill in the questions was 30 minutes. After the completion of questionnaire, the students were individually orientated to OSCE station and asked to do the intravenous medication administration procedure separately in skill lab within the allotted time of 10 minutes. Once the procedure was done the students were asked to occupy the separate room which was arranged priorly away from the classroom to prevent the sharing of the information.

The intervention was given on the same day, for experimental group I the traditional teaching on prevention of medication error was taken in the classroom, it includes 45 minutes session of the lecture method of teaching using the audio-visual aids such as power point, chart, pamphlet, leaflet and blackboard which was followed by a 15 minutes demonstration of intravenous medication administration procedure in the skill lab.

For experimental group II simulation introduction session was given for 15 minutes using the power point presentation in classroom which was followed by simulation-based teaching session for 60 minutes in three phases. In phase-1 all the students were participated in pre-briefing session for 15 minutes in group and after that they were asked to go back to classroom. In phase-II the students were individually undergone the scenario act for 20 minutes and upon completion of the act they were asked to occupy the separate room arranged away from the classroom. The simulation act was evaluated by using the Creighton Competency Evaluation Instrument. Finally in phase-III debriefing session was conducted for 25 minutes involving all the students in group whereas the debriefing questions were asked and explored for each student separately.

Post-test was conducted on 8th day using the same tool as that of the pre-test. The same procedure was implemented for all the 4 selected nursing colleges for the collection of data on the different days (data collection chart was attached in appendices)

Results

Regarding age, majority of the nursing students were in the age group of 20–21 years. In relation to gender, majority of the nursing students were females. Regarding place of stay, majority of the nursing students were days scholars. In relation to number of attempts on pharmacology, majority of the nursing students had one attempt to clear the pharmacology subject.

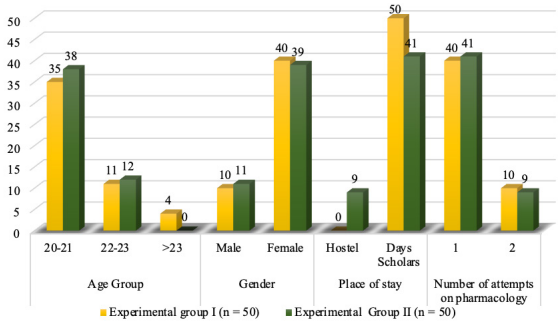

Fig. 1. Frequency and percentage distribution of socio demographic variables of the nursing students

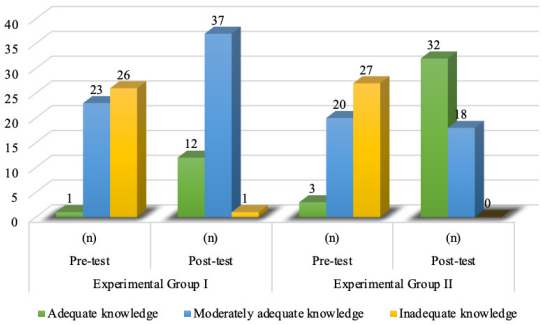

Fig. 2. Comparison of pre and post-test level of overall knowledge on prevention of medication errors within the experimental group I and II

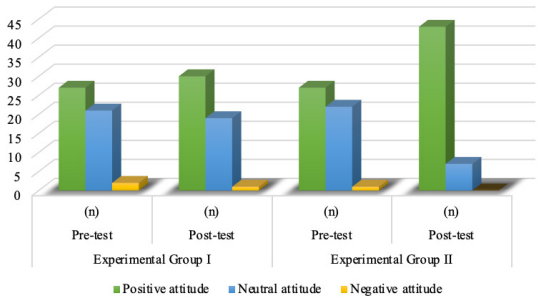

Fig. 3. Comparison of pre and post-test level of attitude towards prevention of medication errors within the experimental group I and II

In experimental group I, majority 26 (52%) of the nursing students had inadequate knowledge in the pre-test whereas only 1 (2%) had inadequate knowledge in the post-test. In experimental group II, majority 27 (54%)

of the nursing students had inadequate knowledge while none of them had inadequate knowledge in the post-test.

In experimental group I, majority 27 (54%) and 30 (60%) of the nursing students had positive attitude in the pre and post-test respectively. In experimental group II, majority 27 (54%) and 43 (86%) of the nursing students had positive attitude in the pre and post-test respectively.

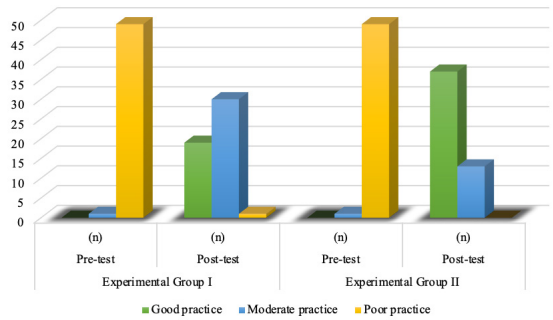

Fig. 4. Comparison of pre and post-test level of practice on prevention of medication errors within the experimental group I and II

In experimental group I, majority 49 (98%) and 1(2%) of the nursing students had poor practice in the pre and post-test respectively. In experimental group ii, majority 49 (98%) of the nursing students had poor practice in the pre-test whereas none of them had poor practice in the post-test (Table 1).

In experimental group I the pre-test mean scores of overall knowledge was 9.16 whereas the post-test mean scores of overall knowledge was 13.28. The post-test mean scores of overall knowledge was higher than that of the pre-test.

In experimental group II the pre-test mean scores of overall knowledge was 9.52 whereas the post-test mean scores of overall knowledge was 15.08. The post-test mean scores of overall knowledge was higher than that of the pre-test.

Comparison of pre and post-test mean scores of attitude towards the prevention of medication errors within the experimental group I and II

In experimental group I, the pre-test mean scores of attitude was 7.26 whereas the post-test mean scores of attitude was 7.52. The computed paired 't' test value 1.218 at p=0.229 revealed that there was no statistically significant difference in the attitude between the pre and post-test. But the mean scores of the post-test was higher than that of the pre-test.

In experimental group II, the pre-test mean scores of attitude was 7.50 whereas the post-test mean scores of attitude was 8.44. The computed paired 't' test value 4.305 at p=0.000 revealed a highly statistically significant difference in the attitude between the pre and post-test. Traditional teaching method and simulation-based teaching method increased the level of attitude among nursing students in the experimental group I and II respectively.

Table 1. Comparison of pre and post-test mean scores of overall knowledge and various aspects of knowledge on prevention of medication errors in experimental group I and II [a]

Aspects of knowledge	Experimental group I n=50				Paired t – test value and p
	Pre-test score		Post-test score		
	Mean	(SD)	Mean	(SD)	
Meaning of medication error	1.12	0.65	1.80	0.45	t=6.263 p<0.001 *** (S)
Adverse drug event	0.28	0.45	0.58	0.50	t=3.130 p=0.003 ** (S)
Causes of medication error	2.02	1.17	2.40	1.03	t =1.719 p=0.092 (NS)
Types of medication error	1.66	1.04	2.16	1.31	t=2.150 p=0.037 * (S)
Rights of medication administration	1.90	1.01	2.42	0.91	t=2.800 p=0.007 **(S)
Strategies for prevention of medication error	1.50	0.91	3.22	1.25	t=7.523 p<0.001 ***(S)
Effects of medication error	0.68	0.47	0.70	0.46	t=0.216 p=0.830 (NS)
Overall, knowledge score	9.16	3.28	13.28	2.63	t=6.458 p<0.001 ***(S)
Aspects of knowledge	**Experimental group II (n=50)**				**Paired t – test value and p - value**
	Pre-test score		**Post-test score**		
	Mean	**(SD)**	**Mean**	**(SD)**	
Meaning of medication error	1.28	(0.83)	1.88	(0.33)	t=4.818 p<0.001 *** (S)
Adverse drug event	0.20	(0.40)	0.54	(0.50)	t=3.351 p=0.002 **(S)
Causes of medication error	2.08	(1.17)	2.98	(1.02)	t =3.870 p<0.001 *** (S)
Types of medication error	1.32	(0.89)	2.68	(1.24)	t=5.915 p<0.001 *** (S)
Rights of medication administration	2.32	(0.93)	2.70	(0.61)	t=2.474 p=0.017 * (S)
Strategies for prevention of medication error	1.54	(1.16)	3.27	(1.34)	t=7.435 p<0.001 *** (S)
Effects of medication error	0.78	(0.42)	0.96	(0.20)	t=2.641 p=0.011 *(S)
Overall, knowledge score	9.52	(3.26)	15.08	(2.64)	t =8.842 p<0.001 *** (S)

[a] S – significant; NS – not significant

Comparison of pre and post-test mean scores of practice on prevention of medication errors within the experimental group I and II

In experimental group I, the pre-test mean scores of practice was 2.73 whereas the post-test mean scores of practice was 6.78. The computed paired 't' test value 12.913 at p<0.001 revealed a highly statistically significant difference in the practice between the pre and post-test (Table 2).

In experimental group II, the pre-test mean scores of Practice was 1.52 whereas the post-test mean scores of practice was 8.19. The computed paired 't' test value

27.674 at p=0.000 revealed a highly statistically significant difference in the practice between the pre and post-test. Traditional teaching method and simulation-based teaching method significantly increased the level of practice among nursing students in the experimental group I and II respectively.

Table 2. Comparison of effective mean scores of overall knowledge, attitude and practice on prevention of medication errors between the experimental groups [a]

Knowledge	Experimental group I n=50		Experimental group II n=50		Independent t – test value and p
	Mean	SD	Mean	SD	
Effective	4.12	4.51	5.61	4.44	t =1.658 p=0.101 (NS)
Attitude	**Experimental group I n=50**		**Experimental group II n=50**		**Independent t – test value and p**
	Mean	SD	Mean	(SD)	
Effective	0.26	1.51	0.94	1.54	t=2.227, p=0.028 *(S)
Practice	**Experimental group I n=50**		**Experimental group II n=50**		**Independent t – test value and p**
	Mean	SD	Mean	SD	
Effective	4.04	2.21	6.67	1.70	t=6.644, p<0.001 ***(S)

[a] S – significant; NS – not significant

The effective mean scores of overall knowledge, attitude and practice of the experimental group II were higher than that of the experimental group I.

In experimental group I, there was a statistically significant correlation between the knowledge and attitude towards the prevention of medication errors as well as between the knowledge and practice (skill) on prevention of medication errors (Table 3).

Table 3. Correlation between the level of knowledge and attitude, knowledge and practice, attitude and practice on prevention of medication error in the experimental group I and II [a]

Experimental group I (n=50)	Attitude	Practice
Knowledge	r=0.330 p=0.019 *(S)	r=0.340 p=0.016 *(S)
Attitude	–	r=-0.065 p=0.656 (NS)
Experimental group II (n=50)	**Attitude**	**Practice**
Knowledge	r=0.125 p=0.393 (NS)	r=0.114 p=0.434 (NS)
Attitude	–	r=-0.210 p=0.144 (NS)

[a] S – significant; NS – not significant

There was a significant association between the level of attitude and the demographic variables gender (Table 4).

Table 4. Association between the post-test level of knowledge, attitude and practice with their selected demographic variable in the experimental group I and II [a]

Knowledge						
Experimental group I (n=50)						
Demographic variables	**Sub variables**	**No.**	**Mean**	**SD**	**F ratio/'t' value**	**p**
Age group	20–21	35	13.03	2.45	0.825	0.444 (NS)
	22–23	11	14.18	2.89		
	>23	4	13.00	3.56		
Gender	Male	10	14.40	2.67	1.528	0.133 (NS)
	Female	40	13.00	2.57		
Place of stay	Hostel	0	–	–	Not applicable	
	Days Scholars	50	13.28	2.63		
No. of attempts on pharmacology	1	40	13.15	2.52	0.696	0.490 (NS)
	2	10	13.80	3.12		
Experimental group II (n=50)						
Age group	20–21	38	14.84	2.73	0.947	0.348 (NS)
	22–23	12	15.67	2.27		
	>23	0	–	–		
Gender	Male	11	14.82	3.22	0.314	0.755 (NS)
	Female	39	15.10	2.48		
Place of stay	Hostel	9	13.89	2.47	1.469	0.148 (NS)
	Days Scholars	41	15.29	2.62		
No. of attempts on pharmacology	1	41	15.15	2.71	0.607	0.547 (NS)
	2	9	14.56	2.30		
Attitude						
Experimental group I (n=50)						
Demographic variables	**Sub variables**	**No.**	**Mean**	**SD**	**F ratio/'t' value**	**p**
Age group	20–21	35	7.54	1.33	0.020	0.980 (NS)
	22–23	11	7.45	1.13		
	>23	4	7.50	1.29		
Gender:	Male	10	7.50	1.43	0.055	0.956 (NS)
	Female	40	7.52	1.24		
Place of Stay	Hostel	0	-	-	Not applicable	
	Days Scholars	50	7.52	1.26		
No. of attempts on pharmacology	1	40	7.57	1.26	0.661	0.544 (NS)
	2	10	7.30	1.34		
Experimental group II (n=50)						
Age group	20–21	38	8.39	0.82	0.677	0.502 (NS)
	22–23	12	8.58	0.90		
	>23	0	-	-		
Gender	Male	11	7.91	0.70	2.508	0.016 *(S)
	Female	39	8.59	0.82		
Place of stay	Hostel	9	7.67	0.87	3.369	0.001 (NS)
	Days scholars	41	8.60	0.74		
No. of attempts on pharmacology	1	41	8.39	0.86	0.895	0.375 (NS)
	2	9	8.67	0.71		
Practice						
Experimental group I (n=50)						
Demographic variables	**Sub variables**	**No.**	**Mean**	**SD**	**F ratio/'t' value**	**p**
Age group	20–21	35	6.79	1.27	0.067	(NS)
	22–23	11	6.67	1.09		
	>23	4	6.93	1.63		
Gender	Male	10	6.72	1.16	0.161	(NS)
	Female	40	6.79	1.27		
Place of stay	Hostel	0	-	-	Not applicable	
	Days scholars	50	6.78	1.24		
No. of attempts on pharmacology	1	40	6.81	1.26	0.351	0.727 (NS)
	2	10	6.65	1.17		

Experimental group I (n=50)						
Age group	20–21	38	8.29	1.14	1.038	0.304 (NS)
	22–23	12	7.87	1.45		
	>23	0	–	–		
Gender	Male	11	8.14	1.36	0.162	0.872 (NS)
	Female	39	8.21	1.20		
Place of stay	Hostel	9	8.78	1.09	1.626	0.111 (NS)
	Days scholars	41	8.06	1.22		
No. of attempts on pharmacology	1	41	8.31	1.12	1.492	0.142 (NS)
	2	9	7.65	1.56		

[a] S – significant; NS – not significant

Discussion

Existing level of overall knowledge on prevention of medication errors among experimental group I and II

Majority 26 (52%) and 27 (54%) of the nursing students had inadequate level of knowledge, whereas 23 (46%) and 20 (40%) had moderately adequate level of knowledge, while only 1 (2%) and 3 (6%) had adequate level of knowledge on prevention of medication errors among experimental group I (traditional teaching method) and II (simulation teaching method) respectively.

The above findings of the study was supported by a descriptive study conducted by Raghavendran et al., to assess the knowledge level of students regarding the prevention of medication errors in a selected nursing college, Kanpur and the result showed that 129 (64.5%) of the students had an inadequate level of knowledge, 49 (24.5%) had a moderately adequate level of knowledge and 22 (11%) had an adequate level of knowledge.[15]

Existing level of attitude towards the prevention of medication errors among experimental group I and II

Majority 27 (54%) of the nursing students had positive attitude in both experimental group I and II, whereas 21 (42%) and 22 (44%) had neutral attitude, while only 2 (4%) and 1 (2%) had negative attitude in the experimental group I and II respectively.

The above findings of the study were supported by Shaju et al., who had conducted a descriptive study to assess the knowledge and attitude regarding medication error among nursing students in a selected college at Mangalore and the results showed that majority 88% of the students had positive attitude whereas 12% had negative attitude towards the medication error.[16]

Existing level of practice on prevention of medication errors among experimental group I and II

In both experimental group I and II, majority 49 (98%) of the nursing students had poor practice whereas only 1 (2%) had moderate practice.

The second objective was to compare the effectiveness between traditional teaching method and simulation-based teaching method on prevention of medication errors.

Knowledge

Comparison of pre and post-test level of overall and various aspects of knowledge on prevention of medication errors within the experimental group I and II

Overall level of knowledge among experimental group I on prevention of medication error, majority 26 (52%) had inadequate knowledge while 23 (46%) had moderately adequate knowledge and only 1 (2%) had adequate knowledge in the pre-test, whereas in the post-test, majority 37 (74%) had moderately adequate knowledge while 12 (24%) had adequate knowledge and only 1 (2%) had inadequate knowledge.

The above findings were supported by a study conducted by Kumar et al., to evaluate the effectiveness of lecture cum demonstration on knowledge and skill regarding cranial nerve assessment among B.Sc. nursing students in Lucknow. Where the results revealed that in the pre-test, majority 80% of the students had inadequate knowledge, 20% had fairly adequate knowledge and none of them had adequate knowledge, while in the post-test none of them had inadequate knowledge whereas 75.5% had fairly adequate knowledge and 24.4% had adequate knowledge.[17]

Effectiveness of the two-teaching methods (traditional teaching method and simulation-based teaching method) on level of knowledge on prevention of medication errors

The computed paired 't' test value 6.458 at $p<0.001$ revealed a highly statistically significant difference between the pre and post-test means scores, indicating that the overall knowledge between the pre and post-test were not similar. Thus, it could be inferred that the traditional teaching method was effective in improving the overall knowledge on prevention of medication errors.

The above findings were supported by the study conducted by Patil et al., to assess the effectiveness of lecture cum Demonstration method on knowledge regarding neurological assessment among undergraduate nursing students from selected colleges of Chandrapur and results findings revealed that the post-test mean score was 18.22 higher than that of the pretest mean scores of 9.1. The calculated t value was 14.95 at $p<0.001$, showed a highly statistically significant difference between the pre and post-test level of knowledge.[18]

In experimental group II the computed paired 't' test value 8.842 at $p<0.001$ revealed a highly statistically significant difference between the pre and post-test means scores, indicating that the overall knowledge between the pre and post-test were not similar. Thus, it could be inferred that the stimulation-based teaching method was effective in improving the overall knowledge on prevention of medication errors.

The above findings were supported by the study designed by Frenzel et al., to evaluate the use of sim-

ulations in preparing students to identify and reduce medication errors and promote patient safety among third-year pharmacy students. The results showed that overall knowledge of post-test score (83.9%) was higher than that of the pre-test score (81.5%).[19]

Effectiveness of traditional teaching method versus simulation-based teaching method on the level of knowledge on prevention of medication errors

In experimental group I the computed independent 't' test value 1.658 at p=0.101 revealed that there was no statistically significant difference in the overall knowledge between the experimental group I and II. The effective mean scores of the experimental group II (simulation-based teaching method) was higher than that of the experimental group I (traditional teaching method), indicating that simulation-based teaching method was more effective than the traditional teaching method.

The above findings were supported by the study conducted by Jyoti et al., to evaluate the effectiveness of simulation-based training versus traditional method of teaching on the retention of birthing care on knowledge and skills among B.Sc. nursing fourth year students and results showed that the knowledge mean score for simulation group (23.05) was higher than traditional teaching group (17.87).[20]

So, the hypothesis H_1 which stated that there will be significant difference between the effective mean of knowledge between experimental group I and II among nursing students on prevention of medication errors was not accepted.

Attitude

Effectiveness of the two-teaching methods (traditional teaching method and simulation-based teaching method) on level of attitude towards the prevention of medication errors

The computed paired 't' test value 4.305 at p<0.001 revealed a highly statistically significant difference in the attitude between the pre and post-test, which indicated that the mean scores of attitude between the pre and post-test were not similar.

Thus, elicited that the attitude towards the prevention of medication errors had significantly increased in the post-test than that of the pre-test. Which clearly showed that the simulation-based teaching method was effective on improving the attitude towards the prevention of medication errors.

The above findings were supported by the study conducted by Frenzel et al., where the results showed that there was a significant improvement in the post-test of Attitude at p<0.05.[19]

Thus, it could be inferred that the traditional teaching method and simulation-based teaching method was effective in improving the level of attitude towards the prevention of medication errors.

Effectiveness of traditional teaching method versus simulation-based teaching method on the level of attitude towards the prevention of medication errors

In experimental group I, the computed independent 't' test value 2.227 at p=0.028 revealed that there was a statistically significant difference in the attitude between the experimental group I and II, indicating that the effective mean scores of attitude between the experimental group I and II were not similar. Thus, it was elicited that the level of attitude towards the prevention of medication errors had significantly increased in the experimental group II than that of the experimental group I, implied that the simulation-based teaching method was more effective than the traditional teaching method.

So, the hypothesis H_2 which stated that there will be significant difference between the effective mean of attitude between experimental group I and II among nursing students on prevention of medication errors was accepted.

Practice

Comparison of pre and post-test level of practice on prevention of medication errors within the experimental group I and II

In experimental group I, 49 (98%) and 1 (2%) of the nursing students had poor practice, likewise 1 (2%) and 30 (60%) had moderate practice in the pre and post-test respectively, while none of them had good practice in the pre-test whereas 19 (38%) had good practice in the post-test.

The above findings was supported by a study conducted by Kumar and Pandey to evaluate the effectiveness of lecture cum demonstration on knowledge and skill regarding cranial nerve assessment among B.Sc. nursing students and the results findings revealed in the pre-test 85.5% of the students had inadequate skills, 14.5% had fairly adequate skills and none of them had adequate skills, whereas in the post-test 9% had inadequate skills, 71.5% had fairly adequate skills and 19.5% had adequate skills.[17]

In experimental group II, 49 (98%) of the nursing students had poor practice in the pre-test whereas none of them had poor practice in the post-test. Similarly, 1 (2%) and 13 (26%) of the nursing student had moderate practice in the pre and post-test respectively. With regard to good practice, none of them had good practice in the pre-test whereas 37 (74%) had good practice in the post-test.

The above findings was supported by a study conducted by Sharma et al., evaluated effectiveness of simulation technique, on practice regarding selected nursing procedure among B.Sc. nursing students and the results showed that in the pre-test (88.8% and 97.5%) had poor practice, (11.3% and 2.5%) had satisfactory practice and none of the students had good practice in intravenous

and intramuscular practice administration, whereas in the post-test none of them had poor practice, (77.5% and 46.4%) had satisfactory practice and (22.5% and 53.6%) had good practice.[21]

Thus, it could be inferred that the shift of level of practice (skill) of majority of the nursing students from poor practice in the pre-test to good practice in the post-test showed the effectiveness of traditional teaching method and simulation-based teaching method in improving the level of practice (skill) on prevention of medication error among nursing students.

Effectiveness of the two-teaching methods (traditional teaching method and simulation-based teaching method) on level of practice in the prevention of medication errors

The study revealed that in experimental group I, the computed paired 't' test value 12.913 at p<0.001 revealed a highly statistically significant difference in the practice between the pre and post-test, indicating that the mean scores of practice between the pre and post-test were not similar

Thus, it was elicited that the practice on prevention of medication errors had significantly increased in the post-test than that of the pre-test. Implying that the traditional teaching method was effective on improving the level of practice on prevention of medication errors.

The above findings was supported by a study conducted by Pandey and Vijaya, evaluated the effectiveness of lecture cum demonstration on knowledge and skill regarding cranial nerve assessment among B.Sc. nursing students and the results findings revealed that the effective mean was 5.82 with calculated t=8.74 at p<0.05 showed a statistically significant difference between the pre and post-test.[17]

In experimental group II, the computed paired 't' test value 27.674 at p<0.001 revealed a highly statistically significant difference in the practice between the pre and post-test, indicating that the mean scores of practice between the pre and post-test were not similar

Thus, it was elicited that the practice on prevention of medication errors had significantly increased in the post-test than that of the pre-test. Implying that the stimulation-based teaching method was effective on improving the level of practice on prevention of medication errors.

The above findings was supported by a study conducted by Sharma et al., evaluated effectiveness of simulation technique, on practice regarding selected nursing procedure among B.Sc. nursing students and the results showed that the mean difference was 1.113 and 1.513 for intravenous and intramuscular administration Practice with calculated t= 23.648 and t= 23.648 at p=0.05 showed a statistically significant difference between the pre and post-test.[21]

Thus, it could be inferred that the traditional teaching method and simulation-based teaching method was effective in improving the level of practice on prevention of medication errors.

Effectiveness of traditional teaching method versus simulation-based teaching method on the level of practice in the prevention of medication errors

In experimental group I the computed independent 't' test value 6.644 at p<0.001 revealed that there was a highly statistically significant difference in the practice between the experimental group I and II, indicating that the effective mean scores of practice between the experimental group I and II were not similar.

Thus, it was revealed that the level of practice on prevention of medication errors had significantly increased in the experimental group II than that of the experimental group I, which implies that the simulation-based teaching method was more effective than the traditional teaching method.

The above findings was supported by the study conducted by Jyoti et al., who evaluated the effectiveness of simulation based training versus traditional method of teaching on the retention of birthing care on knowledge and skills among B.Sc. nursing fourth year students and results showed that the skill mean score for simulation group (37.23) was higher than traditional teaching group (29.23) at t=33.23 at p<0.05 showed a highly statistical significant difference between the two groups.[20]

So, the hypothesis H_3 which stated that there will be significant difference between the effective mean of practice between experimental group I and II among nursing students on prevention of medication errors was accepted.

To correlate the level of knowledge and attitude, knowledge and practice and attitude and practice on prevention of medication errors among nursing students in selected colleges, Puducherry

The study findings revealed that in experimental group I, the significant r=0.330 at p=0.019 and r=0.340 at p=0.016 revealed that there was a statistically significant correlation between the knowledge and attitude, knowledge and practice on prevention of medication errors respectively.

Thus, it could be inferred that when the level of knowledge increased, the level of attitude also increased, similarly when the level of knowledge increased, the level of practice (skill) also increased on prevention of medication errors among experimental group I.

The above finding was supported by the study conducted by the Reddy and Ramesh, where the results showed that there was a positive correlation between post-test level of knowledge and practice at r=0.21 and p<0.05.[22]

In experimental group II, the non-significant r=0.125 at p=0.393 and r=0.114 at p=0.434 revealed that there was

no statistically significant correlation between the knowledge and attitude, knowledge and practice on prevention of medication errors among experimental group I.

The non-significant r=-0.065 at p=0.656 and r=-0.210 at p=0.144 revealed that there was no statistically significant correlation between the attitude and practice on the prevention of medication errors among the nursing student in the experimental group I and II respectively.

So, the hypothesis H_4 which stated that there will be significant correlation between the level of knowledge and attitude, knowledge and practice, attitude and practice among nursing students on prevention of medication errors in the experimental group I and experimental group II was not accepted except the level of knowledge and attitude, knowledge and practice among nursing students in experimental group I.

To associate between the post-test level of knowledge, attitude and practice with their selected demographic variables in the experimental group I and II

The present study results revealed that the association between the post-test level of knowledge and practice with their selected demographic variable in the experimental group I and II revealed a non-significant p value, which showed that there was no statistically significant association between level of knowledge and practice with any of the selected demographic variable in experimental group I and II respectively.

With respect to association between the post-test level of attitude with their selected demographic variable in the experimental group I revealed a non-significant p value, which showed that there was no statistically significant between the level of attitude with their selected demographic variables in the experimental group I.

With regard to the association between the post-test level of attitude with their selected demographic variable in the experimental group II revealed a non-significant p, which showed there was no statistically significant association between attitude and the demographic variables except gender which was statistically significant at p=0.016.

So, the hypothesis H_5 which stated that there will be significant association between post-test level of knowledge, attitude and practice with their selected demographic variables in the experimental group I and experimental group II was not accepted except the post-test level of attitude with their gender demographic variable in the experimental group II.

Nursing implication

Nursing services

- Since the concept of simulation is new in nursing services, it can be utilized for job training and continuing nursing education for nursing personnel. It aids in improving the quality of patient care.
- The requirement for high-quality nursing care focused on patient safety has increased, thus nurses can embrace these novel simulation-based interventional strategies by honoring their ICT abilities to improve their performance at health care settings.
- The use of simulation is proposed to enhance healthcare professional collaboration, interdisciplinary communication, and team training.

Nursing education

- Through simulation, students can learn and practice nursing procedures in a less risky but real-life environment. The safety of patients will be increased by learning through simulation.
- Students will be trained for various nursing procedure through the use of simulations, which also help them to develop their critical thinking and self-reflection skills before going to bed side.
- Effective communication and collaboration can be taught by utilizing simulation through team training, implementation of a standardized approach to communications.
- Simulation can be integrated into Nursing education as an efficient teaching strategy that harmoniously blends nursing theory and practical skills.

Nursing administration

- Policy changes should enable the use of simulation in nursing personnel recruitment and promotion.
- Administrations can plan on holding a continues nursing education and in-service educations to empower faculty in simulation-based activities and thereby promote quality nursing care.

Nursing research

- Encourage Nurses in research activities on Simulation to improve the body of Knowledge for their profession.
- Emphasis on utilization of the Simulation based study results in Practice.

Recommendation

- Replication of the study may be done with the large samples in different settings to generalize the study findings.
- Comparative study can be conducted by using different types of simulation method.
- This study can be conducted by using other teaching methods.
- Comparative study can be conducted on different groups.
- A follow-up study may be taken up to determine the long-term effects of intervention in terms their level of knowledge, attitude and practice.

Conclusion

The study result proved the effectiveness of the traditional teaching method and the simulation-based teaching method in raising the level of knowledge, attitude, and practice in the prevention of medication errors among nursing students in selected colleges of nursing at Puducherry. It also proved that the simulation-based teaching method was more effective than the traditional teaching method. It has been unveiled that simulation-based teaching method was effective which can be utilized as a means to educate the nursing students during their academic performance.

Declarations

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Author contributions

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

Conflicts of interest

The author(s) declare no competing interests.

Data availability

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

Ethics approval

All subjects gave their informed consent for inclusion before they participated in the study. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of Mother Theresa post graduate research nstitute of health science ICE committee.

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ORIGINAL PAPER

Assessment of preoperative anxiety and negative automatic thoughts in patients waiting for corneal transplantation

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ABSTRACT

Introduction and aim. In patients waiting for organ transplantation, increased waiting time can increase anxiety by creating negative automatic thoughts. The aim of this study is to examine the relationship between corneal transplant recipients' negative automatic thoughts and anxiety before organ transplantation.

Material and methods. The population of this correlational descriptive study consisted of 108 patients waiting for cornea transplantation in the Eye Bank unit of a hospital in the south east region of Türkiye. The data were obtained from the patients presented to the ophthalmology clinic for transplantation between September and December 2022. A patient information form, the Negative Automatic Thoughts Scale, and the Trait Anxiety Scale were used to collect the data. Percentage distribution, arithmetic mean, logistic regression and correlation analysis were used in the data analysis.

Results. It was determined that the mean score of the negative automatic thoughts of the transplant patients participating in the study was 100.69±47.83, and the trait anxiety mean score was 53.06±6.07. There was a positive relationship between negative automatic thoughts and trait anxiety at a moderate level, which was statistically significant ($p<0.05$, $r=0.53$).

Conclusion. It was determined in the study that the patients' negative automatic thoughts and trait anxiety were high, and their anxiety increased as the negative automatic thoughts increased.

Keywords. anxiety, corneal transplant, negative automatic thought

Introduction

Organ transplantation is now a routine advanced treatment method applied in many chronic organ diseases. Transplantation is generally defined as the transfer of tissues or organs. Since the number of patients needing transplantation is high and the organ donation is low, the future concerns and uncertainty about the transplantation have a negative effect on them. This general uncertainty causes psychosocial problems.[1,2]

The cornea is a tissue improving the quality of the image on the retina in the human eye. It is a convex, transparent, densely innervated, and sensitive membrane in the anterior part of the eyeball.[3,4] Today, corneal transplant/keratoplasty is one of the most frequently performed transplants worldwide. However, there is still corneal blindness in 4.2 million people, which makes it the fourth leading cause of blindness according to the World Health Organization, and finding corneal donation is a significant challenge.[5] The problem of finding corneal donations is a limiting factor for the total number of keratoplasty procedures performed every year. A global study reveals that 12.7 million people are waiting

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for a corneal transplant, and only one out of 70 will receive a cornea.[6] There are Eye Banks in many provinces of Türkiye with 609 centers to provide corneal transplant services to patients waiting for the cornea. When the current data in Türkiye are examined, the corneal transplantation of a total of 33092 patients were performed, and 32679 patients were waiting for the corneal transplantation at the time of writing.[7]

The increase in the number of transplant candidates and the small number of donors are the reasons for the increased waiting time. Waiting can also lead to negative mental states such as anxiety, agitation, fear, anger, and helplessness.[8-11] The people waiting for organ transplantation are always anxious about facing possible medical issues that will negatively affect organ transplantation or even make it impossible.[10] The increase in waiting time in patients waiting for transplantation creates negative automatic thoughts, which can increase anxiety.

Research questions

Q_1: What is the level of preoperative anxiety and negative automatic thoughts in patients waiting for corneal transplantation?

Q_2: Is there a relationship between preoperative anxiety and negative automatic thoughts in patients waiting for corneal transplantation?

Aim

This study aimed to examine the relationship between the organ transplantation recipients' negative automatic thoughts and anxiety before organ transplantation.

Material and methods

Study design and participants

This correlational descriptive research was performed to examine the relationship between negative automatic thoughts and anxiety states of organ transplantation recipients before corneal transplantation. This study was carried out in the Eye Bank unit of Dicle University Hospitals between September and December 2022. The population of the research consisted of 108 patients waiting for cornea transplantation. The study included the whole population with the non-probabilistic sampling technique, and was completed with 108 patients.

Data collection

The data were obtained via face-to-face interviews with the patients presented to the Ophthalmology clinic for transplantation. It took about 15 minutes to fill out the questionnaires and forms.

Data collection tools

Patient information form

The patient information form includes 10 items that cover the patient's descriptive characteristics (age, occupation, income status, gender, education level, marital status, eye to be operated on, surgery history, waiting time for a donor, the reason for transplantation).

State and Trait Anxiety Scale (STAI)

It is a self-assessment questionnaire developed by Spielberger et al. and consists of short assessments. The STAI includes 40 four-point Likert-type items ranging from "None" to "Completely". The validity and reliability of the STAI in Türkiye were established by Öner and Le Compte. The STAI includes two separate scales, the State Anxiety Score (SAS) and the Trait Anxiety Score (TAS). The SAS requires the person to describe how they feel at a certain moment and under certain conditions and to respond by taking into account their feelings about the situation they are in.

On the other hand, the TAS requires the person to describe how they usually feel. There are two types of expressions in the STAI. While direct statements express negative emotions, reversed statements express positive emotions. The reversed statements in the SAS are the 1st, 2nd, 5th, 8th, 10th, 11th, 15th, 16th, 19th, and 20th items. The reversed statements in the TAS are the 21st, 26th, 27th, 30th, 33rd, 36th, and 39th items. The total score of the reverse statements is subtracted from the total score obtained for the direct statements. A predetermined and constant value is added to this number. This constant value is 50 for the SAS and 35 for the TAS. The last obtained value is the anxiety score of the individual. Higher scores indicate increased anxiety level. In the Turkish validity and reliability study, Cronbach's alpha reliability coefficients were between 0.83 and 0.87 for the TAS and between 0.94 and 0.96 for the SAS.[12]

Negative Automatic Thoughts Scale

The scale was developed by Holland and Kendall in 1980 and its Turkish validity and reliability were established first by Aydın and Aydın in 1990, and then Şahin and Şahin performed the validity and reliability study again in 1992. The scale has 5-point Likert type items. There are no reversed items in the scale. The score range of the scale is 30-150 points, and 67 points and above are evaluated as high for the negative automatic thoughts. The Cronbach alpha internal consistency coefficient of the scale is 0.96.[13]

Statistical analysis

Data analysis was performed using the SPSS 25 (IBM, Armonk, NY, USA) package program for Windows. In the study, statistical methods used in the analysis of data are presented in the Table 1.

Table 1. Statistical methods used in data analysis

Statistical Reasons	Statistical Methods
Distribution of patients' descriptive characteristics	The number, percentage, mean and standard deviation
Normal distribution of data	Test of normality, skewness and kurtosis values
Comparison scale means of between groups	Student T-test, One-Way Analysis of Variance Test
Examine relationship between two variables	Pearson Correlation
Estimate the association of categorical or continuous variables	Logistic Regression

Ethics approval

The ethics committee approval was obtained from Mardin Artuklu University Ethics Committee (#2022/66630), and clinical study permission was obtained from Dicle University Medical Faculty Hospital (#38948411-900-257595). Written informed consent was obtained from the participants after explaining that participation in the study was voluntary and participants who agreed to participate in the study were informed about the purpose, duration, and scope of the study.

Results

The mean age of the patients was 61.25±16.48 years. It was determined that 93.5% were married, 57.4% were male, 26.9% were retired, 80.6% had less income than expenses, 45.4% were primary school graduates (Table 2), 52.8% had surgery on their right eye, 68.5% had a surgery history, and 30.6% need transplantation due to pseudophakic corneal edema (Table 3).

When the descriptive and clinical characteristics of the patients and their mean scores of negative automatic thoughts and trait anxiety were examined, it was determined that the difference between the gender, income status, and negative automatic thought scale means were statistically significant (p<0.05) and the difference between the anxiety score means was not statistically significant (p>0.05). It was determined that the difference of anxiety scale mean scores in educational status was statistically insignificant (p<0.01). There was no statistically significant difference (p>0.05) between the mean scores of negative automatic thoughts and anxiety due to the marital status and occupation. There was no statistically significant relationship found between age and the transplantation waiting time and the mean scores of negative automatic thoughts and anxiety before transplantation (p>0.05) (Table 2).

When clinical characteristics of the patients and their mean scores of negative automatic thoughts and trait anxiety were examined, There was no statistically significant difference between the mean scores of negative automatic thoughts due to the operated eye, surgical history and reason for transplantation (p>0.05). It was determined that the difference of anxiety scale means in the operated eye, surgical history and were statistically significant (p<0.05); but the difference of anxiety score means in the reasons for transplantation was not statistically significant (p>0.05) (Table 3).

Table 2. Mean scores of Negative Automatic Thought Scale and Anxiety Scale according to descriptive characteristics of the patients[a]

Features	Number/ percent		Negative automatic thought	Test and significance	Anxiety	Test and significance
	n	%	$\bar{x} \pm SD$			$\bar{x} \pm SD$
Marital status						
Bachelor	7	6.5	91.85 ± 55.07	t=-0.35	53.00 ± 6,08	t=-0.11
Married	101	93.5	99.39 ± 47.86	p=0.73	53.26 ± 5,55	p=0.91
Gender						
Female	46	42.6	110.80±44.75	t=2.28	53.78±5.60	t=0.85
Male	62	57.4	90.08±48.96	p=0.02*	52.85±5.53	p=0.39
Occupation						
Retired	29	26.9	83.13±47.03		53.96±5.42	
Officer	2	1.9	105.00±63.63		53.50±7.77	
Farmer	6	5.6	72.00±40.49		49.33±4.88	
Self-employment	10	9.3	108.10±50.72	F=1.71	54.60±5.68	F=0.95
Not working	25	23.1	114.88±49.64	p=0.13	53.76±5.62	p=0.44
Housewife	36	33.3	102.11±45.35		52.58±5.61	
Income status						
Income less than expense	87	80.6	105.16±46.98	F=8.05	52.87±5.54	F=2.07
Income equals expense	21	19.4	73.00±44.92	p<0.05	54.80±5.47	p=0.15
Income more than expense	0	0				
Education status						
Illiterate	8	7.4	123.75±29.73		59	
Literate	38	35.2	95.71±45.38		51.76±5.36	
Primary school	49	45.4	104.95±50.88	F=2.27	52.34±5.43	F=6.09
High school	9	8.3	61.33±51.04	p=0.06	56.77±4.84	p<0.01
University	4	3.7	90		59	
	$\bar{x} \pm SS$					
Age	61.25±16.48			r=-0.08 p=0.39		r=-0.03 p=0.69
Transplantation waiting time	5.67±3.93			r=-0.15 p=0.10		r=-0.02 p=0.81

a X – mean, SD – standard deviation, r – correlation, F – One Way Anova, t – student T

The negative automatic thought scale mean score of the transplant patients was 98.90±48.11, and the mean trait anxiety scale score was 53.25±5.55. There was a positive and medium-level relationship between the negative automatic thought and trait anxiety, which was statistically significant (p<0.01) (r=0.53) (Table 4).

The results of the logistic regression analysis regarding the negative automatic thoughts of the patients are shown in Table 4. Among the variables which were found to be significant, a decrease in educational status increased the negative automatic thoughts 0.66 times,

and a one-unit increase in the anxiety scale score increased the negative automatic thoughts 1.34 times (p<0.01) (Table 5).

Table 3. Mean scores of Negative Automatic Thought Scale and Anxiety Scale according to clinical characteristics of the patientsa

Features	Number/ percent		Negative automatic thought	Test and significance	Anxiety	Test and significance
	n	%	$\bar{x} \pm SD$			$\bar{x} \pm SD$
Surgery eye						
Right	57	52.8	101.56±49.00	t=0.60	54.54±5.50	t=2.63
Left	51	47.2	95.94±47.41	p=0.54	51.80±5.30	p=0.01
Surgery history						
Yes	74	68.5	99.00±50.13	t=0.03	54.09±5.59	t=2.38
No	34	31.5	98.70±44.24	p=0.97	51.41±5.06	p=0.01
Reason for Transplantation						
Pseudophakia Corneal edema (PCE)	33	30.6	107.69±49.38		53.87±5.43	
Bullous Keratopathy	18	16.7	91.94±52.34		52.83±5.74	
Keratoconus	3	2.8	49.00±20.29		47.00±1.73	
Dystrophy	6	5.6	89.50±51.11		51.16±6.17	
Herpetic keratitis	5	4.6	100.20±47.69		49.60±5.41	
Graft rejection	11	10.2	76.54±45.02	F=1.32	54.00±5.74	F=1.26
Keratitis	5	4.6	122.20±38.65	p=0.22	54.60±6.18	p=0.26
Nephelion	6	5.6	105.00±36.74		51.83±5.56	
Foreign object penetration	5	4.6	138.00±26.83		56.80±4.91	
DMEK	12	11.1	101.16±48.99		55.08±5.01	
Melting (Ulcer)	4	3.7	80.33±62.05		50.75±5.50	

a X – mean, SD – standard deviation, F – One Way Anova, t – student T, DMEK – descemet membrane endothelial keratoplasty

Table 4. Examination of the relationship between negative automatic thought and anxiety in patients (n=108)a

	Possible range	$\bar{x} \pm SD$	Actual range	Test and significance
Negative Automatic Thought	30–150	98.90±48.11	30–150	r=0.52
Anxiety Score	20–80	53.25±5.55	45–59	p<0.01

a X – mean, SD – standard deviation, r – correlation

Table 5. Results of logistic regression analysis on patients' negative automatic thought statea

Features	B	p	Exp (β)	95% Confidence interval exp (β)	
				Lower	Upper
Gender	-0.32	0.54	0.72	0.25	2.06
Age	-0.01	0.34	0.98	0.95	1.01
Income status	-2.71	<0.01	0.06	0.01	0.33
Surgery history	0.99	0.09	2.70	0.8	8.61
Anxiety score	0.29	<0.01	1.34	1.17	1.54

[a] B – Regression coefficient, Exp (β) – odds range (OR)

Discussion

This is the first study evaluating the levels of preoperative anxiety and negative automatic thoughts and the relationship between them in patients waiting for corneal transplantation. This study has three important results;

Although the anxiety level of the female patients was higher than that of male patients in our study, the relationship was insignificant. Esme et al. stated that there was no statistically significant relationship between gender and state and trait anxiety in their study conducted with lung cancer patients.[14] Bhattacharjee and Banerjee stated in their study that although the trait anxiety of female cancer patients was statistically significantly higher than that of male cancer patients, no significant difference was found between the genders for state anxiety.[15] Courtillié et al. stated that the state anxiety level of female kidney transplant patients was higher than that of male kidney transplant patients.[16] Cardoso et al. also emphasize that being a female is a risk factor in terms of anxiety and depression. Our study result shows similarity with the literature.[17] Bal et al. stated that the followings play a role in the fact that women have higher anxiety levels than men in the general population and that anxiety disorders are more common in women: some biological differences such as differences in the number and structure of serotonin receptors; social learning; attachment patterns; differences in expression of anxiety; and, some other psychosocial factors.[18]

No statistically significant relationship was found between the age and anxiety score mean scores of the pre-transplant patients in our study. Mystakidou et al. also mentioned that there was a negative relationship between age and anxiety.[19] Similarly, Linden, Vodermaier, MacKenzie, and Greig stated that the emotional distress in some types of cancer was inversely proportional to age.[20] Sheppard, Harper, Davis, Hirpa, and Makambi state that there was a negative correlation (r=-0.224) between age and anxiety in their study they conducted with breast cancer patients.[21] Moreover, Weiss Wiesel et al. stated that anxiety decreased with age in elderly individuals with cancer.[22] Srivastava et al. also stated that the young age group, low monthly income, having less financial support, low education level, and being a bachelor were associated with anxiety and depression in cancer patients.[23] It can be said that our study result is different from the results in the literature because the average age of the patients was high and approximately three-quarters of them had a surgery history.

It was seen in our study that there was a positive, medium-level linear relationship between negative automatic thought and trait anxiety, which was statistically significant. In the literature, many studies conducted with different groups support the finding of a positive relationship between automatic thoughts and anxiety. For example, Beck et al. emphasized a positive, linear

significant relationship between anxiety symptoms and anxious automatic thoughts and that this relationship was stronger than the relationship between depressive symptoms and depressive automatic thoughts.[24] Torrente et al. (2014) also stated that there was a statistically significant positive linear high-level relationship between trait anxiety and automatic thoughts, similar to the finding of this study.[25] Alcalar et al. also noticed a positive linear relationship between the automatic thoughts of breast cancer patients and their depression and psychiatric mood disorders.[26] Kara and Acet stated that there was a positive linear significant relationship between the state of anxiety and sub-dimensions of astonishment, negative emotion, and hopelessness of the negative automatic thoughts.[27] Our study results show similarity with the literature.

Study limitations

This study was conducted only with patients in one university hospital in southeastern Türkiye; thus, the results cannot be generalized to the entire society. The present results may serve as a source for future research conducted with patients of different cultural backgrounds. Although it was shown that there is a clear relationship between anxiety and negative automatic thoughts in corneal transplant surgery, since this is an outpatient surgery, no intervention aimed at decreasing anxiety or negative automatic thoughts. This situation requires experimental research, such as a prospective education program.

Conclusion

As a result, it was determined that the negative automatic thoughts and trait anxiety levels of the patients waiting for the corneal transplantation were high. The anxiety increased as the negative automatic thoughts increased. Moreover, in our study, the anxiety level of the female patients was higher than that of the male patients. In the patients waiting for the corneal transplant, their anxieties can be reduced in parallel with providing psychosocial and emotional supports and eliminating negative thought states.

This is the first study evaluating the levels of preoperative anxiety and negative automatic thoughts and the relationship between them in patients waiting for corneal transplantation. In this context, it should be emphasized in training programs that psychological care should be given as much importance as physical care during the corneal transplant nursing care process. Moreover, appropriate nursing care plans should be created to reduce patients' anxiety and negative automatic thought levels during the corneal transplantation process. Finally, more studies should be done on the subject.

Declarations

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Author contributions

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

Conflicts of interest

The authors declare no conflict of interest.

Data availability

Data available on request from the authors.

Ethics approval

The study received ethical approval from Mardin Artuklu University Ethics Committee. Decision date and number: 07.01.2022/21.

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ORIGINAL PAPER

Simplified vs modified (reduced) ultrasound 6 joint score in assessing disease activity in rheumatoid arthritis patients

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ABSTRACT

Introduction and aim. With increasing ultrasound US assessment opportunities for tight rheumatoid arthritis (RA) management, more simplified accurate US-assessment is desired in clinical practice. Aim of the work is assessing modified vs simplified ultrasound 6-joint scores relevance in assessing rheumatoid arthritis disease activity.

Material and methods. Fifty-five RA patients were subjected to detailed history, clinical, and musculoskeletal examination with disease activity assessment by clinical disease activity index, simple disease activity index and disease activity score in 28 joints. Complete blood count, erythrocyte sedimentation rate, C-reactive protein, rheumatoid factor, anti-cyclic citrullinated peptide antibodies were done. Patients underwent US examination (gray-scale and power Doppler) for wrist, 2nd and 3rd metacarpophalangeal and knee joints bilaterally. Synovitis composite score was added. Two US indices were constructed: simplified S6 and modified M6 joint scores.

Results. Statistical significant positive correlations were high between S6/M6 score parameters (total, grey-scale (GS), power doppler (PD), Composite) and disease activity markers. Both M6 and S6 scores differentiated mild-moderate and moderate-severe disease activity patients. However, only S6 score differentiated remission from mild disease activity patients.

Conclusion. Ultrasound 6-joint scores (especially simplified S6) were rapid, easy and sensitive ultrasound tools assessing rheumatoid arthritis disease activity in clinical practice.

Keywords. activity, 6-joint score, rheumatoid arthritis, ultrasound

Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease, mainly involving the small joints of the hands and feet. In the absence of appropriate treatment, RA leads to irreversible joint and tendon damage, disability and premature death.[1] It has been suggested that some patients may experience radiographic progression of joint disease despite being in clinical remission, although this presumably is a carry-over effect of past disease activity. Nevertheless, if clinical assessment of joint swelling is not a sufficiently reliable method to assess patients with RA in a state of remission, more sensitive methods for assessment of disease activity might be needed.[2]

Musculoskeletal ultrasound is capable of evaluating the two elementary findings associated with synovitis: synovial hypertrophy (SH) and synovial fluid/effusion (SF). Both SF and SH are evaluated primarily on gray-scale (GS) ultrasound, while color Doppler (CD) and power Doppler (PD) are utilized to demonstrate activity related to SH . However, a systematic review of the scoring systems used to evaluate synovitis in RA found it difficult to determine the least number of joints that needed to be assessed for a global US score.[3]

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The relevance of US for monitoring RA is well reflected in European League Against Rheumatism (EULAR) recommendations for the use of imaging on clinical management of RA.[4] However, Abo Gabal et al., in their work studying the usefulness of ultrasound 7-joint and 12-joint scores in assessing disease activity in RA patients adding the use of composite synovitis score, reported limitations of both scores and recommended the use of more rapid, objective and sensitive ultrasound score for practical assessment of RA disease activity.[5]

Aim

The aim of the study was to evaluate the relevance of simplified vs modified (reduced) ultrasound 6-joint scores in assessing disease activity in rheumatoid arthritis in clinical practice.

Material and methods

This analytic cross-sectional study was carried out on Fifty-five RA patients fulfilling the 2010 American College of Rheumatology (ACR)/EULAR criteria.[6] Patients were recruited from the rheumatology outpatient clinic and inpatient rheumatology department at Ain Shams university hospital between January 2019–September 2022. Patients having arthritis/arthropathy due to any other rheumatological or systemic diseases were excluded. All patients were subjected to: detailed history and thorough clinical and musculoskeletal examination with assessment of disease activity by clinical disease activity index (CDAI), simple disease activity index (SDAI) and disease activity score in 28 joints (DAS28-ESR).[7,8] Laboratory workup included: Complete blood count (CBC) was measured on a Siemens ADVIA 2120i hematology analyzer (Siemens Healthcare diagnostic, Erlangen, Germany), Erythrocyte sedimentation rate (ESR) was placed into sedimentation measurement stand (BD seditainer Manual ESR BD), C-reactive protein (CRP, 0004956842190c501V9.0) was measured by immuneturbidimetric assay on Roche/Hitachi cobas c systems (GmbH, Mannheim, Germany, reference value <5 mg/L). Rheumatoid factor (RF, 0020764574322c501V8.0) was measured by immuneturbidimetric assay on Roche/Hitachi cobas c systems (GmbH, Mannheim, Germany, reference value <14 IU/mL). Anti-cyclic citrullinated peptide (Anti-CCP, 05115671001V4) antibodies were measured by electrochemiluminescence immunoassay "ECLIA" on Roche diagnostic Cobas e411 (GmbH, Mannheim, Germany, reference value <20 IU/mL).

Ultrasound examination

Systematic ultrasound assessment was performed by a rheumatologist (first author of this study who is Eular certified with 7 year experience in performing musculoskletal ultrasonography) using the Power Doppler PD ultrasound device MyLab^{TM}Six (e-Saote company) with 6–18 MH probe for assessment of small joints of hands and wrist and 3-13 MH probe for larger joints (knees). PD pulse repetition frequency was 500-750 Hz; Doppler frequency was 6.7–11.1 MHz; low wall filters were used.

A systematic multiplanar grey-scale and PD examination of 6 joints: wrist, second metacarpo-phalangeal (2nd MCP) and knee of both sides in modified or reduced score (M6), (330 joints in 55 patients) and wrist, 2nd MCP and 3rd MCP in simplified (S6) score (330 joints in 55 patients), was done.[9,10] The US assessment for each patient, included 12 synovial sites in M6: bilateral wrist (dorsal carpal recesses), bilateral 2nd MCP joint (dorsal and palmar sides) and bilateral knee joint (suprapatellar recess, medial and lateral parapatellar recesses). In case of S6 score 10 synovial sites, bilateral wrist (dorsal carpal recesses), 2nd MCP and 3rd MCP joints (dorsal and palmar sides) were chosen.

Ultrasound scoring system

We then considered each joint as a unique structure, and we assessed the presence of synovitis (synovial proliferation (SP)± synovial effusion (SE)) by B-mode US and PD within the SP in each joint. According to the EULAR/OMERACT definitions, SE and SP were defined. Synovitis (effusion and synovial hypertrophy) on GS images were graded using a 4-grade semiquantitative scale from 0 to 3 as follows: 0 = normal joint (no synovial hypertrophy, no effusion), 1 = mild synovitis (mild synovial hypertrophy, with or without mild effusion), 2 = moderate synovitis (moderate synovial hypertrophy with or without mild or moderate effusion), and 3 = severe synovitis (severe synovial hypertrophy, with or without severe effusion).[11]

Power Doppler synovitis scoring for 6-joint score was evaluated also using a semiquantitative 4-grade scale from 0 to 3 as follows: grade 0 = absence of signal, no intra-articular flow; grade 1 = mild, 1- or 2-vessel signal (including 1 confluent vessel) for small joints and 2 to 3 signals for large joints (including 2 confluent vessels); grade 2 = moderate confluent vessels (>grade 1) and less than 50% of normal area; grade 3 = marked vessel signals in more than half the synovial area (Fig. 1-4).

We added further modification, the higher score obtained for each of the US elementary lesions (US-Synovitis GS and PD) at each synovial site was then considered for the scoring of each joint as a unique structure (composite score).[11]

For M6, sum scores for GS synovitis and PD synovitis were computed in 2 separate scores. The scoring range is 36 for each. Total sum for 6-joint score ranges from 0 to 72. Composite score (the higher of GS or PD scores is used for grading the overall synovitis severity) ranges from 0 to 18. For S6 sum scores for GS synovitis

and PD synovitis were computed in 2 separate scores. The scoring range is 30 for each. Total sum for S6 ranges from 0 to 60. Composite score ranges from 0 to 18.

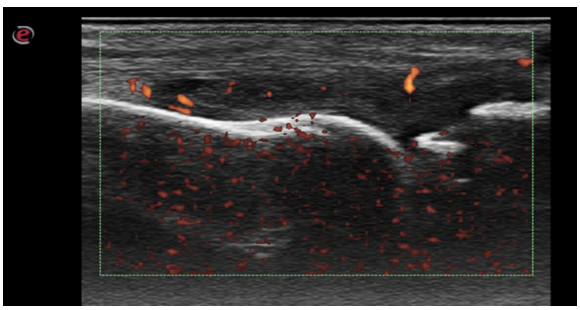

Fig. 1. Ultrasound image (longitudinal power Doppler) of 2nd MCP joint, composite score 2

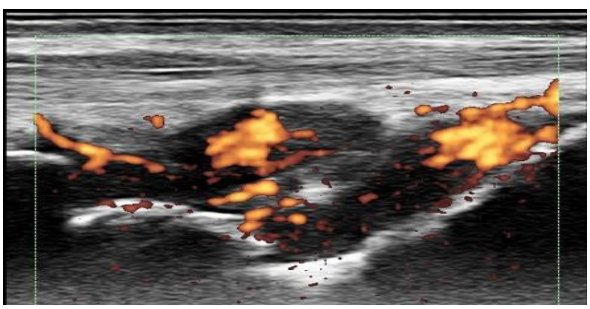

Fig. 2. Ultrasound image (longitudinal power Doppler) of right wrist joint, composite score 3

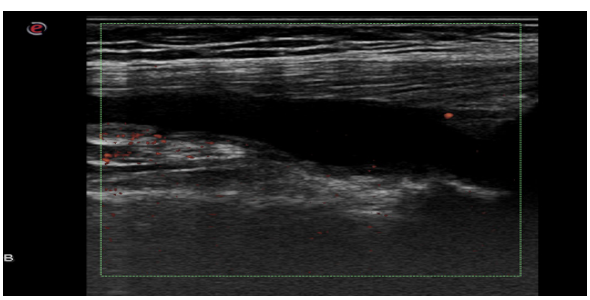

Fig. 3. Ultrasound image (longitudinal power Doppler) of knee suprapatellar recess with effusion and grade 1 synovial thickenning, no Doppler signal

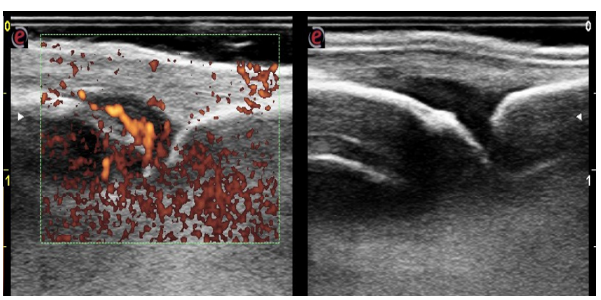

Fig. 4. Ultrasound image of 3rd MCP joint (longitudinal, power Doppler left and gray scale right), composite score 3

Statistical analysis

Data was coded and entered using the statistical package SPSS (IBM, Armonk, NY, USA). Data were summarized using number and percent for qualitative variables, mean and standard deviation for quantitative variables which are normally distributed while median was used for quantitative variables which are not normally distributed. Comparisons of quantitative variables which are not normally distributed between groups were done using nonparametrical Mann-Whiteny and Kruskal-Wallis tests. Multiple comparisons between each two groups were done using Mann-Whitney test with Bonferoni correction. Correlations were done to test for relation between quantitative variables. p values less than or equal to 0.05 were considered as statistically significant.

Ethical approval

All subjects gave their informed consent for inclusion before they participated in the study. Approval of Ain Shams University Ethical Committee was obtained.

Results

Our study was performed on 55 Rheumatoid arthritis patients fulfilling the 2010 American College of Rheumatology (ACR)/EULAR criteria.[6] Table 1 shows RA patients characteristics.

Regarding different extra-articular manifestations among studied patients. Pulmonary fibrosis was diagnosed in 10% of patients, secondary Sjogren's syndrome in 9%, peripheral neuropathy secondary to rheumatoid vasculitis in 7%, carpal tunnel syndrome in 14% (unilateral in 50 % of cases) and rheumatoid nodules in 5.5% of RA patients.

Fifty eight (58.2%) of our patients were on oral corticosteroids and 82% of all patients were on hydroxychloroquine. Concerning conventional synthetic Disease modifying antirheumatic drugs (csDMARDs), 76% of our patients were on leflunomide, 41.8% on methotrexate and 27% on more than one DMARD. None was on biological DMARD. Table 2 presents the total GS synovitis, PD synovitis and composite scores of both S6 and M6 scoring systems.

In Table 3 using ultrasound synovitis composite score, wrist was the commonest joint to be affected in 101/330 examined joints (30.6%) followed by knees in 70/330 (21.21%) then 2nd MCP joints in 55/330 (16.6%) and 3rd MCP joints in 37/330 (11.21%).This was consistent with the known distribution of joint involvement in RA.

Table 4, Figures 5 and 6 show highly statistically significant positive correlations between all M6 score parameters (total, GS, PD, composite) and No TJ, No SJ, VAS, PGA, EGA, ESR, CRP, DAS28 , CDAI and SDAI , $p \leq 0.001$.There was statistically significant positive correlation between M6 GS, PD and composite scores and

Table 1. Characteristics of 55 RA patients*

Variable		No (%)/Mean±SD Range	Variable		No (%)/Mean±SD Range
Sex No (%)	Females	50 (90.9%)	RA duration (years)	Mean±SD	7.35 ± 4.71
	Males	5 (9.1%)		Range	1–20
Age (years)	Mean±SD	41.82 ± 9.09	No. TJ (0–28)	Mean±SD	1.84±2.52
	Range	22–70		Range	1–14
DAS28 (0–9.4)	Mean±SD	3.44±1.3	No. SJ (0–28)	Mean±SD	1.47±2.64
	Range	1.75–7.2		Range	1–10
DAS28 (activity groups)	Remission <2.6	18 (32.7%)	VAS (0–100)	Mean±SD	32.27±19.86
	Mild activity ≥2.6 and ≤3.2	11 (20%)		Range	10–80
	Moderate activity >3.2 and ≤5.1	18 (32.7%)			
	Severe activity >5.1	8 (14.5%)			
CDAI (0–76)	Mean±SD	10.31±8.49	PGA (0–10)	Mean±SD	4.45±1.88
	Range	2–42		Range	2–10
CDAI (activity groups)	Remission ≤2.8	3 (5.5%)	EGA (0–10)	Mean±SD	3.29±1.93
	Mild activity >2.8 and ≤10	32 (58.2%)		Range	1–8
	Moderate activity >10 and ≤22	14 (25.5%)			
	Severe activity >22	6 (10.9%)			
SDAI (0–86)	Mean±SD	11.03±8.76	Rheumatoid F (IU/mL)	Positive	46 (83.6%)
	Range	2.35–44.4		Negative	9 (16.4%)
				Mean±SD	46.88±49.18
				Range	4–250
SDAI (activity groups)	Remission ≤3.3	3 (5.5%)	AntiCCP antibodies (IU/mL)	Positive	50 (90.9%)
	Mild activity >3.3 and ≤11	32 (58.2%)		Negative	5 (9.1%)
	Moderate activity >11 and ≤26	16 (29.1%)		Mean±SD Range	97.13±100.04 4.5–488
	Severe activity >26	4 (7.3%)			
TLC (10^3/mL)	Mean±SD	6.9±2.07	CRP (mg/L)	Mean±SD	7.23±4.26
	Range	3.8–11.3		Range	2–24
HGB (gm/dL)	Mean±SD	11.53±0.95	ESR (mm/hr)	Mean±SD	34.71±20.53
	Range	9–16		Range	9–95
Platelets (10^3/mL)	Mean±SD	308.67±110.42			
	Range	150–600			

* No TJ – number of tender joints, No SJ – number of swallen joints, VAS – visual analogue scale, PGA – patient global assessment, EGA – evaluator global assessment, ESR – erythrocyte sedimentation rate, CRP – C reactive protein, DAS28 – disease activity score, CDAI – clinical disease activity index, SDAI – simple disease activity index, TLC – total leukocyte count, HGB – hemoglobin, AntiCCP – anti cyclic citrullinated protein antibodies

Table 2. Description of ultrasound S6 and M6 scores parameters

S6 score		M6 score	
Variable	Mean±SD Range	Variable	Mean±SD Range
Total S6	11.65±11.28	Total M6	12.89±10.99
0–60	0–46	0–72	2–61
GS synovitis	7.38±6.21	GS synovitis	8.95±6.54
0–30	0–26	0–36	2–34
PD synovitis	4.27±5.27	PD synovitis	3.95±4.9
0–30	0–21	0–36	0–27
Composite score	6.35±4.38	Composite score	6.86±3.77
0–18	0–18	0–18	2–18

Table 3. Examined areas and graded (0-3) parameters of M6 and S6 scores

Examined area			Right		Left	
			score	No./Percent	score	No./Percent
Wrist	Dorsal	GS	0	3 (5.5%)	0	6 (10.9%)
			1	21 (38.2%)	1	22 (40%)
			2	19 (34.5%)	2	19 (34.5%)
			3	12 (21.8%)	3	8 (14.5%)
		PD	0	23 (41.8%)	0	26 (47.3%)
			1	18 (32.7%)	1	15 (27.3%)
			2	6 (10.9%)	2	7 (12.7%)
			3	8 (14.5%)	3	7 (12.7%)
		Composite	0	3 (5.5%)	0	6 (10.9%)
			1	21 (38.2%)	1	20 (36.4%)
			2	18 (32.7%)	2	19 (34.5%)
			3	13 (23.6%)	3	10 (18.2%)
2nd metacarpo-phalangeal	Dorsal	GS	0	36 (65.5%)	0	35 (63.6%)
			1	7 (12.7%)	1	11 (20%)
			2	4 (7.3%)	2	4 (7.3%)
			3	8 (14.5%)	3	5 (9.1%)
		PD	0	43 (78.2%)	0	39 (70.9%)
			1	6 (10.9%)	1	8 (14.5%)
			2	3 (5.5%)	2	7 (12.7%)
			3	3 (5.5%)	3	1 (1.8%)
	Palmar	GS	0	38 (69.1%)	0	33 (60%)
			1	9 (16.4%)	1	12 (21.8%)
			2	4 (7.3%)	2	6 (10.9%)
			3	4 (7.3%)	3	4 (7.3%)
		PD	0	46 (83.6%)	0	45 (81.8%)
			1	4 (7.3%)	1	3 (5.5%)
			2	2 (3.6%)	2	3 (5.5%)
			3	3 (5.5%)	3	4 (7.3%)
		Composite	0	31 (56.4%)	0	24 (43.6%)
			1	11 (20%)	1	19 (34.5%)
			2	3 (5.5%)	2	5 (9.1%)
			3	10 (18.2%)	3	7 (12.7%)
3rd metacarpo-phalangeal	Dorsal	GS	0	38 (69.1%)	0	37 (67.3%)
			1	7 (12.7%)	1	7 (12.7%)
			2	7 (12.7%)	2	6 (10.9%)
			3	3 (5.5%)	3	5 (9.1%)
		PD	0	44 (80%)	0	45 (81.8%)
			1	8 (14.5%)	1	4 (7.3%)
			2	2 (3.6%)	2	3 (5.5%)
			3	1 (1.8%)	3	3 (5.5%)
	Palmar	GS	0	46 (83.6%)	0	44 (80%)
			1	7 (12.7%)	1	10 (18.2%)
			2	1 (1.8%)	3	1 (1.8%)
			3	1 (1.8%)		
		PD	0	50 (90.9%)	0	50 (90.9%)
			1	4 (7.3%)	1	3 (5.5%)
			2	1 (1.8%)	2	2 (3.6%)
		Composite	0	38 (69.1%)	0	35 (63.6%)
			1	7 (12.7%)	1	9 (16.4%)
			2	7 (12.7%)	2	4 (7.3%)
			3	3 (5.5%)	3	7 (12.7%)
Knee	Suprapatellar	GS	0	40 (72.7%)	0	39 (70.9%)
			1	11 (20%)	1	11 (20%)
			2	2 (3.6%)	2	3 (5.5%)
			3	2 (3.6%)	3	2 (3.6%)
		PD	0	52 (94.5%)	0	53 (96.4%)
			1	2 (3.6%)	1	1 (1.8%)
			2	1 (1.8%)	2	1 (1.8%)
	Medial Parapatellar	GS	0	35 (63.6%)	0	37 (67.3%)
			1	14 (25.5%)	1	14 (25.5%)
			2	5 (9.1%)	2	3 (5.5%)
			3	1 (1.8%)	3	1 (1.8%)
		PD	0	51 (92.7%)	0	54 (98.2%)
			1	3 (5.5%)	2	1 (1.8%)
			2	1 (1.8%)		
	lateral Parapatellar	GS	0	21 (38.2%)	0	26 (47.3%)
			1	26 (47.3%)	1	21 (38.2%)
			2	7 (12.7%)	2	7 (12.7%)
			3	1 (1.8%)	3	1 (1.8%)
		PD	0	52 (94.5%)	0	49 (89.1%)
			2	2 (3.6%)	1	5 (9.1%)
			3	1 (1.8%)	3	1 (1.8%)
		Composite	0	19 (34.5%)	0	21 (38.2%)
			1	25 (45.5%)	1	23 (41.8%)
			2	9 (16.4%)	2	9 (16.4%)
			3	2 (3.6%)	3	2 (3.6%)

Table 4. Correlations of M6 and S6 score parameters*

		M6 score				S6 score			
		Total S	GS S	PD S	Composite	Total S	GS S	PD S	Composite
No. TJ	Cor.Co.	0.680	0.672	0.562	0.690	0.723	0.696	0.729	0.695
	p	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
No. SJ	Cor Co.	0.606	0.575	0.641	0.573	0.827	0.797	0.831	0.714
	p	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Dis. Dur.	Cor Co.	0.121	0.027	0.232	0.073	0.074	0.040	0.111	0.049
	p	0.380	0.843	0.088	0.598	0.592	0.771	0.420	0.720
VAS	Cor Co.	0.781	0.713	0.744	0.729	0.860	0.834	0.859	0.817
	p	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
PGA	Cor Co.	0.737	0.673	0.706	0.686	0.742	0.702	0.762	0.705
	p	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
EGA	Cor Co.	0.789	0.709	0.764	0.716	0.812	0.772	0.829	0.766
	p	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
ESR	Cor Co.	0.543	0.432	0.612	0.452	0.596	0.555	0.622	0.501
	p	<0.001	0.001	<0.001	.001	<0.001	<0.001	<0.001	<0.001
CRP	Cor Co.	0.505	0.487	0.479	0.515	0.588	0.574	0.584	0.546
	p	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
RF	Cor Co.	0.018	0.013	0.057	-0.010	-0.032	-0.054	-0.004	-0.087
	p	0.899	0.924	0.680	0.944	0.818	0.693	0.978	0.526
ACCP	Cor Co.	-0.103	-0.124	-0.054	-0.136	0.094	0.118	0.061	0.057
	p	0.455	0.368	0.695	0.323	0.496	0.390	0.656	0.680
TLC	Cor Co.	0.074	0.110	0.146	0.105	0.114	0.080	0.151	0.049
	p	0.592	0.424	0.289	0.446	0.405	0.562	0.271	0.721
HGB	Cor Co.	0.240	0.226	0.076	0.231	0.030	0.051	0.005	0.074
	p	0.077	0.097	0.580	0.090	0.826	0.712	0.972	0.589
PLT	Cor Co.	0.357	0.336	0.371	0.318	0.582	0.561	0.585	0.528
	p	0.007	0.012	0.005	0.018	<0.001	<0.001	<0.001	<0.001
DAS28	Cor Co.	0.772	0.686	0.754	0.708	0.843	0.813	0.849	0.789
	p	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
CDAI	Cor Co.	0.797	0.727	0.730	0.731	0.888	0.862	0.886	0.834
	P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
SDAI	Cor Co.	0.793	0.725	0.726	0.729	0.888	0.863	0.887	0.835
	p	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

* Pearson correlation test, No TJ – number of tender joints, No SJ – number of swallen joints, VAS – visual analogue scale, PGA – patient global assessment, EGA – evaluator global assessment, ESR – erythrocyte sedimentation rate, CRP – C reactive protein, DAS28 – disease activity score, CDAI – clinical disease activity index, SDAI – simple disease activity index, TLC – total leukocyte count, HGB – hemoglobin, AntiCCP – anti cyclic citrullinated protein antibodies, Dis. Dur. – disease duration, RF – rheumatoid factor

platelet count, p≤0.05. Also, there was highly statistically significant positive correlations between all S6 score parameters (total, GS, PD, composite) and no TJ, No SJ, VAS, PGA, EGA, ESR, CRP, DAS28, CDAI, SDAI and platelets, p≤0.001. However, no statistically significant correlations were noted between any of M6 or S6 components and disease duration, TLC, HGB, rheumatoid factor or ACCP-AB titres, p≥0.05.

Table 5 shows comparison between different patient groups (classified according to disease activity measured by DAS28, CDAI and SDAI) regarding S6 score components to determine accuracy and sensitivity in assessing disease activity (when the score differed significantly between patient groups with close disease activities (remission/mild, mild/moderate, moderate/severe). According to DAS28, there was statistical significant difference between patients with remission/mild in total and GS synovitis scores, and between those with moderate/severe in GS synovitis and composite scores, with p<0.05. According to CDAI and SDAI, there was statistical significant difference between patients with mild/moderate disease activity as regard total S6-joint score and all its parameters (GS synovitis, PD synovitis, composite score) with p<0.05.

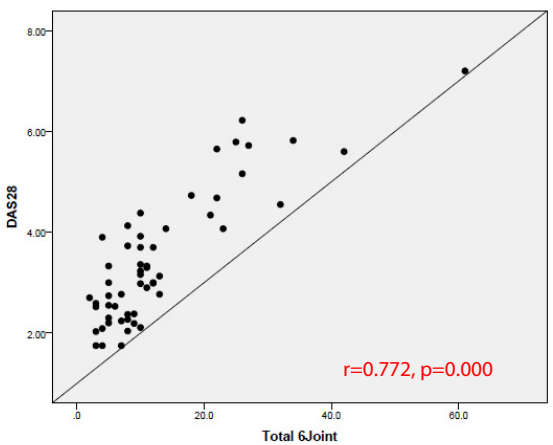

Fig. 5. Correlation between total M6 ultrasound score and DAS-28 ESR

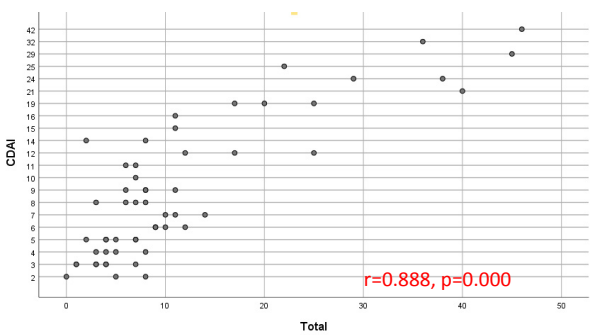

Fig. 6. Correlation between total S6 ultrasound score and CDAI

Table 6 shows that according to DAS28, there was statistical significant difference between patients with moderate-severe disease activity as regard total M6, its GS and PD synovitis scores (but not composite score), with p<0.05. According to CDAI and SDAI , there was statistical significant difference between patients with mild-moderate disease activity in total M6-joint score and all its parameters (GS synovitis, PD synovitis, composite score) with p<0.05.

Table 5. Comparison between patients with different grades of disease activity as regard S6 joint score components (total, GS, PD and composite score)*

	Remission –mild activity	Remission– moderate activity	Remission– severe activity	Mild activity– moderate activity	Moderate– severe activity	Mild activity– severe activity
According to DAS28						
Total S6 score	0.043	0.001	<0.001	1	0.056	0.027
GS synovitis score	0.035	0.006	<0.001	1	0.027	0.046
PD synovitis score	0.256	0.001	<0.001	1	0.066	0.006
Composite score	0.124	0.009	<0.001	1	0.049	0.031
According to CDAI						
Total S6 score	1	0.265	0.009	0.025	0.278	<0.001
GS synovitis	1	0.181	0.007	0.017	0.377	<0.001
PD synovitis	1	0.533	0.015	0.048	0.148	<0.001
Composite score	1	0.228	0.009	0.018	0.348	<0.001
According to SDAI						
Total S6 score	1	0.143	0.018	0.003	0.780	0.002
GS synovitis	1	0.138	0.016	0.009	0.719	0.003
PD synovitis	1	0.229	0.028	0.002	0.747	0.001
Composite score	1	0.162	0.025	0.007	0.912	0.004

* Mann-Whitney and Kruskal-Wallis tests, GS – gray scale, PD – power Doppler, DAS28 – disease activity score, CDAI – clinical disease activity index, SDAI – simple disease activity index

Discussion

Among the different imaging tools described in the European League Against Rheumatism recommendations, US is especially helpful for the following various situations encountered during daily clinical practice: diagnosis of RA, evaluation of disease activity/treatment response/prognosis, and support of remission surveillance.[12] With increasing US assessment opportunities for the tight management of RA, a more simplified, accurate US assessment strategy is desired.[13] We carried out the present study to evaluate the relevance of modified vs simplified ultrasound 6-joint scores in assessing disease activity in rheumatoid arthritis

Most of our patients in this study were females (91%) and males representing only 9%. Similar demographic data were found in El-Gohary et al., (90% females and 10% males) and in Kamel et al. (92% females and 8% males).[14,15] In our study, mean and standard deviation (SD) for the age of our patients were 41.82±9.09 years. Similar results were found in El-Gohary et al. (45.3±12.4), and in Kamel et al. (43.9±10.78).[14,15] Most of the previous figures confirm high incidence of RA in middle aged females. Other studies reported higher mean ages, 66 years in Endo et al. and 53.52±11.81 in Cerqueira et al.[13,4] Different ethnicity or disease durations may account for this.

Table 6. Comparison between patients with different grades of disease activity as regard M6 joint score components (total, GS, PD and composite score)

	Remission –mild activity	Remission– moderate activity	Remission– severe activity	Mild activity– moderate activity	Moderate– severe activity	Mild activity– severe activity
According to DAS28						
Total M6 score	0.246	<0.001	0.003	1	0.049	0.007
GS synovitis score	1	0.045	<0.001	1	0.027	0.003
PD synovitis score	0.446	0.005	<0.001	1	0.052	<0.0015
Composite score	0.663	0.010	<0.001	1	0.069	0.007
According to CDAI						
Total M6 score	1	0.087	0.013	0.001	1	<0.001
GS synovitis	1	0.138	0.023	0.001	1	<0.001
PD synovitis	1	0.562	0.034	0.017	0.406	<0.001
Composite score	1	0.235	0.036	0.001	1	<0.001
According to SDAI						
Total M6 score	1	0.056	0.023	<0.001	1	0.002
GS synovitis	1	0.113	0.046	<0.001	1	0.004
PD synovitis	1	0.240	0.048	<0.001	1	0.001
Composite score	1	0.177	0.094	<0.001	1	0.008

* Mann-Whitney and Kruskal-Wallis tests, GS – gray scale, PD – power Doppler, DAS28 – disease activity score, CDAI – clinical disease activity index, SDAI – simple disease activity index

Measuring disease activity with DAS28, 32.7% of our patients were in remission, 20% had low activity, 32.7% had moderate activity and 14.5% showed severe activity. CDAI and SDAI gave similar findings, about 58% of our patients were having mild activity score, only 5.5% in remission according to both scores, 25.5% and 29.1% of patients had moderate activity and 10.9% and 7.3% of patients had severe activity according to CDAI and SDAI respectively. In 2019, Sivakumaran et al., reported 16.5% of patients with remission, 13.3% had low activity, 38.3 % had moderate activity and 31.6% had severe activity.[16] El-Gohary et al. found that 26% of their patients were in remission as measured by DAS28. Only 6% and 8% were in remission as defined by and SDAI and CDAI.[14] In study of Kamel et al., 8% of patients were in remission, 12% of low activity, 24% of moderate activity, and 56% of high activity as defined by CDAI.[15] Higher percentage of patients in remission and lower percentage of active disease (especially by DAS 28) in our study may reflect better patient compliance, different disease severity, different response to therapy and different ethnicity.

In our study, significant positive correlations between all M6 score parameters and measures of RA disease activity were evident, p≤0.001. There was statistically significant positive correlation between M6 GS, PD and composite scores and platelet count, p≤0.05. Perricone et al., in their study on modified (reduced) 6-joint score reported highly significant positive correlation (p=0.001) between score parameters and DAS28 and CRP.[9] However, DAS-28 scores usually reflect a combination of active and chronic joint changes, better assessment of disease activity by CDAI and its positive correlation with M6 score was more informative in our study.

On the other hand, there was highly statistically significant positive correlations between all S6 score parameters and CDAI and platelets, p≤0.001. Rosa et al., in their study on simplified 6-joint score reported similar correlation with DAS28.[10] In Endo et al., although both DAS28-ESR and DAS28- CRP scores were significantly positively correlated with the 6 joint-GS, and PD scores, such correlations tended to weaken with time after therapy initiation.[13]

In our study, despite equal relevance and sensitivities of both ultrasound scores M6 and S6 in differentiating activity groups with mild-moderate and with moderate-severe disease activities in patients with RA, only S6 score was able to differentiate patient groups with remission vs mild disease activity that may reflect its superiority over M6 score. Also, in M6 score, incorporation of knee joint assessment in place of 3rd MCP joint of S6 score was done. And as for GS synovitis in knee , any grade of knee effusion was considered in our study that may account for high frequency of knee involvement by composite score (70/330, 21%).

In Figus et al., using M6 ultrasound score, ultrasound detected significant differences in the score of joint effusion (SE), synovial hypertrophy (SH), and Doppler signal and significant differences in the joint score of II MCP and wrist (but not for knee joint scores) between oligoarticular PSA and RA, p<0.05. No differences were found between RA and polyarticular PSA.[17] Moreover, previous studies reported good correlation between hand US scores and DAS-28 assessment using three different US scores, which was replicated in the study of Sivakumaran et al.[10,16,18] These findings may support the higher value of S6 (hand joint score) versus M6 score.

However, Perricone et al., stated that they obtained a 6-joint US assessment (M6) that was able to detect 97.7% of patients with 12-joint US-SE, 100% of patients with 12-joint US-SP and 100% of those with 12-joint PD and this 6-joint US score showed a highly significant correlation with changes in DAS-28 (p<0.001).[9]

Abo Gabal et al., in their work studying the usefulness of ultrasound 7 and 12-joint scores in assessing disease activity in RA patients adding the use of synovitis composite score, they reported limitations of both scores as they included assessment of tenosynovitis/paratendinitis which are nonspecific for rheumatoid arthritis, they may result from mechanical injury especially in advanced disease and cannot be used alone as a domain to assess or decide starting, continuing or withdrawing biologics. Twelve score was time consuming and erosions included in 7-joint score were not accurate indicator of disease activity. They reflect structural damage and chronicity of the disease. In contrast, hot erosions or growing erosions over time are the better indicator of pannus activity but were not included in the score.[5]

They also reported that synovitis component of both scores was more informative and accurate in assessing disease activity and combined synovitis score (composite score) recommended by EULAR/OMERACT and Abo Gabal et al., was a significant assessing tool.[5,19] In their study, Abo Gabal et al., mentioned the need for more rapid, objective and sensitive ultrasound score for practical assessment of RA disease activity.[5]

We think that our choice of 6 joints synovitis (GS and PD) score in the present study had avoided these limitations being rapid, easy, including only synovitis, the most important US-detected elementary lesions in RA that reflects disease activity. As in Abo Gabal et al., we added EULAR-OMERACT combined synovitis score (composite score).[5,19] However, its use in our study did not show superiority to any of M6 or S6 GS or PD synovitis scores making its addition to the original M6 or S6 scores components non-essential.

On the other hand, Sivakumaran et al., stated that preselected simplified US scores are less reliable in appreciating the disease burden when compared with an extended protocol for 22 joint US examination, raising clinicians' awareness regarding the need to comprehensively assess multiple hand joints to reliably rule out subclinical inflammation. They stated that the scores including 20 and 22 joints captured more information than the eight-, ten-, and 14-joint scores, even if all the eight US scores they explored correlated very well with the DAS-28 assessment.[16] Also Endo et al., stated that their study was limited by US assessments eliminating the possible involvement of the joints other than bilateral wrist and finger joints using simplified 6 joint score.[13]

Our opinion to solve this issue is to add separate variable joint number ultrasound score (from the remaining 22 joints) that assessing current joints (GS and PD synovitis) showing clinical involvement (symptoms or signs) in previous 2 weeks to be added to standardized assessment using simplified 6 joint score in order not to miss or underestimate RA joint activity.

Conclusion

Ultrasound 6-joint scores (whether modified or simplified) were rapid, easy and sensitive ultrasound tool in assessing disease activity in rheumatoid arthritis in clin-

ical practice. S6 score was superior in differentiating remission from mild disease activity groups.

Recommendation: Further studies are needed on larger scale to establish a cutoff value for ultrasound 6-joint score that differentiates RA patients in remission and at different grades of disease activity as in clinical disease activity indices.

Declarations

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Author contributions

Conceptualization, M.M.A.G.; Methodology, M.M.A.G., M.A.M. and M.A.E.; Software, M.A.M.; Validation, M.M.A.G., A.B.E., M.A.M. and M.A.E.; Formal Analysis, M.M.A.G. and M.A.M.; Investigation, M.A.M., M.A.E. and M.M.A.G.; Data Curation, M.A.M., M.A.E. and M.M.A.G.; Writing–Original Draft Preparation, M.M.A.G. and M.A.M.; Writing–Review & Editing, M.M.A.G., M.A.M. and A.B.E.; Visualization, M.M.A.G. and M.A.M.; Supervision, M.M.A.G.; Project Administration, M.M.A.G.; Funding Acquisition, M.M.A.G. and M.A.M..

Conflicts of interest

The author(s) declare no competing interests.

Data availability

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

Ethics approval

All subjects gave their informed consent for inclusion before they participated in the study. Approval of Ain Shams University Ethical Committee was obtained.

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ORIGINAL PAPER

Exploring barriers to vasectomy adoption among married men in Dadra and Nagar Haveli

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ABSTRACT

Introduction and aim. Vasectomy was introduced in India in 1992 and is the most effective, simple, and safe permanent method of contraception yet its use is very limited among the population. The study's objectives were to assess the knowledge, attitude, and perception towards vasectomy and the barriers to adoption among married men in Dadra and Nagar Haveli.

Material and methods. A cross-sectional hospital-based study was conducted on married male attendants of patients and data were collected on sociodemographic details, knowledge regarding vasectomy, their attitude and perceptions towards vasectomy, and their intention of using vasectomy in the future.

Results. Out of 300 participants, 258 (86%) participants have heard of vasectomy as a contraceptive method, and only one participant has undergone vasectomy. The majority (285; 95%) of the participants agreed that family planning is also a responsibility of males while nearly two-third (185; 61.7%) of them were not willing to undergo vasectomy in the future**.** Procedure-related factors, post-procedure-related factors, social factors, availability of alternate contraceptive methods, and preference for tubectomy were the barriers to the adoption of vasectomy.

Conclusion. There is a need to provide correct information and counseling about vasectomy and non-scalpel vasectomy to eligible couples to increase the acceptance of vasectomy as a safe, effective, and cheaper method of contraception.

Keywords. attitude, barriers, knowledge, married men, perception, vasectomy

Introduction

India initiated the National Family Planning Programme in 1952, encompassing a range of scientifically tested and approved contraceptive methods, supported by strategic implementation ideologies.[1] Among the various methods introduced, sterilization emerged as the most effective permanent solution within the Indian community. Despite vasectomy being a simpler surgical procedure, more cost-effective, and associated with fewer complications, a higher prevalence of tubectomy is shown as compared to vasectomy in India.[2] Between 1960 and 1977, family planning in India predominantly relied on methods like vasectomy and condom use, with men accounting for over 50% of all family planning users. However, attempts to aggressively promote vasectomy during emergencies had adverse consequences for the program's success. Following the shortcomings of this camp-based approach, a combination of positive and negative incentives, along with compulsory sterilization, contributed to a shift in preference towards permanent family planning methods. This shift ultimately led to the widespread adoption of female

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sterilization methods.[3] Vasectomy, which was once the favored choice, now contributes to less than 5% of the total sterilization cases annually. This trend is further supported by data from the NFHS-5 Report, which indicates that vasectomy usage stands at 0.3%, while female sterilization accounts for 37.9%.[4]

To be motivated to use vasectomy services, an individual or couple first needs accurate knowledge of and positive attitudes toward vasectomy.[2] The inclusion of NSV (No-Scalpel Vasectomy) in the National Family Planning Programme of India was officially announced in March 1992. NSV neither requires any cut on the skin with a scalpel blade nor any skin stitch, after the procedure is completed. It is a much less painful and faster procedure than conventional vasectomy. However, over the years, the utilization of male sterilization methods has remained relatively low. One of the reasons for the low and declining use of vasectomy is health professionals' lack of knowledge, misinformation, and personal dislike of vasectomy or untested presumptions about what men thought and wanted.[5]

Aim

This study aims to address the existing research gap by exploring the knowledge, attitudes, and perceptions regarding vasectomy among married men residing in the Dadra and Nagar Haveli district.

Material and methods

Study design

A cross-sectional descriptive study was conducted in a tertiary care hospital in Dadra and Nagar Haveli district. The duration of the study was from August to November 2022.

Study subject

Male attendants of patients coming to the outpatient department, aged 22–60 years, married, have at least one child whose age is above one year, and are willing to give consent were considered as the target population. Study participants with critically ill patients and those who are not willing to give consent were excluded from the study.

Sample size

In the determination of an appropriate sample size for our research study, we drew upon valuable insights from a prior investigation conducted by Shrivastava et al. [6], which reported that 78.9% of the population possessed knowledge about vasectomy. To ensure a robust estimation with a desirable margin of error, we employed the sample size calculation formula, N=4*p*q/d^2, where p represents the proportion (78.9%), and d signifies the absolute error (5%). This meticulous calculation yielded a sample size of 266. In anticipation of potential non-responses, we prudently factored in a 10% non-response rate, thereby culminating in a final sample size of 300 participants for our study.

Sampling procedure

Male participants visiting the outpatient department across various specialties were selected using a simple random sampling method. On a daily basis, the first 2-3 participants were selected randomly and were interviewed using a pre-designed, pre-tested, and semi-structured questionnaire that was originally prepared in English and later translated into Hindi and Gujarati. The questionnaire consisted of three sections. The first section collected sociodemographic details of the participants, such as the number of children they have, and the duration of marriage. The second section focused on contraceptive use and assessed the participants' knowledge regarding vasectomy and No scalpel vasectomy. It also included questions about eligibility criteria for vasectomy, sources of information, and other related topics. The third section aimed to evaluate the participants' attitudes and perceptions towards vasectomy using a three point Likert scale (Agree, Disagree and Don't know). Contraceptive usage was considered if either of the partners was using any method of contraception, including natural methods.

Statistical analysis

The responses were entered into a computer using MS Excel for analysis. Frequencies of all variables were generated to identify any errors or missing data, and data-cleaning procedures were performed accordingly. The data was presented using various descriptive representations, such as tables and charts, as well as inferential statistics. Descriptive statistics, including frequencies, means, and standard deviations (SD), were utilized to summarize the data. To gain insights into the reasons behind the unwillingness of married men to undergo vasectomy, an analysis was conducted on qualitative responses provided by the participants. Through this analysis, the responses were categorized into different themes and categories based on the patterns identified in their answers.

Ethical considerations

Permission was obtained from the Institutional Ethics Committee (DMHS/IEC/2016/214/1237) before commencing the study and written informed consent was obtained from every participant prior to their recruitment into the study. To ensure confidentiality, the identities of the respondents have been kept anonymous. Following the interviews, participants received counseling and comprehensive health education on vasectomy, including its benefits.

Table 1. Socio-demographic information of participants (n=300)

Variables	Frequency (%)
Age group (years)	
22-30	126 (42)
31-40	149 (49.7)
≥41	25 (8.3)
Residence	
Urban	82 (27.3)
Semi-urban	77 (25.7)
Rural	141 (47)
Education	
Primary	20 (6.7)
Upper primary	39 (13)
Secondary	62 (20.7)
Higher secondary	80 (26.7)
Graduate	79 (26.3)
Post graduate	8 (2.7)
Illiterate	12 (4)
Religion	
Hindu	270 (90)
Muslim	26 (8.7)
Christian	4 (1.3)
Occupation	
Unemployed	2 (0.7)
Unskilled	13 (4.3)
Semiskilled	272 (90.7)
Skilled	13 (4.3)
Total family members	
≤4	196 (65.3)
>4	104 (34.6)
Socioeconomic status	
Upper	56 (18.7)
Upper middle	133 (44.3)
Lower middle	79 (26.3)
Upper lower	22 (7.3)
Lower	10 (3.3)
Age of spouse (years)	
18–25	7 (2.3)
26–30	226 (75.3)
31–35	64 (21.3)
>40	3 (1)
Education of spouse	
Primary	18 (6)
Upper primary	37 (12.3)
Secondary	64 (21.3)
Higher secondary	84 (28)
Graduate	52 (17.3)
Post graduate	11 (3.7)
Illiterate	34 (11.3)
Occupation of spouse	
Housewife	279 (93)
Working women	21 (7)
Duration of marriage	
<5	115 (38.3)
6–10	111 (37)
11–15	49 (16.3)
>15	25 (8.3)
Total children born	
≤2	238 (79.3)
>2	62 (20.7)
Total number of sons	
One	173 (57.7)
More than one	60 (20)
None	67 (22.3)

Results

Socio-demographic and reproductive health characteristics

A cross-sectional study was conducted at a tertiary care hospital, involving 300 married men to evaluate their knowledge and attitude towards vasectomy. The mean (±SD) age of participants was 32.6 (±5.6) years. Among the participants, 49.7% (149) fell within the 31–40 age group, and 47% (141) resided in rural areas. Approximately 26.3% of the men had completed their graduation, and the majority of them (90%) identified as Hindu. According to the BG Prasad classification, half of the participants belonged to socioeconomic status (SES) class II. The majority (90.7%) were engaged in semiskilled work. (Table 1)

Regarding their spouses, around 75.3% (226) were aged between 26 and 30 years, and the majority (93%) were homemakers. Around 38.3% (115) of the participants had been married for less than five years, while 79.3% (238) had two or fewer living children at the time of the survey (Table 1).

Contraceptive use and knowledge regarding the vasectomy method

Out of the total participants, 185 (61.7%) reported using contraception. Among the contraceptive users, nearly half of them (89; 48.1%) relied on male condoms, followed by female sterilization (51; 27.5%), natural methods (17; 9.2%), oral pills (14; 7.5%), intrauterine devices (8; 4.3%), and injectable contraceptives (1; 0.5%). Additionally, five participants (5.9%) reported using condoms in combination with other methods. While 258 (86%) participants had heard of vasectomy as a contraceptive option, only one participant had undergone the procedure. Table 2 shows various sources through which the participants obtained information about vasectomy.

Among the total participants, 80 (26.7%) individuals had prior knowledge of non-scalpel vasectomy, and out of those, 34 (11.3%) were aware of its specifics. A majority of participants (184; 61.3%) correctly identified vasectomy as a permanent method of contraception. However, only 34 (11.3%) men were aware of the eligibility criteria for undergoing vasectomy. Among this group, 9 (26.4%) knew that the age criterion is being over 21 years old, 18 (53%) recognized that having two living children is a prerequisite, and 20 (58.8%) participants acknowledged that being married is a requirement. The majority of the participants (256; 85.3%) were unaware of the number of outpatient department (OPD) visits needed for a vasectomy.

Attitude and perception of participants towards vasectomy

The majority (285; 95%) of the participants agreed that family planning is a responsibility that extends to males.

Table 2. Contraceptive use and knowledge regarding vasectomy method (n=300)[a]

Variables	Frequency (%)
Current contraceptive use	
Yes	185 (61.7)
No	115 (38.3)
Type of contraceptive use (N=185)	
Female sterilization	50 (27)
Intrauterine device	8 (4.3)
Male condoms	89 (48.1)
Oral contraceptives	14 (7.5)
Injectable	1 (0.5)
Male sterilization	1 (0.5)
Natural methods	17 (9.2)
Multiple methods	5 (5.9)
Heard of vasectomy as a method of contraception	
Yes	258 (86)
No	42 (14)
Source of information (n=258)*	
Friends/family	158 (61.2)
Doctor/nurse	110 (42.6)
TV advertisements	94 (36.4)
Internet	85 (32.9)
Newspaper	59 (22.8)
Magazine	13 (5)
Radio	6 (2.3)
Others	5 (1.9)
Heard of non-scalpel vasectomy	
Yes	80 (26.7)
No	220 (73.3)
Aware about non scalpel vasectomy	
Yes	34 (11.3)
No	266 (88.7)
Aware of vasectomy as permanent method	
Yes	184 (61.3)
No	116 (38.7)
Aware of eligibility criteria of vasectomy	
Yes	34 (11.3)
No	266 (88.6)
Age range for vasectomy (years) (n=34)	
≤21	1 (3)
≥21	9 (26.4)
Don't know	24 (70.5)
Prerequisite no. of children (n=34)	
Two children	18 (53)
At least 1 son	2 (5.9)
Don't know	15 (44.1)
Marital status (n=34)	
Married	20 (58.8)
Unmarried	4 (11.7)
Don't know	10 (29.5)
OPD visits needed for vasectomy	
≥5	42 (14)
< 5	2 (0.7)
Don't know	256 (85.3)

[a] *Multiple responses recorded

However, nearly half (164; 54.7%) of the participants believed that female sterilization is preferable to male sterilization. Around 22.3% of the participants considered female sterilization to be easier and more commonly practiced. The willingness to recommend vasectomy to their friends, colleagues, or relatives was expressed by 128 (42.7%) participants.

When it comes to post-vasectomy considerations, the majority (204; 68%) of the participants disagreed with the notion that men need to use an additional contraceptive method after undergoing vasectomy. Similarly, 122 (40.7%) participants denied the belief that vasectomy carries long-term health risks (Table 3).

Table 3. Attitude and perception of participants towards vasectomy (n=300)

Indicator	Agree	Disagree	Don't know
1. Family planning is also a responsibility of males	285 (95)	3 (1)	12 (4)
2. Female sterilization is a better method than male sterilization	164 (54.7)	61 (20.3)	75 (25)
3. Willingness to undergo vasectomy in the future	39 (13)	185 (61.7)	76 (25.3)
4. Willingness to recommend vasectomy to friends/colleagues/family members	128 (42.7)	72 (24)	100 (33.3)
5. Vasectomy have a high success rate for contraception	116 (38.7)	31 (10.3)	153 (51)
6. Men need to use another contraceptive method after vasectomy	24 (8)	204 (68.0)	72 (24)
7. Vasectomy has long time health risks	70 (23.3)	122 (40.7)	108 (36)

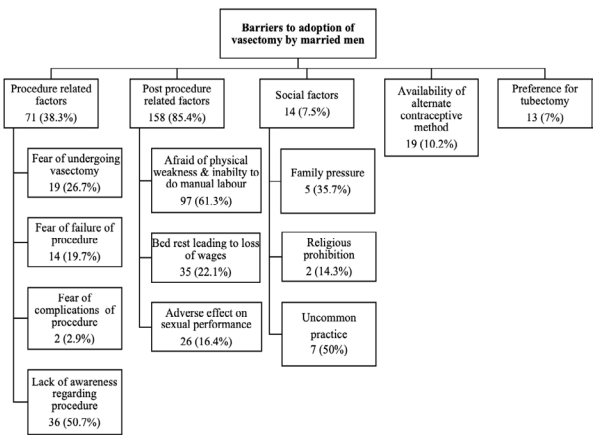

Fig 1. Reasons for unwillingness to undergo vasectomy among married men (n=185)

Barriers to adopting vasectomy

When asked about their willingness to undergo vasectomy, 185 [61.7% (95% CI 56.2-67.5)] of the participants stated that they were not inclined to undergo vasectomy in the future. Figure 1 displays the barriers encountered by married men when considering a vasectomy. The responses were divided into five themes: Procedure-related factors, post-procedure-related factors, social factors, Availability of alternate contraceptive methods, and preference for tubectomy. A significant majority (97; 61.3%) expressed fear of experiencing

physical weakness and being unable to engage in manual labor following vasectomy. Additionally, 35 (22.1%) participants mentioned concerns about having to take bed rest, which could result in the loss of wages. Less than one-third (19; 26.7%) of participants expressed fear of undergoing the surgery itself, while 36 (50.7%) were not well-informed about the procedure and its potential complications. Approximately 10% of participants indicated a preference for alternative contraceptive methods in the future, and 7% mentioned that they would opt for female sterilization for their partners.

Discussion

A cross-sectional descriptive study was conducted at a district hospital to assess the knowledge, attitude, and perceptions towards vasectomy among married men attending the hospital. Among the contraceptive users in our study, only one participant had undergone the procedure and this finding is consistent with the low prevalence rates reported in studies conducted by Madhukumar et al. (0.64%), Safi et al. (1.6%), Srivastava et al. (1.2%), as well as the NFHS-5 data, which reported a prevalence of vasectomy at 0.3%.[1,6-9]

Addressing this knowledge gap is crucial to improve the understanding and acceptance of vasectomy as a viable contraceptive option. While 86% of participants were familiar with vasectomy as contraception, only 80 participants knew of non-scalpel vasectomy, and merely 34 participants were aware of vasectomy. This finding suggests a gap in knowledge and awareness of non-scalpel vasectomy among the participants in our study. In comparison, the study by Garg et al. reported a much higher awareness rate of 97.4%.[10] This discrepancy may indicate variations in educational levels, information dissemination, or healthcare provider counseling across different settings. By addressing the lack of awareness, we can potentially increase the uptake of vasectomy as a reliable family planning option among married men.

Among the 300 participants, the majority of participants (256) lacked knowledge regarding the number of OPD visits needed for the non-scalpel vasectomy. We also found that 18 participants believed that a minimum of two children is required for undergoing vasectomy and 15 participants were uncertain about the minimum number of children required for vasectomy. These findings underscore the lack of clear understanding among the respondents regarding vasectomy.

The success of any family planning program greatly relies on the equal participation and responsibility of both male and female partners. Our study revealed that a majority of participants (285; 95%) acknowledged the importance of male involvement in family planning and recognized that it is a shared responsibility for the well-being of the family. This finding can be attributed to the increased education and awareness about family planning within Indian communities, leading to a better understanding of their respective roles.

Our study found that nearly half of the participants (164; 54.7%) believed that female sterilization is preferable to male sterilization. Our findings were consistent with the study done by Madhukumar et al., where they also observed that 50% of males believed that tubectomy is better than vasectomy and the study by Sood et al. also found that almost 53% of men in their study believed that tubectomy was a simpler procedure therefore their partners should undergo sterilization instead.[7,11]

To assess their attitude towards vasectomy, participants were asked about their willingness to adopt vasectomy in the future and it was found that nearly two-thirds (185; 61.7%) of them were not willing to undergo vasectomy in the future. Similar findings were observed in studies conducted by Nesro et al. (76%), Ayeli et al. (80.4%), Safi et al. (89.3%), Sood et al. (89%).[8-12]

In our study, the high rejection rate was due to post-surgery-related myths and stigmas (85.4%) related to vasectomy. The majority of participants expressed fear regarding physical weakness and the inability to perform manual labor (61.3%) after the procedure. Similar concerns were noted by Madhukumar et al. (72%) and Shrivastava et al. (67.6%).[6,7] Participants also expressed worries about the need for prolonged bed rest following the surgery (22.1%), which could result in wage loss. Our findings align with those of Shrivastava et al. (38.2%) in a separate study.[6] Additionally, a common myth associated with vasectomy was a perceived loss of libido after the procedure, as observed in studies conducted by Shrivastava et al. (78.8%), Madhukumar et al. (76%), and Ayele et al. (25.4%).[6-8] We also found that 16.4% of participants were concerned about potential adverse effects on sexual performance. These findings highlight the importance of addressing and debunking these misconceptions to promote informed decision-making regarding vasectomy. The unwillingness to undergo vasectomy was also because of a lack of awareness regarding the procedure (50.7%) and which is in alignment with studies done by Srivastava et al. (70%), and Ayele et al. (74%).[6,8] Participants also reported fear of undergoing surgery (26.7%) in our study. Ayele et al. also found that 34% of men were scared of the surgery in North West Ethiopia.[7] The fear of failure of the procedure was also observed in 19% of participants in our study. Sood et al. also found that 52% of the men had fear of failure of vasectomy as it brings a bad name to wives in Punjab.[11] Fear of complications (3%) of the procedure after the surgery was another factor observed in our study which was coherent with studies done by Ayele et al. (35.7%), Desmennu et al. (26%) and Nesro et al. (16%).[8,12,13] These findings indicate the importance of addressing fears and providing adequate information to alleviate concerns and promote informed decision-making regarding the procedure.

Religious prohibitions were also a concerned factor in willingness for vasectomy in our study. Approximately 14% of the participants reported that their religious beliefs forbid vasectomy, which aligns with findings from studies conducted in Punjab (70.5%) and North West Ethiopia (34.7%). Social stigma and family pressure (35.7%) were found to be deciding factors to undergo vasectomy in our study.[8,11] Almost 75% of the respondents reported that the vasectomy procedure may cause community mistreatment according to a study done by Chinnaiyan et al.[14] These consistent observations highlight the need to consider and address religious and social perspectives when discussing and promoting vasectomy as a contraceptive option.

When asked about their intention to recommend vasectomy to others, 128 participants (42.7%) expressed their willingness to recommend the procedure and share their knowledge about it with friends, colleagues, and relatives. However, despite the positive attitude towards vasectomy, a significant portion of participants held reservations regarding certain aspects. While 68% (204) disagreed with the need for another contraceptive method post-vasectomy, only 38.7% (116) agreed that vasectomy has a high success rate. Additionally, 40.7% (122) believed that there might be some long-term health risks associated with vasectomy. These findings align with the study conducted by Safi et al., where 42.2% of participants expressed concerns about the health risks associated with non-scalpel vasectomy.[9] These findings suggest the need to address these misconceptions regarding vasectomy with counseling services.

Study limitations

The present study had limitations in terms of generalizability, as the findings are based on the opinions of participants who exhibited better health-seeking behavior by seeking medical care at the hospital.

Conclusion

The prevalence of vasectomy remains extremely low in various regions and states of India due to the presence of misconceptions, myths, lack of knowledge, and social stigmas surrounding the procedure. To boost the family planning program and increase acceptance of vasectomy, it is crucial to provide accurate information and education to the population through healthcare professionals and various media platforms. Efforts should be made to normalize the concept of vasectomy and reduce stigmas associated with it among the general population as well as healthcare professionals themselves. The government can play a vital role by initiating and promoting awareness programs to enhance knowledge and understanding about vasectomy among the population.

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Declarations

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Author contributions

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Conflicts of interest

All authors declare that they have no conflicts of interest.

Data availability

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

Ethics approval

Permission was obtained from the Institutional Ethics Committee (DMHS/IEC/2016/214/1237) before commencing the study and written informed consent was obtained from every participant prior to their recruitment into the study. To ensure confidentiality, the identities of the respondents have been kept anonymous. Following the interviews, participants received counseling and comprehensive health education on vasectomy, including its benefits.

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ORIGINAL PAPER

Behaviors of pregnant women regarding travel – the case of Türkiye

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ABSTRACT

Introduction and aim. With the development of transportation facilities and options, people can now travel easily. With women having a greater presence in the workforce, pregnant women can work until the eighth week before childbirth. This study was conducted to examine the behaviors of pregnant women regarding travel.

Material and methods. The data for this cross-sectional and descriptive study were collected from 519 pregnant women who voluntarily participated in the research and met the research criteria. The data were analyzed using the SPSS 24 software package.

Results. The average age of the participating pregnant women was 27.49±0.17, with 63.8% residing in urban areas, 74.8% being primiparous, and 47.6% being in the third trimester of pregnancy. A decrease in travel and car usage during pregnancy. The use of bus, train/tram/metro, taxi, bicycle/scooter, and motorcycle decreased during pregnancy, while use of car, plane, and ship/ferry increased. 81.9% of women always wore a seat belt during pregnancy. There was a statistically significant difference between the educational level of women and their car usage ($p<0.005$). It was found that women wore seat belts more frequently in the third trimester. As the number of pregnancies increased, the frequency of seat belt usage decreased.

Conclusion. A decrease in the frequency of travel was observed among women during pregnancy. The levels of seat belt usage and correct seat belt fastening were unsatisfactory.

Keywords. car usage, pregnancy, travel, seat belt

Introduction

With the development of transportation facilities and options, people can now travel easily. With women having a greater presence in the workforce, pregnant women can work until the eighth week before childbirth. In fact, if there is no problem until the 37th week of pregnancy with the medical report that she can work, the expectant mother can continue to work. Pregnancy is a natural process that causes significant anatomical, physiological, and psychological changes in a woman's body.[1,2] The consequences of these physical changes that occur as pregnancy progresses include the seat belt not fully encompassing the mother, and a decrease in the distance between the abdomen and the steering wheel during car usage and travel. Due to the potential for trauma during motor vehicle usage and travel in pregnancy, extra caution should be exercised during this period.[3]

Traumas experienced during pregnancy pose a threat to the lives of both the mother and the fetus. It is reported that women experience trauma at a rate of 5-8% throughout their pregnancies, resulting in fetal loss.[4] Particularly, motor vehicle accidents or collisions

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can increase the risk of preterm labor, early membrane rupture, hypertension, phlebitis, uterine and placental injuries; therefore, travel distances longer than 180 km after the 28th week of pregnancy are not recommended for pregnant women.[3] The total number of crash induced injuries during pregnancy led to 3.6 more deaths (fetal/neonatal) than deaths among newborns and infants.[5] The most important factor that contributes to mild effects on the mother and fetus is the use of seat belts. However, when seat belts are incorrectly fastened, they can cause uterine rupture and fetal deaths due to the compressive effect of sudden flexion.[8] The use of seat belts alone can reduce fetal and maternal injuries by 50%.[6]

Pregnant women are increasingly active in their work lives and travel for work or leisure purposes. The most suitable period recommended for travel is the second trimester (14-26 weeks). During pregnancy, the incidence of superficial and deep venous thrombophlebitis increases due to the elevation of coagulation factors and the venous dilation caused by progesterone.[7] For short-distance travel, it is recommended to prefer private vehicles, and for long distances, air travel should be the first choice.[8] When traveling by car, taking a break every two hours and walking for at least 10 minutes during breaks should be ensured to exercise the thigh muscles.[7] Until the 28th week of pregnancy, women can travel without any documents, and between the 28th and 36th weeks, they can travel with a "fit to fly" report. However, boarding an aircraft is prohibited after the 36th week.[8] It is recommended to choose seats on the aisle side to allow movement.[9] Additionally, flights are not recommended for high-risk pregnancies such as presence of anemia, cervical insufficiency, or bleeding.[7] Bus travel is not the preferred option during pregnancy as it causes the legs to remain inactive for prolonged periods.[10] If necessary, pregnant women should sit on the aisle side, move at regular intervals, and perform leg exercises.[8,11]

Aim

This study was conducted to examine the behaviors of pregnant women regarding travel and car usage.

Material and methods

Ethical approval

Permission was obtained from the Institutional Review Board of the researcher's affiliated university (Date: 27.11.2020, No: 2014/08-13) for the implementation of the study. Informed voluntary consent was obtained from the participating pregnant women.

Study design and participants

This study is a cross-sectional and descriptive research. The population of the study consisted of pregnant women. The minimum sample size required for the study was calculated as 385 using the unknown population sample formula (n=t 2.p.q/d 2) with a 95% confidence interval (d=0.05), t=1.96, p=0.5, and q=0.5. Accordingly, 519 pregnant women were included in the study.

Collection of data

To collect data on the behaviors of pregnant women regarding travel and car usage, a questionnaire was prepared by the researchers through a literature review.[12,13] Participants completed the questionnaire online through self-reporting. The survey form was shared online through various social platforms (Twitter, Facebook, Instagram, WhatsApp, email, etc.) between December 2020 and June 2021. Participants were allowed to log in once from the same computer ID number via the survey.com website. The study included all pregnant women living within the borders of Türkiye, aged 18 years and above, and without high-risk pregnancies. And also, the data was collected during the COVID-19 pandemic period, but the travel habits of pregnant women, independent of the pandemic, were questioned.

Data collection tools

The questionnaire consisted of two sections: the first section included five questions on demographic characteristics (age, place of residence, educational status, gestational age, and number of pregnancies), and the second section consisted of 37 questions on pregnant women's behaviors related to travel and car usage (pre-pregnancy and post-pregnancy travel frequency, car usage frequency, discomfort experienced during travel, most common sitting position in the vehicle, seat belt usage, use of additional safety systems or restrictions, resting and break durations during travel, etc.), making a total of 42 questions.

Data analysis

The dependent variable of the study was pregnant women's car usage and travel behaviors, and the independent variables consisted of their sociodemographic characteristics (age, educational status, place of residence, number of pregnancies, gestational age). The data were analyzed using the Statistical Package for Social Sciences (SPSS) version 24 (IBM, Armonk, NY, USA). Descriptive statistics such as frequencies, percentages, means, and standard deviations were used, and the chi-square test was employed for comparing dependent and independent variables. The significance level was set at $p<0.05$.

Results

The mean age of the participating pregnant women was 27.49±0.17 (minumum:18, maximum:42). Among them, 63.8% lived in urban areas, and 69.4% had a bachelor's

degree. In our study, 74.8% of the women were primiparous, and 47.6% were in the third trimester (Table 1).

The comparison of women's travel frequency before pregnancy and during pregnancy is presented in Table 2. It shows a decrease in travel frequency during pregnancy.

Table 1. Demographic and obstetric characteristics of pregnant women

Variables	n (%)
Place of residence	
City	331 (63.8)
Town	165 (31.8)
Village/Small town	23 (4.4)
Educational status	
Primary/Secondary School	20 (3.9)
High school	93 (17.9)
Bachelor's degree	360 (69.4)
Postgraduate degree	46 (8.9)
Gestational week	
First trimester (1-13 weeks)	106 (20.4)
Second trimester (14-26 weeks)	166 (32)
Third trimester (27-42 weeks)	472 (47.6)
Number of pregnancies	
1	388 (74.8)
2	106 (20.4)
3	22 (4.2)
4 or more	3 (0.6)
Total	519 (100)

Table 2. Comparison of women's travel frequency before pregnancy and during pregnancy

Variables	Travel frequency before pregnancy	Travel frequency during pregnancy
	n (%)	n (%)
Everything	47 (9.1)	21 (4)
Every 2-3 days	58 (11.2)	21 (4)
Once a week	74 (14.3)	65 (12.5)
Once a month	198 (38.2)	126 (24.3)
Once every 2 months	13 (2.5)	25 (4.8)
Once every 3 months	5 (1)	2 (0.4)
Once a year	9 (1.7)	8 (1.5)
Twice a year	14 (2.6)	3 (0.7)
Three to four times a year	7 (1.3)	4 (0.8)
I don't travel	94 (18.1)	244 (47)
Total	519 (100)	519 (100)

When the means of transportation used by women before and during pregnancy are analyzed, the use of buses (20.6%), trains/trams/metro (7.3%), taxis (9.2%), bicycles/scooters (1.3%) and motorcycles (1.7%) with pregnancy decreased. On the other hand, the use of car (66.1%), airplane (12.2%), and ship/ferry (4.0%) is higher during pregnancy (Table 3). In addition, women stated that the safest means of transportation were cars (51.1%) and airplanes (9.4%) during pregnancy.

The problems experienced by women while using different transportation methods are shown in Table 4. The most common issues reported while driving a car were back pain (12.5%), nausea/vomiting (10.8%), and fear for the safety of their babies (8.9%).

Table 3. Transportation methods used by women before and during pregnancy

Variables	Travel frequency before pregnancy	Travel frequency during pregnancy
	n (%)	n (%)
Car	237 (50.9)	265 (66.1)
Bus	96 (20.6)	11 (2.8)
Taxi	43 (9.2)	33 (8.2)
Train/Tram/Metro	34 (7.3)	23 (5.7)
Airplane	33 (7.1)	49 (12.2)
Ship/Ferry	9 (1.9)	16 (4)
Motorcycle	8 (1.7)	–
Bicycle/Scooter	6 (1.3)	4 (1)
Total	466 (100)	401 (100)

Table 4. Problems experienced by women while using transportation methods

Variables	Car	Bus	Ship/ Ferry	Train/ Tram/Metro	Airplane	Taxi
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Back pain	65 (12.5)	5 (1)	–	–	–	3 (0.6)
Nausea/vomiting	56 (10.8)	5 (1)	–	–	–	3 (0.6)
Fear for the safety of the baby	46 (8.9)	6 (1.2)	–	1 (0.2)	3 (0.6)	5 (1)
Fatigue	38 (7.3)	3 (0.6)	–	–	1 (0.2)	3 (0.6)
Increased stress	31 (6.0)	4 (0.8)	–	–	–	3 (0.6)
Hot flashes	29 (5.6)	5 (1)	–	–	–	5 (1)
Feeling of bloating	28 (5.4)	3 (0.6)	–	–	–	–
Headache	24 (4.6)	3 (0.6)	–	–	1 (0.2)	–
Fear for personal safety	16 (3.1)	2 (0.4)	–	–	–	2 (0.4)
Fear of losing control	14 (2.7)	2 (0.4)	1 (0.2)	–	–	1 (0.2)
Palpitations	9 (1.7)	2 (0.4)	–	–	–	2 (0.4)

It was seen that women who used cars as a means of transportation during pregnancy changed their seating positions from the driver's seat to the passenger seat or the back seat. The preferred seat for women who used cars for transportation was the passenger seat both before pregnancy (29.9%) and during pregnancy (38.7%). It was also found that women preferred to sit in the back seat after becoming pregnant when using taxis. Among women who traveled by plane during pregnancy, the majority chose window seats and seats in the aisle (Table 5).

The frequency of car and seat belt usage by women before and during pregnancy is presented in Table 6. According to the results, there was a decrease in the frequency of car usage during pregnancy. Additionally, an increase in seat belt usage was observed during pregnancy. 81.9% of women stated that they always wear a seat belt during pregnancy (Table 6).

Table 5. Seat preference by women before and during pregnancy

Variables	Before pregnancy	During pregnancy
	n (%)	n (%)
Car		
Passenger seat	155 (29.9)	201 (38.7)
Driver's seat	76 (14.6)	41 (7.9)
Back seat	6 (1.2)	23 (4.4)
Bus		
Front seat by the window	17 (3.3)	5 (1)
Front seat by the aisle	11 (2.1)	1 (0.2)
Middle seat by the window	27 (5.2)	2 (0.4)
Middle seat by the aisle	13 (2.5)	2 (0.4)
Back seat by the window	18 (3.5)	1 (0.2)
Back seat by the aisle	10 (1.9)	–
Train/Tram/Metro		
Seat by the window	29 (5.6)	19 (3.7)
Seat in the aisle	5 (1.0)	4 (0.8)
Ship/Ferryboat		
Upper deck/open area	8 (1.5)	2 (0.4)
Lower deck/enclosed area	1 (0.2)	14 (2.7)
Airplane		
Seat by the window	26 (5)	32 (6.2)
Middle seat	4 (0.8)	6 (1.2)
Seat by the aisle	3 (0.6)	11 (2.1)
Taxi		
Back seat	37 (7.1)	31 (6)
Front passenger seat	6 (1.2)	2 (0.4)

Table 6. Frequency of car and seat belt usage before and during pregnancy

Variables	Before pregnancy	During pregnancy
	n (%)	n (%)
Car usage		
Never	184 (35.5)	255 (49.1)
Rarely	96 (18.5)	82 (15.8)
Occasionally	103 (19.8)	99 (19.1)
Frequently	39 (7.5)	26 (5.0)
Always	97 (18.7)	57 (11.0)
Seat belt usage	12 (2.3)	12 (2.3)
Never	23 (4.4)	17 (3.3)
Rarely	31 (6.0)	29 (5.6)
Occasionally	42 (8.1)	36 (6.9)
Frequently	411 (79.2)	425 (81.9)
Total	519 (100)	519 (100)

When examining how pregnant women fasten seat belts, the majority (65.3%) place the belt under their abdomen, with support on their shoulders and hips. The rate of women using a seat belt adjuster, which helps position the seat belt, is 5.4%. Only 19.3% of pregnant women are aware of the existence of this device (Table 7).

37.2% of women always take a break on their journey every 2-3 hours during their pregnancy, and 27.8% of them stroll for 10 minutes (min). 33% of the pregnant women were informed about travel during pregnancy (Table 8). 49.9% of women received this information about travel from an obstetrician, 27.6% from the internet, 10% from a nurse, 9.4% from magazines/books, and 2.1% from newspapers.

Table 7. Seat belt fastening position and seat belt adjuster usage during pregnancy

Variables	n (%)
Seat belt fastening position	
Using only the diagonal part of the seat belt	99 (19.1)
Positioning the seat belt over the shoulders and hips, above the abdomen	69 (13.3)
Positioning the seat belt under the abdomen with support on the shoulders and hips	339 (65.3)
Not using a seat belt	12 (2.3)
Seat belt adjuster usage	
Yes	28 (5.4)
No	491 (94.6)
Awareness of Seat belt Adjuster	
Yes	100 (19.3)
No	419 (80.7)
Total	519 (100)

Table 8. Behaviors of pregnant women during travel

Variables	n (%)
Taking a break every 2-3 hours	
Never	33 (6.4)
Rarely	49 (9.4)
Occasionally	97 (18.7)
Often	147 (28.3)
Always	193 (37.2)
Walking for 10 minutes during each break	
Never	51 (9.8)
Rarely	77 (14.8)
Occasionally	129 (24.9)
Often	118 (22.7)
Always	144 (27.8)
Received information about travel during pregnancy	
Yes	174 (33.5)
No	345 (66.5)
Total	519 (100)

There was no statistical difference between car usage of women according to gestational week, place of residence, and number of pregnancies ($p>0.05$). The proportion of women who always drive a car during pregnancy was 10.4% in the first trimester, 10.8% in the second trimester, and 11.3% in the third trimester. A statistically significant difference was found between driving status according to education level ($p<0.05$). Accordingly, women with postgraduate education had a higher car usage rate (32.6%), which constituted a statistical difference compared to primary school/middle school (0%), high school (6.5%), and undergraduate (10%) education levels.

There was no statistically significant difference in seat belt usage among women based on gestational week, place of residence, and number of pregnancies ($p>0.05$).

The proportion of women who always used a seat belt during pregnancy was 82.1%, 79.5%, and 83.4% in respective trimesters. It was found that women wore seat belts more frequently in the third trimester (p>0.05). When examining seat belt usage based on educational level, women with undergraduate (84.7%) and postgraduate education (89.1%) had the highest rates, while women with primary school/middle school (50.0%) and high school (74.2%) education levels had lower rates (p>0.05). The frequency of seat belt usage decreases as the number of pregnancies increases (84.5%, 74.5%, 72.7%, and 66.7% respectively).

Discussion

The findings of this study, which aimed to examine the behaviors of pregnant women regarding travel and car usage in Türkiye, indicate a decrease in travel and car usage frequency during pregnancy. In our study, it was found that nearly one-fifth (19.1%) of women occasionally drove a car during pregnancy. In contrast to our study, Auriault et al. reported a decrease in daily car usage frequency after the 6th month of pregnancy.[12] The level of car usage among pregnant women may vary depending on their educational levels, employment status, and lifestyle.

In the literature, it has been emphasized that women should make an appropriate choice among the types of transportation during pregnancy, depending on the place and duration of travel. It has been highlighted that the most common types of travel during pregnancy are automobiles and planes, however, ship travel can also be preferred.[14] In this study, the use of buses, trains/tramways/metro, taxis, bicycles/scooters, and motorcycles decreased during pregnancy, while the use of cars, planes, and ships/ferries increased. Consistent with our findings, a study conducted in the United States also indicated that pregnant women mainly walked to their workplaces (11.4–12.5%) or used buses, subways, or trains (6.8–14.3%).[15]

It was determined that pregnant women experienced the most, low back pain, nausea/vomiting, and fear that something would happen to their baby while driving. Low back pain and nausea/vomiting are common physical problems during pregnancy while driving and traveling.[16] Furthermore, women have concerns about avoiding things that could harm their babies during pregnancy. Because of this extreme attention to safety, it is reasonable to assume expectant mothers to use seat belts more widely to protect their babies. However, some pregnant women may believe that in the event of a possible traffic accident during pregnancy, seat belts would exert excessive pressure on their bellies, causing harm to their babies and even leading to miscarriages/deaths.[17-19] Therefore, pregnant women should be provided with information to ensure comfortable and safe car usage and travel during pregnancy, as well as to reduce/eliminate misconceptions.

In our study, it was observed that women who used cars as a means of transportation during pregnancy would switch from the driver's seat to the front passenger seat or the rear seat. The most preferred seat for women using cars for transportation was the front passenger seat, both before and during pregnancy. In the literature, sitting in the front seat or driver's seat after the second trimester while traveling by car is not recommended for pregnant women because it reduces the distance between the abdomen and the steering wheel. Therefore, it may be a good option for pregnant women to prefer the back seat.[7] In this study, similar to the literature, we found that pregnant women preferred to sit in the back seat more when using a taxi. Air travel is a rarely preferred mode of transportation without obstetric or medical complications. During air travel, it is recommended that pregnant women prefer the seats in the aisle to stand up and stretch their legs during the flight.[3] However, contrary to the literature, it was observed in this study that pregnant women who used airplanes mostly chose seats by the window (6.2%). Therefore, it is considered beneficial to provide pregnant women with more detailed information about travel during pregnancy in prenatal education sessions.

The use of seat belts during pregnancy is important for the safety of both the mother and the fetus.[20] The American Academy of Family Physicians stated that four out of five unborn babies who died in a car accident would have survived if their mothers had worn a seat belt.[21] In our study, it was determined that there was a decrease in the frequency of driving and an increase in the frequency of using seat belts with pregnancy. It was observed that the majority of pregnant women (81.9%) in the study always wore seat belts during pregnancy. Consistent with our findings, several other studies have reported that nearly all pregnant women in France (90–100%), the United Kingdom (98%), the United States (96%), Malaysia (90%), and Ireland (74.6%) wore seat belts while traveling.[12,17,18,22,23] To ensure that all pregnant women wear seat belts during pregnancy, women should be informed about the importance of wearing seat belts during pregnancy. Considering the evidence highlighting the benefits of seat belt usage during pregnancy in reducing maternal and neonatal mortality and morbidity rates, it is essential for all pregnant women to correctly wear seat belts in motor vehicles.[19] American College of Obstetricians and Gynecologists recommends that pregnant women fasten the lap belt snugly below the abdomen over the hips and pelvis, position the shoulder belt across the chest (between the breasts) and over the middle of the collarbone (away from the neck), and never place the shoulder belt under the arm or behind the back. It is recommended to wear both the lap belt and the shoulder

strap during pregnancy.[20,21] In our study, the majority of pregnant women (65.3%) placed the seat belt under the abdomen, supporting the shoulders and hips. Similarly, in another study, more than 80% of pregnant women reported that they placed their seat belts under the abdomen.[12] In a study conducted with pregnant women who received care from health centers in the USA, it was found that the majority (72%) used their seat belts correctly.[18] In Ireland, 47.4% of pregnant women placed their seat belts correctly.[23] It was reported that 29% of pregnant women in Malaysia positioned their seat belts correctly.[17] In the United Kingdom, only 12.8% of pregnant women correctly positioned both the shoulder and lap belt at the same time.[22] In line with these findings, it is seen that not all pregnant women wear their seat belts correctly. Furthermore, it was seen that the number of pregnant women who used the seat belt apparatus for positioning the seat belt was very low (5.4%). Only one-fifth of women were aware of the existence of this apparatus. Contrary to our finding, in the study of Auriault et al. in France, half of the women are aware of an additional safety device that helps position the seat belt.[12] The literature suggests that pregnant women often fail to correctly position seat belts and may not be sufficiently familiar with the use of seat belt adjusters, emphasizing the need to provide pregnant women with information and counseling in this regard.[24]

When traveling in cars, pregnant women are advised to avoid prolonged sitting due to venous stasis and the potential risk of thromboembolism. They are recommended to limit car travel to a maximum of six hours per day and take a break of at least 10 minutes every two hours during car journeys.[14] In accordance with the literature, it was found that one-third (37.2%) of the pregnant women included in our study regularly took a break every 2-3 hours during their journey, and about one-third (27.8%) took a 10-minute stroll during the breaks. A study conducted with 543 pregnant women and 521 postpartum women in Los Angeles found that women spent two hours on average in car travel.[25] In our study, one-third of the pregnant women (33%) received information on travel during pregnancy. Half of the women (49.9%) received it from an obstetrician, 27.6% from the internet, 10% from a nurse, and 9.4% from a magazine/book. In Ireland, only 22% of pregnant women reported that they received advice about travel during pregnancy.[23] It has been found that those who receive information mostly get this information from radio/television/internet. In this regard, it is clearly evident that healthcare professionals should provide information on travel during pregnancy, and public awareness campaigns through mass media platforms are needed.

When the proportion of women who always wear seat belts during pregnancy (82.1%; 79.5%; 83.4%) is examined, it is seen that seat belts are used the most in the third trimester. In parallel with our research, Auriault et al. reported that the rate of using seat belts increases with the month of pregnancy (91.7% in six-month and 96.9% in nine-month).[12] In the study conducted by Faradila et al., it was found that pregnant women wear seat belts most frequently during the first trimester, and they do not prefer to use it due to the difficulties brought by the seat belt as the pregnancy progresses.[17] Seat belt wearing status according to pregnancy trimesters may vary depending on the socio-cultural characteristics of pregnant women.

In this study, the level of use of car was higher in pregnant women with postgraduate education (32.6%). In addition, the rate of women who always use seat belts during pregnancy was found mostly in the third trimester and in pregnant women with a high level of education. Acar and Weekes similarly found that pregnant women with a university degree used seat belts more.[22] In a study by Faradila et al., it was reported that there was no correlation between the education level of pregnant women and their seat belt-wearing status.[17] It is thought that this finding is due to the higher awareness of traffic accidents with the increase in their education level.

In this study, it was found that the frequency of wearing seat belts diminished as the number of pregnancies increased. In a different study, no relationship was found between the number of pregnancies and the use of seat belts.[17] It was believed that the main reason underlying this finding might be due to different variables such as mother-infant attachment.

Study limitations

The strong aspect of the study is that it covers pregnant women throughout Türkiye. However, the findings are only valid for the group in which the research was conducted and cannot be generalized to the whole population. In the study, the self-report method was used in order for pregnant women to respond easily. However, the inclusion of illiterate pregnant women with the self-report method is a limitation of the study. One of the limitations is that only women with access to the internet and social media participated in the research. Furthermore, the scarce number of studies on this subject in the literature limited the discussion.

Conclusion

There has been a decrease in the frequency of use of cars and travel by women with pregnancy. It has been found that the pregnant women's level of using seat belts and wearing seat belts correctly is not sufficient. Preventive efforts are needed to make women more aware of the importance of wearing seat belts correctly during pregnancy. To achieve this, health professionals, especially nurses and midwives, should definitely organize training sessions about travel during pregnancy in prenatal training. Furthermore, in order to inform society about

travel during pregnancy, public service announcements about this topic should be broadcast from the mass media. It is also recommended to enhance the literature by conducting different studies related to the usage of car and travel during pregnancy.

Declarations

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Author contributions
Conceptualization, Ş.K.E., R.D. and G.A.; Methodology, Ş.K.E., E.C.E., R.D. and G.A.; Software, Ş.K.E. and E.C.E., Validation, Ş.K.E. and E.C.E., Formal Analysis Ş.K.E.; Investigation, Ş.K.E., R.D. and G.A.; Resources, Ş.K.E., E.C.E., R.D. and G.A.; Data Curation, Ş.K.E., R.D. and G.A.; Writing – Original Draft Preparation, Ş.K.E. and E.C.E., Writing – Review & Editing, Ş.K.E. and E.C.E., Visualization, Ş.K.E. and E.C.E., Supervision, Ş.K.E. and E.C.E., Administration, Ş.K.E. and E.C.E., Funding Acquisition, Ş.K.E., E.C.E., R.D. and G.A.

Conflicts of interest
The authors declare that no conflicts exist.

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

Ethics approval
Permission was obtained from the Institutional Review Board of the researcher's affiliated university (Date: 27.11.2020, No: 2014/08-13) for the implementation of the study. Informed voluntary consent was obtained from the participating pregnant women.

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ORIGINAL PAPER

Determinants of distress levels in high-risk pregnant women – cross-sectional study

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ABSTRACT

Introduction and aim. Global and national care recommendations indicate that women with high-risk pregnancies should receive personalized and qualified care during this period. This study was conducted to determine the distress levels in high-risk pregnant women and affecting factors.

Material and methods. The cross-sectional this study was conducted with total of 416 high-risk pregnant women who met the inclusion criteria in the obstetrics clinic of a training and research hospital. The study data were collected with data collection form and "Tilburg Pregnancy Distress Scale (TPDS)."

Results. The mean TPDS total score of high-risk pregnant women was 18.25±6.85. It was found that planning pregnancy, gravida, and diagnosis of gestational hypertension, systemic diseases, and gestational diabetes in the present pregnancy was associated with pregnancy-specific distress ($p<0.05$; $\beta=0.291$, $\beta=0.158$, $\beta=0.272$, $\beta=0.137$, $\beta=0.116$, respectively).

Conclusion. It is advised that health professionals assess the distress levels of high-risk pregnant women and give personalized care during prenatal period.

Keywords. distress, health professional, high-risk pregnancy, nursing care

Introduction

A high-risk pregnancy is defined as a pre-pregnancy or current pregnancy-related condition that causes higher risk of maternal, fetal, or neonatal problems than normal during the antepartum, intrapartum, or postpartum period.[1] Although the diagnostic criteria vary, the literature has reported the prevalence of high-risk pregnancies from 6 to 33%.[2-4] The recent data in Türkiye indicate that 10–15% (130,000 per year) out of 1.3 million births include high-risk pregnancies.[5] Globally, three out of every four women die from perinatal causes such as severe hemorrhage, infection, preeclampsia and eclampsia, delivery complications, and unsafe abortion.[6] On the other hand, negative maternal-fetal outcomes such as caesarean delivery, intrauterine fetal death, neonatal intensive care follow-up, neonatal death, low birth weight, and stillbirth are reported in women with high-risk pregnancies.[4,7]

Psychological distress has also been reported besides the negative maternal-fetal outcomes in women with high-risk pregnancies.[8-11] Psychological distress is defined as a state of emotional suffering characterized by symptoms of depression and anxiety. In psychological distress, depression and anxiety symptoms co-occur with common somatic complaints and medically unex-

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plained syndromes.[12] However, the distinguishing characteristics of psychological distress include exposure to a stressful event that threatens physical or mental health, failure to cope with this stressor effectively, and emotional anguish resulting from this ineffectiveness.[13] The literature has documented more psychological distress experienced by women with high-risk pregnancies. Of pregnant women with congenital heart failure (CHF), 39% had traumatic distress, 22% had depression, and 31% had anxiety.[8] Yuksel et al. and Gözüyeşil and Arıöz Düzgün reported that the distress levels were high in pregnant women who were at risk in their current pregnancy.[9,14] A meta-analysis reported that 34% of pregnant women who were hospitalized due to obstetric complications had depression and 29% had anxiety, and compared to the general obstetric population, pregnant women who were followed up in the hospital due to their obstetric complications showed clinical symptoms of depression and anxiety two times more than the prevalence of depression and anxiety.[10] Some meta-analysis results also reported that the risk of developing depression was approximately 1.5 times higher in pregnant women who had pre-gestational diabetes or gestational diabetes compared the ones who did not.[11] Prenatal distress of women with high-risk pregnancies are reported to be affected by factors such as the level of education, perception of income-expenses, the education level of the spouse, the place where they lived for the longest period of childhood, wanting the pregnancy, and the number of children.[14] It is not certainly known whether the distress experienced in the prenatal period in women with high-risk pregnancies develops due to effects of some socio-demographic and obstetric variables of these women or only due to the current diagnosed risk.

Maternal distress experienced in the antenatal period causes low birth weight, intrauterine growth retardation (IUGR), preterm labor as well as cardiometabolic, respiratory and neurodevelopmental negative maternal-fetal-childhood health outcomes.[15] High-risk pregnancy threatens maternal-fetal health globally, and women with medical/obstetric risks are reported to have more mental health problems during pregnancy.[5,6,9-11]

It is known that the antenatal period increases the susceptibility to psychological health problems.[15] It is reported that pregnant women who are at risk during pregnancy are likely to experience mental health problems.[16-18] Although it is emphasized in studies that pregnancy complications can lead to physiological health risks up to maternal-fetal death, antenatal mental health problems are associated with the mother's postpartum mental health problems, and the negative cognitive and mental development of newborns in following life.[5,6,19-21] Global and national care recommendations indicate that women with high-risk pregnancies should receive personalized and qualified care during this period.[22,23] Although it is known that stressful situations such as high-risk pregnancy cause more psychological health problems, and their possible negative effects can be predicted within the framework of what is currently known, limited information is available on the distress in pregnancy among women with high-risk pregnancies.[5,6,8-10,14,19-21,24] Moreover, there seems to be a gap in the literature about which high-risk pregnancies have pregnancy-related distress levels and whether factors other than the current risk diagnosis during pregnancy have effects on pregnancy-specific distress. The current study is considered to have important scientific contributions that this study will contribute to filling an important gap in the literature and these results are believed to be useful for health authorities to make strategic plans and/or to create a road map to be used in practice.

Aim

This study was conducted to determine the distress levels in high-risk pregnant women and affecting factors.

Material and methods

Study design, sample size, and sampling strategy

This cross-sectional study was conducted in the perinatology clinics of a training and research hospital in Türkiye. Population of this study consisted of pregnant women hospitalized in obstetrics clinics with the diagnosis of high-risk pregnancy between March 2017 and March 2018. High risk factors identified for the decision to be admitted to the perinatology clinic; it includes hypertensive diseases, severe hyperemesis gravidarum, IUGR, polyhydramnios/oligohydramnios, diabetes mellitus, preterm/postterm labor, multiple pregnancy, Rh isoimmunization, systemic diseases (asthma, heart diseases, liver or kidney problems) and antenatal bleeding. Random sampling method was used in the study. Power analysis was performed to determine the sample size of the study. According to the study conducted by Bacacı in Türkiye, it was necessary to include at least 414 high-risk pregnant women, with a 5% difference at 90% power and 5% margin of error.[25] 457 pregnant women were invited to the study, and 41 pregnant women were not included in the study because they filled out the data collection forms incompletely. The study was completed with the participation of 416 pregnant women who met the inclusion criteria and agreed to participate in the study. The study included pregnant women who (1) were ≥18 years old, (2) were diagnosed with a high-risk pregnancy, (3) were ≥ in 12th gestational week, (4) were hospitalized for ≥3 days, (5) had a healthy fetus, and (6) were able to understand and answer the questions.

Instrument

The "Data Collection Form" and the "Tilburg Pregnancy Distress Scale (TPDS)" were used to collect data.

Data collection form

The form was prepared by the researchers in line with the relevant literature.[8,22,23,25-27] It included 19 questions about pregnant women's socio-demographic (age, education level, employment status, family type, etc.) and obstetric (gravida, gestational week, parity, current high-risk pregnancy diagnosis, etc.) features.

Tilburg pregnancy distress scale (TPDS)

TPDS was developed by Pop et al. in 2011 to diagnose pregnancy-specific distress.[28] Turkish validity and reliability of the scale was performed by Çapık and Pasinlioğlu in 2015.[29] The 16-item scale is responded on a 4-point Likert scale and included options ranging from "quite often" (0 points) and "never" (3 points). Items 3, 5, 6, 7, 9, 10, 11, 12, 13, 14, and 16 are scored reversely, and the total and subscale total scores are calculated. The subscales of the scale are "Negative Affect" and "Partner Involvement". Scores to be obtained from the scale range between 0 and 48 points, and the scores to be obtained from the negative affect and partner involvement subscales are 0-33 and 0-15 points, respectively. The scale has a cut-off point, and a total score of ≥28 indicates that pregnant women are at risk of experiencing distress. Cut-off points for partner involvement and negative affect subscale are ≥10 and ≥22, respectively. Cronbach's Alpha value was 0.83 in the original form of the scale,[29] while it was found 0.95 in the current study.

Data collection procedure

Each participant was given information about the study before the data collection forms were administered to the pregnant women, and data were gathered after their verbal and written consent was received. After the purpose, scope, ethical sensitivities and possible benefits of the study were explained to the pregnant women, the study process was started with the pregnant women who agreed to participate in the study and signed the informed consent form. The researchers obtained data from pregnant women through face-to-face interviews at the perinatology clinics of the training and research hospital where the study was carried out. Filling in the data collection tools was completed in approximately 10-15 minutes.

Ethical approval

The study was started after receiving ethics committee approval from the Non-Experimental Research Ethics Committee and written permission from the Provincial Health Directorate (decision no: 2017/61-35, date: February 10, 2017).

Data analysis

SPSS software was used to analyze the data (v. 24.0, IBM Corp., Armonk, NY, USA). Before the analysis was done, the Kolmogorov-Smirnov test was used to determine whether the data were distributed normally. Descriptive statistics are presented as a numbers (n), percentages (%), and mean and standard deviations (mean±SD). The independent sample t-test was used to compare two independent groups, the analysis of variance test (ANOVA) was used to compare three or more independent groups, and Tukey or Tamhane's T2 posthoc tests were used to determine which group indicated a statistical difference in those with ≥3 variables based on variance homogeneity. Multiple linear regression analysis (Stepwise model) was performed to determine the relationship between TPDS and the pregnant women's variables. A 95% confidence interval and a statistical significance of $p<0.05$ were used for all the findings.

Results

The majority of participating women with high-risk pregnancies (27.9%) were aged between 25 and 29, with a mean age of 28.96±6.06 years. It was found that 42.1% of the pregnant women were illiterate/primary school graduates, 91.6% were unemployed, and 78.4% had been married for ≥3 years.

Table 1. The distribution of the descriptive and obstetrics characteristics of the pregnant women and TPDS total mean scores according to descriptive and obstetrics characteristics (n=416)#

Characteristics	n	%	Mean±SD	Test and *p* (t/F)
Age groups (years) (mean±SD=28.96±6.06, min–max=18–44)				
≤ 24	109	26.2	17.66±7.44	
25–29	116	27.9	18.65±6.92	F=0.988
30–34	106	25.5	17.77±6.32	p=0.398
≥ 35	85	20.4	19.08±7.39	
Education status				
Illiterate/Primary school graduate	175	42.1	17.55±7.18	
Secondary school graduate	110	26.4	18.64±7.04	F=1.121
High school graduate	108	26	18.95±5.94	p=0.34
University graduate	23	5.5	18.52±7.18	
Employment status				
Employed	35	8.4	19.49±5.27	t=1.4
Unemployed	381	91.6	18.14±6.97	p=0.168
Spouse's education status				
Illiterate/Primary school graduate	145	34.9	17.90±7.25	
Secondary school graduate	102	24.5	18.76±6.5	F=0.322
High school graduate	129	31	18.29±6.58	p=0.809
University graduate	40	9.6	18.10±7.21	
Spouse's employment status				
Employed	408	98.1	18.28±6.77	t=0.523
Unemployed	8	1.9	17±10.69	p=0.601
Duration of marriage (years)				
1-2	90	21.6	17.62±6.93	t=-0.99
≥ 3	326	78.4	18.43±6.82	p=0.323
Family type				
Nuclear	342	82.2	18.4±6.72	t=0.952
Extended	74	17.8	17.57±7.39	p=0.342

Place of residence stayed longest				
Provincial center	305	73.3	18.33±6.91	F=0.24
County	88	21.2	18.24±6.74	p=0.787
Village or Town	23	5.5	17.30±6.57	
Perception of monthly income level				
Expenses less than income	214	51.4	18.26±7.07	
Expenses equal to income	183	44	18.15±6.75	F=0.185
Expenses more than income	19	4.6	19.16±5.26	p=0.831
Planning pregnancy				
Yes	319	76.7	17.24±6.49	t=-5.693
No	97	23.3	21.6±6.96	p<0.001
Gestational week (mean±SD=30.30±5.24, min-max=13-39)				
13-27 weeks	85	20.4	17.09±6.42	t=-1.756
≥28th week	331	79.6	18.55±6.93	p=0.08
Gravida				
1[a]	105	25.2	18.85±6.48	
2[b]	95	22.8	16.23±6.42	F=4.116
3[c]	89	21.5	18.21±6.91	p=0.007
≥ 4[d]	127	30.5	19.31±7.15	[a,b], [b,d]
Parity				
Nulliparty	139	33.4	18.34±6.36	
1	105	25.2	17.10±6.86	F=1.69
2	100	24.1	18.55±6.89	p=0.169
3	72	17.3	19.36±7.655	
Interpregnancy interval				
Primigravid	105	25.2	18.85±6.48	F=0.764
< 24 month	197	47.4	18.26±6.90	p=0.466
≥ 24 month	114	27.4	17.70±7.09	
Multiple gestation				
Yes	14	3.4	18.14±6.33	t=0.062
No (singleton pregnancy)	402	96.6	18.26±6.87	p=0.95
History of abortion				
Primigravid	105	25.2	18.85±6.48	F=0.623
Yes	120	28.9	17.52±6.91	p=0.537
No	191	45.9	18.85±6.48	F=0.623
History of stillbirth				
Primigravid	105	25.2	18.85±6.48	F=0.623
Yes	29	7.0	17.52±6.91	p=0.537
No	282	67.8	18.11±6.98	
History of high-risk pregnancy in previous pregnancies				
Primigravid[a]	105	25.2	18.85±6.48	F=3.975
Yes[b]	87	20.9	19.68±6.71	p=0.02
No[c]	224	53.9	17.42±6.97	[b,c]
High-risk pregnancy diagnosis in current pregnancy				
Risk of premature birth[a]	136	32.7	15.76±6.60	
Preeclampsia[b]	47	11.3	17.57±5.39	
Gestational diabetes[c]	42	10.1	19.26±6.77	F=7.715
Gestational hypertension[d]	64	15.4	21.78±6.57	p<0.001
Systemic diseases[e]*	57	13.7	20.42±6.53	[a,c], [a,d]
Placenta previa[f]	21	5.0	18.24±6.84	[a,e]
Premature rupture of membrane[g]	49	11.8	17.84±6.96	

[#] SD – standard deviation, TPDS – Tilburg Pregnancy Distress Scale, F – One-way ANOVA test, t – Independent sample t-test, * – systemic diseases: heart disease, kidney disease, liver disease, and asthma; all pregnant women have gone antenatal care visit; the letters a, b, c, d, e, f and g indicate the group that makes the difference

The majority of the pregnant women's spouses (34.9%) were illiterate/primary school graduates, and almost all of them were working (98.1%). Besides, 82.2% had a nuclear family, 73.3% resided in the provincial center, more than half (51.4%) had income less than expenses, and 23.3% reportedly did not plan their pregnancy. The mean gestational week was 30.30±5.24 weeks, 20.4% of them were in the 2nd trimester (13–27 weeks), and 79.6% of them were in the 3rd trimester (≥28th weeks). It was found that 25.2% of the pregnant women had their first pregnancy, 33.4% never gave birth, the period between the previous pregnancy and the current pregnancy was <24 months for 47.4%, and almost all of them (96.6%) had singleton pregnancies. It was also found that 28.9% of pregnant women had abortions, 7% had a history of stillbirth, and 20.9% had a history of high-risk pregnancy in previous pregnancies. Clinical diagnoses showed that 32.7% had a risk of premature birth, 11.3% had preeclampsia, 10.1% had gestational diabetes, 15.4% had gestational hypertension, 13.7% had systemic diseases (heart disease, kidney disease, liver disease, and asthma), 5.0% had placenta previa, and 11.8% had premature rupture of membranes. The total TPDS score averages indicated no statistically significant differences according to age of women with high-risk pregnancies and the age of their spouses, education level and employment, marriage duration, family type, place of residence stayed longest, perception of monthly income level, gestational week, parity, interpregnancy interval, multiple gestations, and history of stillbirth (p>0.05). A statistically significant difference was found between the TPDS total mean score and planning of pregnancy, gravida, history of abortion, and high-risk pregnancy in the previous pregnancy (p<0.05). The mean TPDS total mean scores of pregnant women with gestational diabetes, gestational hypertension, and systemic diseases indicated a statistically significant difference (p<0.05) (Table 1).

Table 2. The mean scores of TPDS scale and subscales of the pregnant women (n=416)*

TPDS total and subscales	Min-Max	Mean±SD
Negative affect subscale	0–28	13.13±5.67
Partner involvement subscale	0–15	5.12±2.93
Total	1–36	18.25±6.85

* SD – standard deviation, TPDS – Tilburg Pregnancy Distress Scale

Participating women's negative effect and partner involvement subscales and the TPDS total mean scores were found 13.13±5.67, 5.12±2.93, and 18.25±6.85, respectively (Table 2).

Table 3 shows the results of a multiple linear regression analysis of the variables associated with TPDS total mean scores of women with high-risk pregnancies. Anal-

ysis results showed that the effect of being diagnosed with a high-risk pregnancy in the presence of participating women's all existing variables, planning pregnancy, gravida, gestational hypertension in the current pregnancy, systemic diseases, and gestational diabetes indicated a statistically significant difference (p<0.001). The TPDS total mean scores of those who did not plan their pregnancy compared to those who did were approximately 5 points (p<0.001) higher, mean scores of those with primigravida were 2.5 points (p=0.001) higher compared to those with second pregnancy, and compared to those at risk of premature birth in their current pregnancy, the mean scores of those with gestational hypertension were 5 points (p<0.001) higher, mean scores of those with systemic diseases were 3 points (p=0.004) higher, and mean scores of those with gestational diabetes were 3 points (p=0.013) higher.

Table 3. Multiple linear regression analysis results for TPDS total scores (n=416)#

Variables	B	SE	β	t	p	%95 CI for β	
						Lower Bound	Upper Bound
Constant	10.388	1.075	–	9.661	<0.001	8.274	12.502
Planning pregnancy *No vs. Yes*	4.710	0.770	0.291	6.118	<0.001	3.197	6.224
Gravida *Primigravid vs. 2. pregnancy*	2.483	0.48	0.158	3.319	0.001	1.012	3.954
High-risk pregnancy diagnosis in current pregnancy							
Gestational hypertension vs. The risk of premature birth	5.152	0.882	0.272	5.839	<0.001	3.417	6.887
Systemic diseases vs. The risk of premature birth	2.721	0.934	0.137	2.911	0.004	0.884	4.557
Gestational diabetes vs. The risk of premature birth	2.634	1.053	0.116	2.501	0.013	0.564	4.705

#TPDS – Tilburg Pregnancy Distress Scale, B – unstandardized regression coefficient, SE – standard error, β – standardized, t – independent sample t-test value, 95% CI – 95% confidence interval, n=416, R=0.411, R^2=0.169, Adjusted R^2=0.159, F=16.668, and p<0.001, * – systemic diseases: heart disease, kidney disease, liver disease, and asthma; stepwise model was used

Discussion

Several negative maternal-fetal outcomes can be documented in high-risk pregnancies during the antenatal, innatal, and postnatal periods.[4,7] The pregnancy process itself could cause women to experience psychological changes.[15] Pregnant women who are at risk during pregnancy are reported to experience a variety of mental health problems.[9,14,24,25] The mean TPDS total, negative affect, and partner involvement subscale scores of pregnant women with high-risk pregnancies were found to be 18.25±6.85, 13.13±5.67, and 5.12±2.93, respectively, in the current study. A study conducted with women with high-risk pregnancies reported the mean TPDS total, negative affect, and partner involvement subscales scores as 29.05±11.6, 23.17±9.8, and 5.88±4.8, respectively, and their pregnancy-related distress levels were higher than the ones reported in this study.[14] In a study, the rate of maternal psychiatric symptoms was found 48.5% in high-risk pregnant women.[18] The study conducted by Woods et al. reported that 18.9% of pregnant women with ≥2 chronic diseases and 32.3% of women with complications experienced high levels of stress; they were found to have experienced psychological stress during pregnancy approximately 3 times [OR=3.1, 95% CI=1.8–5.5] and 1 time [OR=1.2, 95% CI=0.72–1] more, respectively.[25] In two studies conducted, prenatal distress levels were found to be higher in pregnant women with high-risk/problems with their current pregnancy.[9,24] According to a meta-analysis, pregnant women who were followed up in the hospital due to obstetric problems had two times more anxiety and depression symptoms than the general obstetric population, and around three out of every ten pregnant women had depression and anxiety (34% and 29%, respectively).[10] Studies reports that pregnant women with pregnancy complications experience various mental health problems, ranging from stress to depression, and have psychiatric symptoms. The current study has revealed that high-risk pregnant women have psychological distress, and have presented that risky pregnant women experience negative affect and that partner involvement is important for these pregnant women.

Women who have high-risk/current pregnancy-related problems are reported to experience more prenatal distress than those who do not.[14,30] Furthermore, various socio-demographic and obstetric variables that may represent a risk associated with pregnancy have been associated with prenatal distress.[14] In addition to the risk in the currently diagnosed pregnancy, factors such as education level, perception of income-expenses, spouse's education level, place of residence for the longest period of childhood, wanting the pregnancy, and the number of children could have effects on pregnancy distress.[14] This study found that unplanned pregnancy, primigravida and three or more pregnancies, gestational diabetes mellitus, gestational hypertension and systemic disease increase the distress level of high-risk pregnant women. Regression analysis presented that the most important determinants of distress levels in high-risk pregnant women were unplanned pregnancy, primigravida, hypertension during pregnancy, systemic disease and gestational diabetes mellitus. The study conducted by Gözüyeşil and Arıöz Düzgün detected that the difference between wanting the pregnancy and the TPDS total mean scores was statistically significant (p<0.05). A study determined that approximately 4 (39%) in every 10 pregnant women with

CHF experienced traumatic distress, 3 (31%) experienced anxiety and 2 (22%) experienced depression.[8] According to the study conducted by Lee et al., the risk of depression in pregnant women who had pregestational or gestational diabetes is approximately 1.5 times greater [RR=1.430, 95% CI=1.251–1.636] than in those who did not.[11] Our findings show that, in addition to the association between pregnancy distress and gestational hypertension, systemic diseases and gestational diabetes, unplanned pregnancy and high-risk pregnancy in the second pregnancy are effective in pregnancy-specific distress in women. Our research result is consistent with previous studies reporting that various obstetric factors have an impact on the mental health of high-risk pregnant women. It also points out that hypertension, diabetes and systemic complications during pregnancy may be important health risks that increase the distress levels of high-risk pregnant women. On the other hand, it suggests that the importance of pregnancy planning for the mental health of high-risk pregnant women is noteworthy and that those experiencing their first pregnancy may be more prone to maternal psychological health risks.

Study limitations

This study has some limitations. It was conducted in a single center in Türkiye the results can therefore be generalized only to pregnant women living in that province and having similar characteristic features. Secondly, limitation is due to the self-reporting of the levels of psychological distress which may not always be aligned with objective assessment by health professionals. The restrictions also include that the study was conducted only on a sample of high-risk women with pregnancy – without a control sample (women with physiological pregnancy).

Implications for health care practice

Women who are followed up in the hospital due to high-risk pregnancies during the prenatal period should be provided with optimal care to preserve and improve maternal, fetal, and neonatal health. We consider that provision of care by health professionals for the mental health needs of high-risk pregnant women in line with their risk diagnosis may increases maternal well-being. It is recommended to evaluate the pregnancy-related distress in the women with high-risk pregnancies and to manage the care process in cooperation with psychiatry/psychological counselling clinics when deemed necessary.

Conclusion

The current study detected that women with high-risk pregnancies experienced pregnancy-related distress. It has been found that pregnant women of almost all ages experience psychological distress, regardless of their education level, employment status, spouse's education level and employment status, duration of marriage, family type, place of residence for the longest time, and income level. Obstetric factors such as gestational age, parity, interval between the last two pregnancies, multiple pregnancy, abortus and stillbirth history have been detected to be ineffective on the distress levels of high-risk pregnant women. It has been determined that unplanned pregnancy, high-risk pregnancy history in the previous pregnancy, gestational hypertension, systemic diseases and gestational diabetes are factors that increase the distress of high-risk pregnant women of all ages. Moreover, when all known sociodemographic and obstetric variables were taken into account, it was concluded that the most important determinants of the distress levels of high-risk pregnant women were unplanned pregnancy, primigravida, gestational hypertension, gestational diabetes mellitus and systemic disease.

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Declarations

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Author contributions

Conceptualization, P.K. and E.N.; Methodology, P.K. and E.N.; Formal Analysis, P.K. and E.N.; Data Curation, P.K. and Z.Ç.; Writing – Original Draft Preparation, P.K.; Writing – Review& Editing, P.K. and E.N.; Supervision, E.N.

Conflicts of interest

No potential conflict of interest was reported by the authors.

Data availability

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

Ethics approval

The study was started after receiving ethics committee approval from the Non-Experimental Research Ethics Committee and written permission from the Provincial Health Directorate (decision no: 2017/61-35, date: February 10, 2017).

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ORIGINAL PAPER

Assessment of serum creatinine, urea, and aminotransferase levels among methamphetamine addicted individuals in Khartoum State

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ABSTRACT

Introduction and aim. Methamphetamine-use disorder is a pressing global public health issue. In Sudan, the escalating methamphetamine (METH) consumption has become a significant social and health problem. This study aims to evaluate liver and kidney biomarkers in methamphetamine addicts in Khartoum state

Material and methods. The study was an analytical prospective cross-sectional hospital-based study. One hundred participants were enrolled in this study, fifty were cases (methamphetamine addicts), and others were healthy non-METH users as a comparative group.

Results. METH users had a mean age of (27±7) years and had been using METH for an average of (14±9) months. Urea and creatinine levels were also significantly elevated in METH users compared to non-users, with $p<0.001$ and $p=0.044$, respectively. Their aspartate transaminase (AST) and alanine transaminase (ALT) levels were significantly higher compared to non-users, with $p<0.001$.

Conclusion. There was significant increases in creatinine, urea, and aminotransferases levels in the case group. ALT showed a moderate positive correlation with abuse duration, while AST showed no significant correlation. Urea and creatinine levels had strong and moderate positive correlations with abuse duration, respectively.

Keywords. aminotransferases, creatinine, methamphetamine, urea

Introduction

Methamphetamine (METH) nowadays is at the top of abused drugs worldwide. It is a widely-abused and addictive psychostimulant.[1] It has become a major social problem causing a substantial economic burden. METH is associated with many disorders and it is metabolized mainly in the liver and excreted by the kidneys.[2] Urea is the non-protein nitrogen compound found in the blood at the highest concentration. It serves as the primary excretory product of protein metabolism. The liver synthesizes urea through the enzymatic conversion of amino groups and free ammonia produced during protein breakdown, a process known as the urea cycle.[3]

Creatinine is synthesized from creatine and creatine phosphate within muscle tissue and is subsequently released into the bloodstream at a consistent rate

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proportional to the individual's muscle mass. The concentration of creatinine in the plasma is inversely correlated with the glomerular filtration rate, which serves as an indicator of renal filtration function. Despite its limitations, plasma creatinine is frequently employed as a means to evaluate renal filtration function.[3]

Aspartate aminotransferase (AST) is ubiquitously present in various tissues such as the liver, brain, pancreas, heart, kidney, lung, and skeletal muscle. In the event of tissue damage in any of these organs, AST is released into the circulatory system.[4]

Alanine aminotransferase (ALT) is predominantly localized in the liver. Elevated levels of ALT are consistently regarded as a matter of concern; however, they do not necessarily imply a serious condition. An increased ALT level may suggest mild or severe liver impairment.[5]

Neurotoxic doses of METH can induce liver necrosis and increase blood levels of ammonia.[4] additionally, one of the kidney's primary functions is the filtration and discarding of waste products from the blood. The measurements of elevated blood urea and creatinine indicate decreased renal function, (reduced clearance).[2,6]

Aim

Therefore, this study was conducted to measure creatinine, urea, and aminotransferases in methamphetamine addicted individuals.

Material and methods

The study was a prospective analytical cross-sectional, hospital-based study conducted in Khartoum during the period from 1st June 2022 to 1st October 2022. Before the examination, participants were given a verbal explanation of the study's goals and procedures. They were then asked to sign a formal consent form. The study was approved by University of Medical Sciences and Technology (IRB UMST/EG/2022/18, approval date 11/01/2022), and all participants were provided with a verbal explanation of the study's objectives and procedures and they were verbally approved.

This study aimed to compare aminotransferase, urea, and creatinine levels among methamphetamine users (case group) and healthy non-users (as a control group).

A total of one hundred participants were recruited for this research investigation, with fifty individuals classified as cases (ICE addicts) undergoing a rehabilitation program for ICE addiction (the period of rehabilitation was not considered). The remaining fifty participants were selected as a control group, consisting of healthy individuals without any history of substance abuse.

Participants with autoimmune diseases, take oral contraceptives, cancer, renal failure, dehydration, liver disease, alcoholics, pregnant women, those with any disorder affect aminotransferase, urea, and creatinine levels, and those who are not willing to agree to participate in the study were excluded.

Data for this study were obtained using a structured questionnaire administered to the participants. Additionally, the history of any previous drug abuse was extracted from the patients' medical records. Then about three mL of venous blood samples were collected in a plain container from all participants using disposable syringes after clearing the skin with 70% alcohol. Then samples were separated by centrifugation at 3000 RMP for three minutes, and serum was obtained.

Aminotransferases were quantified utilizing the kinetic method as per the SPINREACT (Barcelona, Spain) protocols, employing a spectrophotometer BTS 305. Urea levels were determined using the enzymatic urease method following the BioScien (Egypt) procedures with a spectrophotometer BTS 305. On the other hand, creatinine concentrations were assessed using the kinetic Jaffe reaction in accordance with the spectrum procedures using a spectrophotometer BTS 305. The precision and accuracy of all methods used in this study were checked by commercially prepared control (normal and pathological control sera).

Statistical analysis

The data was entered and organized in a Microsoft Office Excel 2010 spreadsheet. The Statistical Package for the Social Sciences software (version 22.0; IBM SPSS Inc.) was used for analysis. The information collected from the questionnaire was coded as variables. The normality of the data was tested using the Kolmogorov-Smirnov test. Descriptive and inferential statistics, including analysis of independent variables, were then conducted.

Results

This was prospective analytical cross sectional study to estimate serum urea, creatinine, AST and ALT levels in METH users. The study enrolled 50 METH users (case group) and 50 non-users (compare group). The mean age of the case group was (27±7) years, while the mean age of the control group was (29±9) years (Table 1). The mean duration of METH abuse was (26±5) months, with a minimum of 3 months and a maximum of 48 months.

Table 1. Distribution of the study group according to age and duration

		Number	Minimum	Maximum	Mean	SD
METH users	Age	50	18	47	27	7
	Duration (months)	50	3	48	26	5
Non users	Age	50	23	43	29	9

The mean creatinine level was significantly higher in case group (1±0.1 mg/dL) when compared to control group (0.8±0.02 mg/dL, p=0.04), whereas the mean urea

level was significantly higher in METH user (42±4 mg/dL) compared to non-users (20±0.5 mg/dL, p<0.001) (Table 2).

The mean ALT level was significantly higher in METH users (37.8±10.6 U/L) compared to the non-users (24.7±2.4 U/L, p<0.001), also AST level was significantly higher in METH users (41.7±9.1 U/L) compared to the non-users (28.7±4.2 U/L, p<0.001).

Table 2. Mean difference of creatinine, urea, ALT and AST levels between users and non-users*

	Creatinine (mg/dl)		Urea (mg/dl)		ALT (U/L)		AST (U/L)	
	M±SD	p	M±SD	p	M±SD	p	M±SD	p
METH users n=50	1.0±0.1	0.04	42±4	<0.001	37.8±10.5	<0.001	41.7± 9.0	<0.001
Non users n=50	0.8±0.02		20±0.5		24.7±2.4		28.7±4.17	

* Data are expressed as mean±SD

There was no significant mean difference of creatinine among tetrahydrocannabinol (THC) and Tramadol (TML) positive and negative group p=0.08 and p=0.79 respectively, whereas mean urea level is higher in THC positive group when compared to THC negative group p=0.04, but no significant difference in the mean of urea among TML positive and negative group (p=0.63) (Table 3 and Table 4).

Table 3. Mean difference of creatinine, urea, ALT and AST levels between THC positive and negative*

	Creatinine (mg/dl)		Urea (mg/dl)		ALT (U/L)		AST (U/L)	
	M±SD	p	M±SD	p	M±SD	p	M±SD	p
THC positive n=15	0.8±0.06	0.08	30 ±12	0.02	36.4±3.18	0.399	40.3 ±8.0	0.447
THC negative n=35	0.8±0.02		46 ±35		38.4±12.4		42.3±9.5	

* Data are expressed as mean±SD

Table 4. Mean difference of creatinine, urea, ALT and AST levels between TML positive and negative

	Creatinine (mg/dl)		Urea (mg/dl)		ALT(U/L)		AST(U/L)	
	M±SD	P	M±SD	P	M±SD	p	M±SD	p
TML Positive n= 2	1.1±0.15	0.79	34 ±18	0.63	41±2.82	0.292	36.5±0.7	0.414
TML Negative n = 48	1.0±0.12		42 ±31		37.7±10.7		41.9±9.2	

There was no significant mean difference of ALT among THC and TML positive and negative group p=0.399 and p=0.292, respectively.

Additionally, there was no significant mean difference of AST among THC and TML positive and negative, p=0.447 and p=0.414, respectively.

There was a moderate positive correlation between creatinine and duration of addiction (r=0.496, p<0.001), and a strong positive correlation between urea level and duration of abuse (r=0.877, p<0.001) (Table 5).

There was a week positive correlation between AST levels of the METH users and duration of use, r=0.196, the correlation was statistically insignificant, p value 0.174. Moreover, there was a moderate positive correlation between ALT levels of the METH users and duration of use, r=0.300, the correlation was statistically significant, p=0.034.

Table 5. Correlation between urea, creatinine, ALT and AST with duration of use among the case group, n=50

	Variables	Urea (mg/dl)	Creatinine (mg/dl)	ALT (U/L)	AST (U/L)
Duration of use (months)	Correlation coefficient	0.877	0.496	0.300	0.196
	Sig. (2-tailed)	<0.001	<0.001	0.034	0.174
	Number	50	50	50	50
	Strength	Strong	Moderate	Weak	Weak
	Direction	Positive	Positive	Positive	Positive

Discussion

The misuse of METH places a significant financial strain on families, resulting in a range of societal issues. Moreover, it gives rise to various detrimental health effects such as heart problems, liver and kidney failure, as well as neurodegenerative disorders.[7] Hepatocellular enzyme serum concentrations and creatinine, urea levels can be utilized as selective biomarkers for hepatic tissue damage and kidney damage respectively.[8]

This study aimed to measure creatinine, urea, AST and ALT serum levels among METH addicted individuals in Khartoum state.

In the present study, there were significant increase in urea and creatinine levels among METH users when compared to control group. Similar observation has been recorded by Basile et al., and Baradhi et al.[9,10] They suggested multiple factors that contribute to the development of acute kidney injury in cases of methamphetamine intoxication. These include vasoconstriction, instability in hemodynamics, depletion of fluid volume, hyperthermia, and the development of pigment nephropathy due to rhabdomyolysis.[11,12]

Nephrotoxicity can occur with both prescribed medications and illicit substances. Methamphetamine is an example of a drug that can cause nephrotoxicity, potentially resulting in kidney failure. This failure can manifest as either chronic over a prolonged period or acute suddenly. Drug-induced nephrotoxicity, including methamphetamine use, is a frequently observed cause of sudden kidney failure.[10]

Furthermore, the study revealed significant increase in hepatocellular enzymes (AST and ALT) in METH addicted case group when compared to the non-users control group. These findings were in accordance with

results of Halpin et al. and Chian et al. their studies included individuals who exhibited a diverse array of clinical manifestations, including both acute and chronic hepatitis, as a consequence of methamphetamine addiction.[13,14] Also the findings were in match with a study performed by Shannon et al., who concluded that liver injury can be an effect of methamphetamine abuse.[13]

Possible mechanism of liver injury in methamphetamine includes; lipid peroxidation and toxic effects on liver cells.[15]

However, the present findings were inconsistent with Nazari et al. and Zhao et al., who stated that, there were no differences in ALT between the two groups.[16,17]

This may be attributed to variations in the research design (differences in sample size, and participant demographics) and contextual factors (geographical location and time period of the study) which may influence the outcomes. Moreover, the study showed that there was a strong positive correlation between urea levels and duration of the METH abuse and a moderate positive correlation between creatinine levels and duration of METH abuse

Additionally, there was a moderate positive correlation between ALT levels and duration of use. METH-induced hepatocellular damage maybe the consequence of the direct effects of the drug on the liver and the relatively prolonged duration of abuse.

The study encountered several limitations. Firstly, the stigma surrounding the study population presented challenges in data collection. Additionally, the timing of sample collection proved to be a significant obstacle. Furthermore, the study faced limitations due to the limited availability of admitted addicts for inclusion. Moreover, the lack of complete patient history and data accessibility further constrained the study.

Further investigation is strongly recommended, encompassing a comprehensive assessment of renal and hepatic functionalities. To accurately gauge the extent of renal impairment, it is advised to employ more specific renal markers such as cystatin C, estimated glomerular filtration rate, neutrophil gelatinase–associated lipocalin, and kidney injury molecule-1. Additionally, measuring ammonia levels is recommended to evaluate the severity of liver damage. Also, it is highly recommended to enhance public awareness regarding the use of METH, the contributing factors to its abuse, and the associated health complications.

Conclusion

There was significant increase in creatinine, urea, ALT and AST levels among METH addicts, strong positive correlation between duration and urea levels, and moderate positive correlation between the duration and creatinine and ALT level. However, AST levels showed no significant correlation with the duration of abuse.

Declaration

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Author contributions

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

Conflicts of interest

The authors have no conflicts of interest to declare.

Data availability

All datasets are available upon request from the corresponding author.

Ethics approval

The study was approved by the ethical committees of the University of Medical Sciences and Technology (IRB UMST/EG/2022/18, approval date 11/01/2022). Verbal consents were obtained from all participants, and approval was granted by the hospital administration through the office of the medical director.

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ORIGINAL PAPER

Effect of clinic-based and telemonitored home-based intervention on pain intensity, functioning and quality of life in patients with knee osteoarthritis

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ABSTRACT

Introduction and aim. The study assessed and compared the effects of clinic-based and telemonitored home-based interventions on pain intensity, functioning and quality of life in patients with knee osteoarthritis (KOA).

Material and methods. Forty-two patients were recruited purposely and randomly allocated into clinic-based (CBG) and telemonitored home-based group (THG) equally. The CBG and THG received isometric exercises to strengthen the quadriceps and hamstring muscles using theraband. THG received the exercise at home they were monitored on phone thrice in a week, while the CBG did the exercise in the hospital. The subjects performed four sets of eight repetitions three days in a week for eight weeks. Pain intensity, functioning and health related quality of life (HRQoL) were assessed at pretreatment, 6th and 8th week of intervention. Data was analyzed with descriptive and inferential statistics. Alpha level was set at 0.05.

Results. There was a significant reduction ($p<0.001$) among pre-treatment, 6^{th} and 8^{th} week intervention in pain intensity, functional pain intensity and quality of life of CBG and THG. CBG showed significant reduction ($p<0.001$) in pain intensity, function and increase in HRQoL than THG at 8^{th} week.

Conclusion. Clinic-based and telemonitored home-based interventions were both effective in the management of KOA but clinic-based intervention was better than telemonitored home-based intervention.

Keywords. clinical-based, knee osteoathritis, telemonitored, tera-band

Introduction

Osteoarthritis is the most prevalent musculoskeletal disease worldwide among people older than 60 years and it accounted for 3–9% of years lived with disability worldwide, one of the significant contributors to years lived with disability among the musculoskeletal conditions and fourth leading cause of years lived with disability globally.[1,2] It is estimated that by 2030, the proportion of people with OA will have risen from 20% to 30% in those aged 60 years or over.[3] Increasing life expectancy, decreasing physical activity and increasing body weight are all considered as underlying factors. Being a leading

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musculoskeletal cause of disability in elderly persons all over the world and a major cause of physical limitations and reduced quality of life, OA, among other types of arthritis, is the most common type of joint disease in adults worldwide.[4,5] Osteoarthritis can affect any joint, but preferentially affects the knee, hand, hip and spine. Knee osteoarthritis (KOA) accounts for approximately 85% of the burden of osteoarthritis worldwide.[6] KOA is a common progressive multifactorial joint disease and is characterized by chronic pain and functional disability.[2] Thus, individuals with KOA suffer from progressive increased impact on their activities of daily living, which leads to losses in labor relations, leisure, social life, and sleeping quality, leading also to important decrease in their quality of life.[7]

Physiotherapy treatment for KOA involves therapeutic exercises which are used in almost all treatment sessions in the management of KOA.[8] Exercise therapy is particularly helpful in decreasing pain and improving joint motion, for which high-quality evidence has been available in the past decade.[9,10] Exercise therapy consisting of strengthening exercise and general aerobic exercise is now seen as one of the key elements of OA management.[2] Literature has recommended that patients with symptomatic OA of the knee to participate in self-management programs, strength training, low-impact aerobic exercise, and neuromuscular education, and to engage in physical activity consistent with national guidelines.[11]

Telemonitoring is a convenient way for patients to avoid travelling and to perform some of the more basic work of healthcare for themselves.[12] The objective of telemonitoring is to allow patients and medical experts to carry on their sessions through telecommunication networks as if they are in the same place.[13] The usual pattern of managing patients with KOA requires patients to keep attending the clinic for one-on-one sessions with the physiotherapists. However, patients who live far away from the clinics may find it difficult to attend clinic regularly due to distance and cost of transportation.[14] In order to address these problems which could make treatment ineffective, telephysiotherapy which entails the use of telecommunications technology as a medium for providing information for therapeutic exercises to patients at homes that are at a distance from the physiotherapy clinics should be considered.[15] The applications of telemonitoring and its effectiveness have been documented in rehabilitation of stroke and patients with total knee replacement.[16,17] The efficacy of telephone-based rehabilitation technology on the outcomes of pain and physical function and quality of life in patients with KOA were documented.[18] Bennell et al. investigated the clinical and cost effectiveness of adding nurse-delivered telephone coaching to a physiotherapist-delivered physical activity intervention for people with KOA. Better outcomes were achieved in group of patients that received physiotherapy treatment with nurse-delivered telephone coaching than those that received only physiotherapy treatment. The usual pattern of managing patients with KOA requires patients to keep attending the clinic for one-on-one sessions with the physiotherapists. However, patients with KOA often require multiple clinic visits for care and are often faced with accessibility challenges of cost, distance and transportation.[14] In order to address these challenges, effectiveness of telemonitored home-based intervention and clinic-based intervention be considered in the management of patients with KOA. Although, similar studies have been reported in the literature, most of these works were carried out where there are better facilities in terms of stable electricity, consistent internet with uninterrupted signal and adequate funding.[16-18] It is imperative to carry out the study in a location where some of the aforementioned challenges in the management of patients with knee osteoarthritis are bedeviling the management.

Aim

The objectives of the study were to evaluate the effect of clinic-based and telemonitored interventions on pain intensity, function and quality of life and to compare the effects of such in patients with knee osteoarthritis.

Material and methods

Participants

Participants for this study were individuals diagnosed with osteoarthritis of the knee joint referred to Out-patient Physiotherapy Clinic of State Hospital Abeokuta.

Inclusion criteria

Eligible for participation in this study were:

i. Patients diagnosed with osteoarthritis of the knee of not less than six weeks.

ii. Patients that have means of communication via mobile telephone.

iii. Patients who understands English or Yoruba language.

Exclusion criteria

Excluded from participation in this study were:

i. Participants that have any other knee joint diseases.

ii. Participants with history of knee joint trauma.

iii. Participants with history of knee surgery or arthroplasty.

Sampling technique

A purposive sampling technique was used for this study to recruit subjects with knee osteoarthritis.

Research design

The research design was Quasi experimental study

Sample size determination

The sample size for the study was determined using the formula.[15]

$$n = \frac{c \times \pi1(1-\pi1) + \pi2(1-\pi2)}{(\pi1-\pi2)^2.}$$

Where:
n is the size per group
C is 7.9 for 80% power
$\pi1 = 0.25$ (proportion estimate)
$\pi2 = 0.65$ (proportion estimate)

Therefore:

$$n = \frac{7.9 \times 0.25(1-0.25) + 0.65(1-0.65)}{(0.25-0.65)^2}$$

Thus, n (size per group) = 20.49
= 21

This was increased to 25 per group because of attrition (25 participants for clinic-based group and 25 participants for telemonitored home-based group).

Randomization

The randomization of the participants to the clinic-based group and telemonitored home based group was shown in Fig 1

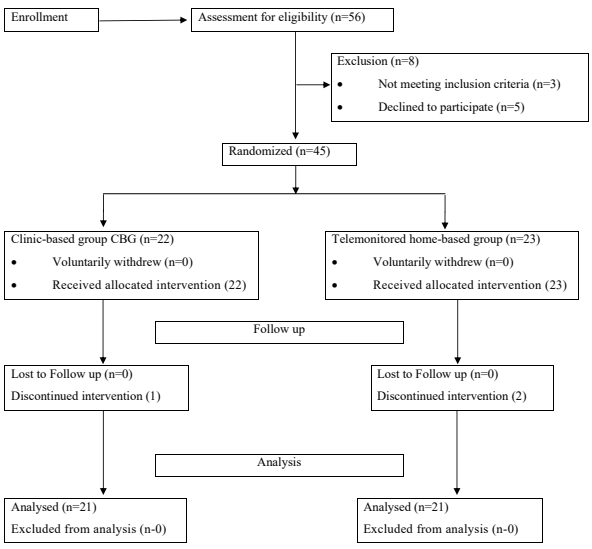

Fig. 1. CONSORT patient flow for the study

Instruments

The instruments that were used for this study include:

Quadruple Visual Analog Scale (QVAS)

This was used to assess pain intensity experienced by the participants at the time of assessment, typical or average pain, pain at its best, and pain at its worst, respectively.[21] It consists of 4 items with each item numbered 0 to 10. With 0 indicating no pain and 10 is worst possible pain. A Yoruba translated version of the QVAS was used for participants who had preference for the Yoruba language.

The Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC)

WOMAC is a self-administered health-status instrument for patients with KOA, consists of 24 items within three subscales: pain (5 items), stiffness (2 items) and physical function (17 items). This questionnaire which is valid, reliable and responsive was developed by Bellamy.[22] All WOMAC subscales (pain, stiffness, and physical function) were internally consistent with Cronbach's coefficient alpha of 0.91, 0.81, and 0.84, respectively. The Yoruba version of WOMAC translated from English by Ojoawo and Akinwunmi was used for participants who do not understand English.[23]

WHOQoL-BREF

This instrument comprises 26 items, which measure the following broad domains: physical health, psychological health, social relationships, and environment. WHOQoL-BREF has been shown to display good discriminant validity, content validity and test-retest reliability. Their sensitivity to change is currently being assessed.[1] The WHOQoL-BREF is a sound, cross-culturally valid assessment of QoL, as reflected by its four domains: physical, psychological, social and environment.[24]

Theraband

This was used to strengthen the muscles around the knee joint. The band is made by Fixtur Displaysm FDS Illinois. It consists of 5 sizes resistance band in different colors made from rubber latex.

Mobile phone

This was used to communicate with the participants on home treatment.

Tape rule

15 0cm or 60 inches long China made butterfly tape rule was used to measure waist and hip girth measurement.

Stadiometer

This consists of height meter and weighing scale made by Maney Medical Technology Co. Ltd, Shanghai to measure the height and weight of participants.

Procedure

Ethical approval was obtained from the Health Research and Ethics Committee of Institute of Public Health, Obafemi Awolowo University Ile-Ife, Nigeria and Department of Planning Research and Statistics, Ogun state Ministry of Health. The eligible patients were duly

informed of the rationale and procedure for the study and were enlightened about the aim of the research in improving Physiotherapy services to patients with knee osteoarthritis. Informed consent was obtained from each patient and confidentiality was ensured.

Each participant was then randomly allocated into one of the two groups without considering the severity of the condition in order not to introduced bias using fish bowl methos Fig 1. An opaque envelope with 50 small papers was used for the randomization. Twenty-five papers were inscribed with CG while the remaining twenty-five papers inscribed with TG. Subjects were asked to pick a paper from the envelope as they were coming into the department. Those that picked CG were allocated to clinic-based intervention group (CBG) and those that picked TG were allocated into telemonitored home-based intervention group (THG).

Clinic-based group intervention

Assessment of the patient was done at pretreatment using WOMAC, WHOQoL-BREF and QVAS. Participant's weight, height, waist and hip circumference were measured using appropriate instrument (Fig. 2). Strengthening exercises was administered to this group, three times a week for 8 weeks in the clinic. Patient sat comfortably of a chair with arm. Theraband was anchored at one end to the chair's foot and the other end of the band to the ankle of the patient. Patient was instructed to push the theraband by extending the leg. The ability to push the band and sustain it with a bearable resistance was used as a requirement for the choice of the band. Once the specific band has been chosen, patient then used it for strengthening both the harmstrings and quadriceps muscles using is the procedure of Lee et al.[25] For the quadriceps strengthening, the participant sat on a chair with the knee maintained in 90 degree flexion and the theraband was locked with the foot of the chair on one end with the band wrapped around the ankle on the other end as shown in plate 1. The patient extended the knee while the theraband stretched out, thereby strengthening the quadriceps muscle (Fig. 3). The stretching was sustained for 10 second with a period of wrest for 4 second, 10 repetitions were carried out for a session. The patient was also asked to lie prone on the treatment bed, the theraband was attached to the foot of the bed on one hand and wrapped ankle joint on the other hand. The limb was flexed at the knee, thereby strengthening the hamstring muscle. The stretching was sustained for 10 second with a period of wrest for 4 second, 10 repetitions were carried out for a session. They were reassessed at the end of third, sixth and eight week of intervention.

Telemonitored home-based group intervention

Assessment of the patient was also done at pretreatment using WOMAC, WHOQoL-BREF and QVAS. Participant's weight, height, waist and hip circumference were measured using appropriate instrument. They were required to perform the same exercise programmes given to the CBG at home three times a week for eight weeks. The exercises were demonstrated to them by the Physiotherapist in the clinic and they were asked to perform the exercise to be sure it is well understood. The participants were also provided with the pictorial representation of the exercises and this was taken home by each participant as a guide to ensure compliance and adherence. Mobile telephone monitoring through calls and SMS was ensured two times a day, a wakeup call of 30 minutes before the exercise and a call during the exercise to monitor their compliance. This was done on three occasions of the treatment programmes in a week. They were provided with log-book for proper documentation of the treatment procedure. This group of patients only reported to the clinic at the end of third, sixth and eight week for reassessment.

Fig. 2. A patient with the application of theraband at the starting point to perform quadriceps muscle strengthening exercise

Fig. 3. A patient with the application of theraband at the final point performing quadriceps muscle strengthening exercise

Outcome measures

Pain intensity was assessed with QVAS; WOMAC was used to assess the functional pain, stiffness and physical function, and WHOQoL-BREF was used to measure the quality of life. These measurements were carried out

pretreatment, third week, six week and eight weeks of the intervention.

Data analysis

The data was analyzed using Statistical Package for Social Sciences SPSS (IBM version 23, Armonk, NY, USA). Descriptive statistics and inferential statistics of Mix Method ANOVA was used to compare the mean value of pretreatment, 3rd, 6th and 8th week treatment of pain intensity, function pain, stiffness, physical difficulty and quality of life within the groups. Mixed Method ANOVA was also used to compare the pain intensity, function pain, stiffness, physical difficulty and quality of life of clinic-based and telemonitored home-based intervention between the groups. A post hoc analysis was employed where necessary. Alpha level was set at 0.05 was considered as statistically significant.

Results

Physical characteristics of participants

Presented in Table 1 is the physical characteristics of the subjects. The mean age, body mass index (BMI) and WHR of CBG were 55.55±8.53yrs, 28.01±6.51kg/m² and 0.87±0.04 respectively while the mean age, BMI and WHR of THG were 62.94±05.67 years, 28.95±5.92 kg/m² and 0.94±0.12 respectively. There was no significant difference (p>0.05) between weight and Body mass index of CBG and THG .

Table 1. Descriptive statistics of participants in CBG and THG (n=42)*

Variables	CBG (n=21) Mean±SD	THG (n=21) Mean±SD	Total group (n=42) Mean±SD	t	p
Age (yrs)	55.55±8.53	62.94±5.67	59.05±8.13	-3.110	0.004
Height (m)	1.65±0.04	1.58±0.08	1.62±0.07	3.567	0.001
Weight (kg)	76.55±18.57	72.06±15.38	74.42±17.05	0.807	0.425
BMI (kg/m²)	28.01±6.51	28.95±5.92	28.46±6.17	-0.466	0.644
Hip Cir (cm)	101.85±11.51	111.22±6.66	106.29±10.53	-3.026	0.005
Waist Cir (cm)	89.20±12.96	104.50±16.72	96.45±16.58	-3.169	0.003
WHR	0.87±0.04	0.94±0.12	0.90±0.09	-2.350	0.024

* CBG – clinic-based group, THG – telemonitored home-based group, SD – standard deviation, BMI – body mass index, Hip Cir – hip circumference, Waist cir – waist circumference, WHR – waist to hip ratio

Within group effect of telemonitored home-based intervention on pain intensity, function and quality of life

Shown in Table 2 is mixed method ANOVA comparing the mean value of the pretreatment, 3rd week, 6th week and 8th week intervention on pain intensity, function and quality of life of participants in THG. The results revealed a significant difference (p<0.001) in the physical function, functional pain and joint stiffness between pretreatment and each of 6th and 8th week intervention

Table 2. Within group effect of telemonitored home-based intervention on pain, intensity, function and quality of life (n=21)*

Variables	Pre	Week 3	Week 6	Week 8	Chan	p
PI	56.46 (33.87)[a]	55.76 (34.57)[a]	53.61 (28.46)[a]	49.63 (19.72)[a]	5.83	0.219
HQoL	79.65 (8.84) [f]	79.06 (8.54)[f]	80.61 (8.22)[f]	82.61 (9.40)[f]	2.96	0.415
FPain	8.06 (2.36) [g]	7.17 (2.94)[h]	5.11 (2.02)[i]	4.00 (1.94)[j]	4.06	<0.001
JStifnes	3.56 (1.38)[k]	3.44 (1.29) [l]	2.67 (0.97)[m]	2.11 (0.75)[n]	1.45	<0.001
Phyl Dif	33.22 (8.82)[o]	30.11 (12.46)[p]	22.77 (5.26)[q]	19.55 (3.60)[r]	3.67	<0.001

* PI – pain intensity, HQoL – health related quality of life, FPain – functional pain intensity, J Stiffness – joint stiffness, Phyl Diff – physical difficulty, Post Hoc analysis using LSD of mean values with the same superscript (a, b, c, d, e, f, g, h, i, j, k l, m, n, o, p, q, r) shows no significant difference but means with different superscript shows a significant difference

Within group effect of clinic-based intervention on functional pain, joint, stiffness and physical difficulty

Shown in Table 3 is the Mixed Method ANOVA comparing the mean value of the pretreatment, 3rd week, 6th week and 8th week of functional pain, stiffness and physical difficulty of participants in CBG. There was a significant difference (p<0.001) in functional pain when pretreatment, 6th week and 8th week intervention was compared. There was also a significant difference (p<0.001) in physical difficulty when pretreatment, 6th week and 8th week of intervention were compared. There was also a significant difference in functional pain and physical difficulty when 6th week and 8th week intervention was compared.

Table 3. Mix-method ANOVA, comparing the pretreatment, 3rd 6th and 8th weeks of pain intensity, functioning, quality of life, functional pain, joint stiffness and physical difficulty of clinical based group (n=21)*

Variables	Pre	Week 3	Week 6	Week 8	Chan	p
PI	72.50 (31.77)[a]	65.61 (38.16)[b]	78.67 (40.17)[a]	64.98 (43.63)[c]	5.46 (2.14)	0.05
HQoLife	79.65 (8.84)[h]	79.06 (8.54)[h]	80.61 (8.22)[h]	82.61 (9.40)[d]	1.96 (2.13)	<0.001
FPain	7.75 (4.56)[a]	6.90 (2.99)[b]	4.20 (2.89)[c]	3.00 (2.55)[e]	4.75 (3.24)	<0.001
JStifnes	3.15 (1.18)[g]	2.60 (0.50)[h]	2.00 (1.03)[j]	1.50 (0.96)[k]	1.65 (0.89)	<0.001
Phyl Dif	26.90 (14.25)[l]	23.70 (12.19)[m]	13.40 (8.49)[n]	11.70±8.23[p]	4.20 (1.45)	<0.001

* PI – pain intensity, HQoL – health related quality of life, FPain – functional pain intensity, J Stiffness – joint stiffness, Phyl Diff – physical difficulty, Post Hoc analysis using LSD of mean values with the same superscript (a, b, c, d, e, f, g, h, i, j, k l, m, n, o, p, q, r) shows no significant difference but means with different superscript shows a significant difference

Between group effect of clinic-based intervention and telemonitored home-based intervention on pain intensity, function and quality of life

Presented in Table 4 is the mixed method ANOVA comparing the mean value of the pretreatment, 3rd week, 6th

Table 4. Mix-method ANOVA, comparing the mean CBG and THG values of outcome measures*

Variables	CBG n=21					THG n=21					
	Pre	Week 3	Week 6	Week 8	Change	Pre	Week 3	Week 6	Week 8	Change	p
PI	72.50 (31.77)[a]	65.61 (38.16)[a]	78.67 (40.17)[a]	64.98 (43.63)[a]	7.52 (1.23)	56.46 (33.87)[a]	55.76 (34.57)[a]	53.61 (28.46)[a]	49.63 (19.72)[a]	5.83	0.219
HQoLife	84.50 (8.20)[h]	85.95 (8.40)[h]	87.50 (7.69)[h]	90.20 (8.23)[h]	5.40 (2.13)	79.65 (8.84)[h]	79.06 (8.54)[j]	80.61 (8.22)[k]	82.61 (9.40)[l]	2.96	<0.001
FPain	7.75 (4.56)[a]	6.90 (2.99)[b]	4.20 (2.89)[c]	3.00 (2.55)[c]	4.75 (3.24)	8.06 (2.36)[a]	7.17 (2.94)[b]	5.11 (2.02)[d]	4.00 (1.94)[f]	4.06	<0.001
JStifnes	3.15 (1.18)[g]	2.60 (0.50)[h]	2.00 (1.03)[j]	1.50 (0.96)[k]	1.65 (0.89)	3.56 (1.38)[g]	3.44 (1.29)[i]	2.67 (0.97)[j]	2.11 (0.75)[k]	1.45	<0.001
Phyl Dif	26.90 (14.25)[l]	23.70 (12.19)[m]	13.40 (8.49)[n]	11.70 (8.23)[p]	4.20 (1.45)	33.22 (8.82)[l]	30.11 (12.46)[m]	22.77 (5.26)[o]	19.55 (3.60)[q]	3.67	<0.001

* pre – pretreatment, PI – pain intensity, HQoL – health related quality of life, FPain – functional pain intensity, JStiffness – joint stiffness, Phyl Diff – physical difficulty, Post Hoc analysis using LSD of mean values with the same superscript (a, b, c, d, e, f, g, h, i, j, k l, m, n, o, p, q) shows no significant difference but means with different superscript shows a significant difference

week and 8th week of pain intensity, function and quality of life of participants in CBG and THG. There was no significant difference (p>0.05) in pain intensity in pretreatment, 3rd week, 6th week and 8th week when CBG and THG were compared. There was a significant difference (F=20.859 p<0.001) in physical function in 3rd week, 6th week and 8th week when CBG and THG were compared. However, physical function improves significantly (p<0.001) in 8th week of CBG than THG.

Discussion

This study investigated the effect of clinic-based and telemonitored home-based intervention on pain intensity, functioning and quality of life in patients with knee osteoarthritis and compared the effects of clinic-based and telemonitored home-based intervention on those variables. Forty-two patients with knee osteoarthritis participated in the study. The participants of this study were on the average age of 60years; this was in line with the age prevalence of knee OA as documented in several studies.[26,27] Also, the mean body mass index BMI of the participants in this study was in over weight range. These physical characteristics profile of the participants, showed that majority of the participants are overweight. In essence, it implies that knee osteoarthritis is associated with increase BMI. Being overweight is a key factor for knee OA and provides substantial grounds for concern of disease severity and productivity losses.[28-30] Fowler-Brown et al. found that a 5 kg/m^2 increase in BMI was associated with a 32% increase in the probability of OA[28]. In a study, Felson et al. reported that obese individuals have 1.5 to 2 times the risk of developing knee OA as their leaner counterparts.[31] The weight of the body especially from the knee joint above constitute a greater percentage weight of the body. This is the location where there are intestinal structures and thoracic component. If these are added with excess body fat, it creates a greater weight for the knee joint promoting knee OA. It was observed from the result that there was a significant difference between the clinic-based and telemonitored home-based groups regarding the age, height, waist, hip and waist to hip ratio. Considering the age, though there was a difference but the age was still within the range of adulthood which could not really affect the exercise or intervention of the participants. Again, the hip, waist and waist to hip ratio are function of central obesity, the mean values of each of these variables were not too outrageous. More so, the exercises were in sitting position which may not have much relationship with the central adiposity.

It was observed from the study that there were significant differences in physical function of patients with knee osteoarthritis in the clinic-based group between pretreatment and sixth week, between pretreatment and eight week of intervention. A significant difference was also observed in functional pain between pretreatment and sixth week, between pretreatment and eight week of intervention. However, functional pain improved better in 8th week than 6th week. These results were consistent with the outcome of a study conducted by Odole et al.[18] They investigated the effects of a 6-week telemonitored program on pain intensity and physical function of patients with osteoarthritis of the knee. Statistical significant were found in physical function of patients between baseline and fourth week, baseline and sixth week of intervention. Exercise therapy has been found to reduce pain, improve physical function and quality of life in patients with knee osteoarthritis. Muscle strengthening exercises are important given that muscle weakness is almost universal in people with knee OA and is related to higher pain levels and reduced function.[32,33] A further benefit of strength training is the resulting increase in levels of physical activity that may come with increased muscle strength. In a study conducted by Maly and colleagues, they concluded that the feeling of stiffness in osteoarthritis is related with self-efficacy for physical activity, and stiffness also shows a moderate association with physiologic predictors of the risk of falls in older adults.[34] However, knee stiffness had significantly correlated with physical disabilities and it is an important symptom associated with knee OA and so, health care providers can improve physical activity of OA patients with training muscle strength exercises. Similarly, in this study there was an improvement in the quality of life of participants in the clinic based group between pretreatment and eight weeks. This improvement in the quali-

ty of life could most probably be as a result of improved functional activity level. Rétsági et al. in their study on relationship between physical performance and quality of life and the level of physical activity among the elderly reported a positive relationship between physical activity level and quality of life.[35,36]

More so, it can be observed in the study a significant improvement in the physical function of participants in the telemonitored home-based group between the pretreatment and eight weeks. A study conducted by Odole and Ojo, reported a significant improvement between the pretreatment and sixth week of the intervention.[18] This study was not in accordance with their study as improvement was only recorded between the pretreatment and eight week of intervention. Although, there was a significant improvement in functional pain between pretreatment and 6th week; pretreatment and 8th week in the telemonitored home-based intervention. Functional pain also improved better in the 8th week than in the 6th week of intervention in the THG. This was in accordance with the study conducted by Azma et al.[36] The specialist monitoring on phone of the quality of exercises, and assurance given to them on the efficacy of exercises could be responsible for the improvement recorded.

However, comparing the mean values of the pretreatment, 3rd week, 6th week and 8th week of functional pain, physical function and quality of life in CBG and THG, participants in the CBG showed a better global improvement than the THG. This could be as a result of the psychological effects of being seen by a professional. The self-confidence they developed and the motivation given to them by the professional maybe responsible for the global improvement.

This study shows that the outcomes of functional pain, physical difficulty and quality of life in patients with osteoarthritis of the knee under clinic-based group (physiotherapist administered knee exercises) are comparable to those in the telemonitored home-based group (self-administered knee exercises and telephone monitoring) following eight weeks of intervention.

Study limitations

The outcome measures used in the study were based on the reports of the patients. It was on the trust that patients reported the actual levels of their perception of all the outcome measures. Their telemonitoring was on the phone not on the video. It was on the trust as well that patients reported the performance of the exercises at home.

Conclusion

In conclusion, clinic-based and telemonitored home-based interventions were effective in the management of knee osteoarthritis but the clinic-based intervention proved more effective.

It is recommended that any of clinical based and home based theraband exercises can be recommended or combined for patient with KOA for a better result.

Declarations

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Author contributions

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

Conflicts of interest

No conflict of interest from the authors.

Data availability

Data is available at request.

Ethics approval

Ethical approval was obtained (ERC/2021/07/15) from the Health Research and Ethics Committee of Institute of Public Health, Obafemi Awolowo University Ile-Ife, Nigeria and Department of Planning Research and Statistics, Ogun state Ministry of Health.

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ORIGINAL PAPER

Expression of vascular endothelial growth factor and microvessel density in oral squamous cell carcinoma and its correlation with various clinico-pathological parameters

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ABSTRACT

Introduction and aim. Angiogenesis, which is accomplished by capillary sprouting, is the process by which new vessels are created from pre-existing ones. In tumor, once their initial blood supply is depleted, a tumour is unable to grow without additional blood flow. Additionally, a tumor's microvasculature, or microvessel density (MVD), increases along with its capacity to produce angiogenesis. We aimed to observe the relationship between the expression of vascular endothelial growth factor (VEGF) and MVD (using CD34) in oral squamous cell carcinoma (OSCC).

Material and methods. The expression of VEGF and CD34 antibodies was analysed using immunohistochemistry method on 50 cases of histopathologically proved OSCC. The expression was correlated with clinicopathological parameters.

Results. A significant correlation was observed between VEGF expression and gender, LVSI. No correlation between any other factors and the difference in VEGF expression was statistically significant. Similarly, the MVD expression was not found to be statistically significant in any of the pathological parameters.

Conclusion. VEGF positivity as well as MVD were found to be independent of the tumor pathology. Tumor MVD was found to be independent of the expression of VEGF. Further studies in a larger study group may establish a significant association so that antiangiogenic targeted therapy may be initiated.

Keywords. microvessel density, oral squamous cell carcinoma, vascular endothelial growth factor

Introduction

Cancer of the lips and oral cavity (CLOC) is one of the most common types of cancer in the world. In 2020, over 177,000 people died from CLOC, with Southeast Asia having the highest number of deaths. India also has a high number of CLOC cases, accounting for over 10% of all cancer cases in the country.[1]

Oral squamous cell carcinoma (OSCC) is the most common type of CLOC.[2] It is more common in older adults and men.[3,4] Although there have been advances in diagnosis and treatment, the 5-year survival rate for OSCC remains low, at about 50-60%.[5] This is largely because OSCC often spreads to the lymph nodes. Recent research has identified a number of cellular events that play a role in tumor progression, which may lead to new treatment options in the future.[6]

Tumors need blood vessels to grow and spread. Angiogenesis is the process by which new blood vessels form from existing ones. This process is called capillary sprouting. The number of blood vessels in a tumor is linked to how aggressive the tumor is, and the number of blood vessels in a tumor has been shown to be an

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independent prognostic factor. Once a tumor's original blood supply is used up, it cannot grow beyond 1-2 mm without a new blood supply. The initial 1-2 mm zone is the farthest distance that oxygen and nutrients can diffuse from blood vessels.[7]

Therefore, angiogenesis is essential for tumor progression and metastasis. Although angiogenesis is difficult to measure directly in human tumors, there is increasing evidence that MVD may be considered as an indirect marker of neoangiogenesis. Although the most common antibodies used for microvessel staining so far are those against Von Willebrand factor VIII, CD31, and CD34, but CD34, a glycoprotein found in the membrane of endothelial cells (ECs), is considered to be highly sensitive for endothelial cells and produces minimum background staining.[8]

Vascular endothelial growth factor (VEGF) is the most important factor for the formation of new blood vessels in tumors. However, tumors do not start forming new blood vessels right away. This is called vascular quiescence. Vascular quiescence is ended by the "angiogenic switch", which is when the tumor starts to produce angiogenic factors. The angiogenic switch is "on" when there are more angiogenic factors than antiangiogenic factors, and it is "off" when the two types of factors are balanced.[8,9,10]

Aim

Thus this study was undertaken to evaluate the immunohistochemical expression of VEGF and CD 34 in OSCC and correlate the expression with histologic features of the tumor. The rationale behind this was that if the expression could be demonstrated in OSCC, this could offer an additional therapeutic strategy in the form of newer antiangiogenic drugs to prevent and treat cancer that are used as an adjunct to the currently available modalities.

Material and methods

This hospital-based retrospective study was carried on cases of OSCC from October 2018 to September 2022 that fit the selection criteria were included in the study. The Institutional Ethics Committee approved the study protocol (meeting date; 18/06/2021, decision number; 2021/3309). Written informed consent could not be obtained due to the retrospective nature of the study.

Inclusion

Patient with both sex, age range from 18 to 80 years and histologically confirmed radical excised cases of oral squamous cell carcinoma were included.

Exclusion

Recurrent OSCC, punch and incisional biopsy cases, unknown T-stage and N-stage cases, cases with neoadjuvant treatment, and poorly preserved cases were excluded from this study.

Histopathological diagnosis

Archival blocks from the pathology department were retrieved between October 2018 and September 2022. Relevant patient data were obtained from the hospital database. Histological evaluation with tumor grading was performed according to the World Health Organization (WHO) criteria. Tumor depths of invasion, lymphovascular invasion, and perineural invasion were recorded. Pathological staging was then performed according to the AJCC 8th edition. All included cases were grouped into two categories based on depth of invasion: ≤1 cm and >1 cm.

Immunohistochemistry procedure

Immunohistochemical evaluation of VEGF and CD34 was performed on 4–5 µm thick formalin-fixed paraffin-embedded tissue sections on poly-L-lysine-coated slides.

After deparaffinizing in three changes of xylene for 5 minutes each and rehydrating in a graded series of alcohol, the microwave antigen retrieval was performed using Tris EDTA (target retrieval buffer, pH 9) at 700 watts for 5 minutes, 600 watts for 5 minutes, and 600 watts for 5 minutes. Then, the slides were washed with Tris-buffered saline (TBS, pH 7.4). An endogenous peroxidase block was performed by adding 100 µL of peroxidase block to each tissue section and incubating at room temperature for 8–10 minutes. The slides were then washed in TBS for 5 minutes. Primary antibodies against CD34 (monoclonal mouse antibodies; ProTaqs Cat. No. 401602092; Quartett GmbH, Germany) and anti-VEGF (monoclonal mouse antibodies; clone VG-1; Diagnostic Biosystems, The Netherlands) were added to each tissue section (100 µL) and incubated in a humid chamber for 45 minutes. After washing in TBS for 5 minutes, HRP polymer (100 µL) was added to each tissue section, and the slides were kept in a humidity chamber for 30 minutes. After washing, a freshly prepared DAB solution was added and incubated for 15 minutes. The slides were then washed with wash buffer and distilled water. The counterstain was done with Harris hematoxylin for 1 minute, followed by washing with running tap water. Dehydration was done with a graded series of isopropyl alcohol (70%, 85%, and 100%) for 5 minutes each, followed by a xylene wash. Finally, the slides were mounted in DPX. Positive and negative controls were included in each batch.

Expression or scoring

VEGF

VEGF was expressed as a cytoplasmic stain in the tumor cells. The stained slides were interpreted as described by

Soini et al.[11] Scoring was based on the intensity of immunostaining in the lining endothelial cells (ECs; I) and the percentage of positive cells (P). The final immunostaining score was determined by the sum of the intensity of immunostaining (I) and the P. Final scores ranged from 0 to 7.

MVD

Vascular hotspots were evaluated under low magnification, and microvessel counting was performed manually under high power. The average was calculated for statistical evaluation. The mean of all microvessel counts was calculated as 21. All specimens were classified as "LOW MVD" for values ≤21 and "HIGH MVD" for values >21.

Statistical analysis

Measurement data were expressed as the mean ± standard deviation. Count data were expressed as percentages. Associations between VEGF and MVD expression and clinicopathologic factors were tested using the chi-square test. To assess the correlation between VEGF and MVD, the Karl-Pearson correlation coefficient was calculated. A p value of <0.05 was defined as statistically significant. Stata Version 15.1 software was used (StataCorp LLC, Texas, USA).

Results

A total of 50 cases were included, with males predominating over females. The male-to-female ratio was 4.5:1. The mean age of the patients was 55 years, with an age range of 28 to 80 years. Buccal mucosa (54%, 27/50) was the most common tumor site, followed by tongue (34%, 17/50), gingivobuccal sulcus (10%, 5/50), and lip (2%). Most of the cases (84%, 42/50) were grade I. Most of the cases (64%, 32/50) had a depth of invasion (DOI) of 1 cm. Only 8% (4/50) of the cases showed evidence of lymphovascular invasion. Almost equal proportions of cases had and did not have evidence of perineural invasion (PNI). The majority of cases (34.0%, 17/50) were T2, followed by 30% (15/50) cases of T4. An equal number of cases (50%, 25/50) had and did not have nodal metastasis (Table 1).

VEGF expression in OSCC

VEGF expression was found in 47 (94%) cases, of which 28 (56%) had strong expression and 19 (38%) had weak expression. A significant correlation was observed between VEGF expression and sex and LVSI. No other factors were significantly correlated with the difference in VEGF expression (p>0.05).

MVD expression in OSCC

Overall, equal percentages of low and high MVD were observed. There was no significant correlation between MVD and clinicopathologic parameters (Table 2).

Table 1. Correlation between VEGF expression and clinicopathological parameters

Clinicopathological parameters		Numbers (%)	VEGF		p
			Weak	Strong	
Age	<60	36 (72)	14	22	0.219
	>61	14 (28)	8	6	
Sex	Male	41 (82)	15	26	0.021
	Female	9 (18)	7	2	
Site	BM	27 (54)	8	19	0.091
	Gingivobuccal sulcus	5 (10)	3	2	
	Lip	1 (2)	1	0	
	Tongue	17 (34)	10	7	
Grade	Well differentiated	42 (84)	19	23	0.132
	Moderately differentiated	7 (14)	2	5	
	Poorly differentiated	1 (2)	1	0	
Depth of invasion	DOI< I cm	32 (64)	14	18	>0.999
	DOI> I cm	18 (36)	8	10	
Lymphovascular invasion	Present	4 (8)	4	0	0.044
	Absent	46 (92)	18	28	
Perineural invasion	Present	24 (48)	11	13	0.257
	Absent	26 (52)	11	15	
T stage	T1	11 (22)	5	6	>0.999
	T2	17 (34)	8	9	
	T3	7 (14)	4	3	
	T4	15 (30)	5	10	
N stage	0	25 (50)	11	14	0.973
	1	6 (12)	2	4	
	2a	4 (8)	2	2	
	2b	9 (18)	4	5	
	3b	6 (12)	3	3	

Table 2. Correlation between MVD expression and clinicopathological parameters

Clinicopathological parameters		Numbers (%)	MVD		p
			Low	High	
Age	<60	36 (72)	16	20	0.533
	>61	14 (28)	8	6	
Sex	Male	41 (82)	19	22	0.721
	Female	9 (18)	5	4	
Site	BM	27 (54)	11	16	0.277
	Gingivobuccal sulcus	5 (10)	2	3	
	Lip	1 (2)	0	1	
	Tongue	17 (34)	11	6	
Grade	Well differentiated	42 (84)	20	22	0.845
	Moderately differentiated	7 (14)	3	4	
	Poorly differentiated	1 (2)	1	0	
Depth of invasion	DOI< I cm	32 (64)	16	16	0.774
	DOI> I cm	18 (36)	8	10	
Lymphovascular invasion	Present	4 (8)	3	1	0.340
	Absent	46 (92)	21	25	
Perineural invasion	Present	24 (48)	12	12	>0.999
	Absent	26 (52)	12	14	
T stage	T1	11 (22)	4	7	0.127
	T2	17 (34)	11	6	
	T3	7 (14)	1	6	
	T4	15 (30)	8	7	
N stage	0	25 (50)	10	15	0.795
	1	6 (12)	4	2	
	2a	4 (8)	2	2	
	2b	9 (18)	5	4	
	3b	6 (12)	3	3	

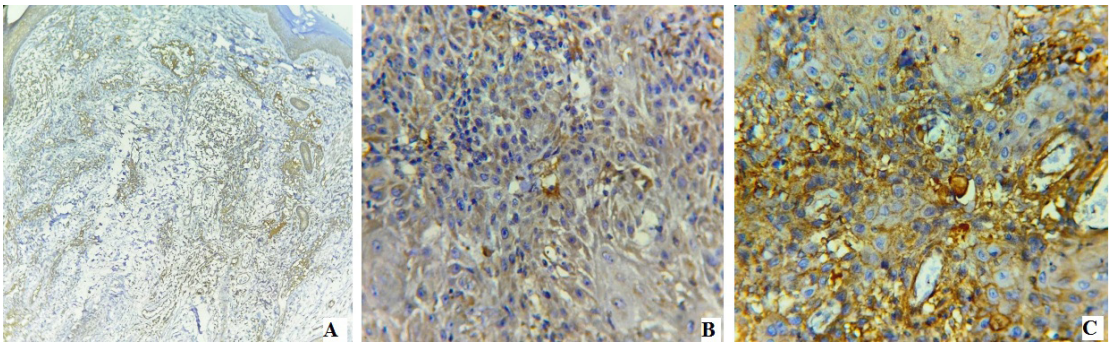

Fig. 1. A: Microsection shows strong cytoplasmic positivity of VEGF in tumour cells in angiosarcoma of skin which was taken as positive control (400x). B: Microsection shows weak cytoplasmic positive staining of VEGF in oral squamous cell carcinoma cells (400x). C: Microsection shows strong cytoplasmic positive staining of VEGF in oral squamous cell carcinoma cells (400x)

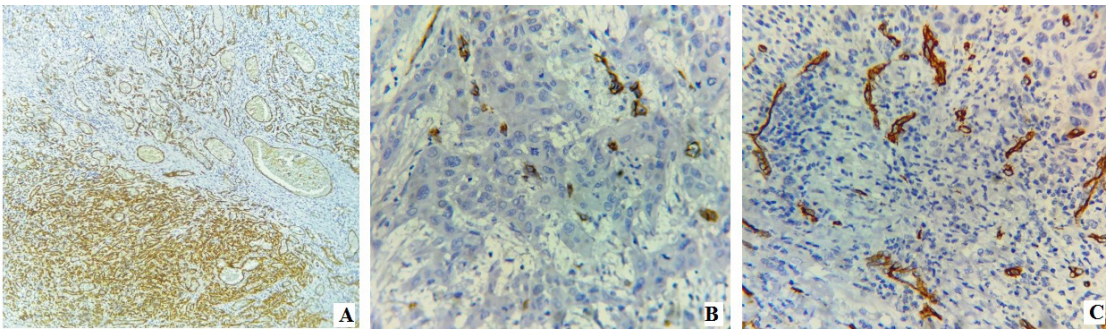

Fig. 2. A: Microsection shows strong membranous positivity of CD34 in endothelial cells of capillary hemangioma which was taken as positive control (400x). B: Microsection shows membranous staining of CD34 in endothelial cells of oral squamous cell carcinoma indicating low MVD (400x). C: Microsection shows membranous staining of CD34 in endothelial cells of oral squamous cell carcinoma indicating high MVD (400x)

The comparison of MVD scores with respect to VEGF expression

In our study, the majority of cases showed strong VEGF expression, among which 57.1% (16/28) showed strong MVD. Among tumor with absent VEGF expression, 66.7% (02/02) cases show low MVD. Low MVD was seen in 52.6% (10/19) of cases of tumor with weak VEGF expression. There was no significant correlation between MVD and VEGF expression (p=0.593) (Fig. 1 and 2).

Discussion

Tumors recruit new blood vessels from the existing circulation (angiogenesis), which contributes to tumor invasion and metastasis. Studies in the literature provide evidence that VEGF expression is necessary for neoangiogenesis, which is essential for tumor growth and metastasis.[12] Based on this evidence, we hypothesized that oral cavity tumors express VEGF for their growth and that MVD increases with increasing VEGF expression. To test our hypothesis, we evaluated VEGF expression and MVD using the CD34 marker in 50 OSCC cases. Both VEGF expression and MVD were correlated with known clinicopathological parameters.

Although we found strong VEGF expression in most OSCC cases with age ≤60 years, buccal mucosa, well-differentiated tumor, and DOI<1 cm, the difference was not statistically significant. Only sex and lymphovascular invasion (LVI) showed a significant correlation. All LVI-positive cases expressed VEGF, but 60.9% of LVI-negative cases also showed strong VEGF expression. We could not find similar studies that correlated VEGF expression with LVI.

We had the most cases in grade I, with 54.7% expressing strong VEGF expression. However, we could not establish a statistically significant correlation. Our study is consistent with previous reports.[7,13,14]

Astekar et al. found in their study that VEGF expression decreased from well-differentiated to poorly differentiated OSCC, but others have found a significant correlation between VEGF expression and tumor grade.[15] In their study, all poorly differentiated OSCC specimens and most moderately differentiated OSCC specimens expressed VEGF significantly with moderate to strong intensities, in contrast to our findings. They opined that the tumor cells in poorly and moderately differentiated OSCC exhibit angiogenic phenotypes, which could reflect a deregulated genotype.[7,16] Of our cases, 34% were classified as T2 and 14% as T3. We did not find a significant correlation between tumor stage and VEGF expression, but Sappayatosok et al. and Li et

al. did.[14,17] Larger sample sizes may be needed to establish a correlation in our study.

In our study, 50% of cases showed nodal metastasis, but the correlation between N stage and VEGF expression was not significant (p=0.973). Similar findings were observed by Nadir et al., but others have found a significant correlation between lymph node metastasis and VEGF expression.[14,17-19]

The current data showed no significant correlation between MVD and clinicopathological factors, which is in agreement with previous reports.[12,15,20]

When correlating MVD with age, we did not observe a statistically significant correlation (p=0.533). Of cases ≤60 years, 55.6% showed high MVD. Our findings are consistent with a previous report in which no statistically significant correlation was found.[21] However, Shahsaveri et al. found a significant correlation between MVD and age (p=0.029).[22] Two studies found a significant correlation between tumor grade and MVD (p<0.02, respectively).[23,24] We found no significant correlation between grade, DOI, LVI, or PNI with MVD. The possible reason for this lack of association in our study is the smaller sample size. We had the most cases in the T2 stage, but we did not observe a significant correlation between MVD and tumor stage (p=0.127). Our findings are consistent with those of a previous study.[15] However, Shieh et al. and Sappayatosok et al. found significant correlations (p<0.0001 and p<0.005, respectively).[17,26]

Artese et al. and Miyahara et al. found a significant correlation between MVD and lymph node metastasis.[27,28] In the current study, half of the cases showed nodal metastasis. We did not find a significant correlation between MVD count and nodal status (p=0.795). Our findings are consistent with those of Sappayatosok et al.[17] However, Elmorsy et al. found a significant correlation between MVD and nodal stage (p<0.001).[25]

Few studies have correlated MVD with VEGF. In our study, we did not find a significant correlation between VEGF and MVD (p=0.593). However, Astekar et al. found a significant correlation (p<0.001) in a similar study.[15] In our study, none of the clinicopathological parameters studied showed a significant correlation with MVD. However, some of the studies mentioned above found significant correlations with a few parameters. In future studies with larger sample sizes, MVD may help establish associations with a range of clinicopathological parameters. The density of tumor blood vessels measured in studies is primarily based on areas selected from the peripheral or central part of the tumor, or an even assortment of hotspot areas. This is another reason for the dissimilarity between reports. For achieving a precise result, more samples with more harmonized assessment methods are needed. Without strict standardization in methodology, conflicting results on the correlation between reports will continue.

In the present study, the most important limitation was the number of cases. By increasing the counts, more detailed results will be achieved.

Conclusion

Our study results demonstrate that VEGF expression in OSCC is independent of most clinicopathological variables, with the exception of patient sex and LVI. MVD (indirectly measured by CD34 expression) in OSCC cases is also uncorrelated with known clinicopathological factors and tumor VEGF expression. Further studies in a larger cohort may establish a significant association, which could be useful for applying antiangiogenic targeted therapy.

Declarations

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Author contributions

Conceptualization, S.K.H.; Methodology, N.K.J.; Formal Analysis, Investigation, S.K.H., R.P and N.K.J.; Data Curation, S.K.H. and N.K.J.; Writing – Original Draft Preparation, S.K.H., N.K.J., and R.P.; Supervision, S.H.K and R.P.

Conflicts of interest

The authors declare that there are no financial or other relations that could be construed as a potential conflict of interest.

Data availability

Datasets analyzed in this study are available from the corresponding author upon reasonable request.

Ethics approval

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.

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ORIGINAL PAPER

Surgical management of upper cervical esophagus stricture caused by ingestion of corrosive substances – a single-center experience

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ABSTRACT

Introduction and aim. Corrosive strictures of the upper cervical esophagus and hypopharynx are hard to treat in the operating room because there is a high chance of aspiration during swallowing after a high-up or proximal esophageal anastomosis. In this cases, we aimed to evaluate the role of intraoperative dilatation of the proximal hypopharyngeal and cervical esophageal stumps during surgery.

Material and methods. Patients who underwent surgery and had upper cervical esophageal and hypopharyngeal strictures from corrosive substance ingestion were included.

Results. Out of total 27 patients, 10 had a cricopharyngeal or proximal cervical esophageal stricture with a long segment thoracic esophageal stricture that was treated with intra-operative dilatation (IOD) of the proximal hypopharyngeal stump. IOD was done in two cases with Hegar's dilator and in three cases with wire-guided Savary Gillard dilators. In 74% (20/27) of the cases, the colon was frequently used as an esophageal substitute, while the stomach was only used in 10 cases. On follow-up, none of them developed repeated aspirations or required a tracheotomy.

Conclusion. IOD of the proximal hypopharyngeal and cervical esophageal stumps during surgery for corrosive upper cervical esophageal or cricopharyngeal strictures helps to save the proximal stump and avoid frequent hospital stays and multiple surgeries.

Keywords. corrosive injury of the esophagus, dilatation, esophageal replacement surgery, esophageal stricture

Introduction

Dysphagia, or difficulty swallowing, is a common symptom of an esophageal stricture, which is the abnormal narrowing of the esophageal lumen. It is a severe side effect of numerous disease processes and underlying causes. In developing nations, chronic strictures brought on by the consumption of corrosive substances are underreported. These strictures affect the upper cervical esophagus and hypopharynx.[1] Even though esophageal replacement surgery is the best option when multiple endoscopic dilations don't work, it is a very difficult procedure because the anastomoses at this high level are prone to anastomotic leakage, early postoperative stenosis, and disruption of the deglutition mechanism, which leads to repeated aspirations.[2-4] Additionally, maintaining the continuity of the upper digestive tract through esophageal substitution at healthy tissue margins compromises the strength of the swallowing mechanism and

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frequently necessitates performing a tracheostomy to ensure the avoidance of recurrent aspirations.

Aim

Here, we are presenting retrospective clinical information about patients who had corrosive-related esophageal and hypopharyngeal strictures that developed over time, along with our surgical approaches for treating them.

Material and methods

In this study, patient who went to the Department of Surgical Gastroenterology with chronic strictures of the upper cervical esophagus and hypopharynx from drinking corrosive substances and then had esophageal replacement surgery from 2009 to 2020 were included.

Patient-relevant information such as age, sex, duration of symptoms, radiological findings, and initial resuscitative measures was extracted from the hospital record. Preferred surgical approaches were done as per the patient's condition or clinical presentation. Prior to any management approaches, the planned route of reconstruction, its benefits, and the potential complications were explained to the patients, and informed consent (001/NEW/EC/INST/2023/15947) was obtained from the patient or their legal guardians. Prior to surgery, all patients were evaluated with an endoscopy.

A patient who had high stricture but normal lumen intraoperatively underwent intraoperative dilatation and esophageal reconstruction surgery.

Feeding jejunostomy was done in all cases in order to take care of nutrition prior to reconstruction surgery.

Surgical procedure

An oblique left cervical incision was made along the anterior border of the sternocleidomastoid muscle to access the cervical esophagus. The prevertebral fascia was found after the carotid sheath, and its contents were laterally retracted. The recurrent laryngeal nerve in the tracheoesophageal groove was carefully avoided by direct pressure. The upper thoracic esophagus was bluntly mobilized from the superior mediastinum, while the cervical esophagus was encircled by a No. 10 feeding tube and gently pulled upward.

Intraoperative dilatation protocol

The cervical esophagus was divided at the thoracic inlet in all cases, and the lower cut end was transfixed with a silk suture. Ten patients with a cricopharyngeal or proximal cervical esophageal stricture and a long-segment thoracic esophageal stricture were treated with intra-operative dilatation of the proximal hypopharyngeal stump. Intra-op dilatation was done in the initial 4 cases with Hegars dilators (1 for gastric pull-up and 3 for colon pull-up) and in the subsequent 6 cases with wire-guided Savary Gillard dilators (all for colon pull-up). Of these 3 cases, in two, the guide wire was directly passed to the cervical esophagus under laryngoscope guidance, and in one, the guide wire was placed under the guidance of a pediatric endoscope. In all 10 cases, one finger was used by the assistant at the lower cut end of the cervical esophagus (Fig. 1 and 2). After dilatation of the proximal esophageal stump, the esophagus is anastomosed to the terminal ileum in an end-to-end fashion with interrupted 4-0 monofilament sutures (PDS). All knots were placed on the inside, with the exception of the final three or four sutures placed on the anterior surface. After being pulled straight, the graft was stitched to the left crus using several 2-0 silk sutures in order to prevent late redundancy and abdominal visceral herniation. For the colo-gastric anastomosis, the distal end of the colon graft is transected about 10 cm distally from the hiatus. To enable the colo-colonic anastomosis, the divided colon's two ends were moved. The two-layer technique was used to perform both a cologastric (and colo-jejunal, in the event that the stomach was severely damaged) and a colo-colostomy.

In all cases, a midline incision was made in the abdomen, from the xiphisternum to 5cm below the umbilicus. The omentum was dissected free from the transverse colon, and the ascending colon, hepatic flexure, and cecum, along with the ileocecal junction, were fully mobilized from the retroperitoneum. The colon graft's vascular supply is then evaluated. The middle colic vessels and their arcade with the right colic and ileocolic vessels are checked under trans illumination by lifting the whole mobilized right colon vertically. Vascular supply through this arcade and in the marginal vessels was again checked after clamping the illeo-colic, right colic, and ileal (if required) arteries with a vascular bulldog clamp (before its division). The middle colic vessels served as the graft's foundation. Utilizing an umbilical tape that had been cut to the length between the left ear and the xiphoid, the necessary length of the colon was determined. The site on the terminal ileum (up to 7-8cm in all cases) where the umbilical tape reaches was marked with a silk stitch. An appendectomy was done. In all of our cases, the graft (right colon or stomach) was brought up substernally in the anterior mediastinum up to the left neck. In all the cases, we enlarged the thoracic inlet by removing the medial aspect of the left clavicle from the sternoclavicular joint. To prevent diaphragmatic obstruction of the graft, the diaphragm was resected laterally for several centimeters on each side of the midline at the lower end of the retrosternal route. Although prior coronary artery bypass surgery may make the creation of a substernal window hazardous and a relative contraindication, we have not encountered any such situation in our series.

Postoperative follow-up was done. The quantitative variables were summarized using the mean and standard deviation, while the qualitative variables were summarized using frequencies and percentages.

Results

A total of 27 cases were included, out of which 16 were male and 11 were female. Our study population's mean ± SD age was 27±7.12, with a range of 17–37 years. Twenty patients had ingested acids, and only seven had a history of alkali ingestion. Suicidal intent was associated with ingestion in 17 (63% of cases) and accidentally in 10 (37% of cases).

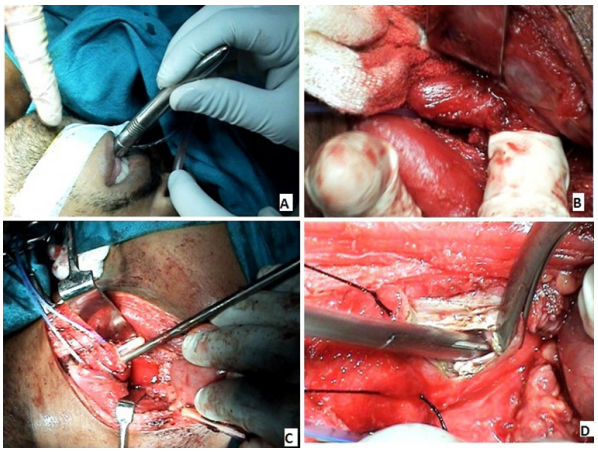

Fig. 1. A: Ante grade Dilatation with Hegar's dilator, B: Finger guidance at lower end, C and D: Esophageal stricture, stomach normal, whole stomach gastric pull up, retrosternal route

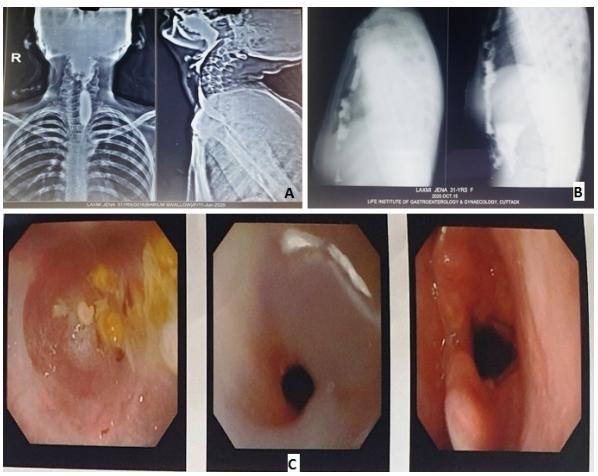

Fig. 2. A and B: Upper esophageal stricture, C: Upper GI endoscopy showing esophageal stricture

All of the patients, with the exception of one (a 32-year-old woman), had not undergone surgery while their injuries were still acute. During the acute stage of the injury, she had undergone surgical treatment (a feeding jejunostomy). For all patients, there was an average delay of 4 to 14 months between the injury and the reconstruction. The time frame was between 6 and 9 months in the vast majority of cases (79%). There were 4.3±1.5 hours of operating time on average. After clinical evaluation, a total of 22 patients underwent esophageal replacement surgery (Fig. 1 and 2).

In 74% (20/27) of the cases, the colon was frequently used as an esophageal substitute, whereas in 26% of the cases, the stomach was used. All of them took the substernal route.

In this study, 17 people only had esophageal reconstruction surgery, while 10 people with a cricopharyngeal or proximal cervical esophageal stricture and a long segment thoracic esophageal stricture were treated with a single-stage approach, such as intra-operative dilatation of the proximal hypopharyngeal stump and then esophageal reconstruction surgery (Fig. 3).

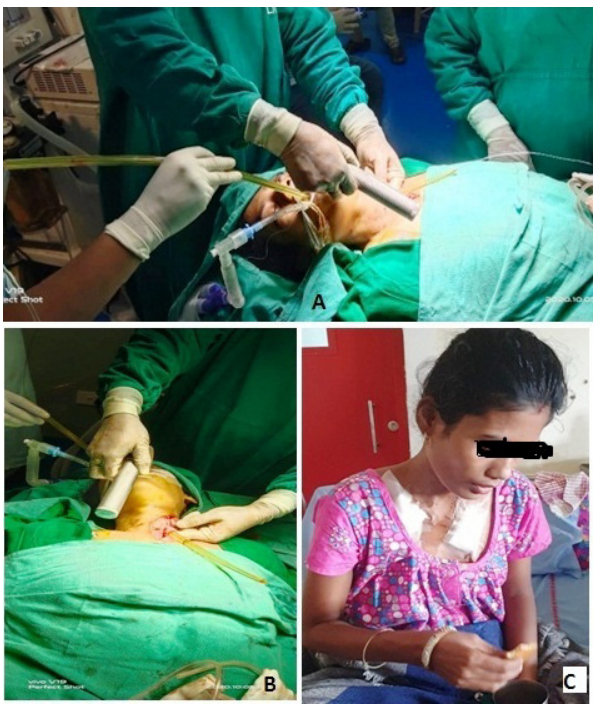

Fig. 3. A and B: Single-stage approach such as intra-operative dilatation of the proximal hypopharyngeal stump by wire-guided Savary Gillard dilators and subsequent esophageal reconstruction surgery of a 32 years old lady, C: Post operative period was uneventfull

Intra-op dilatation was done in 4 cases with Hegars dilators (1 for gastric pull-up and 3 for colon pull-up) and 6 cases with wire-guided Savary Gillard dilators (all for colon pull-up). The clinical management details of corrosive esophageal stricture are shown in Table 1.

From the post-operative day onward, enteral feeding was initiated in every patient through a feeding jejunostomy. A contrast study on post-operative day 10 revealed no anastomotic leakage. So all were started on both a semi-solid and liquid diet on post-operative day 10. But one patient developed a minor leak after

two days of starting an oral diet, which subsided spontaneously within seven days with only external compression. None of our patients developed pulmonary aspiration or required a tracheostomy in the post-operative period. The condition of all the patients at the time of discharge was stable and satisfactory.

Table 1. The clinical management details of corrosive esophageal stricture

Clinical parameters	Esophageal reconstruction surgery only	Intraoperative dilatation+esophageal reconstruction surgery
Total numbers	17	10
Male:female	10:7	6:4
Ingestion (acid:alkaline)	13:4	7:3
Intention (suicide:accident)	11:6	6:4
Time range (injury to surgery)	4–14 months	5–13 months
Intraoperative dilator used		
Hegars dilator	–	4
Savary Gillard	–	6
Post-operative complication (minor leak subsided with extrinsic compression only)	1	0
Post operative dysphagia	5/17	2/10
Dilatation frequency		
Once	1	1
Two time	2	–
Three time	1	1
>3 time	1	–
Dilatation duration	7–24 months	8–24 months

During the follow-up period (2.6 months to 12 years), one patient required endoscopic dilatation of the proximal esophageal stump three times over a 24-month period; another patient required dilatation only once after an 8-month period, but neither developed repeated aspirations nor required a tracheotomy.

Discussion

Due to the relative ease of access to caustic chemicals, ingesting them and the accompanying corrosive damage to the aero digestive tract are common occurrences in developing countries.[5-6]

Acids, or alkalis, are often the caustic chemicals that are consumed. Most caustic ingestions occur in Western nations where alkaline materials predominate, whereas acid-related injuries are more frequent in some developing nations like India, where sulfuric and hydrochloric acids are readily available.[7]

Acids and alkalis can cause a variety of tissue injuries. Coagulation necrosis and liquefactive necrosis are the underlying mechanisms of acidic and alkaline damage, respectively. Corrosive injuries may only affect the stomach or esophagus. In 20% to 62.5% of cases, esophageal and stomach injuries coexist.[6] For a very long time, it was thought that the esophageal mucosa was more susceptible to injury from alkalis than the stomach mucosa was, which was more susceptible to harm from acids.[8-9]

However, in this study, both alkali and acid were found to be associated with upper gastrointestinal injuries. Unlike the previous report, we also found that the vast majority of caustic ingesting cases were suicidal, as opposed to accidental occurrences, and almost all fatal results occurred in individuals who had that purpose.[10-11] The majority of the patients in this study were young adults, and 63% of the cases involved suicidal intent. Accidental consumption of corrosive substances was also noted.

Recognition and treatment of corrosive injuries must occur quickly for success. Sadly, despite all efforts, maintaining an esophageal lumen is not always possible. The most severe side effect of corrosive oesophageal injury is esophageal stricture, which is even higher in some other accounts, developing in between 10 and 30% of individuals who eat caustic substances.[12-14]

Consuming caustic compounds can cause damage ranging from modest mucosal erythema to stomach and esophageal transmural necrosis with viscous perforation. Esophageal strictures may develop after the primary damage has healed. The cornerstone of treatment for established strictures is therapeutic dilatation after the initial damage or until fibrosis stops developing. Yet, a lot of these individuals are either resistant to dilation or need it frequently, which seriously impairs their quality of life. Replacement surgeries are the next step in management when recurrent dilatation fails to reduce symptoms or when the patient refuses to undergo repeated dilatations.[9]

Even though esophageal replacement surgery is the best way to treat upper cervical esophageal and hypopharyngeal strictures caused by drinking caustic substances, it is a very hard procedure because high-up or proximal esophageal anastomoses are prone to anastomotic leakage, early postoperative stenosis, and disruption of the deglutition mechanism, which can lead to recurrent aspirations.[3,11,15] Also, replacing the esophagus at healthy tissue boundaries to restore the continuity of the upper digestive tract affects the integrity of the swallowing mechanism and often requires a tracheostomy to keep the person from aspirating again and again.

Several surgical treatments are available, depending on the specific circumstances. The location of the stricture, the time since the corrosive injury, its proximity to the laryngeal inlet, the condition of the larynx and airway, the length of the stricture, and the existence or absence of strictures further downstream all affect the treatment method.

Ananthakrishnan et al. discussed their experience managing pharyngoesophageal strictures in 51 patients.[16] Based on the severity of the pharyngeal stricture and the degree of related distal esophageal involvement, they separated the patients into groups and established treatment recommendations for each group. After inhaling air, they

used computed tomography to examine the stricture distal to the throat. For patients with pharyngoesophageal strictures, an esophago-coloplasty was then performed to achieve distal continuity after a cervical esophagostomy was first performed on the right side and repeatedly dilated to obtain appropriate dilatation.

They advised that for a reconstruction to be successful, there must be a precise hypopharyngeal opening, a wide anastomosis, a suitable esophageal substitute, a patent esophageal route, and an airway. A decent esophageal substitute must have the following characteristics: isoperistalsis, a sufficient blood supply, an appropriate size and length, and little or no surrounding fatty tissue. Moreover, the swallowing mechanism's anatomical and functional integrity ought to be kept close to normal.

In our study, we did intraoperative dilation of the proximal hypopharyngeal and cervical esophageal stumps during surgery for upper corrosive strictures. We did this to avoid problems with swallowing and breathing after surgery, as well as frequent hospital stays and multiple surgeries. All of the people in our study did well with the surgery, which is the same as what Yannopoluset al. said about their study.[15] Both problems with deglutination and problems with breathing were prevented, and normal esophageal function was restored.

In a different study, Karunkar et al. stated about their experience with how to treat 15 patients with difficult pharyngoesophageal strictures that affected the larynx.[11] In their investigation, five patients received further colonic interposition, and ten patients were recovered with dilatation using end-less string insertion. A remarkable case was reported involving an 82-year-old female patient with a corrosive esophageal stricture. For over 40 years, she successfully managed her condition through home self-bougienage. This prolonged self-dilation therapy resulted in a maintained good quality of life, manageable symptoms, and excellent nutritional status. The authors concluded that self-dilation, with proper patient training, can be a safe and effective treatment option, obviating the need for frequent and costly hospital admissions for endoscopic esophageal dilation.[17]

The timing of an esophageal replacement operation after a corrosive injury is still being debated. A study found that scarring continued for 6 months after a corrosive event in the esophagus. To avoid the risk of anastomotic stenosis caused by an operation performed too soon, when the scar has not fully formed, surgical intervention must be performed nearly 6 months after the acute event.[11,18] In this study, in the majority of cases (79%), the surgery was performed 6–9 months after the acute event. In this study, we did not find anastomic stenosis.

Although the stomach, colon, and jejunum have all been used in esophageal substitution, we preferred the colon because the colon is long enough to replace the esophagus, and because of its resistance to acid, it is less likely to develop late complications like esophagitis and stricture. A study has shown that although the liberal blood supply of the stomach makes it the most reliable organ for esophageal replacement, in most cases, the stomach is not a suitable candidate for esophageal substitution because it is usually moderately or severely injured by caustic agents.[9] Long-term gastroesophageal reflux, the potential for ulceration, anastomotic stenosis, and progressive dysfunctional propulsion are all drawbacks. When an anastomosis is done in the neck because of diffused oesophageal injuries, the stomach is never long enough to reconnect the esophagus. Instead, many patients have to have a partial gastrectomy or gastric bypass because the stomach was damaged by acid.[18,19] In our study, five patients underwent gastric pull-ups, provided that they had strictures in the lower esophageal segment and that their stomachs were all healthy.

Whether the residual esophagus should be removed following colonic interposition is still up for debate. On the connection between esophageal injury and carcinoma, numerous studies have been conducted. There has been no evidence to suggest that the esophagus that has been scarred and damaged has a higher incidence of carcinoma.[20,21] In our study, we did not remove any of the patients' remaining esophagus, and we detected no incidence of carcinoma during the 12-year follow-up.

Conclusion

During surgery for upper corrosive strictures, dilatation of the proximal hypopharyngeal and cervical esophageal stumps was found to help keep the proximal stump, which stops problems with swallowing and breathing after surgery. This single-stage approach, such as intraoperative dilatation and esophageal reconstruction, will help to avoid frequent hospital admissions and multiple surgical sessions. Further study is needed to substantiate its role.

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Author contributions

Conceptualization, H.C.M.; Methodology, H.C.M. and J.R.M.; Validation, S.D.; Formal Analysis, S.B.D.; Resources, S.B.D. and S.D.; Writing – Original Draft Preparation, S.B.D. and S.D.; Writing – Review & Editing, H.C.M., J.R.M., S.B.D. and S.D.

Conflicts of interest
The authors have no conflicts of interest to declare.

Data availability
Data available on request from the authors.

Ethics approval
All subjects gave their informed consent for inclusion before they participated in the study (001/NEW/EC/INST/2023/15947). The study was conducted in accordance with the Declaration of Helsinki.

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ORIGINAL PAPER

Identification of *Brucella melitensis* from camel's blood by vitek2 and real time polymerase chain reaction

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ABSTRACT

Introduction and aim. Brucellosis is a zoonotic disease. Experimental clinical and laboratory diagnosis is still facing problems in identifying the organism. The present study will diagnose a *Brucella* infection in camel blood in Qatar using serological assays. Isolation and identification were performed on a camel blood sample. Brucella in bacterial isolates was determined by real-time polymerase chain reaction (RT-PCR) as a gold standard test.

Material and methods. A total of 220 samples, 200 random serum samples, and 20 EDTA blood samples were selected among the above-mentioned random samples, and 20 serum samples from camel handlers were collected from Al Shahaniya province, Qatar. The Rose Bengal test (RBT), buffered antigen plate agglutination test (BAPAT), and enzyme linked immunosorbent assay (cELISA) for the monoclonal antibody in serum samples were performed using commercially available kits. For the molecular detection of *Brucella*, conventional PCR and real-time PCR (GPS kit) were used for the genus-specific insertion sequence IS711. *Brucella melitensis* (MICROBOSS Hightech GmbH kit) was used to identify subspecies.

Results. The results identified by vitek2 compact (30%) showed *B. melitensis* in 6 samples out of 20 isolates. Both conventional (66.67%) and RT-PCR (83.33%) analyses supported this, demonstrating the presence of *Brucella*. These tests also showed that *Brucella* species were present in Rose Bengal 182/200 (91%), BAPAT 182/200 (91%), and cELISA (90%) 180/200 in camel serum.

Conclusion. To conclude, the prevalence of brucellosis in dromedary camels is higher in this region, and as a matter of urgency, measures should be taken to control the disease.

Keywords. Brucella melitensis, brucellosis, camels, conventional PCR, RT-PCR, serological assays

Introduction

Brucellosis is a global zoonotic infection. *Brucella* species infect sheep, goats, cattle, deer, elk, pigs, dogs, camels, and the environment as well as humans.[1] People and camels get brucellosis from gram-negative bacteria in the genus *Brucella* when they come into contact with large or small ruminants that are infected with *Brucella abortus* or *Brucella melitensis*.[2,3] Brucellosis is propagated not only to people in contact with infected animals but also polluted products; the portal entry into the body takes many routes, for example, raw milk into the digestive tract, intact skin, mucous membranes, and through respiration. *B. melitensis* is the foremost cause of human brucellosis (94% of cases); B. abortus

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is significantly less common (6%), indicating that it is a significant pathogen for humans worldwide.[4,5] *Brucella* species are facultative intracellular gram-negative bacteria that cause brucellosis, which is depicted by abortion in camels and undulant fever, arthritis, endocarditis, and meningitis in humans.[6] Human brucellosis is most widespread in countries reported for *Brucella*, and in people who arrive back from indigenous areas.[7-9] There are no vaccines against brucellosis for humans.[10] Although brucellosis infection is of considerable significance in animals, the ailment in humans is less acknowledged, despite information that it is related to considerable and prolonged morbidity.[11,12] The almost asymptomatic epidemiology of the disease requires the necessary and precise exploration of the species, which is the core demand to reach the objective.

Brucella are special coccobacilli that can live inside cells and prefer placental trophoblasts. They are also known to infect the rough endoplasmic reticulum and reticuloendothelial tissues in the spleen, liver, and blood vessels, which is a major public health concern.[13-17] Numerous Brucella species, some of which are OIE-registered, such as those that affect cattle (*B. abortus*), sheep, goats, camels (*B. melitensis*), and swine (*B. suis*), are known to infect various animal species.

Serological tests are the fundamental brucellosis screening tests. Standard methods of brucellosis testing are laborious and tedious, pose a risk of infection, and can generate discordant results. Culturing brucellosis is challenging. The Rose Bengal test (RBT), the buffered antigen plate agglutination test (BAPAT), and enzyme-linked immunosorbent assays (cELISA) are used to detect Brucella species. To overcome the genetic diversity of *Brucella* species, molecular methods are used to diagnose brucellosis; hence, polymerase chain reaction techniques are adapted.[13,18,19]

Aim

This study aimed to conduct serological screening, isolation, and identification of *Brucella* species in camels' blood. To detect *Brucella* subspecies in bacterial DNA by real-time PCR as a confirmatory test.

Material and methods

Collection of samples

A total of 220 samples of dromedary camels, 200 camel serum samples, and 20 serum samples from camel handlers were randomly collected from different farms located in the Al Shahaniya province, Qatar. None of the animals tested had been administered with any vaccine; collected samples were used for the agglutination of Brucella antibodies. Twenty EDTA blood samples of seropositive camels were used for isolation techniques, and the positive isolates were subjected to IS711 RT-PCR analysis.

Serological assays

Rose Bengal test

5 mL of blood was collected aseptically from the jugular vein of camels and subjected to centrifugation to separate the serum at 5000 rpm for 5 min. On a white porcelain plate, 50 µL of serum and 20 µL of RBT reagent were mixed gently and spread out to about 2 cm in diameter. The plate was then moved around gently on a rocker (Boeco, Germany) for 4 minutes and viewed under light to see if the substances mixed. A visible precipitation was recorded as positive. Control negative and control positive were included in the tests.[20]

Buffered antigen plate agglutination test

Buffered antigen plate agglutination test (BAPAT) was done according to Mahmoud et al., adding 30 µL reagent with 80 µL serum and agitated gently for 8 min at intervals to test the agglutination reaction.[21] Agglutination was considered positive, indicating the presence of specific antibodies to Brucella. To check the sensitivity of the reaction, all positive samples were repeated with different dilutions and examined.

Detection of Brucella using cELISA

cELISA was performed for all the serum samples collected using the Svanova kit (Svanova-Sweden) in the DS2ELISA machine (automated) as per the given protocol. The same tests were performed for 20 human serum samples as well. The optical density was read at 450 nm in automated DS2ELISA. The status of the sample is determined as ≥30% positive or <30% negative.

Isolation of bacteria

Twenty EDTA blood samples were collected aseptically from the jugular veins of positive camels in Oxoid signal blood culture bottles and sent to the lab in an ice box.[22] Culture systems were incubated anaerobically at 37 °C, and the cultures that showed growth within 3 days were incubated both aerobically and anaerobically and examined daily for a week by allowing the blood broth mixture to flow over. The loop of growth is streaked on Tryptic soy agar and Brucella agar and incubated for 3–14 days. It was found to have a smooth, yellow, honeycomb-like colony structure both in aerobic and anaerobic incubation (5% CO_2).

A small colony was used for gram-staining and found to be gram-negative, pink-coloured cocci. Then the colony was subcultured on TSA, MacConkey, and sheep blood agar enriched with 5% horse serum plates to ensure bacterial growth before considering it negative. The isolates recovered were identified according to the conventional method prescribed by the vitek 2 compact automated system (bioMérieux, Marcy-l'Étoile, France). The isolate was subcultured on TSA plates and incubated at 37°C. After growth, it was stored at 4°C until used for identification.

Identification of Gram-negative bacteria

A small colony was used for Gram staining and viewed under an Olympus microscope. Pink-coloured cocci were found to be gram-negative bacteria. The Vitek 2 compact GN card, which contains 64 biochemical tests, was used to identify organisms, grade the isolates from acceptable to excellent identification, and give the details of the biochemical tests.[23]

Preparation of bacterial inoculum

The Densicheck was standardized first with (0, 0.5, 2.0, and 3.0 mL) and the blank set with 3 mL of saline. According to the kit, a suspension of each isolate was made by mixing the bacterial colonies in 0.45% NaCl saline at pH 5 and standardizing it with Densicheck (BioMérieux, Marcy-l'Étoile, France) at a level of 0.5–0.63 McFarland in an opacity tube. The time taken for preparation and card filling must be less than 30 minutes.[24] The card was filled, sealed, and inserted into the reader, which is subjected to kinetic colorimetric measurement. The results were obtained after 8 to 10 hours for gram-negative bacteria.

Bacterial DNA extraction

Based on the identification of the organism as *B. melitensis* by the vitek 2 compact, DNA was extracted from the isolate using a three-step approach (heat shock: suspend the isolate in 200 µl of phosphate-buffered saline (PBS) and centrifuged three times at 10000 rpm for 10 min (wash), heat inactivation was done at 100°C for 10 min, and it was cooled immediately). Then 30 µL of DNA was extracted. Qiagen DNA mini kit (Hilden, Germany), followed by Kingfisher Duo Prime (Thermo Fisher Scientific, Waltham, Massachusetts, USA), according to references. DNA quantification was done by a Quawell UV-VIS spectrophotometer (Q5000) and a Nanodrop 8 spectrophotometer (NDE2200281, Thermo Fisher Scientific, Waltham, Massachusetts, USA).

Real-time PCR and conventional PCR assay

The RT-PCR reaction was performed in triplicate in 96-well 0.1 µL plates (qPCR 96-well plates, Micro Amp TM, Applied Biosystem). Bacterial DNA was analyzed by RT-PCR with the IS711 primer probe. Amplification of the Brucella DNA genus was in Bayeta et al.[25] Using (forward: GCTTGAAGCTTGCGGACAGT) and (reverse: GGCCTACCGCTGCGAAT), probe (5'-6-FAM-AAGCCAACACCCGGCCATTATGGT-TAMRA 3') (Invitrogen, Thermo Fisher Scientific, Waltham, Massachusetts, USA). Total mix volume was 15 µL/sample containing: Master mix 3 µL (Applied Biosystems, Waltham, Massachusetts, USA), 0.3 µL for each forward and reverse primer, 0.1 µL of the labelled probe, and 3.5 µL DNA and water to make up the total volume. Quant-studio 7flex RT-PCR (Applied Biosystems, Waltham, Massachusetts, USA) is used with the following thermal profile: The reaction mixture was initially incubated for 5 minutes at 95°C. Amplification was performed for 40 cycles at 95 °C for 30 s, followed by annealing and extension at 60 °C for 1 min. A GPS kit (Genetic PCR Solutions-Dtec-QPCR Test, Lot 002600320217, Spain) was used to analyze the same DNA samples twice, once with diluted DNA and once without diluted DNA. Subspecies were done using the MicroBOSS Hightech GmbH kit, and optimisation was done repeatedly, which confirmed the amplification curve for *B.melitensis* at Ct 21.8 to be positive. Samples exhibiting sigmoid curves below 35 threshold cycles (CTs) were considered positive, and negative controls of Brucella were included in each run to detect any contamination or amplification failure. The instrument automatically set the threshold and additionally confirmed electrophoresis.

The conventional PCR reaction was performed in duplicate in microtiter plates using a verity 96-well thermal cycler system with the following run conditions: Following 40 cycles of 72°C for 32 s, 54°C for 30 s, and the 4°C hold stage previously mentioned for typing, there will be 1 cycle of 95°C for 10 min, 1 cycle of 95°C for 15 min.[17] The total reaction mixture volume is 25 µL, containing 12.5 µl of TaqManTM Universal Master Mix (Applied Biosystems, Waltham, Massachusetts, USA). 1 µL of each primer, 2 µL of bacterial DNA as a template, and nuclease-free water sum up to a total reaction volume of 25 µL. 1.5% Agarose gel electrophoresis served to verify the PCR product. 3 µL loading buffer (Invitrogen), 15 µL PCR product, ladder 5 µL with 50 bp ladder (Thermo Fisher Scientific, Waltham, Massachusetts, USA), adjust the mode to 100 V and time 30 min. visualized in UV trans illuminator (spectroline). The Amplicon size was matched with the ladder.

Ethics approval

Experiment has been approved by administration of Tharb camel Hospital.

Results

The serological test results were obtained from 220 serum samples: 200 camel serum and 20 camel handlers serum. Out of 200 serum samples, 182 were strong positives; the RBT was found to be strong visibly; mild agglutination (18 weak positives) was confirmed as a weak positive under the agglutination viewer; and the camel handlers serum (20 suspected mortal samples) was found to be negative in all the tests. Among 182 seropositive samples, 20 were selected for blood culture (EDTA blood sample) of camels who had a previous history of brucellosis. When the samples were examined for brucellosis using the RBT, 182 samples (91%) out of a total of 200 tested strong positive for the presence of *Brucella spp*., and 18 samples were weak positive. When cELISA was performed to detect the IgM antibodies against *Brucella spp.* Out of 180 (91%) samples, positive results are shown in Table 1.

Table 1. Total number of positive results per serological test and real-time PCR used for the detection of brucellosis in camels*

Tests	Total no. of samples	Sample type	Positive	Negative	Suspected	%	
						Positive	Negative
RBT	200	Camel sera	182	0	18	91	0
RBT	20	Human sera	0	20	0	0	100
BAPAT	200	Camel sera	182	0	18	91	0
BAPAT	20	Human sera	0	20	0	0	100
ELISA	200	Camel sera	180	0	18	90	0
ELISA	20	Human sera	0	20	0	0	100
Blood culture	20	Camel blood	6	14	0	30	70
RT-PCR IS711	6	Camel blood	5	1	0	83.33	16.67
RT-PCR GPS	6	Camel blood	2	4	0	33.33	66.67
Conventional-PCR IS711	6	Camel blood	4	2	0	66.67	33.33

* RBT – Rose Bengal test, BAPAT – buffered antigen plate test, ELISA – enzyme immunosorbent assay, RT-PCR – real-time polymerase chain reaction, PCR – polymerase chain reaction

Table 2. Number of positive results per isolate for the detection of brucellosis in blood samples of camels

Sample no.	Sample Type	Incubation Period	Gram stain	Media/Agar used
1	Blood	5-days aerobic	*Gram-negative bacteria*	TSA/MC
2	Blood	3-5-days aerobic/ anaerobic	*Brucella melitensis*	TSA/MC
3	Blood	5-days aerobic	*Brucella melitensis*	TSA/MC
4	Blood	5-days anaerobic	*Brucella melitensis*	TSA/MC
5	Blood	4-days aerobic/ anaerobic	*Brucella melitensis*	TSA/MC
6 (repeat sample)	Blood	4-days aerobic/ anaerobic	*Brucella melitensis*	TSA/MC

Table 1 depicts the findings of the RBT, BAPAT, ELISA, RT-PCR, and conventional PCR assays. The issues of RT-PCR analysis show the presence of Brucella species in 6 samples (83.33%) out of 20 samples. The results of serological assays showed the presence of Brucella species. Similar to RBT (91%), ELISA (90%), and BAPAT assay (91%) in the camels' samples. The growth in the oxoid signal culture system depicts the presence of the organism, which was dressed on different agar plates and incubated. After 3–7 days, colony growth was tested with gramme stain. The positive results of bacterial isolates are illustrated in Table 2. Bacterial DNA was uprooted, quantified, and analyzed. The results of RT-PCR analysis using the IS711 manual show the manifestation of Brucella species in 5 samples (83.33%) out of a total of 6 bacterial DNAs (Fig. 1).

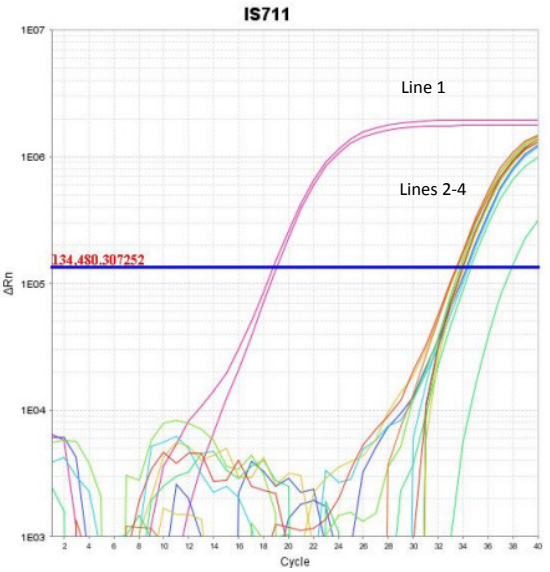


Fig. 1. IS711 Real-time PCR amplification; Ct 21.8 (Line 1 – control positive and lines 2-4 – bacterial DNA)

Table 3 reveals the presence of Brucella species in the insulate with other organisms, which was re-cultured to yield the pure isolate. The presence of Brucella species in (3%) was linked by RT-PCR analysis (Fig. 2). 182 samples (91%) out of the total 200 samples show the presence of Brucella *spp.*

Table 3. Presence of *Brucella spp.* and other organism in the isolate*

Serial no.	Identified	Gram-positive	Gram-negative	Serology
1	*Brucella melitenisis*	NIL	No other Gram-negative	RBT (182) = +++
2	NIL	NIL	NIL	RBT (18) = ++
3	NIL	NIL	NIL	
4	*Brucella melitenisis*	NIL	*Spingomonas paucimobils*	BAPAT (182) = + +++
5	NIL	NIL	*Burkholdaria gladioli*	BAPAT (18) = ++
6	*Brucella melitenisis*	NIL	*Aeromonas salmonicidas, Oligella ureolytica*	Cellist (180) = +++
7	*Brucella melitenisis*	NIL	No other gram-negative	cELISA (20) = N
8	*Brucella melitenisis*	NIL	NIL	
9	*Brucella melitenisis*	Staphylococcus cohini	NIL	

* NIL – no organism found, RBT – Rose Bengal test, BAPAT – buffered antigen plate test, ELISA – enzyme immunosorbent assay

According to the Rose Bengal test, 182 samples (91%) showed the presence of *Brucella* species. By BAPAT assay, 180 samples (90%) reveal the presence of

Brucella species. by ELISA. The results of colony growth on colourful agar plates are depicted in Figure 3, which shows isolates used for bacterial DNA isolation.

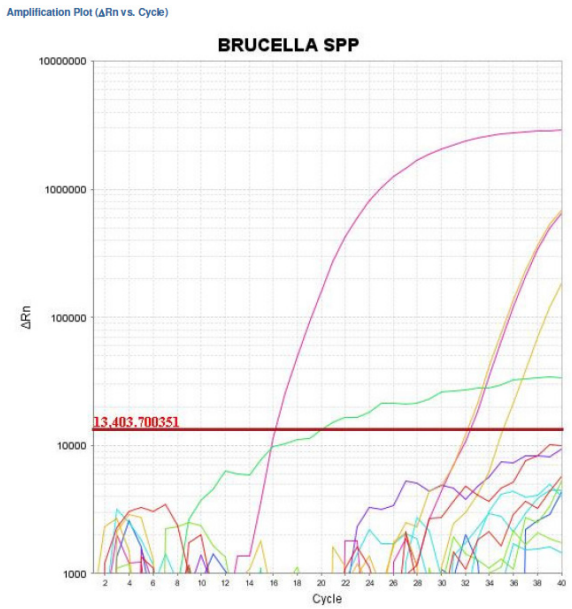


Fig. 2. Real-time PCR amplification curves of the *Brucella* spp. in the bacterial DNA of camels

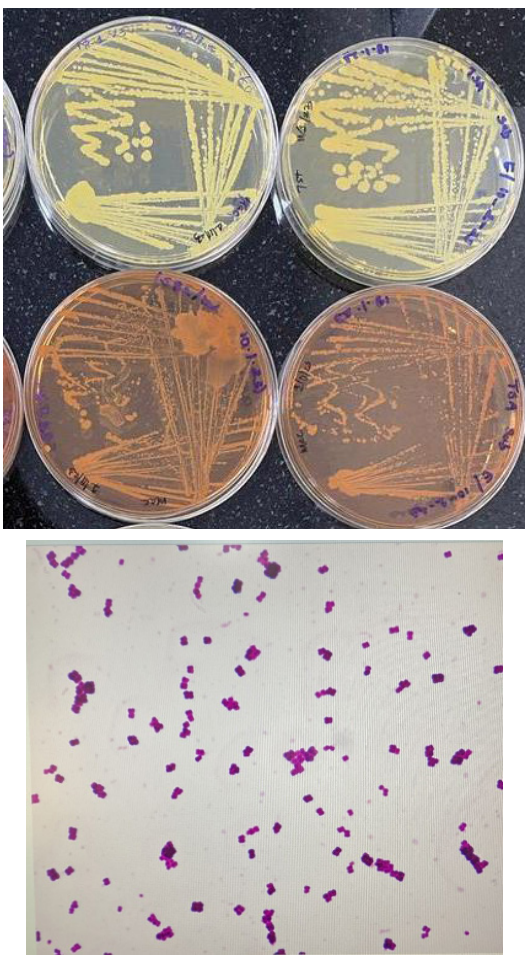


Fig. 3. *Brucella* colony growth in different plates

The bacterial DNA samples that gave a positive result with the IS711 manual were used to identify the subspecies. The sub-species was identified using MicroBoss for *B. melitensis*. Optimisation was done, and the amplification curve for *B. melitensis* was attained at Ct=21.8 and control positive curve was obtained at Ct=23.02. This indicates the presence of *Brucella* species and sub species in the given bacterial DNA.

The PCR product was confirmed by 1.5% agarose gel electrophoresis loaded into the gel, and the amplicon was matched with the ladder. The issues were delved into independently, and RT-PCR yielded positive results (Fig. 4). A repeated analysis was done with the tackle. In this trial, BMEII0466 with the following sequence garbling external membrane protein was used both conventionally and by RT-PCR (Fig. 5)

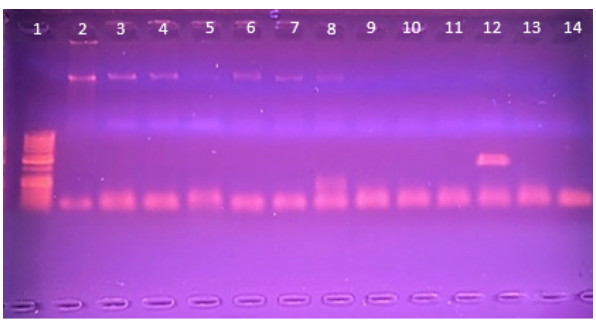
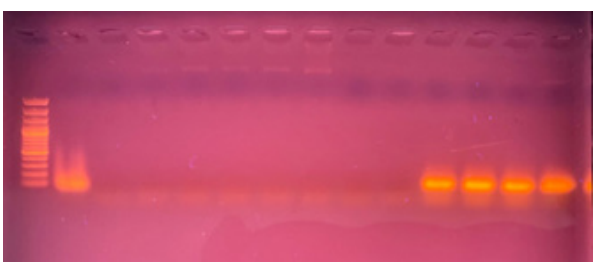


Fig. 4. Agarose gel electrophoresis of PCR products

Result of real-time PCR on *Brucella* isolates. Lane 1, 50bp plus DNA ladder (Invitrogen); lines 2 to 13 are *Brucella* spp. isolates; line 14, reference positive control.



BMEII0466	5′-cy5- CCTCGGCATGGCCCGCAA-BHQ-2-3′
BMEII0466(f)	TCGCATCGGCAGTTTCAA
BMEII0466(r)	CCAGCTTTTGGCCTTTTCC

Fig. 5. Number of positive results conventional and RT-PCR used for the detection of *B. melitensis* [BMEII0466] ref

Discussion

Brucellosis is a neglected transmittable disease affecting all domestic and wild animals and humans with important economic and public health importance.[26,27] Dependable identification of the applicable *Brucella* species is challenging with any individual approach and available serology.[28] The camel handlers lab personnel might be asymptotic without any affiliated symptoms

when being a carrier. There are few studies available on the outcomes of asymptomatic brucellosis and most of them are case reports.[29] However, we still hope to give a clinical reference for opinion and treatment to strengthen active webbing and surveillance.[30] Webbing and identification of serological tests is the top tool for effective epidemiological analysis.[31]

Our findings demonstrated that the highest prevalence of *Brucella* in camel serum and 182 samples showed that the existence of *Brucella* (91%) out of 200 samples. The BAPAT assay, 182 positive and 18 suspected samples, confirmed the presence of *Brucella* (91%). The ELISA assay, 180 camel sera and 18 suspected samples, demonstrated the presence of *Brucella* (90%). The existence of *Brucella* DNA as showed by the RT-PCR was regarded as evidence for the potential risks for the consumers who utilizes products of *Brucella* infected camels. The prevalence of brucellosis is comparatively high in imported camels.[7]

In peer reviewed papers that describe the prevalence of bovine brucellosis in animals, only data are available on perceptivity and particularity of the serological tests in Egypt. The asemantic infection leads to revocation in female camels, orchitis and epididymitis with frequent sterility in males, because of the localization of *Brucella* within reproductive organs.[32,33] These issues reveal an advanced circumstance of brucellosis among the camels in this region. Indeed though detailed reports on camel brucellosis aren't reported yet from Qatar, advanced prevalence of the complaint in camels is reported from the neighboring regions. A previous report discusses 15 camels that were set up to be infected with *Brucella* in Jordan.[34] Whereas, in another study conducted in Sudan revealed that 40 tested samples were positive for *Brucella* when 2,000 camel serum and milk samples were examined.[35] Bacterial infections are transmitted to humans from other sources where antibiotics are used for various purposes and should cause emerging resistant strains.[36,37] Detection of brucellosis in cattle and humans relies on the ways employed for discovery and identification of the pathogens; subspecies, due to inheritable diversity and analogous to other contagious and non-infectious conditions of brucellosis in camels, are asymptomatic and require individual ways to be estimated. The results indicate that the blood samples of camels shows the presence of *Brucella* species for brucellosis.[38] Molecular methods like PCR are often applied to detect brucellosis.[39] In this discussion, the RT-PCR test detected *Brucella*. Followed by the tract of results of RT-PCR in aborted camels as verified and reported by Al-Majali et al.[40]

Numerous researches were published on the detection of *Brucella* by PCR, both from pure culture and from field samples mostly of cattle origin.[41-44] Diagnostic techniques are important for control and extinction of brucellosis; isolation provides the specific diagnosis and it is considered to be the gold standard method.[45] The demerits of the culture technique is that it is tedious, hazardous, and lacks sensitivity and specificity. The serological tests are the tools used for detection of *Brucella* infection.[46] The RT-PCR assay is a valued diagnostic tool when culture fails or serological results are indecisive in brucellosis detection[47] Intermediated screening must be done.

The complications and death due to infection cause economic and social damage. It is a main public threat, in developing and low income countries due to human and camel relations. This disease spreads to the local community via the habit of consuming milk and meat and there is a lack of awareness regarding brucellosis among nomadic people, which reduces the rate of reproduction by causing fetal loss in pregnant women, abortion in camel, still births, infertility, and swollen testes. This affects the sale value of camels, marketing of milk, meat and wool. Since camel racing is the main entertainment of the Middle East, it will affect the economic status of the countries. Therefore the uncontrolled spread of disease affecting animals, humans and environment needs effective molecular diagnostic tools.

Conclusion

The present study was concluded by serological tests and cELISA. The blood samples of seropositive camels were subjected to the isolation technique, followed by the identification of *Brucella*. The presence of bacterial DNA was confirmed for *Brucella* species and subspecies by gold-standard tests like RT-PCR. The prevalence of *Brucella* in camels is higher in this region, and drastic steps must be taken to control the spread of disease from camel to camel as well as from camels to humans. Further studies are required to identify *Brucella* species by sequencing.

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Declarations

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Author contributions

Conceptualization, K.M. and M.R.; Methodology, K.M; Software, U.S.; Validation, S.S, S.M. and K.M.; Formal Analysis, K.M.; Investigation, S.M.; Resources, S.M.M.; Data Curation, U.S.; Writing – Original Draft Preparation, K.M.; Writing – Review & Editing, U.S.;

Visualization,S.M.M .; Supervision, M.R.; Project Administration, K.M.

Conflicts of interest
No conflicts of interest.

Data availability
All the data in this study is completely incorporated in the manuscript.

Ethics approval
Experiment has been approved by administration of Tharb camel Hospital.

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ORIGINAL PAPER

Unveiling challenges in cadaveric dissection for medical education – a study of student perspectives

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ABSTRACT

Introduction and aim. Cadaveric dissection is the mainstay of learning anatomy by medical students. Even though newer teaching learning methodologies have been implemented to facilitate learning anatomy, cadaveric dissection remains the most preferred method by students. The objective of this study was to analyze the student perspective of different aspects of learning anatomy with their positive and negative experiences and to get a better understanding of their opinions and their experience.

Material and methods. In this cross-sectional study, a pretested and validated questionnaire was used to collect information from the students after approval from the Institutional Ethics Committee. Informed consent was obtained from every student prior to their participation in the study.

Results. Three hundred seventy-seven students took part in this study and the response rate obtained was 63.5%. The majority of the students stated that cadaveric dissection has helped them in learning basic anatomy and had deepened their understanding of complexity of human body and has helped in better performance in clinical applications. The smell of formaldehyde and eye irritation was cited as the major reasons for finding dissection sessions challenging. Most of the students agreed that counselling before dissection sessions will help to alleviate the emotional reactions to cadavers.

Conclusion. The students unanimously agreed that cadaveric dissection sessions are the best way to learn basic anatomy complemented with newer teaching tools like prosection and computer-based approaches. However, the problems encountered by the students like smell and irritation of formaldehyde need to be addressed with usage of alternative techniques.

Keywords. anatomy, cadaveric dissection, medical curriculum, medical education

Introduction

Anatomy is considered the cornerstone and fundamental basis of clinical medicine.[1] The practice of dissecting cadavers (CD) traces its origins back to 300 BC, and by the 15th century, it had evolved into a vital method for exploring the intricate structures of the human body.[1,2] The inclusion of cadaver dissection in the traditional undergraduate medical curriculum is of great significance, and as a result, it has continued to be a pivotal component of anatomical education for the past four centuries.[3]

Anatomy has traditionally been taught through a range of methods, including lectures, practical sessions using models, prosected materials, and cadaveric dissection. In recent times, newer approaches such as computer-assisted learning models, interactive computer-based software, and radiological images have been introduced. Many universities worldwide have adopted modern

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integrated curricula that incorporate problem-based learning (PBL), case-based learning (CBL), team-based learning (TBL), and other computer-assisted teaching methods in their undergraduate anatomy courses.[4-8]

Nevertheless, cadaver dissection remains the most superior method for learning human anatomy. This is because it enables students to grasp the surface anatomy of various structures, gain a tactile understanding of tissues and organs, and appreciate the prevalence of anatomical variations.[9]

Over the years, the majority of students have shown enthusiasm for participating in dissection activities. However, some students have found the experience of dissection to be quite distressing and have chosen to avoid it. In response to this behavior, it is essential for faculty to recognize, acknowledge, and validate the emotional distress experienced by these students. This recognition is crucial because such distress can potentially hinder the acquisition of the necessary competencies and skills.[10-12]

Aim

The objective of this study was to investigate the perceptions and attitudes of medical students at a tertiary care teaching institute in South India, drawn from diverse cultural backgrounds. The study focused on their views and attitudes regarding cadaveric dissection and other contemporary advancements in anatomical learning methods

Material and methods

A cross-sectional study design was used. The main objective of this study was to probe medical student perspectives on cadaveric dissection learning sessions. The study was conducted among medical undergraduate students of a tertiary care teaching centre in Tamilnadu, India. This is one of India's largest private universities in the field of science and technology, and students from various social and cultural backgrounds are trained here. All the medical students of the university from the second year to the final year were included in the study. The students in this university get the opportunity to perform dissection under the guidance of faculties. There are weekly 12 hours of dissection for the students, with two hours each day. The data was collected during the period from January to March 2021. All medical students who had direct contact with the use of cadavers for learning during their first year of medical school training and who signed the informed consent form to participate in the study were enrolled. Approval was granted by the Ethics Committee of SRM Medical College Hospital & Research Centre, SRM Institute of Science and Technology No. 2179/IEC/2020.

The study tool consisted of a questionnaire consisting of 17 questions which were pretested and validated with the experts of the department of Anatomy and among five students. The questions were developed by reviewing different related literature articles. The questionnaire consisted of 5 questions about the agreement or disagreement regarding positive experience relating to dissection sessions and application of dissection related in their clinical training and other aspects of their attitude. There were five questions about negative experience regarding dissection like fear of infection, mutilating bodies or eye irritation. Rest of the questions were about scope for improvement of the learning sessions.

A total of 377 medical students from second year to internship were included in the study. After obtaining formal permission from the Institutional Ethics Committee and consent from the participants, they were told about the objectives and relevance of the study before they filled out the questionnaires containing 17 items which was circulated through Google form link.

The actual sample size was calculated using the single population proportion formula with the following assumptions: p=0.5 (prevalence), 95% confidence interval, and 5% margin of error. The total number of students was 594, and a 10% non-response rate was applied, yielding a final sample size of 259. The convenience sampling method was used to select the samples.

Data was downloaded from Microsoft Excel (Microsoft, Redmond, Washington, USA). The principal investigator reviewed all the collected data for consistency and completeness. Using SPPS v21.0 (IBM, Armonk, NY, USA), suitable percentage and proportions were calculated in interpretation of the results obtained.

Results

The questionnaire was sent to 594 students and 377 (63.5 %) completed the questionnaire. The mean age of the students was 21±1.6 years. Table 1 shows the demographic features of the study participants.

Table 1. Demographic features of the study participants

Variables		n (%)
Age (in years)	Less than 18	0
	18-24	299 (79.3)
	> 24	78 (20.7)
Sex	Male	204 (54.1)
	female	173 (45.9)
Attendance in dissection	Regular	315 (83.6)
	Irregular	62 (16.4)
Year of study	Phase II	138 (36.6)
	Phase III	160 (42.4)
	Internship	79 (20.9)

All of the participants (n=377, 100%) strongly agreed that cadaver-based learning of gross anatomy had helped them to develop and enhance empathy and compassion which helped during training in clinical settings. A majority of the participants (n=371, 98.4 %)

strongly agreed that cadaver dissection had deepened their understanding of anatomy with respect to complexity and variability of the human body and provided an effective learning experience that reinforced lecture and textbook material. Also, 98% (n=370) of the participants strongly agreed that the anatomical knowledge gained through dissection could be applied in training in clinical skills/examination of patients. The participants endorsed that CD has also improved their power of critical thinking and professionalism. As a result, it is reasonable to state that performing on cadavers generate positive experiences for the further medical education. Table 2 depicts the positive experiences of cadaveric dissection.

Table 2. Positive experiences of cadaveric dissection

Attitude area	Agree; n (%)	Disagree; n (%)
Help to develop and enhance empathy and compassion which is helpful during clinical posting	377 (100)	0
Deepen the understanding of anatomy with respect to complexity and variability of the human body	371 (98.4)	6 (1.6)
Provide an effective learning experience that reinforced lecture and textbook material.	371 (98.4)	6 (1.6)
Anatomical knowledge gained through dissection could be applied in training in clinical skills/ examination of patients	370 (98)	7 (2.0)
Improved critical thinking power and professionalism	377 (100)	0

Upon analyzing the reasons for finding the dissection challenging, multiple factors were rooted out as depicted in Figure 1. A majority of the participants agreed that the odor of formaldehyde is a major limiting factor (n= 348; 92.3%). More than half of the participants complained of eye irritation as well (n=226; 60%). Other factors which were accounted for were fear of infections (n=190; 50.3 %), fear of mutilating bodies (n=115; 30.5%) and a dislike of touching cadavers (n=42; 11.1 %).

Upon further analysis of factors that were related to the conduct of the cadaver-based learning of anatomy, 73.7% (n=278) of the participants revealed that long durations of dissections were a disadvantage. Being uncomfortable with the peer groups was also put forth as a reason by a small fraction of participants (n=58; 15.4 %). A few participants also expressed that the anxiety and stress related to dissection negatively impact learning (n=15; 4%). All of the participants agreed that they were contented with the current teaching methodologies followed for learning anatomy (n=377, 100%).

The solutions for the above-mentioned challenges were also proposed. Counselling before entering the dissection room concerning the possible emotional reactions to cadaver was considered as critical by all of the participants (n=377; 100%). They also insisted that need of proper use of safety equipment (gloves and masks) should be emphasized throughout the period (n = 348; 92.3%). A majority of the participants (n=342; 90.2%) agreed that suitable measures should be adopted to overcome the unpleasant odor (e.g. face masks). The participants also emphasized the requirement for proper management of molds/fungus on cadavers (n=190; 50.4%) and maintaining general cleanliness of the dissection hall (n=148; 39.3%). Figure 1 depicts the major negative experiences/ challenges during the dissection sessions.

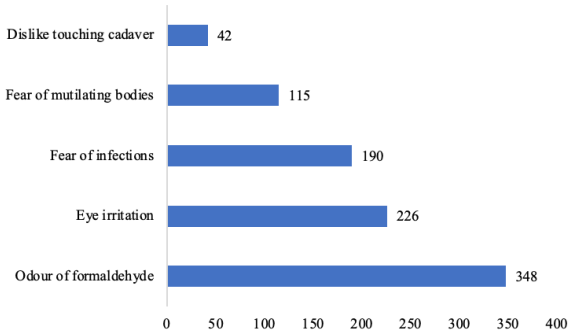

Fig 1. Negative experiences/challenges during cadaveric dissection

Regarding teaching-learning methodology, all of the participants agreed that small group teaching prior to discussion can be considered for better understanding of structures (n=377; 100%). They also facilitated the usage of newer teaching tools and technologies along with CD for making learning anatomy enlightening. However, 100% of the students strongly agreed that cadavers could not be replaced fully with any other advances in learning anatomy.

Discussion

The purpose of this study was to examine medical student perceptions and attitudes towards cadaveric dissection and other latest advances in methodologies for learning anatomy. Anatomy knowledge is extremely important in the practice of medicine. Hence cadaveric dissection is considered to be an indispensable part in medical education since centuries.

In this study, the majority of students had favorable attitudes toward the utility of cadaveric dissections in the teaching process of anatomy. Cadaveric dissection, according to the students, deepens their understanding of anatomy, improves their clinical examination skills, increases their respect for the human body, and delivers a better perception of patient examination skills while also making learning interesting. This perspective conveyed by the participants in this study is similar to that expressed in an Australian study by Dissabandara et al.[13] Furthermore, Abass Alhassan et al. stated that approximately 87.9% of the participants in their study regarded cadaveric dissection as essential and integral in the science of human anatomy.[14]

Previous research has shown that, while the versatility of cadaveric dissection in the modern medical education system is being challenged, those who actively participated in cadaveric dissection performed quite well in written and oral examinations.[13,15] According to the findings of several studies, dissections promote deeper learning by providing students with a significant chance to study the exact complexity of human body tissue and their clinical significance.[16,17]

In this study, the major perceived downsides of cadaveric dissection were the smell of formalin, eye irritation, and fear of infection. Furthermore, the students expressed apprehension about mutilating bodies. The majority of students, however, universally agreed that cadaveric dissection was the most crucial tool for learning for anatomy in the first year.[18,19] This finding is line with the observations of previous studies. Several studies found that most students reported experiencing anxiety and stress, which had a negative impact on their learning.[20,21]

The problem in recognizing structures is typical with students who are being introduced to dissection for the very first time. However, this challenge is believed to foster critical thinking among students, which is an essential element of the Problem Based Learning which is quite often followed in the latest competency based medical curriculum. According to studies, sufficient pre-dissection preparedness using lectures, model-based sessions, and appropriate tutor assistance during the dissection session usually alleviates such problems. The students also recommended that counselling before entering dissection room for the first time about the potential emotional reactions to cadaver is essential. They also insisted that need of proper use of safety equipment (gloves and masks) should be emphasized throughout the period as the same is not provided free of cost to the students. Majority of the participants also agreed that suitable measures should be adopted to overcome the unpleasant odor. The participants also emphasized the requirement for proper management of molds/fungus on cadaver due to inadvertent climatic conditions of high humidity and maintaining general cleanliness of the dissection hall. Even though students consider dissection as an integral part in learning medicine, long hours of dissection per week may have been physically challenging in their initial medical school years.

The hallmark finding from this study was the student's inclination towards cadaveric dissection based learning and a qualitative insight into this perspective was brought into light like, the traditional belief of necessity of exposure to cadaver in the first year of medical school and feeling of 'being a doctor' upon contact with cadaver. These findings lead us a dilemma for implementation of latest technologies in learning anatomy of human body. Hence the ideal technique with will be CD coupled with latest technologies which will make anatomy education enlightening.

Conclusion

Cadaveric dissection is indispensable in teaching and learning anatomy. Better facilitatory sessions and newer teaching methodologies like flipped classroom sessions will help in improving the student's attitude towards dissection which in turn will help the students to perceive an effective learning experience, perform well in examinations and as well as to apply this knowledge in clinical training.

Declarations

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Author contributions

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

Conflicts of interest

The authors have no conflicts of interest to declare.

Data availability

Available, subject to request to authors.

Ethics approval

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of SRM Medical College Hospital & Research Centre, SRM Institute of Science and Technology No. 2179/IEC/2020.

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ORIGINAL PAPER

Hemodialysis dose and long-term COVID-19 outcomes – a retrospective cohort study

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ABSTRACT

Introduction and aim. Long-term outcomes of COVID-19 pose a global challenge, particularly impacting individuals with underlying health conditions, including those who have undergone hemodialysis (HD). The study aimed to investigate the relationship between preexisting dialysis dose, measured by single pool Kt/V (spKt/V), and long-term outcomes of COVID-19 in patients undergoing HD.

Material and methods. Demographic, clinical, and laboratory parameters following COVID-19 recovery, and long-term outcomes, including the presence of COVID-19 sequelae, hospitalization, and all-cause mortality during a year after COVID-19 were retrospectively analyzed.

Results. Out of the 195 patients included, there were 108 males (55.4%) and 87 females (44.6%), with a median age of 56 (44-63) years and a dialysis duration of 49 (31.3–85.2) months. Patients with spKt/V<1.4 had a significantly increased risk of long-term COVID-19 sequelae (HR 9.1, 95% CI: 3.4; 24.6), hospitalization (HR 7.6, 95% CI: 3.9; 14.6), and all-cause mortality (HR 8.5, 95% CI: 2.9; 25.8) within one year after COVID-19 recovery compared with those with spKt/V≥1.4. spKt/V cutoff point of ≤1.3 emerged as a significant risk factor for one-year hospitalization and mortality within our cohort.

Conclusion. Suboptimal dialysis dose, as indicated by spKt/V < 1.4, is associated with adverse long-term COVID-19 outcomes in patients undergoing HD. Optimizing dialysis adequacy may mitigate these risks. Further research is needed to validate these findings and explore interventions to improve outcomes in this vulnerable population.

Keywords. dialysis dose, hemodialysis, hospitalization, long COVID, mortality

Introduction

The COVID-19 pandemic has left an indelible mark on global healthcare systems, with millions of individuals affected by the severe acute respiratory syndrome caused by the novel coronavirus (SARS-CoV-2).[1] While much attention has been rightfully directed towards preventing the spread of the virus and treating acute cases, an emerging concern lies in the long-term health consequences experienced by survivors of COVID-19, particularly those with pre-existing chronic kidney disease (CKD) who require hemodialysis (HD).[2,3]

Patients undergoing HD are at higher risk of contracting COVID-19 and related complications due to multifactorial risks, including underlying comorbidities, immunosuppression, and frequent dialysis facility visits, which contribute to their increased vulnerability.[2–4] Vaccination against SARS-CoV-2 has been found

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to be highly effective in preventing severe illness, hospitalization, and death in the HD population.[5,6] However, recent studies have suggested that even fully vaccinated individuals, including patients undergoing HD, may still develop long-term COVID, a condition characterized by persistent symptoms that extend beyond the acute phase of COVID-19.[6,7]

Long-term COVID presents a wide range of symptoms that vary in severity and persist for extended periods, spanning weeks, months, or even years.[3,8] Ongoing research is diligently investigating the trajectory of long-term COVID, yet the precise mechanism remains generally unknown.[9,10] Potential factors being explored include the activation of autoimmune antibodies, reactivation of latent viruses, the persistence of SARS-CoV-2 in other organs, and direct organ damage resulting from chronic immune dysregulation.[10] Consequently, there is a current hypothesis that chronic pre-infection inflammation could be a potential contributor to the development of long-term COVID.[9,10]

The measurement of single-pool Kt/V (spKt/V) is a standard practice in clinical settings to gauge dialysis adequacy and is integral in determining the overall health and clinical outcomes of patients receiving HD treatment.[11,12] Specifically, for patients on a thrice-weekly HD regimen, it is recommended to achieve a target spKt/V of 1.4 per HD session, with a minimum delivered spKt/V of 1.2.[13] However, realizing this goal is not always feasible due to various factors, including vascular access issues, individual patient variability, comorbidity burden, and practical constraints.[11] Falling below the recommended spKt/V of 1.2 has been associated with persistent chronic inflammation and increased oxidative stress, factors that may potentially amplify the inflammatory response triggered by a COVID-19 infection.[14,15] This, in turn, could contribute to the emergence of long-term COVID symptoms in patients undergoing HD.

Despite the implications of suboptimal HD doses, a comprehensive examination of the relationship between HD dose and long-term COVID-19 outcomes has been lacking.

Aim

To address this knowledge gap, we conducted a retrospective cohort study investigating the impact of HD doses on long-term COVID-19 outcomes. Our hypothesis posited that reduced HD doses would be associated with worse long-term COVID-19 outcomes in patients receiving maintenance HD. To test this hypothesis, we assessed the relationship between pre-existing spKt/V and 1-year COVID-19 clinical outcomes, including the presence of long COVID sequelae, hospitalization following recovery from COVID-19, and all-cause mortality.

Material and methods

Study design and setting

This retrospective cohort study was conducted in collaboration between two dialysis centers: Dialysis Medical Center LLC "Link-Medital" in Odesa, Ukraine, and Dialysis Medical Center LLC "Nephrocenter" in Kyiv, Ukraine, in the scientific partnership with the State Institution "Institute of Nephrology of the National Academy of Medical Sciences of Ukraine," also located in Kyiv, Ukraine. The study was carried out as a part of the Institute's ongoing project titled "Exploring the Mechanisms of Development and Identifying Therapeutic Targets for Post-COVID Syndrome in Dialysis Patients," which is registered under the National Study Registration Number 0122U000144. The study adhered to the principles outlined in the Declaration of Helsinki and took place from May 2021 to May 2023. The study protocol received approval from the Institute Ethics Committee (protocol number: 2-2021, dated April 6, 2021).

Study participants

The study included individuals aged 18 and above who had undergone HD for a minimum of three months before acquiring COVID-19. The confirmation of COVID-19 diagnosis involved either the detection of SARS-CoV-2 RNA through real-time reverse transcription polymerase chain reaction (RT-PCR) in nasopharyngeal swab specimens or the observation of imaging results consistent with COVID-19. Excluded from the study were patients who were younger than 18, had a dialysis duration of less than three months before their COVID-19 diagnosis, had been hospitalized in the three months leading up to the study, received kidney transplants during the study period, were undergoing immunosuppressive therapy, had systemic or malignant diseases, or were currently experiencing acute inflammatory conditions.

All the patients were dialyzed three times a week for 4 hours using Fresenius 5008S High Volume HDF System. The HD procedure involved the use of bicarbonate-based dialysate, volumetric ultrafiltration control, and single-use synthetic (polysulfone) dialyzers. The median blood flow rate was 300 mL/min, and the dialysate flow rate was 500 mL/min. Heparin was administered as the standard anticoagulant.

Data collection

We collected a comprehensive dataset from eligible participants, covering various facets including demographic information, a three-month average of pre-existing spKt/V values, and crucial clinical and laboratory indicators. spKt/V was measured using the second generation ln formula:

$$spKt/V = -\ln(R - 0.008 \cdot T) + \\ + (4 - 3.5R) \cdot Weight\ loss/V,$$

where 'R' is the ratio of post-dialysis to pre-dialysis blood urea; 'V' is total body water volume; 'T' is dialysis session time, or treatment time in hours; 'ln' represents the natural logarithm with a base of 'e,' where 'e' has a value of approximately 2.718.[12,16]

The collected indicators included body mass index (BMI), anuria status, comorbidities (such as diabetes mellitus, hypertension, and a history of cardiovascular disease), the type of vascular access used for dialysis, hemoglobin levels, platelet counts, D-dimer, serum electrolyte concentrations, C-reactive protein (CRP) levels, intact parathyroid hormone levels (iPTH), ferritin, albumin, and cholesterol levels. The results of the first laboratory investigation conducted following COVID-19 recovery were utilized for all the data. This investigation took place at a median of 32 (28–37) days after the onset of COVID-19.

Body mass index (BMI) was calculated as weight in kilograms divided by the square of the height in meters. Anuria was defined as daily diuresis of less than 100 mL. The measurement of data involved the use of an automatic analyzer "Flexor Junior" (Vital Scientific, Netherlands) for biochemical measurements, and hematological parameters of blood were determined using an "ABX Micros-60" (Horiba Medical, France). iPTH levels were assessed through an immunoradiometric assay, and electrolyte levels were measured using standard autoanalyzer techniques.

In addition, we meticulously documented the details of COVID-19 infections, capturing specific information such as the date of infection onset, the vaccination status before contracting COVID-19, radiologic findings (the percentage of lung damage on computed tomography [CT] images), the necessity of oxygen therapy during the acute phase of the disease, the presence of long-term COVID-19 sequelae, any cases of hospitalization following COVID-19 recovery, and instances of mortality. The identification of long-term COVID sequelae involved a meticulous review of medical records, focusing on whether patients self-reported post-COVID symptoms or if documented medical conditions could explain these symptoms.

Outcome measures and their definitions

The primary outcome measures included:

The presence of long-term COVID sequelae

Long-term COVID sequelae encompassed a range of persistent symptoms and health issues that linger beyond the acute phase of COVID-19. They included but were not limited to respiratory, neurological, cardiovascular, or other medical conditions arising as a result of the initial COVID-19 infection and could not be explained by alternative diagnoses.

The incidence of hospitalization following recovery from COVID-19

This outcome referred to instances where participants required hospitalization for medical treatment or care after their recovery from COVID-19 infection. It aimed to assess the need for hospital-based medical intervention following the resolution of the initial COVID-19 illness.

The rate of all-cause mortality within one year following COVID-19 infection

This measure entailed recording the number of deaths due to any cause among the study participants within a year after contracting COVID-19.

Statistical analysis

All statistical analyses and graphs were performed using the MedCalc Statistical Software version 22.007 (MedCalc Software Ltd, Ostend, Belgium). Continuous variables were presented as medians (M) with interquartile ranges (Q25-Q75) due to non-normal distribution. Categorical variables were expressed as counts and percentages. To assess the differences between groups based on dialysis dose (spKt/V<1.4 vs. spKt/V≥1.4), we employed the chi-squared (χ^2) test for categorical variables and independent t-tests or Mann-Whitney U tests for continuous variables, as appropriate. Correlation analysis was done using the Pearson test. We employed Kaplan-Meier survival curves to assess the one-year survival probability of patients in relation to dialysis dose. Log-rank tests were conducted to determine the statistical significance of differences between the two dialysis dose groups. A multivariate Cox proportional hazards regression model was used for the primary outcomes of interest (long-term COVID-19 sequelae, one-year hospitalization, and all-cause mortality). We calculated hazard ratios (HR) with 95% confidence intervals (CI) to evaluate the associations between dialysis dose and these outcomes. In the analyses, we adjusted for potential confounders, including patient age, dialysis vintage, diabetes status, a history of cardiovascular disease and vaccination, and the requirement of oxygen support during the acute phase of COVID-19. To evaluate the predictive ability of spKt/V values for COVID-19 outcomes, we performed a Receiver Operating Characteristic (ROC) analysis. We assessed the area under the curve (AUC) and determined optimal cutoff values to maximize sensitivity and specificity for predicting the outcomes.

Results

Patient presentation and initial COVID-19 outcomes

Out of the initial cohort of 246 eligible patients who survived the acute phase of COVID-19, we excluded 51 patients due to them meeting exclusion criteria (Fig. 1).

Consequently, our study comprised 195 patients, with a median dialysis vintage of 49 (31.3–85.2) months. The average age of the study population was 56 (44–63)

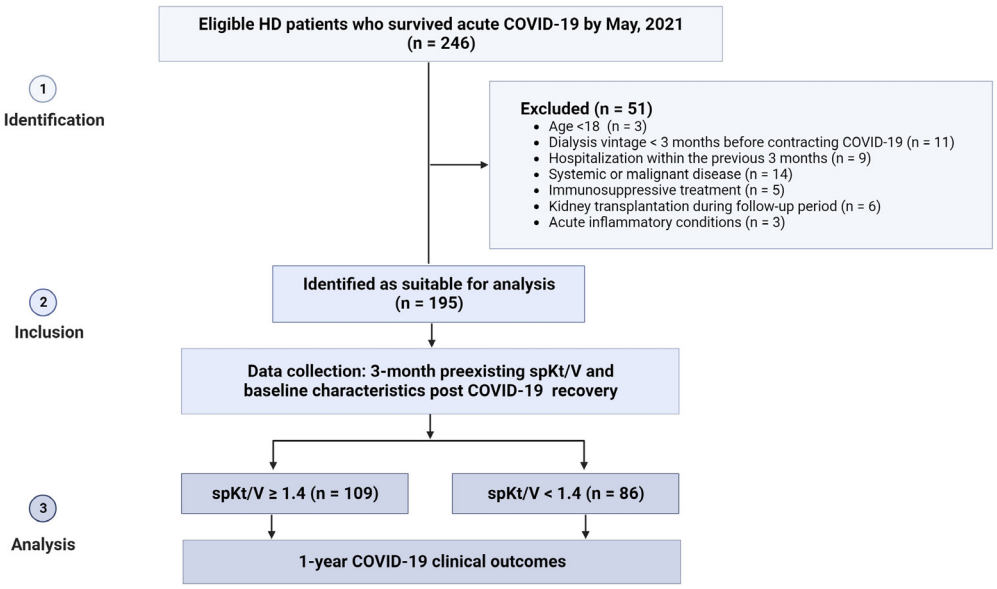

Fig. 1. The study flowchart

years, with 108 males, accounting for 55.4% of the cohort. The most prevalent comorbidities among the participants were hypertension, affecting 189 (97%) of them, followed by obesity (BMI ≥ 30 kg/m^2) in 41 (21%), cardiovascular disease in 31 (15.9%), and diabetes in 26 (13.3%).

During the acute phase of COVID-19, the most commonly reported symptoms were fever (131/67.2% of patients), cough (115/59%), and fatigue (103/52.8%). Interestingly, 24 (12.3%) of patients remained asymptomatic. Additionally, 77 (39.5%) of the patients underwent lung CT scans, and only 4 (5.2%) of them exhibited significant pulmonary damage of ≥50%. Nevertheless, 38 (19.5%) of the patients required hospitalization with supplemental oxygen.

Notably, 25 (12.8%) of the patients had received full vaccination against COVID-19 using either the Pfizer-BNT-162b2 or Moderna-mRNA-1273 vaccine before contracting the virus.

The 3-month average dialysis dose before the onset of COVID-19 infection exhibited a range from 1.1 to 2, with a median spKt/V value of 1.4 (1.3–1.5). Notably, 109 patients (55.9%) had spKt/V levels exceeding 1.4, while 84 patients (43.1%) fell within the range of spKt/V values between ≥1.2 and < 1.4; and only a small fraction, consisting of 2 patients (1.02%), fell below the target level of spKt/V (<1.2), primarily due to issues related to inadequate vascular access.

To further analysis, we categorized patients based on their attainment of the target spKt/V value. As demonstrated in Table 1, patients with spKt/V <1.4 displayed higher blood pressure, CRP, BMI, and D-Dimer levels in comparison to patients with spKt/V ≥1.4.

Expectedly, they exhibited lower levels of Hb and calcium. In addition, this group showed a higher preva-

Table 1. Characteristics of the study cohort stratified by 3-month average dialysis dose preceding COVID-19 infection*

	Patients with spKt/V ≥ 1.4 (n=109)	Patients with spKt/V < 1.4 (n=86)	p
Demographic and routine clinical data			
Male gender, n (%)	57 (52.3%)	51 (59.3%)	0.33
Age, years	56 (46–62)	56 (42.5–64)	0.87
Dialysis vintage, months	45.5 (29–103.5)	49 (36.5–66)	0.91
Temporary vascular access, n (%)	2 (1.83%)	5 (5.82%)	0.14
Hypertension, n (%)	104 (95.4%)	85 (98.8%)	0.12
Systolic blood pressure, mm Hg	140 (120–145)	140 (130–150)	0.02
Diastolic blood pressure, mm Hg	75 (71.3–80)	80 (75–90)	0.04
Diabetes mellitus, n (%)	9 (8.2%)	17 (19.7%)	0.02
Cardiovascular disease, n (%)	12 (11.1%)	19 (22.1%)	0.03
BMI, kg/m^2	24.2 (21.8–27.5)	28.6 (23.8–31.5)	<0.0001
spKt/V	1.45 (1.4–1.56)	1.3 (1.22–1.36)	<0.0001
Hb, g/L	105 (94.5–115)	98 (85.2–112)	0.007
Serum albumin, g/L	38.5 (36.1–41.6)	36.7 (35.5–40.7)	0.12
Calcium, mmol/L	2.26 (2.16–2.35)	2.19 (2.02–2.29)	0.03
Phosphorus, mmol/L	1.58 (1.29–1.96)	1.72 (1.48–2.02)	0.08
iPTH, ng/L	307.0 (129.3–587.5)	224.0 (126.0–557.9)	0.49
Total cholesterol, mmol/L	4.9 (3.6–5.6)	4.6 (3.8–5.3)	0.63
CRP, mg/L	10.2 (4.1–16.9)	12.7 (6.9–18.8)	0.04
Ferritin (ng/mL)	301 (159.3–476.5)	322 (235.5–401)	0.21
Platelet count (/mm^3)	214.0 (172.2–248.2)	202.0 (182–224)	0.31
D-Dimer (ng/mL)	420 (263–890.7)	522 (257.5–906.3)	0.03
Initial COVID-19 outcomes			
Vaccinated status for COVID-19, n (%)	16 (14.7%)	9 (10.5%)	0.38
Area of lung lesions on CT images, %	15 (11.3–30)	25 (15–40)	0.02
Hospitalization with oxygen supply, n (%)	14 (12.8%)	24 (27.9%)	0.008

* BMI – body mass index, CRP – C-reactive protein, CT – computed tomography, Hb – hemoglobin, iPTH – intact parathyroid hormone, spKt/V – single-pool Kt/V

lence of obesity, diabetes, and a history of cardiovascular disease. They also presented a more extensive degree of pulmonary damage and a higher rate of hospitalization requiring oxygen support during the acute phase of COVID-19. Notably, there was an inverse correlation observed between the extent of pulmonary damage on CT scans and the patients' preexisting spKt/V, as illustrated in Fig. 2.

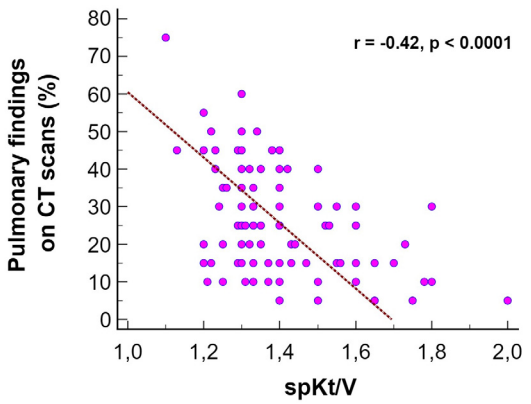

Fig. 2. Correlation between the percentage of COVID-19-associated pulmonary involvement and preexisting spKt/V in patients undergoing HD

Preexisting spKt/V and long-term COVID-19 outcomes

Over the one-year follow-up period, long-term COVID-19 sequelae manifested in 146 patients, accounting for 74.8% of the cohort. Additionally, 37 (18.9%) patients required hospitalization, and 13 patients (6.7%) died. Among those hospitalized during the long-term COVID period, cardiovascular events were the leading cause, responsible for 14 (38.8%) cases, followed by infections in 11 (29.7%) cases, and gastrointestinal bleeding in 4 cases (10.8%). Cardiovascular events accounted for 5 (38.5%) cases of death, sepsis for 4 (30.7%) cases, stroke for 2 (15.4%) cases, pulmonary embolism for 1 (7.7%) case, and 1 (7.7%) case remained classified as unknown.

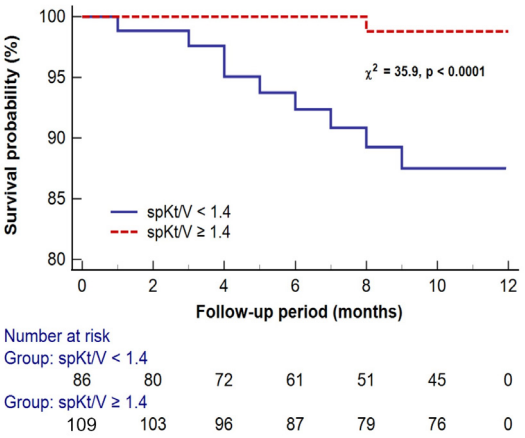

Fig. 3. Survival probability of patients undergoing HD one year after COVID-19, stratified by attainment of the target spKt/V level prior to infection

Patients with spKt/V levels below 1.4 exhibited a notably higher prevalence of long-term COVID-19 sequelae (χ^2 = 50.2, p < 0.0001), hospitalization rate (χ^2 = 36.7, p < 0.0001), and mortality (χ^2 = 12.8, p = 0.003) when contrasted with those having spKt/V values of 1.4 or greater. The Kaplan-Meier analysis revealed a significantly higher one-year survival probability following COVID-19 in patients with spKt/V ≥1.4 (Fig. 3).

In the multivariate Cox-regression analysis, adjusted for patient age, dialysis vintage, diabetes, a history of cardiovascular disease, vaccination status, and severe acute-phase COVID-19 (defined as the need for oxygen support), preexisting spKt/V <1.4 was associated with a 9-fold higher risk of experiencing long-term COVID-19 sequelae (HR 9.1, 95% CI: 3.4; 24.6), a 7-fold increase in the likelihood of hospitalization within one year following COVID-19 (HR 7.6, 95%

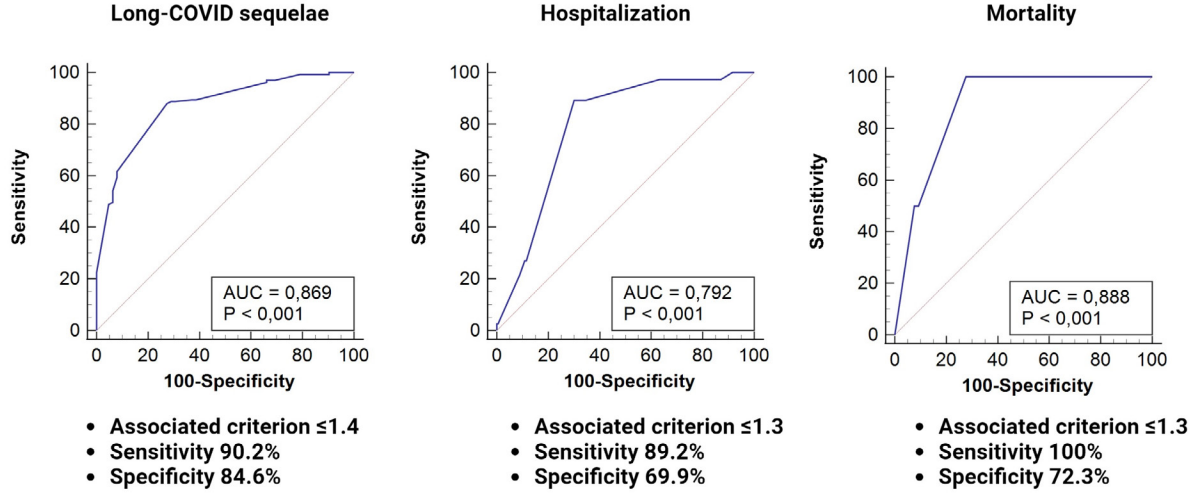

Fig. 4. ROC analysis for COVID-19 outcome prediction using spKt/V value in patients undergoing HD

CI: 3.9; 14.6), and an 8-fold rise in mortality risk (HR 8.5, 95% CI: 2.9; 25.8).

The ROC analysis was employed to discern the predictive accuracy of specific spKt/V cut-off points in relation to long-term COVID-19 outcomes. The findings of this analysis revealed that for the anticipation of the long-COVID syndrome, a spKt/V cut-off point of less than 1.4 demonstrated notable sensitivity and specificity. However, when it came to predicting long-term hospitalization and one-year mortality among patients undergoing HD, the ROC analysis indicated that a spKt/V cut-off point ≤1.3 exhibited superior predictive performance (Fig. 4).

Discussion

The present study sought to elucidate the intricate relationship between preexisting dialysis dose, as indicated by spKt/V values, and the long-term outcomes of COVID-19 in the cohort of patients treated with HD. Our hypothesis stems from recognizing that dialysis adequacy encompasses a multifaceted array of factors extending beyond urea removal alone. It involves considerations such as the quality of vascular access, the presence of comorbidities, fluid balance management, correction of malnutrition, acidosis, and anemia, maintenance of electrolyte homeostasis, and addressing chronic inflammation.[17–20] Within this context, a reduced dialysis dose can be seen as a reflection of this intricate interplay of factors, potentially contributing to unfavorable COVID-19 outcomes in patients undergoing HD. However, to the best of our knowledge, only two studies have delved into the relationship between dialysis dose and COVID-19.[21,22] Kooman et al. illustrated that deviating from target dialysis doses is a potential risk factor for 30-day COVID-19-related mortality in the HD population.[21] The second study has recently explored whether differences exist in dialysis adequacy between HD patients with COVID-19 and those without infection.[22] Nevertheless, neither of these reports has furnished evidence of a long-term association between dialysis dose and COVID-19 outcomes, including the specific Kt/V values as predictors.

In line with the aforementioned studies, our findings underscore the critical importance of optimizing dialysis doses to mitigate both immediate and long-term adverse outcomes in COVID-19 patients.[21,22] We found an inverse relationship between spKt/V values and the extent of pulmonary damage observed on CT scans. Furthermore, patients with preexisting spKt/V values below 1.4 were at a heightened risk of developing long-term COVID-19 sequelae, experiencing extended hospitalization during a year after COVID-19 recovery, and facing an increased risk of all-cause mortality when compared to patients receiving spKt/V≥1.4. Our data specifically indicated that a spKt/V cutoff point of ≤1.3 emerged as a significant risk factor for one-year hospitalization and mortality within our cohort.

The evaluation of dialysis adequacy through spKt/V urea is a widely utilized method to assess the administered dialysis dosage.[23] Although acknowledged for its limitations, it is recommended by the Kidney Disease Outcomes Quality Initiative (KDOQI) guidelines as a meaningful metric for evaluating the impact of dialysis on patient survival.[13,24] According to these guidelines, patients receiving HD three times a week should aim for a target spKt/V of 1.4 per session, with a minimally acceptable delivered dose set at spKt/V of 1.2. In our patient cohort, the majority (55.9%) achieved spKt/V values of 1.4 or higher, while 43.1% received at least the minimal dose (spKt/V >1.2 and <1.4), and merely 1% fell below the minimal dialysis threshold (spKt/V < 1.2). Consequently, it may be reasonably inferred that the adequacy of dialysis should not have a significant influence on COVID-19 outcomes within our cohort. However, exceeding the recommended dialysis dose has been associated with reduced mortality rates and decreased risk of hospitalization.[25] Hence, achieving the target or surpassing the minimal dose requirement might hold the potential to mitigate the risks of COVID-19-associated hospitalization and mortality, as demonstrated in our study.

The association of suboptimal or reduced dialysis dose with increased risk of long-term COVID-19 outcomes can be explained by a convergence of factors. First, patients undergoing HD typically present with compromised immune systems, as even a single hemodialysis session can contribute to immune deficiency.[26–28] This susceptibility renders them more severe COVID-19 and more unfavorable long-term clinical outcomes compared to the general population.[29,30] Insufficient dialytic efficacy further weakens this already compromised immune response, potentially exacerbating the severity of the infection. While the long-term consequences of COVID-19 in the HD population are not yet fully understood, studies have shown that COVID-19 can involve persistent sequelae and other clinical complications that persist for weeks to months after initial recovery.[3,8] Moreover, recent evidence has shown that the adequacy of HD can independently predict seroconversion in patients receiving HD following anti-COVID vaccination.[31] This highlights the potentially far-reaching impact of dialysis adequacy, extending its implications beyond immediate COVID-19 outcomes.

Our findings revealed an increased risk of one-year hospitalization and mortality in patients with preexisting spKt/V <1.4, regardless of factors such as patient age, dialysis vintage, diabetes, history of cardiovascular disease, vaccination status, and severe acute-phase COVID-19. Although there is limited description of long-term COVID-19-associated mortality in patients

treated with HD, existing studies provide some insights. For instance, Och et al. have reported a mortality rate of 37.4% and 39.3% at 3 and 6 months, respectively, in their longitudinal cohort study.[8] Another prospective study found a 35.7% mortality rate over 12 months among 56 HD patients with COVID-19.[32] These studies indicate that elevated mortality rates in COVID-19 patients on HD extend beyond the initial hospitalization, persisting into the first year after diagnosis. In our study cohort, the mortality rate was notably low (6.7%), considering the generally high dialysis adequacy. It's important to note that direct comparison with the mentioned studies is challenging due to the absence of Kt/V data in their reports. Given the intricate interplay of long COVID, comorbidities, and cardiovascular and all-cause mortality in HD patients, establishing a specific timeframe after COVID-19 infection when mortality can be solely attributed to long COVID is challenging. The multifactorial nature of mortality in this population requires a comprehensive assessment of individual patient characteristics, encompassing the presence of long COVID symptoms, the severity of comorbidities, and the impact of persistent inflammation.

Second, reduced spKt/V has been shown to be associated with toxins accumulation and potentially indicates the systemic inflammatory stress determined by increased CRP levels, up-regulation of proinflammatory cytokines and chemokines, and downregulation of anti-inflammatory cytokines.[14,20,27] Chronic inflammation is a well-documented characteristic in patients on HD, often stemming from various factors like the accumulation of uremic toxins, oxidative stress, and constant exposure to dialysis membranes, among others.[33] Intriguingly, in the context of COVID-19, chronic inflammation at baseline may have a somewhat paradoxical effect, potentially offering a degree of protection against severe outcomes in patients undergoing HD.[34] However, this amplified immune response can be a double-edged sword. On the one hand, it might help combat the virus more effectively. On the other hand, it can lead to an excessive and dysregulated inflammatory response, known as a "cytokine storm," which is associated with severe lung damage and complications seen in severe cases of COVID-19.[35] Our finding of an inverse association between spKt/V values and pulmonary damage observed on CT scans indirectly supports this hypothesis. Suboptimal or reduced dialysis dose, indicating insufficient toxin removal, contributes to the systemic inflammatory state observed in HD patients, heightening susceptibility to severe lung injury upon COVID-19 infection. This aligns with previous research suggesting that uremic toxin indoxyl sulfate plays a toxicophysiological role as a mediator in the kidney-lung axis.[36] Moreover, our previous report demonstrated that increased preexisting serum indoxyl sulfate was associated with poor COVID-19 outcomes in HD patients, including the severity of pulmonary damage observed on CT scans.[4] Although our study did not directly measure cytokine levels due to its retrospective nature, a notable increase in CRP levels was observed in patients with spKt/V <1.4 compared to those with spKt/V ≥1.4. This finding also implies a potential association between dialysis adequacy and inflammatory markers. Additionally, we previously demonstrated a high cytokine concentration in patients with both preexisting hypertension and long-COVID sequelae, indirectly suggesting a negative impact of chronic inflammation on long-term COVID-19 outcomes in this patient population.[37] Nonetheless, this complex interaction between chronic inflammation, dialysis adequacy, and long-term COVID-19 outcomes requires further investigation for a comprehensive understanding.

Third, the influence of a reduced dialysis dose on long-term COVID-19 outcomes can be viewed through the lens of comorbidity burden. A notable proportion of HD patients contend with comorbidities, encompassing conditions such as hypertension, cardiovascular events, and diabetes.[38,39] In our study cohort, a higher prevalence of these concurrent health issues was observed in patients with Kt/V<1.4 compared to the group achieving the Kt/V target. This observation emphasizes that our study cohort is not an exception and higher comorbidity burdens were evident in patients with suboptimal dialysis doses. These concurrent health issues introduce a multitude of complexities into the landscape of dialysis dosing. Challenges such as fluid overload, suboptimal vascular access, hemodynamic instability, and the use of multiple medications may hinder the efficient removal of uremic toxins during dialysis.[40–43] Also, comorbidities can exert a toll on a patient's nutritional status, potentially resulting in shifts in muscle mass and protein level.[44] Collectively, these factors contribute to a diminished dialysis dose, heightened mortality risk, and an increased susceptibility to severe acute COVID-19, and long-term COVID-19 outcomes.[19,39,45-48]

The study has several noteworthy limitations that should be acknowledged. First, its retrospective nature relies on historical data, which may introduce recall bias. Patients interpreted and reported their symptoms differently, potentially introducing bias in self-reported persistent symptoms and other subjective measures relevant to long-term COVID-19 outcomes. This variability in patient reporting could have impacted the accuracy of the collected data. Future studies could benefit from standardized data collection methods and cross-verification of self-reported symptoms with medical records or other objective measures to mitigate this variability in patient reporting. Second, the study primarily identifies associations between dialysis dose and long-term COVID-19 outcomes but does not establish causation.

Moreover, the relatively small sample size and regional characteristics of the study population may limit the generalizability of the findings to a broader HD population. Variations in patient demographics, healthcare practices, and resources at different centers could potentially influence the results. Larger, multicenter studies with diverse populations would enhance generalizability and provide a more comprehensive understanding of the associations. Third, despite efforts to exclude certain patients based on specific characteristics and comorbidities, there is a possibility of selection bias, and the excluded patients may have had different outcomes not considered in the analysis. Conducting sensitivity analyses or providing additional details on the characteristics of excluded patients could offer insights into the potential impact of selection bias on study outcomes. Additionally, the study's data covers a specific time frame, clinical practices and treatments for COVID-19 may have evolved during this period, potentially impacting outcomes. Furthermore, while the study focused on whether patients met a spKt/V target before contracting COVID-19, it did not investigate potential variability in dialysis dose over time, which could also influence outcomes. Future research could explore how changes in dialysis dose and inflammatory markers over time might impact long-term COVID-19 outcomes, providing a more nuanced understanding. Finally, due to the lack of information on socioeconomic status in the patient's medical records and the treatment of the acute phase of COVID-19 in specialized hospitals where data collection was not possible, the study did not account for factors such as socioeconomic status and the specific medications used in the acute treatment of COVID-19, which could potentially influence the observed results.

Nonetheless, despite the limitations our study is the first to shed light on the association between dialysis dose and one-year COVID-19 outcomes, underscoring the significance of optimizing dialysis therapy to protect patients undergoing HD from the potential long-term consequences of COVID-19. Based on these insights, we propose practical recommendations for physicians:

- prioritize regular monitoring of Kt/V levels aiming to achieve and maintain values at or above recommended targets;
- customize dialysis plans to cater to individual patient requirements, considering factors such as comorbidities, hypervolemic and nutritional statuses, suboptimal vascular access, hemodynamic instability, and others;
- reinforce infection prevention measures, including advocacy for COVID-19 vaccination;
- finally, impart education to patients on the paramount importance of adherence to dialysis prescriptions for overall improved outcomes.

Conclusion

In conclusion, our study highlights the crucial role of target dialysis dose, as measured by spKt/V values, in shaping the long-term outcomes of COVID-19 in patients undergoing HD. Achieving target spKt/V levels appears to confer protection against COVID-19 outcomes, including long-term sequelae, extended hospitalizations, and mortality. spKt/V threshold of ≤1.3 as a significant risk factor for both one-year hospitalization and mortality within our cohort. Further research is warranted to explore the intricate relationship between dialysis adequacy and COVID-19 outcomes, potentially offering new avenues for enhancing the care and prognosis of these patients.

Declarations

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Author contributions

Conceptualization, N.S.; Methodology, N.S..; Software, N.S.; Formal Analysis, N.S.; Data Curation, A.R. and L.S.; Writing – Original Draft Preparation, N..S. and A.R.; Writing – Review & Editing, N.S.

Conflicts of interest

The authors declare no competing financial support or interests.

Data availability

The data used in the study is available upon reasonable request to the corresponding author.

Ethics approval

The study protocol was approved by the Ethics Committee of the State Institution "Institute of Nephrology of the National Academy of Medical Sciences", Kyiv, Ukraine (protocol number: 2-2021, dated April 6, 2021).

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ORIGINAL PAPER

The content of lactoferrin and interleukin-8 in breast milk of patients with lactational mastitis

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ABSTRACT

Introduction and aim. Information concerning lactoferrin and interleukin-8 (IL-8) local levels in breast milk are not numerous and requires further research. The aim of this study was to determine the content of lactoferrin and interleukin-8 in the breast milk of patients with lactational mastitis, and to identify new potential markers for assessing the activity of the inflammatory process in the mammary gland.

Material and methods. This study analyzed the breast milk of 30 women with lactostasis (group I), 37 women with lactational mastitis (group II) and 30 healthy lactating women (age 26±5 years old). The milk content of lactoferrin and interleukin-8 (IL-8) was determined by enzyme-linked immunosorbent assay.

Results. The average value of lactoferrin in breast milk of healthy women was 4.78±0.47 mg/mL, exceeding levels in group I 1.8 times ($p<0.05$). The level of lactoferrin in group II exceeds the control values 3.1 times ($p<0.05$). The content of IL-8 in breast milk of women in group I was 7.3 times higher than the control (3.63±0.12 pg/mL, $p<0.05$). In lactational mastitis, the concentration of IL-8 in breast milk exceeded the group I 13.9 times ($p<0.05$) and was 1.9 times higher than group I ($p<0.05$).

Conclusion. The analysis has revealed an increase of lactoferrin and IL-8 in breast milk of the test groups, which indicates the activation of non-specific protection.

Keywords. breast milk, interleukin-8, lactational mastitis, lactoferrin, lactostasis

Introduction

An urgent problem in the postpartum period of childbirth is the development of lactational mastitis, which begins with stagnation of milk (lactostasis), infection (in 70-80% of cases, the causative agent is *Staphylococcus aureus*), and a decrease in the overall stability of the body. Lactational (postpartum) mastitis is inflammation of the parenchyma of the mammary gland, which is associated with the lactation process. Lactational mastitis most often develops as a result of long-term pathological lactostasis. Lactostasis is a dysfunctional state of the lactating mammary gland, where there is a discrepancy between the processes of milk formation and milk yield.[1]

An insufficient immune response causes protracted disease, periods of disability enlarge, and the risk of chronic pathological process increases.[1-3] An important

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issue is the preservation of lactation, since breast-feeding provides not only harmonious physical and psycho-emotional development of the child, but is also considered an integral part of the reproductive health of a woman.[4,5]

Breast milk is a natural unique biological product that contains a wide range of biologically active substances and protective factors, which include immunoglobulins, lysozyme, lactoferrin, oligosaccharides, immunocompetent cells, as well as cytokines/chemokines (TNF-α, IL-1β, IL-2, IL-6, IL-8, IL-10, IFN-γ, etc.).[6,7] There is an assumption that anti-inflammatory cytokines protect a child's body, and that inflammatory cytokines, especially interleukin-8 (IL-8), play a role in protecting the mammary gland against infection. The focus of inflammation is provided by interleukin-8, which activates adhesion and degranulation of neutrophils, and enhances the phagocytic effect.

Lactoferrin (LF) plays a special role in non-specific protection, has bacteriostatic and bactericidal effects mainly on gram-positive flora, and performs fungicidal, antiviral, immunomodulatory and other functions.[8] Currently, information on the local level of LF and IL-8 in the development of the inflammatory process in the mammary gland is not numerous and requires research.

Aim

To determine the content of lactoferrin and interleukin-8 in the breast milk of patients with lactational mastitis, and to identify new potential markers for assessing the activity of the inflammatory process in the mammary gland.

Material and methods

The selection of women for the examination was carried out in the surgery departments of the Clinical Emergency Medical Hospital of the city of Lviv in Ukraine. Women who gave birth for the first time, and in whom inflammatory process in the mammary gland occurred within three or four weeks after childbirth, participated in the study. The results of the anamnesis, clinical examination, special instrumental studies (ultrasound of mammary glands), and data from bacterial analysis were used to make the diagnosis. The women in this study were chosen based on convenience criteria. Breast milk was collected in the morning on an empty stomach on the first day of the patient's admission to the hospital.

Laboratory investigations were performed between 2016 and 2020 in the diagnostic laboratory of the Department of Clinical Laboratory Diagnostics of Danylo Halytsky Lviv National Medical University, Lviv, Ukraine. Research was performed with safety measures for the health of patients, respecting their rights, human dignity, and moral and ethical norms in accordance with the principles of the Helsinki Declaration of Human Rights, the Council of Europe Convention on Human Rights and Biomedicine and the relevant Laws of Ukraine. This study was approved by the Ethical Committee of the Danylo Halytsky Lviv National Medical University, Ukraine (meeting minutes No. 2 dated February 15, 2016) and written informed consent was obtained from mothers if they agreed for breast milk sampling.

Breast milk was collected by extracting 5 mL into a sterile test tube. Immediately after sampling, the milk was centrifuged at 2000×*g* for 10 min.[9] From the middle layer of the centrifuged sample (the upper layer contains the supernatant fats), 1 mL of liquid was taken and 500 μL was placed into Eppendorf tubes. LF and IL-8 content in the obtained milk was analyzed by an enzyme-linked immunosorbent assay (ORG 284, Lactoferrin by Organtec; Human IL-8 ELISA Kit by Diaclone) using the STAT FAX 303 plus analyzer according to the manufacturer's guidelines. Statistical processing of the obtained data was based on the method of variation statistics, using the STATISTICA 6.0 (Statsoft, Tulsa, Oklahoma, USA) program.

Results

The study analyzed the breast milk of 97 women aged 18 to 36 (average age: 26±5 years old). Patients were divided into two comparison groups. Group I included 30 women with lactostasis. Group II consisted of 37 women who developed lactational mastitis. The control group included 30 clinically healthy lactating women.

The results of the LF content in breast milk of women with a dysfunctional breast condition are presented in Fig. 1.

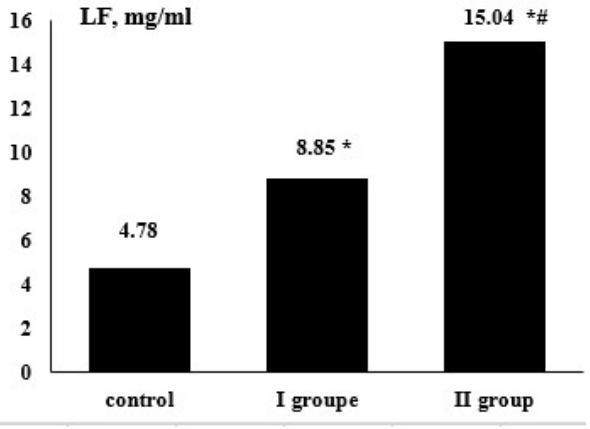

Fig. 1. The content of LF in breast milk of women in groups I and II, (mean), * – probability of difference in indicators compared to the control group ($p<0.05$); # – probability of difference in indicators compared to group I ($p<0.05$).

The average LF value in breast milk of practically healthy women who made up the control group is

4.78±0.47 mg/mL. In group I, the concentration of this marker is 8.85±0.3 mg/mL, which is 1.8 times higher than the control value ($p<0.05$). The level of LF in breast milk of women in group II is 15.04 ±0.53 mg/mL, which exceeds the control values by 3.1 times ($p<0.05$).

The study of breast milk of patients with lactational mastitis indicates changes in IL-8 production. The obtained results are presented in Fig. 2.

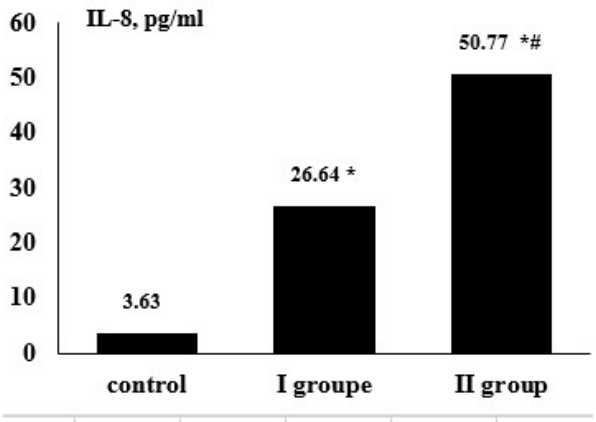

Fig. 2. The content of IL-8 in breast milk of women in groups I and II, (mean), * – probability of difference in indicators compared to the control group ($p<0.05$); # – probability of difference in indicators compared to group I ($p<0.05$)

The content of IL-8 in breast milk of women in group I is 26.64±1.68 pg/mL, which is 7.3 times higher than the control values (3.63±0.12 pg/mL, $p<0.05$). In breast milk of women in group II, the level of IL-8 (50.77±1.58 pg/mL) is 13.9 times higher than in the control group ($p<0.05$). The test group analysis reveals a 1.9-fold increase of IL-8 in breast milk with the development of lactational mastitis as compared to the indicator of women who developed lactostasis ($p<0.05$).

Discussion

Lactoferrin iron-binding glycoprotein, which is synthesized by mucous membrane epithelial cells, is contained in the secondary granules of neutrophils, the secretions of almost all exocrine glands. The highest LF content was found in breast milk, and its expression is controlled by prolactin.[8,10] Early functional changes in the mammary gland in lactostasis are accompanied by an increase in LF expression, which is likely to reveal the protective reaction of epithelial cells. The comparison of the LF content in the test groups reveals an increase in the production of this marker with the development of lactation mastitis by 1.7 times relative to the indicator of women in group I ($p<0.05$). The obtained results provide for the activation of local immunity, indicate pronounced inflammatory changes in the mammary gland.[11]

The non-specific protection of the body from infectious agents is known to activate various mechanisms and factors. As an anti-infective protection first line component, lactoferrin exhibits a bacteriostatic effect by sequestration of free iron. The lack of the necessary substrate inhibits the growth of bacterial colonies. The bactericidal effect of lactoferrin is due to the direct interaction of the protein molecule with the microorganism membrane, which leads to its destruction.[10,12,13]

It is worth noting a key role of lactoferrin in the modulation of the immune response. At the molecular level, in the presence of this glycoprotein, the expression of cytokines changes, in particular, pro-inflammatory interferon-γ, interleukins (IL-1β, IL-8, TNF-α), and the production of IL-5 and IL-10 is reduced. In addition, lactoferrin binds iron, which accumulates in damaged tissues, and catalyzes the formation of toxic hydroxyl radicals, that is, it has an anti-inflammatory effect.[14,15]

IL-8 is produced mainly by endothelial cells, monocytes, and macrophages under the influence of bacterial endotoxins and cytokines (IL-1, TNF-α, IL-6, etc.). IL-8 acts as a chemotactic factor for neutrophils, enhances their adherence to endothelial cells, promotes penetration from the vascular bed into infected tissue.[7,10] The analysis of the test group reveals a 1.9-fold increase in IL-8 levels in breast milk associated with the onset of lactational mastitis, as compared to women who developed lactostasis ($p<0.05$), indicating the involvement of the inflammatory process and indicating destructive processes in the breast parenchyma under the influence of activated neutrophils.[16]

High concentrations of the studied markers in lactostasis indicate the initial stages of the development of the inflammatory process. As a result of the absence of milk outflow, a favorable environment for the reproduction of bacteria is formed. Lactic acid fermentation occurs in milk, which leads to the destruction of the epithelium of the milk ducts and alveoli. When the pressure in the mammary gland increases, blood circulation is disturbed, venous congestion occurs, and this creates additional conditions for the development of infection. Untimely elimination of lactostasis leads to the development of mastitis.[1]

With the development of the inflammatory process, of paramount importance is the migration of neutrophils and other cells from the blood into breast milk through the epithelium of the milk alveoli. In the presence of IL-8, the processes of activation and degranulation of neutrophils occur, they are accompanied by the generation of reactive oxygen species, nitric oxide, the release of lactoferrin, lysozyme into the intercellular medium. Such mechanisms are likely to protect the surrounding tissues from destruction, contribute to the rapid completion of the inflammatory process.[7,8,16]

Conclusion

The study reveals an increase in the production of lactoferrin and interleukin-8 in breast milk of both groups as compared to healthy women. With the development of lactation mastitis, the level of lactoferrin exceeds the control values 3.1-fold, and 1.7-fold in women with lactostasis ($p<0.05$). The mean concentration of IL-8 is 13.3 times higher in the control group and 1.9 times higher in women with lactostasis ($p<0.05$). The established levels of lactoferrin and IL-8 in milk can serve as predictive markers for assessing the degree of inflammation. The results obtained in patients with lactation mastitis indicate the activation of non-specific protection, the ability to counteract the destructive effect of infectious factors.

Declarations

Funding

The study was conducted at the researchers' own expense.

Author contributions

Conceptualization, N.D. and I.H.; Methodology, N.D.; Software, S.T.; Validation, L.L., V.A. and N.D.; Formal Analysis, O.B.; Investigation, N.D.; Resources, N.L..; Data Curation, S.T.; Writing – Original Draft Preparation, V.A. Writing – Review & Editing, S.Z.; Visualization, L.S.; Supervision, L.L.; Project Administration, V.A.; Funding Acquisition, N.D.

Conflicts of interest

Authors declare no conflict of interest.

Data availability

The presented data are available from authors upon request.

Ethics approval

All subjects gave their informed consent for inclusion before they participated in the study. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of Danylo Halytski Lviv National Medical University, Ukraine (meeting minutes No. 2 dated February 15, 2016).

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ORIGINAL PAPER

Assessment of hepatorenal biochemical indices in male Sprague Dawley rats preceding concurrent oral administration of Ghana alcoholic bitters and natural cocoa powder

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ABSTRACT

Introduction and aim. There has been a surge in the consumption of Ghanaian alcoholic bitters. Ghanaian alcoholic bitters are formulated from a maceration of not less than three plant parts making the organic composition very complex. There appear to be no studies on the effect of Ghanaian alcoholic bitters on the hepatorenal biochemistry indices. The study aimed to assess the effects of alcoholic bitters and cocoa powder on the liver and kidney serum biochemistry.

Material and methods. Twenty-four healthy male Sprague Dawley rats, age 11–12 weeks, weighing 150–250 g were used. The rats were randomly assigned to four groups (n=6). At the end of the experimentation, a blood sample was taken by cardiac puncture and centrifuged to obtain the serum for biochemical assays and analysis.

Results. The liver enzymes showed no significant difference between the treatment and control groups. There were higher mean values for total bilirubin and direct bilirubin for alcoholic bitters and natural cocoa powder groups respectively than the control group and the co-administration of alcoholic bitters and natural cocoa powder group.

Conclusion. The study concludes that alcoholic bitters consumption might cause injury to the liver and kidney resulting in anomaly of the hepatorenal indices from rat blood serum biochemistry.

Keywords. biochemistry, Ghanaian alcoholic bitters, hepatorenal indices, natural cocoa powder, Sprague Dawley rat

Introduction

Ghanaian alcoholic bitters are alcohol-based preparations obtained by macerating fresh or dried plant parts such as the bark, root, leaves and/or seeds in alcohol of 18% to 45% concentration.[1-3] The Ghanaian alcoholic bitters are formulated from a combination of not less than three plant parts making the organic composition of these drinks very complex. Most of the alcoholic bitters used plant parts from *Xylopia aethiopica*, *Anthocleista nobilis* and *Khaya senegalensis* for the concoction preparation.

Alcoholic bitters are widely consumed all over Ghana and neibouring countries including, Côte d'Ivoire, Togo and Nigeria.[4] This high consumption is linked to the claimed health benefits consumers intend to derive from its intake. Excess use of alcoholic bitters negatively

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affects human health, and is a serious worldwide problem.[5] The reasons for drinking alcoholic beverages vary, and they include being part of a standard diet, medical objectives, relaxing effects, euphoric effects, recreational purposes, artistic inspiration, alleged aphrodisiac benefits, and happiness.[6] Alcoholic bitters, however, play a direct role in the production of reactive oxygen and nitrogen species, creating an environment prone to oxidative stress.[5] Among the most severe health problems caused by alcohol, is its adverse effect on the liver and kidney. The damage to the liver and kidney is mostly detected when there are anomalies of hepatorenal indices from liver and kidney function tests. These hepatorenal indices include aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and gamma-glutamyl transpeptidase (GGT), total protein, albumin, creatinine, urea and bilirubin.[7] For instance, alcoholic herbal bitters was found to cause a combined elevation in urea and creatinine and bilirubin suggesting a moderate to severe form of kidney and liver damage in rats.[7] There have been no studies conducted on the effect of Ghanaian alcoholic bitters on the hepatorenal indices. This study's findings would serve as the baseline for diagnosing hepatorenal disorders using these biochemical indices. This would be easier, more affordable and less riskier than performing biopsies for liver and kidney tissues which is invasive.

Furthermore, there is no intervention that could counteract the oxidative stress on the liver and kidney tissues preceding Ghanaian alcoholic bitters consumption. Therefore, utilizing natural cocoa powder known to be a rich source of flavonoid, theobromine,[8] and several important natural antioxidants could provide protection for the body against free radicals.[9]

Aim

The aim of this study is to determine if simultaneous consumption of Ghanaian alcoholic bitters with natural cocoa powder has a protective effect on the liver and kidneys. The aim was accomplished by assessing liver and kidney damage through changes in the hepatorenal biochemical indices using Sprague Dawley rats.

Material and methods

Study design

This experimental study used Sprague Dawley rats as a model for evaluation of Ghanaian alcoholic bitters consumption and its effect on the hepatorenal biochemical indices. This experimental research design using animals was used to increase human knowledge and significantly offer solutions to questions in biological and biomedical sciences.[10] The authors followed the criteria for the alcohol model because the Ghanaian herbal alcoholic bitters are a mixture of at least three plant parts (concoction) in alcohol with a bitter, sour or bittersweet flavor.[11]

Alcoholic bitters

A popularly used alcoholic bitters in Ghana made with *Xylopia aethiopica, Anthocleista nobilis* and *Khaya senegalensis* in 42% (v/v) alcohol was selected for this study.

Natural cocoa powder

Natural cocoa powder (NCP) was purchased from the manufacturer. The content of the natural cocoa powder as stated in the manusfacturer's information sheet is shown in Table 1.

Table 1. Composition of Natural coacoa powder according to manufacturer's specification

Component	Amount (g/100 g)
Total dietary fiber	34.3
Total carbohydrate	15.5
Protein	24.0
Moisture	6.0
Free fatty acid	1.2
Theobromine	1.2
Total ash	5.7
Acid insoluble ash	0.1

A solution of the NCP (20% (w/v)) was freshly prepared daily with warm water. The procedure for the preparation of the natural cocoa solution (NCS) was as follows:

- 1 g of the NCP was measured using a chemical weighing scale,
- 5 mL of warm water was added to the 1 g NCP to make a natural cocoa solution (NCS),
- The 20% (w/v) NCS solution was given to the rats through oral administration.

Animals

Twenty-four healthy Sprague Dawley rats of 11–12 weeks of age and weighing 150–250 g were acquired from the Center for Scientific Research into Plant Medicine, Mampong, Ghana. They were housed in the laboratory animal house of the Department of Anatomy, University of Health and Allied Sciences, Ho Teaching Hospital, for two weeks to achieve acclimatization before the commencement of the experiment. They were kept in cages at a laboratory temperature condition of 24–27°C and a 12-hour light-dark cycle. The animals were fed on grower mash and had free access to water ad libitum throughout the study. All the animals were treated according to the National Institute of Health Guidelines for the care and use of laboratory animals (NIH, Department of Health, and Human Services Publication no. 85-23, revised 1985). The study was approved by the Committee on Human Research, Publication, and Ethics of the School of Medicine and Dentistry, Kwame Nkrumah University of Science and Technology, with reference number: CHRPE/RC/205/17. Only male rats were used for the study because female rats are more

susceptible to alcoholic hepatotoxicity and are more likely to die early.[12]

Experimental design

The 24 mature male albino rats were randomly assigned to four groups (n=6) as follows:

1. Group A: This was the control group given 1.5 mL of physiological saline through oral administration every day for 63 days
2. Group B or the alcoholic bitters (ALC_B) group received a daily dose of 1000 mg/kg of 42% (v/v) alcoholic bitters
3. Group C or the NCP group – the third experimental rats were given 1000 mg/kg body weight of a 20% (w/v) NCS
4. Group D: Alcoholic Bitters + NCP (ALC_B + NCP) – the group D rats were given Alcoholic Bitters + NCS at a dosage of 1000 mg/kg of 42% (v/v) of alcoholic bitters and 1000 mg/kg of 20% (w/v) of NCS daily.

All treatments were administered between 9:00–11:00 am by oral administration daily for 63 days following a recommendation by Srikanth et al. and Siervo et al.[13-14] The body weight of each rat was determined using an animal weighing balance on the first day to serve as a baseline and repeated weekly throughout the entire period of the treatment.

Determination of parameters for biochemical assays

Twenty-four hours after the last treatment, the rats were put under light thiopental sodium anaesthesia and blood samples were taken by cardiac puncture. The blood samples were collected into plain tubes for liver and renal biochemical assays. The blood samples in the plain tubes were centrifuged at 4,000 rpm for 15 minutes and the supernatant (serum) was separated and stored at a temperature of –4°C until it was used for assaying. The serum biochemistry was performed with the VITROS® 5600 Integrated System (Ortho Clinical Diagnostics, Raritan, New Jersey, USA). Parameters that were determined for the liver function test were aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma-glutamyl transpeptidase (GGT), total bilirubin (T-BIL), direct bilirubin (DBIL), indirect bilirubin (I-BIL), total-protein (T-PROT), albumin and globulin. The renal function test parameters were serum sodium (Na^+), serum potassium (K^+), chloride (Cl^-), blood urea nitrogen (BUN) and serum creatinine.

Data analysis

Statistical analyses were carried out using the IBM Statistical Package for Social Sciences (SPSS) software (SPSS 24.0 version, Inc., Chicago, IL, USA) and GraphPad Prism 8.4.2 (GraphPad Software, Inc., San Diego, CA, USA). The values were expressed in means±standard error of the mean (SEM). Normal distribution was tested with the one-sample Kolmogorov-Smirnov test and Shapiro-Wilk normality test. Mean differences among the control and treatment groups were tested with the analysis of variance test (ANOVA), followed by Tukey's Multiple Comparison (TMC) Test. The level of statistical significance was determined at $p < 0.05$ (95% confidence interval).

Results

Descriptive statistics of the weight of organs of the Sprague Dawley rats

Table 2 presents the descriptive statistics from a one-way analysis of variance (ANOVA) of the weight of the liver and the kidneys of the rats post-treatment. The weight of the liver in the control group was 6.54±0.58 g. This was higher than observed for alcoholic bitters and in the NCP groups (6.27±0.50 and 6.19±0.47 g) respectively, but lower than in the ALC+NCP (6.55±0.32 g) group. However, the differences were not statistically significant (p=0.9255). The mean weight of the right and left kidneys in the control group were 0.60±0.05 g and 0.66±0.87g espectively. There was no statistically significant variations of means of the liver and kidney indices among the groups (p>0.05).

Table 2. Weights of organs of Sprague Dawley rats treated with alcoholic bitters and NCP*

Organs	Control	ALC_B	NCP	ALC_B+NCP	p
Liver (g)	6.54±0.58	6.27±0.50	6.19 ±0.47	6.55 ±0.32	0.926
Right kidney (g)	0.60±0.05	0.63 ±0.03	0.76 ±0.09	0.67 ±0.03	0.476
Left kidney (g)	0.66±0.87	0.65 ±0.05	0.70 ±0.09	0.64 ±0.01	0.733

* Data recorded in mean±standard error of the mean, ALC_B – alcoholic bitters, NCP – natural cocoa powder, ALC_B/NCP – alcoholic bitters + NCP, statistically significant difference (p<0.05)

Descriptive statistics and ANOVA of liver serum biochemical indices of Sprague Dawley rats

Table 3 shows the mean values and one-way analysis of variance (ANOVA) of the liver function test results of the Sprague Dawley rats fed with alcoholic bitters and natural cocoa powder. The four liver enzymes; AST, ALT, ALP and GGT, demonstrated no statistically significant differences between the groups. There was, however, a numerically higher mean value of GGT (53.80±27.74 U/L) of ALC_B than the other treatment groups as well as the control group. Also, the mean values of ALT (121.50±40.89 U/L) and ALP (339.00±64.31 U/L) of the control group were numerically higher than in the treatment groups. The AST/ALT ratio was not statistically different between the groups (p>0.05). Regarding the bilirubin parameters, the total and direct bilirubin

showed no significant difference between all the groups. There were, however, higher mean values for total bilirubin (43.81±24.2 µmol/L) and direct bilirubin (21.84±0.2 µmol/L) for the ALC_B than the control group and the ALC_B + NCP group. The indirect bilirubin mean values for the ALC_B and NCP were significantly higher (p=0.0021) than the control group. On the protein indices, total protein, albumin, and Alb/Glob ratio indicated no significant variations between the groups. The globulin level (38.33±1.71 g/L) of the ALC_B group was significantly higher (p=0.0124) than the NCP, ALC_B + NCP and the control groups.

Table 3. Liver serum biochemical indices of Sprague Dawley rats fed with alcoholic bitters and NCP*

Parameters	Control	ALC_B	NCP	ALC_B+NCP	p
AST (U/L)	147.40±9.03	325.20±46.51	300.30±85.05	286.80±70.82	0.152
ALT (U/L)	121.50±40.89	96.40±19.31	103.67±45.24	43.33±15.38	0.437
GGT (U/L)	10.25±0.25	53.80±27.74	11.00±0.30	11.67±1.67	0.333
ALP (U/L)	339.00±64.31	251.80±61.37	215.00±95.28	215.00±52.37	0.571
AST/ALT ratio	3.14±1.69	2.80±0.65	2.73±0.82	2.55±0.19	0.996
T/Bilirubin (µmol/L)	13.00±5.37	43.81±24.2	8.99±2.59	5.48±3.32	0.357
D/Bilirubin (µmol/L)	13.00±5.39	21.84±0.2	7.78±0.51	3.41±2.23	0.271
I/Bilirubin (µmol/L)	0.39±0.39	5.82±0.69	4.23±0.45	2.25±2.24	**0.002**[ab]
T/protein (g/L)	66.26±3.94	77.33±5.46	78.67±4.81	76.50±3.94	0.095
Albumin (g/L)	35.00±1.91	58.80±21.68	40.67±4.06	34.33±2.60	0.592
Globulin (g/L)	31.28±2.2	38.33±1.71	37.77±1.18	37.23±2.31	**0.012**[ade]
Alb/Glob ratio	1.15±0.05	1.00±0.06	1.00±0.03	1.05±0.02	0.199

***** Data recorded in mean±standard error of the mean, ALC_B – slcoholic bitters, NCP – natural cocoa powder, ALC_B+NCP – alcoholic bitters + NCP, a – control versus ALC_B, b – control versus NCP, c – control versus ALC_B + NCP, AST – aspartate aminotransferase, ALT – alanine aminotransferase, GGT – gamma glutamyl transpeptidase, ALP – alkaline phosphatase, Alb/Glob – albumin/globulin ratio, statistically significant difference (p<0.05)

Descriptive statistics and ANOVA of kidney serum biochemical parameters of Sprague Dawley rats

Table 4 shows the renal biochemical parameters of the Sprague Dawley rats that received the alcoholic bitters and the natural cocoa powder for the period of the treatment. The one-way analysis of variance (ANOVA) of the renal parameters showed no statistically significant variations among the groups. There was no significant difference in the means of urea nitrogen between the treatment groups and the control group. However, the mean creatinine level (50.37±4.11 mmol/L) of the co-administered group was found to be statistically significantly higher than the rats that received alcoholic bitters only ((ALC_B) and control group).

Table 4. Renal serum biochemical Indices of Sprague Dawley rats fed with alcoholic bitters and NCP*

Parameters	Control	ALC_B	NCP	ALC_B+NCP	p
Sodium (mmol/L)	139.75±0.85	137.6±2.42	136±0.1	137.67±1.2	0.58
Potassium (mmol/L)	7.50±0.81	8.36±1.63	8.83±0.96	5.70±0.81	0.451
Chloride (mmol/L)	101.75±0.75	101.40±2.8	105±0.04	100.67±1.2	0.7
Urea nitrogen (mmol/L)	7.40±1.38	5.70±0.74	8.30±0.66	5.33±0.18	0.18
Creatinine (mmol /L)	33.36±2.89	37.06±3.26	39.73±4.6	50.37±4.11	0.006[ce]

***** Data recorded in mean±standard error of the mean, ALC_B – slcoholic bitters, NCP – natural cocoa powder, ALC_B+NCP – alcoholic bitters + NCP, statistically significant difference (p<0.05)

Discussion

The biochemical profile including the liver function test and kidney function test were assessed in the current study. The present study revealed no stastitically significant difference in the values of the liver enzymes (AST, ALT, GGT and ALP) of the rats fed with alcoholic bitters, natural cocoa powder and the co-administered groups. The findings of the present study disagree with a previous study by Johnson et al. who observed a significant increase in AST, ALT, GGT and ALP in rats that received alcoholic bitters.[15] Another study by Adias et al. reported a dramatic rise of AST and GGT among chronic alcohol drinking participants in Nigeria compared with their nondrinking counterparts.[16] Similarly, a previous report in Ghana among 60 total abstainers, 56 social drinkers and 100 alcoholics, indicated that GGT and AST were sufficiently sensitive to detect chronic alcoholics and that the serum GGT and AST showed a progressive increase with increasing alcohol intake.[17] The enzymes AST and ALT were also found to increase in a study by Shair et al.[18] The present study, though not statistically significant, it showed the AST and GGT values were numerically higher in the alcoholic bitters group than the control, NCP only and co-administered groups. Typically, elevated ALT and AST levels reflect the extent of hepatocellular injury while elevated serum ALP and GGT levels indicate the extent of impaired bile flow or cholestasis.[19] In particular, GGT is well known as a marker of alcohol consumption and correlates to alcohol consumption.[20] For instance, GGT was reported to be the most significant associations and ALT level the weakest relationship in alcohol consumption.[21] This could be the reason for the increased level of GGT in the present study. And while AST and ALP did not increase after alcoholic bitters treatment, these enzymes may not be sufficiently sensitive to detect minimal acute hepatotoxicity.[22] Gamma-glutamyl transpeptidase is present in the cell membranes of the tissues of certain organs, including the liver, kidney, spleen, pancreas and heart. Chronic alcohol use causes those cells to become inflamed and necrotic causing an increase in the amount of GGT to leak out.[23] The finding of the high

GGT of this study could therefore be a demonstration of hepatocellular injury and/or other organs in the rats that received the alcoholic bitters treatment. The differences in finding of the present study and the previous studies could be due to the pattern of drinking and type of drinks consumed. Aside from this, alcoholic liver disease has a multi-factorial aetiology, including environmental factors and genetics and might not be exactly same between animal and human studies.[24] Stranges et al. reported the possibility of intermittent heavy use of alcohol to have a greater influence on GGT blood levels than a regular one.[21] In this study, alcoholic bitters were regularly administered to rats and of the same type and this might be the reason for the lack of a significant increase in GGT because according to Bellentani et al., drinking alcohol outside mealtimes and drinking multiple different alcoholic beverages increase the risk of developing alcohol induced liver damage.[25] The postulation is that, heavy drinkers tend to have a raised mean cell volume, high GGT, but only a slightly raised serum alkaline phosphatase.[26] The mean GGT for the rats in the alcoholic bitters group in the present study was numerically higher than the control group, natural cocoa powder and co-administered natural cocoa powder and alcoholic bitters groups as well as previous studies using similar rats.[27,28] This indicates that cocoa might offer a protective effect on the liver of the rats which were fed with the cocoa and alcohol bitters. It is also confirmed in natural cocoa powder treated rats not having significant increase of the liver enzymes and this corroborates previous similar study, where consumption of cocoa extract alone showed no significant difference compared to control group for the liver enzymes.

Furthermore, this study found that the total bilirubin (direct and indirect) was significantly higher in the rats that received alcoholic bitters than the control and the other treatment groups. This supports previous studies by O'Malley et al. and Tanaka et al. where increased mean serum total bilirubin following alcohol consumption were observed.[29,30] The high total bilirubin level might be due to alcohol being a competitive inhibitor of bilirubin conjugation. Consistent with this possibility, both indirect (unconjugated) bilirubin and direct (conjugated) bilirubin of rats fed with the alcoholic bitters are significantly higher than those in the natural cocoa powder and co-administered natural cocoa powder and alcoholic bitters groups. Thus, while hepatic clearance of ethanol is primarily catalyzed by alcohol dehydrogenase, the microsomal ethanol-oxidizing system and aldehyde dehydrogenase, a minor, but forensically significant clearance pathway, involves ethanol conjugation with glucuronic acid.[29,31] The pathway for glucuronidation of ethanol involves UDP-glucuronosyltransferase 1A1 (UGT1A1), which is also primarily responsible for glucuronidation of bilirubin.[29] Ethyl glucuronide (EtG) is a direct phase-II metabolite of ethanol formed through the UGT1A1 catalyzed conjugation of ethanol with glucuronic acid.[32] Thus, forensic scientists have been using the advantageous properties of EtG in studying drunk driving cases, covert alcohol use among psychiatric inpatients and multiple other situations in which alcohol consumption was thought to play a role.[31] The high bilirubin observed in the present study among rats fed with alcoholic bitters might be due to slight hemolysis. For instance, Padmini and Sundari reported that erythrocytes from alcoholics have significantly decreased resistance to hemolysis in comparison to non-alcoholics.[33] However, the rats that received the co-administration of alcoholic bitters and natural cocoa powder has low bilirubin values. This indicates that cocoa plays a role in the excretion of bilirubin.

The kidney is the site of accumulation of chemicals; hence urea and creatinine are sensitive and reliable biochemical indices for evaluation of renal function.[34] In the present study, the means of urea nitrogen and creatinine of the group treated with alcoholic bitters alone were higher than the natural cocoa powder only, co-administered and control groups. This finding corroborates several previous study reports where higher than normal values of the parameters were recorded in rats.[29,35,36] The elevated serum creatinine is an indication of injury of the kidney due to the alcoholic bitters intake and hence the bitters can be said to have a reno-toxic effect on the kidneys of the rats. Thus, urea is formed in the liver, and is excreted by the kidney and elevation of it indicate kidney injury, with resultant reduced glomerular filtration.[37.]

The rat that received co-administered alcoholic bitters and natural cocoa powder showed no elevation of urea nitrogen, creatinine as well as electrolytes. This implies that the natural cocoa powder preserved the renal integrity and did not affect their capacity to excrete these ions. A previous study reported the capability of cocoa to prevent or reduce the complications in chronic kidney disease.[38]

Study limitations

The authors wish to acknowledge some limitations of the study which could be considered in future similar studies. The concentration of the Ghanaian alcoholic bitters, and the natural cocoa powder were not given in difference doses to difference groups. That could determine the effective dosage of the natural cocoa powder. Furthermore, the antioxidants and methylxathine quantities were not determined in the natural cocoa powder that was used, though only theobromine was stated in the manufacturer's information sheet. The study was also limited in the fact that the constituents of the Ghanaian alcoholic bitters were not determined.

Conclusion

This study found no significant difference of the liver enzymes between control and treatment groups. There was, however, numerically high values of AST and GGT in Sprague Dawley rats treated with Ghanaian alcoholic bitters indicating injury to the liver. The Ghanaian alcoholic bitters also appeared to have a reno-toxic effect as there were elevated serum creatinine in Ghanaian alcoholic bitters fed rats. The natural cocoa powder could offer a protective effect on the liver and kidney of the rats which were fed with both alcoholic bitters and cocoa powder together. Further studies on different doses of alcoholic bitters and natural cocoa powder are recommended.

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Declarations

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Author contributions

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

Conflicts of interest

The authors declared no conflict of interest

Data availability

The datasets used during the current study are available from the first author on reasonable request.

Ethics approval

Ethical approval was obtained from the Committee on Human Research, Publication, and Ethics of the School of Medicine and Dentistry, Science Kwame Nkrumah University of Science and Technology, with reference number: CHRPE/RC/205/17.

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ORIGINAL PAPER

Stigma and its relationship with life satisfaction in patients with type 2 diabetes mellitus

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ABSTRACT

Introduction and aim. This study aims to determine the level of stigma in patients with type 2 diabetes mellitus (T2DM) and evaluate its relationship with their life satisfaction.

Material and methods. This is a descriptive and cross-sectional study conducted in a single tertiary care center. Two hundred and ten patients ≥18 years old diagnosed with T2DM were included in this study. The relationship between the "Type 2 diabetes stigma assessment scale" and the "Satisfaction with life scale" was assessed using Pearson correlation analysis.

Results. The average age of the participants was 54.85±15.81 years. The majority of patients reported adherence to diabetes treatment and having comorbidities. Stigma levels were higher in patients ≤65 years, those with less than a high school education, and those receiving insulin or oral anti-diabetic treatment, but lower with a T2DM diagnosis duration of ≤ 5 years ($p<0.05$). Life satisfaction was influenced by age, education status, economic status, adherence to treatment, dietary compliance, and the presence of comorbidities. A moderately negative relationship between stigma level and life satisfaction was identified.

Conclusion. The study's results indicate that an increase in stigma level is associated with a decrease in life satisfaction in patients with type 2 diabetes mellitus.

Keywords. life satisfaction, stigma, type 2 diabetes mellitus

Introduction

It is estimated that there are approximately 537 million adults aged between 20–79 years with type 2 diabetes mellitus (T2DM) diabetes worldwide. This constitutes the vast majority of diabetes cases (over 90%) and affects more than 220 million individuals globally.[1] In Türkiye, it is estimated that 42% of the adult population has either diabetes or prediabetes.[2] Diabetes is a disease that can lead to psychological, social, and psychosocial issues for patients. The psychological well-being of individuals with diabetes can adversely affect the management of the disease. Stigma, one of the factors influencing psychological well-being, is a significant problem experienced by a high proportion of individuals with diabetes.[3-4]

Stigma is defined as "a significant deterioration of one's reputation or devaluation."[5] Individuals with chronic illnesses can experience stigma due to their conditions.[4,6] In the literature, while there are numerous studies on the medical aspects and physical complications of diabetes, relatively few studies have focused on the stigma faced by patients with T2DM due to their diabetic status.[3] A multinational study reported that one out of every five people with diabetes experienced discrimination.[7] In a study by Abdoli et al., participants stated experiencing stigma in all as-

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pects related to diabetes and feeling deprived of a normal life.[8]

Experienced or perceived stigma can significantly damage a person's social identity. Health-related stigma serves as a barrier to seeking help and participating in health services, hindering efforts to improve health.[9-10] Stigma in diabetes is associated with poor disease management. It can present a barrier to accessing diabetes-related services, employment, and marriage opportunities, thereby hindering patients with diabetes from playing an active role in society.[1] Increased stress due to experiencing stigma can negatively impact quality of life by leading to behavioral changes in managing the disease and non-adherence to treatment.[11-13]

Life satisfaction refers to a cognitive/judgmental process and is defined as the overall assessment of individuals' quality of life according to their chosen criteria. It is one of the fundamental elements that people need to be happy and find meaning in their lives.[14] Based on the literature, studies were found reporting that high levels of stigma in type 2 diabetes were associated with less participation in recommended diabetes self-management behaviors, including diet and physical activity behaviors.[15-17] These negative judgments can affect individuals' self-care behaviors and change their life satisfaction.[4,18] Perksy et al. reported in their study that stigma was associated with reduced self-care, increased symptoms, and reduced life satisfaction in individuals with type 2 diabetes.[18] However, there is not enough study examining the level of stigma in patients with T2DM and its relationship with life satisfaction.

Research questions:

1. What is the level of stigma and life satisfaction in patients with T2DM?
2. Is there a relationship between stigma and life satisfaction in patients with T2DM?

Aim

This study examined the level of stigma in patients with T2DM and its relationship with life satisfaction.

Material and methods

This is a descriptive and cross-sectional study. Data were collected through surveys between January and March 2023. The surveys were conducted face-to-face with individuals and took an average of 10-15 minutes. The population of the study consisted of patients diagnosed with T2DM in an education and research hospital in Istanbul. The hospital offers 60 internal medicine beds, 20 of which are set aside for diabetic patients, with roughly 80–100 diabetes mellitus patients admitted per month. The sample size for the study was determined as 207 patients, calculated using the formula n=t2pq/d2 based on unknown population parameters. All patients who were admitted to the clinic with the diagnosis of T2DM on the dates of the study and who met the inclusion criteria of the study were included. Patients aged 18 and above, diagnosed with T2DM, and willing to participate in the study were included. Patients with communication problems and those who did not agree to participate were excluded from the study. The study was completed with 210 participants.

Data collection

Data were collected using the "Data collection form," which was developed based on the literature, the "Type 2 diabetes stigma assessment scale (DSAS-2)," and the "Satisfaction with life scale (SLS)."

Data collection form

This form consists of 20 questions about the participants' socio-demographic characteristics and conditions related to diabetes (duration of illness, comorbidities, family history of diabetes, etc.).

Type 2 diabetes stigma assessment scale (DSAS-2)

A scale developed by Browne et al. and adapted to Turkish by İnkaya and Karadağ is based on a 5-point Likert-type scale and comprises 19 items.[5,19] The scale has three subscales: 1) Treated differently, 2) Blame and judgment, and 3) Self-stigma. The total stigma score ranges from 19 to 95 points, with a higher score indicating a higher level of stigma. The Cronbach's alpha value for this scale was determined to be 0.82. In this study, the Cronbach's alpha values were found to be 0.94 for the total stigma level, 0.81 for the treated differently subscale, 0.89 for the blame and judgment subscale, and 0.79 for the self-stigma subscale.

Satisfaction with life scale (SLS)

This scale provides a general assessment of individuals' life satisfaction. Developed by Diener, Emmons, Larsen, and Griffin and adapted to Turkish by Dağlı and Baysal, the scale is a 7-point Likert-type (1: Strongly disagree – 7: Strongly agree) and consists of five items.[14,20] A higher score on the scale indicates higher life satisfaction. The Cronbach's alpha value for this scale is 0.88, and it was found to be 0.89 for this study.

Ethical considerations

This study adheres to the ethical principles outlined in the Declaration of Helsinki. The necessary permissions were obtained from the institution where the study was conducted. The University Institutional Review Board approved the study (IRB date and number: 14.10.2022/2022.135). Both verbal and written informed consent were obtained from participants who met the study criteria. Participants were assured that their responses would remain anonymous and confidential.

Table 1. Socio-demographic and clinic data of the participants (n=210)*

Age	Mean ± SD (Min.–Max.)	54.85±15.81 (19–93)	
		n	%
BMI (kg/m²)	Normal	49	23.3
	Overweight	100	47.6
	Obesity	61	29.0
Gender	Female	126	60.0
	Male	84	40.0
Marital status	Married	163	77.6
	Single	47	22.4
Educational status	Literate	57	27.1
	Primary	80	38.1
	High school	42	20.0
	Bachelor's degree	31	14.8
Economic status	Income more than expenses	68	32.4
	Income partially covers expenses	78	37.1
	Income less than expenses	64	30.5
Working status	Working	98	46.7
	Not working	112	53.3
Home status	Alone	27	12.9
	Living with family	183	87.1
Family history of diabetes	Yes	129	61.4
	No	81	38.6
Duration of illness	≤ 5 years	61	29.0
	6-10 years	61	29.0
	≥ 11 years	88	42.0
Type of diabetes treatment	Insulin	66	31.4
	OAD	67	31.9
	Insulin + OAD	77	36.7
Treatment adherence	Yes	103	49.0
	Partially	71	33.8
	No	36	17.1
Compliance to diet	Yes	64	30.5
	Sometimes	80	38.1
	No	66	31.4
Regular exercise	Yes	37	17.6
	Sometimes	84	40.0
	No	89	42.4
Comorbidity	Yes	124	59.0
	No	86	41.0
Regular physician follow-up	Yes	135	64.3
	No	75	35.7

* OAD – oral antidiabetic drug

Statistical analysis

Data analysis was conducted using SPSS Statistics 24.00 software. Continuous variables were presented as means ± standard deviation (SD), and categorical variables were expressed as percentages. A comparison of patients' DSAS-2 and SLS scores across age, economic status, educational status, presence of comorbidities, treatment adherence, diet compliance, and duration of illness was performed using an independent sample t-test and one-way analysis of variance (ANOVA). Tukey's HSD and LSD tests were employed for post hoc analysis. Pearson correlation analysis was conducted to assess the relationships between continuous variables. Two-sided p values <0.05 were considered statistically significant.

Results

The mean age of the study participants was 54.85±15.81 years. The majority of patients were female (60%), married (77.6%), not employed (53.3%), and living with their families (87.1%). Additionally, the majority had a family member with a diabetes diagnosis (61.4%), and 42% had a T2DM diagnosis for 11 years or longer. The other socio-demographic and clinical characteristics of the participants are presented in Table 1.

The DSAS-2 total score mean for the patients was determined to be 62.32±18.13. When evaluating the DSAS-2 subscales, the mean score for "treated differently" was 20.09±5.77, "blame and judgment" was 22.71±7.36, and "self-stigma" was 19.52±5.65 (Table 2). Statistically significant higher DSAS-2 scores were found in patients aged 65 and under, those with less than a high school education, and those receiving insulin or oral anti-diabetic (OAD) treatment. Further, patients with a T2DM diagnosis duration of 5 years or less had significantly lower DSAS-2 mean scores compared to others (p<0.05) (Table 3).

Table 2. Type 2 diabetes stigma assessment scale and satisfaction with life scale scores of the participants*

	n=210	Mean	SD	Min	Max
DSAS-2	Treated differently	20.09	5.77	6	30
	Blame and judgment	22.71	7.36	7	35
	Self-stigma	19.52	5.65	6	30
	Total Score	62.32	18.13	19	95
SLS	Total Score	12.33	4.27	5	25

* DSAS-2 – type 2 diabetes stigma assessment scale, SLS – satisfaction with life scale

The total mean score for the SLS among participants was 12.33±4.27 (Table 2). Statistically significantly higher SLS scores were found in patients under 65 years of age, those with a high school education or higher, those with a good income, those adhering to treatment, those adhering to the diet, and those without comorbidities alongside diabetes (p<0.05) (Table 3).

Life satisfaction total scores exhibited moderately negative correlations with both DSAS-2 total scores (r=-0.45, p<0.01) and all DSAS-2 subscales (respectively treated differently r=-0.44, p<0.01; blame and judgment r=-0.41, p<0.01; self-stigma r=-0.46, p<0.01) (Table 4).

Table 3. Factors affecting participants' levels of stigma and life satisfaction[a]

	Type 2 diabetes stigma assessment scale								Satisfaction with life scale	
	Treated differently	Test, p	Blame and judgment	Test, p	Self-stigma	Test, p	Total score	Test, p	Total score	Test, p
Age										
> 65 year	19.64±6.01	-2.116*	22.09±7.58	-2.064*	19.09±5.96	-1.879*	60.82±18.91	-2.265*	12.68±4.40	2.014*
≤ 65 year	21.36±4.87	0.03	24.45±6.42	0.04	20.74±4.48	0.03	66.56±15.07	0.02	11.34±3.71	0.04
Educational status										
< High school	20.93±5.38	2.835*	23.58±6.93	2.366*	20.30±5.19	2.628*	64.81±16.86	2.835*	11.79±4.02	-2.570*
≥ High school	18.51±6.17	0.005	21.08±7.88	0.01	18.07±6.18	0.01	57.66±19.57	0.009	13.36±4.55	0.01
Economic status										
More than expenses	19.21±6.10	1.322 π	21.34±7.55	1.862 π	18.55±6.02	0.754 π	59.44±19.04	1.414 π	13.69±4.44	6.346 π
Partially covers expenses	20.74±5.43	0.27	23.62±7.03	0.16	19.97±5.51	0.47	64.33±17.23	0.25	12.13±4.10	<0.001
Less than expenses	20.23±5.79		23.06±7.43		19.69±5.42		62.98±18.08		11.14±3.92	
Diabetes treatment										
Insulin	19.20±6.29	5.000 π	21.76±8.05	6.213 π	18.77±6.09	3.265 π	59.73±19.74	5.204 π	12.21±4.27	0.922 π
OAD	19.10±5.51	0.01	21.04±6.41	<0.001	18.78±5.37	0.04	58.93±16.70	0.01	12.90±4.70	0.40
Insulin + OAD	21.71±5.22		24.97±7.02		20.82±5.31		67.51±16.87		11.95±3.85	
Treatment adherence										
Yes	20.00±6.13	0.218 π	22.88±7.79	0.187 π	19.40±6.06	0.156 π	62.28±19.33	0.152 π	13.09±4.15	4.393 π
Partially	20.67±5.29	0.80	23.06±6.85	0.83	20.00±4.49	0.86	63.72±16.53	0.86	10.75±4.75	0.01
No	19.93±5.53		22.28±7.03		19.46±5.39		61.68±17.28		12.04±3.97	
Compliance to diet										
Yes	18.44±5.92	3.880 π	21.48±7.77	1.333 π	18.09±5.83	3.192 π	58.02±18.70	2.698 π	13.72±4.32	5.044 π
Partially	20.79±5.74	0.02	23.03±7.45	0.27	19.85±5.36	0.04	63.67±17.96	0.07	11.74±4.32	0.01
No	20.84±5.47		23.43±6.90		20.40±5.57		64.66±17.40		11.71±3.96	
Comorbidity										
Yes	19.40±5.78	-1.457*	22.08±7.41	-1.031*	18.99±5.63	-1.146*	60.47±18.14	-1.239*	11.84±4.07	2.033*
No	20.57±5.74	0.15	23.15±7.32	0.30	19.90±5.65	0.25	63.61±18.08	0.22	13.05±4.46	0.04
Duration of illness										
≤ 5 years	18.52±5.84	3.232 π	20.80±6.93	2.942 π	18.05±5.68	3.195 π	57.38±17.76	3.290 π	13.29±5.18	2.693 π
6-10 years	20.75±5.42	0.04	23.46±7.18	0.06	20.47±5.38	0.04	64.69±17.31	0.03	12.34±3.71	0.07
≥ 11 years	20.71±5.82		23.51±7.59		19.89±5.66		64.11±18.46		11.66±3.82	

[a] OAD – oral antidiabetic drug * – independent samples T-test, π – one-way analysis of variance (ANOVA) p<0.05

Table 4. Relationship between participants' levels of stigma and life satisfaction[a]

n=210		SLS Total	DSAS-2 Total	Treated differently	Blame and judgment	Self-stigma
SLS Total	r	1				
DSAS-2 Total	r	-0.45*	1			
Treated differently	r	-0.44*	0.97*	1		
Blame and judgment	r	-0.41*	0.97*	0.91*	1	
Self-stigma	r	-0.46*	0.96*	0.91*	0.87*	1

[a] DSAS-2 – type 2 diabetes stigma assessment scale, SLS – satisfaction with life scale, r – Pearson correlation *p< 0.01

Discussion

Determining the level of stigma in patients with diabetes is crucial for nurses to understand their patients comprehensively and contribute to individualized diabetes management strategies.[6,10] In this study, both stigma and life satisfaction were found to be at a moderate level among patients with T2DM. The study's results indicate that an increase in stigma level is associated with a decrease in life satisfaction in patients with T2DM.

For patients with T2DM, accepting that they have a chronic illness and need to change their lifestyle can be challenging, and the pressure and stigma they face from people around them can lead to psychosocial problems.[21] Stigma, with its prejudiced attitude and behaviors that may lead to discrimination against the individual, further complicates disease management.[17] In a study by Himmelstein and Puhl with 1212 T2DM patients in the U.S. (2021), participants reported frequent experiences with diabetes-related stigma, including blame and judgment, self-stigma, and differential treatment. Additionally, the same study found that participants experienced high levels of stigma.[3] Similar studies have indicated that patients with T2DM experience significant levels of stigma to varying degrees.[4-5,22] This study also aligns with the literature, showing that patients experience stigma. Zhang et al.'s study involving 453 young and middle-aged patients with T2DM showed that the duration of diabetes, monthly income, and insulin treatment were significant factors influencing stigma. Age and education level did not affect stigma, but patients with a shorter duration of diabetes in the study group reported higher stigma levels.[22] In Hansen et al.'s study, patients with a shorter period of diabetes also had higher levels of stigma.[23] These results have been associated with insufficient knowledge about the disease, leading to poor disease control and individuals being more susceptible to stigmatization in their surroundings. In this study, the participants' levels of stigma were found to be moderate. It was observed that patients under 65, those with a high school education

or higher, those using both insulin and OAD, those not adhering to their diet, and those with a disease duration of 6 years or longer had higher stigma levels – this suggests that the level of stigma in individuals with T2DM may vary depending on cultural differences and individual characteristics.

Stigma in patients with diabetes is more related to the treatment process than the symptoms of the disease.[6] Insulin injections, blood sugar monitoring, dietary restrictions, hypoglycemic attacks, and more can contribute to an individual's experience of diabetes-related stigma.[10] Stigma experiences have negative implications for treatment, including insulin appraisals.[24] In Liu et al.'s study involving 12,000 participants, it was noted that patients using intensive insulin experienced stigma more frequently.[10] In line with the literature, in this study, patients using both insulin and OAD, those not adhering to the diabetes treatment, and those with a disease duration of six years or longer exhibited significantly higher levels of stigma. These results may be associated with the parallel increase in stigma with the duration of diabetes diagnosis, which could lead to both effective and unsuccessful disease management.

The life satisfaction levels of the participants in this study were found to be at a moderate level. In Rodríguez-Almagro et al.'s study, patients with T2DM had a moderate quality of life, with a lower quality of life observed in young and female participants compared to the other group.[25] In other studies in the literature, lower education levels and longer disease durations have been associated with lower life satisfaction.[26-28] In this study, no statistically significant difference was observed between genders; however, patients under 65 years of age, those with higher education levels, those with better economic conditions (income more than expenses), those who adhered to treatment and diet, and those without additional diseases had higher life satisfaction. These results suggest that patients who manage their disease and adhere to treatment have higher life satisfaction. Furthermore, this research found that an increase in stigma level is associated with a decrease in life satisfaction in patients with T2DM. In their study, Kato et al. emphasized the relationship between self-stigma level and self-esteem.[4] Besides the challenges in managing the disease, the prejudiced attitudes reflected by the social environment can lead individuals to distance themselves from society and self-stigma.

Study limitations

The study's limitations include its single-center design and the use of surveys, which may introduce selection bias. Treatment adherence, compliance to diet, and regular exercise was assessed without the use of a scale. Data regarding these parameters were obtained based on the patient's declaration.

Conclusion

In this study, it was determined that patients with T2DM had moderate levels of both stigma and life satisfaction. The study's results revealed that an increase in stigma level is associated with a decrease in life satisfaction in patients with T2DM.

Recognizing, monitoring, and evaluating self-stigma symptoms in patients is crucial for both nurses working in diabetes clinics and diabetes education nurses – this can contribute to enhancing patients' self-care motivation and reducing complications associated with the disease. Identifying psychosocial factors that may lead to stigma in patients, reducing disease stigma, developing coping strategies for dealing with negative emotions, providing counseling to reduce disease-related stress, and promoting diabetes knowledge among the public to prevent discrimination should be targeted to achieve this goal.

Declarations

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Author contributions

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

Conflicts of interest

The authors declare that there is no conflict of interest regarding this article.

Data availability

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

Ethics approval

The University Institutional Review Board approved the study (IRB date and number: 14.10.2022/2022.135).

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ORIGINAL PAPER

Patterns and characteristics of traumatic dental injuries in children – a retrospective study in a dental hospital in Kolkata, India

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ABSTRACT

Introduction and aim. Traumatic dental injuries (TDIs) in children can have aesthetic, functional, and psychological consequences. This retrospective study aimed to investigate the epidemiology and patterns of TDIs in a hospital-based population.

Material and methods. Patient records from the Department of pediatric and Preventive Dentistry in a dental college and hospital in Kolkata, India, were reviewed. A total of 381 patients, aged 1 to 12 years, who presented one or more traumatized anterior teeth between September 2018 and August 2022, were included. Data on age, sex, mechanism of injury, type of trauma, dentition type, and number of affected teeth were collected. The World Health Organization classification system was used to classify the type of dental trauma.

Results. Among the study population, there were 235 males (61.7%) and 146 females (38.3%), with a mean age of 7.67±3.87 years. Falls were the most common cause of trauma (52.2%). The maxillary central incisors were the most frequently affected teeth in both primary and permanent dentitions.

Conclusion. Falls were the most common cause of trauma and the maxillary central incisors were the most commonly affected teeth in children.

Keywords. anterior teeth, child, dental trauma, epidemiology, primary/permanent teeth

Introduction

Traumatic dental injuries (TDIs) pose a significant public health problem due to their potential to cause aesthetic, functional, and psychological disturbances, particularly in children. These injuries account for approximately 5% of all bodily traumatic injuries and display a wide-ranging prevalence of anterior tooth injuries globally, ranging from 4% to 58%.[1,2] The variation in prevalence can be attributed to various factors, such as differences in trauma classification, study types, sample sizes, diagnostic criteria, and cultural behaviors.[2-5]

While TDIs can affect individuals of any age, they are more commonly observed among children and young adults, with the maxillary central incisors and lateral incisors being particularly vulnerable due to their position and exposure.[6-10] Previous studies have indicated a higher incidence of dental trauma in patients with inadequate lip coverage and class II malocclusion.[10-12] Typically, traumatic injuries affect a single tooth, although severe cases may involve multiple teeth. These injuries often lead to discomfort and hinder patients' daily routines. Therefore, there is a need for early intervention and proper management to preserve the affected teeth and reduce potential complications.

The aetiology of dental trauma varies depending on the age group. Falls are the most frequent cause in toddlers and preschool children, while sports-related injuries and interpersonal violence predominate among school-age children and adolescents.[8,13,14]

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Aim

Research suggests that the profiles of dental trauma differ across ethnicities and geographic locations. Unfortunately, limited data are available on dental trauma in the pediatric population in the eastern part of India.

The primary aim of this study is to investigate the characteristics of traumatic dental injuries (TDIs) in children aged one to twelve years, with a focus on demographic factors, injury etiology, and types of dental trauma. Among children who visited a tertiary care hospital in Kolkata, India.

Material and methods

A retrospective analysis of TDI in a pediatric population treated at the Department of Pediatric and Preventive Dentistry, Dr. R. Ahmed Dental College and Hospital, Kolkata, India, between September 2018 and August 2022 was conducted. Approval for the study was obtained from the Institutional Ethics Committee (IEC/DCH/279 dated 28/9/2022). The present study was performed in accordance with the Declaration of Helsinki, and the research adheres to the guidelines outlined in the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) statement.

Selection of patients and data collection

Clinical records of all patients aged 12 years or younger with traumatic injuries to the maxillary and mandibular incisors and canines were reviewed. Both primary and permanent anterior teeth with trauma were considered. Over a four-year period, 388 patients were examined in our department. Incomplete medical records, such as missing data of radiographs, were excluded. Additionally, patients with syndromes, craniofacial anomalies, neuromuscular diseases, physical, or mental retardation were excluded from the study, resulting in the exclusion of seven clinical records. Finally, 381 (235 males and 146 females) patient files were scrutinized and categorized into two age groups: 1-6 years and 7-12 years.

The recorded data included age at the time of injury, gender, mechanism or cause of injury, type of trauma, type of dentition, and the number of affected teeth. Mechanisms of injury were categorized into falls, road traffic accidents, play or sports-related injuries, bicycle accidents, trauma from a tube well handle, interpersonal violence, and animal-related injuries. A tube well is a type of water well that uses a long, narrow pipe (the tube) to draw water from underground sources, mainly in rural areas. A tube well handle is a part of the equipment used to manually operate tube wells. Mishaps, such as slips or improper handling during its operation, can result in the handle colliding with the face, with a particular focus on the mouth region. Such inadvertent contact may induce blunt force trauma, potentially leading to traumatic dental injuries. The type of dental trauma was classified in accordance with the slightly modified World Health Organization (WHO) classification system as proposed by Andreasen et al.[9]

According to this classification, dental trauma included: enamel infraction, enamel fracture, enamel-dentin fracture, complicated crown fracture, uncomplicated crown-root fracture, complicated crown-root fracture, and root fracture and periodontal tissue injuries: concussion, subluxation, intrusion, extrusion, lateral luxation, and avulsion. Teeth affected by trauma were numbered according to the internationally recognized two-digit Federation Dentaire Internationale numbering system. Lip coverage was assessed according to Burden.[11] If the upper incisors were covered by the lip while at rest, it was deemed as adequate lip coverage. Conversely, lip coverage was considered inadequate if most of the upper incisors were visible, or if there was noticeable tension in the lip during closure.

Statistical analysis

The collected data were analyzed using Microsoft Excel 2010 (Redmond, WA, USA) and Epi Info 7 (CDC-INFO, Atlanta, GA, USA). Comprehensive descriptive analyses, including frequencies, proportions, and percentages, were conducted. To determine the statistical significance of the findings, the chi-square test was employed, with a significance level set at $p<0.05$.

Results

The average age of participants in this research was 7.20±3.43 years, with a range of one to 12 years. Among the surveyed individuals, 235 were male (61.7%), and 146 were female (38.3%), resulting in an overall male-to-female ratio of 1.6:1. Specifically, in the 1-6 years age group, the male-to-female ratio was 1.2:1, which increased to 1.8:1 in the 7–12 years age group.

Table 1. Characteristics of patients with traumatic dental injury

Characteristics				n	%
Number of patients	Male	1–6 years	n=84	233	61.2
		7–12 years	n=149		
	Female	1–6 years	n=67	148	38.8
		7–12 years	n=81		
	Total			381	100
Number of Affected teeth	Primary teeth			274	42.7
	Permanent teeth			367	57.3
	Total			641	100
Lip coverage	Adequate			175	45.9
	Inadequate			206	54.1
	Total			381	100

Among the 381 patients, 214 (63.3%) fell within the 7 to 12-year age group, while 167 (36.7%) were in the 1–6-year age group. A total of 641 affected teeth (274

Table 2. Etiology of patients with traumatic dental injury according to gender*

Etiology	Male		Female		Total		Chi-square test (p)	Primary teeth		Permanent teeth		Chi-square test (p)
	n	%	n	%	n	%		n	%	n	%	
Fall	123	52.3	76	52.1	199	52.2	12.95 p=0.04 Df=6	181	66.1	141	38.4	59.59 p<0.001 Df=6
Play	47	20.0	15	10.3	62	16.3		28	10.2	73	19.9	
RTA	24	10.2	24	16.4	48	12.6		40	14.6	65	17.7	
Bicycle	15	6.4	12	8.2	27	7.1		11	4	32	8.7	
Tube well	9	3.8	11	7.5	20	5.2		10	3.7	23	6.3	
IPV	16	6.8	6	4.1	22	5.8		2	0.7	31	8.5	
Animal	1	0.5	2	1.4	3	0.8		2	0.7	2	0.5	
Total	235	100	146	100	381	100		274	100	367	100	

* IPV – interpersonal violence, Chi Square test=12.95, Df=6 p= 0.04

primary teeth and 367 permanent teeth) were observed. Therefore, the number of affected teeth per individual was 1.68. In 206 (54.1%) children with TDI, inadequate lip coverage was observed (Table 1).

Among a total of 274 traumatized primary teeth, 153 (55.8%) were in males and 121 (44.2%) in females. Similarly, of the 367 permanent teeth with TDI, 246 (67%) occurred in males and 121 (33%) in females. The incidence of affected teeth was significantly higher (p=0.004) in males than females.

The distribution of etiology by gender and dentition type is shown in Table 2. Falls were the most common causes of TDI in primary (66.1%) as well as permanent teeth (38.4%). Overall, falls were responsible for 52.2% of TDI cases. The other causes included play or sports-related injuries (16.3%), road traffic accidents (12.6%), bicycle accidents (7.1%), trauma from a tubewell handle (5.2%), interpersonal violence (5.8%), and animal-related injuries (0.8%).

As shown in Table 3, the maxillary central incisors were the most commonly affected teeth in this survey, accounting for 69% (n=189) of primary teeth and 77.7% (n=285) of permanent teeth. Among the 274 primary teeth with TDI, 232 (84.7%) were maxillary teeth. Similarly, 334 (91%) maxillary teeth were affected with TDI out of 367 permanent teeth. The occurrence of TDI was significantly higher in maxillary teeth compared to mandibular teeth (Chi Square= 28.46, df=2, p<0.001).

Table 3. Distribution of traumatic injuries according to primary and permanent tooth type

Tooth type		Primary teeth		Permanent teeth	
		n	%	n	%
Maxillary	Central incisor	189	69	285	77.7
	Lateral incisor	34	12.4	42	11.4
	Canine	9	3.3	7	1.9
Mandibular	Central incisor	22	8	24	6.6
	Lateral incisor	12	4.4	7	1.9
	Canine	8	2.9	2	0.5
	Total	274	100	367	100

Occurrence of TDI was significantly higher in maxillary teeth compared to mandibular teeth (Chi Square=28.46, Df=2, p<0.001).

Table 4 presents the distribution of traumatic dental injuries based on etiology and tooth type. Fall and road traffic accidents (RTA) were the two main etiologic factors of primary teeth trauma, whereas fall and play were the two main causes of TDI in permanent teeth.

The distribution of traumatic injury types is shown in Table 5. Periodontal tissue injuries (n=173, 63.1%) were more common in primary teeth compared to hard tissue injuries (101, 36.9%), whereas hard tissue injuries (n=198, 53.9%) predominated over periodontal tissue injuries (n=169, 46.1%). Hard tissue injuries occurred more commonly in permanent teeth, whereas periodontal tissue injuries were more commonly seen in primary teeth (p<0.05).

Table 4. Distribution of traumatic dental injuries according to etiology and tooth type

	Primary teeth (n=274)						Permanent teeth (n=367)					
	Maxillary			Mandibular			Maxillary			Mandibular		
Etiology	Central incisor	Lateral incisor	Canine	Central incisor	Lateral incisor	Canine	Central incisor	Lateral incisor	Canine	Central incisor	Lateral incisor	Canine
Fall	127	22	4	12	9	7	112	10	1	12	5	1
Play	16	3	0	7	2	0	64	8	0	1	0	0
RTA	26	8	3	1	1	1	42	14	6	2	1	0
Bicycle	9	1	1	0	0	0	22	7	0	1	1	1
Tube well	8	0	0	2	0	0	20	1	0	2	0	0
IPV	2	0	0	0	0	0	25	2	0	4	0	0
Animal	1	0	1	0	0	0	0	0	0	2	0	0
Total	189	34	9	22	12	8	285	42	7	24	7	2

Table 5. Distribution of types of traumatic injuries according to primary and permanent tooth

	Primary teeth		Permanent teeth		Total	
Type of TDI	n	%	n	%	n	%
Hard tissue injuries						
Enamel infraction/Enamel fracture	17	6.2	53	14.4	70	10.9
Enamel-dentin fracture	12	4.4	71	19.3	83	12.9
Complicated crown fracture	42	15.3	46	12.5	88	13.7
Uncomplicated crown-root fracture	3	1.1	17	4.6	20	3.1
Complicated crown-root fracture	23	8.4	8	2.2	31	4.8
Root fracture	4	1.5	3	0.8	7	1.1
Periodontal tissue injuries						
Concussion	3	1.1	0	0	3	0.5
Subluxation	60	21.9	59	16.1	119	18.6
Intrusion	25	9.1	17	4.6	42	6.6
Extrusion	19	6.9	23	6.3	42	6.6
Lateral luxation	18	6.6	30	8.2	48	7.5
Avulsion	48	17.5	40	10.9	88	13.7
Total	274	42.7	367	57.3	641	100

Table 6 shows the distribution of patients according to the number of teeth affected with TDI. In 47% (N=78) of children, only one primary tooth was affected. Similarly, 46.5% (N=100) of children had only one affected permanent tooth.

Table 6. Distribution of patients according to number of injured teeth*

Type of teeth	Single tooth fracture		Two teeth fracture		Three Teeth fracture		Four Teeth fracture		Five Teeth fracture		Total Teeth fracture	
	n	%	n	%	n	%	n	%	n	%	n	%
Primary	78	47	73	44	10	6	5	3	0	0	166	100
Permanent	100	46.5	84	39	27	12.5	2	1	2	1	215	100

* n – number of children with dental trauma

Discussion

Dental traumas are more common in children and adolescents, primarily due to factors such as their active lifestyles, risk-taking behaviors and participation in sports. Engaging in sports and recreational activities exposes them to a heightened risk of accidents, including those impacting their teeth. Furthermore, since children are still refining their motor skills and coordination, they may be more susceptible to accidents such as falls and collisions.

This study findings reveal a higher incidence of TDIs in males compared to females, regardless of the dentition involved. This is in agreement with results of previous studies indicating a higher incidence of TDIs in males.[14-16] The male to female ratio of 1.6:1 suggests that males may be more vulnerable to dental trauma due to differences in behavior, physical activity levels, and risk-taking tendencies. Additionally, the gender ratio in favor of males increased progressively with age in this survey. According to Collao-González et al. the less marked gender difference in preschool children might be due to age-related childhood activities.[17] On the other hand, greater male predominance in TDI in older children and adolescents may be due to their increased engagement in outdoor activities, sports, and incidents involving violence

Consistent with previous studies, falls were identified as the leading cause of dental trauma, in this survey although the percentage of falls as a cause of dental trauma tends to decrease from younger to older children.[17,18] Younger children, especially preschoolers, are still developing their motor skills and coordination. They are more prone to tripping, stumbling, or falling due to their limited balance and coordination compared to older children. The finding that falls were the primary cause of dental trauma underscores the importance of creating safe environments and promoting injury prevention measures, particularly in settings where children are prone to falls, such as playgrounds and sports facilities. Educating parents, caregivers, and teachers about potential risks and implementing safety measures can help reduce the occurrence of falls and subsequently lower the incidence of TDIs.

The maxillary central incisors were identified as the most frequently affected teeth in both primary and permanent dentitions, consistent with previous research, highlighting their vulnerability to trauma.[19] The anterior teeth, especially the maxillary central incisors, are more exposed and prominent, making them more susceptible to direct trauma. In contrast, primary teeth are often more protected by surrounding soft tissues and the position of the lips, offering some level of safeguard against external forces. The longer lifespan of permanent teeth exposes them to a greater cumulative risk of trauma over time. As individuals age, the likelihood of exposure to traumatic events increases, contributing to a higher incidence of permanent tooth fractures.

The study also investigated the types of dental injuries observed. Luxation injuries were the most common type in the primary dentition. This is consistent with previous studies highlighting the increased mobility of primary teeth, making them more prone to displacement without fracture.[19-22] Complicated crown fractures were the most common type of injury in the permanent dentition. Complicated crown fractures consist of both crown fracture and pulp involvement, indicating a more severe form of dental trauma. The higher prevalence of complicated crown fractures in the permanent dentition can be attributed to the increased strength and rigidity of permanent teeth.

Additionally, this study found a lower prevalence of adequate lip coverage compared to studies conducted in Iraq.[23] Adequate lip coverage was observed in 45.9% of children in the study. This finding shows the need for awareness and education regarding the prevention

of TDIs. Adequate lip coverage can act as a protective cushion during falls or accidents, reducing the impact on the anterior teeth. Promoting lip protection during physical activities, sports, and play can help minimize the risk of dental injuries.[17-25]

Approximately 53% of the participants in this study experienced dental trauma involving two or more affected teeth, aligning closely with the findings of Choi et al.[26] This outcome highlights detrimental impact of dental trauma on multiple adjacent teeth.

This study has some limitations. Firstly, the survey was conducted in a single dental hospital in Kolkata, India, which may limit the generalizability of the findings to a broader population. Additionally, potential biases in retrospective data, age group specificity, exclusion of patients with syndromes, craniofacial anomalies, neuromuscular diseases, physical, or mental retardation may also affect the representation of certain demographic groups. Finally, the use of a hospital-based sample may introduce a bias towards more severe cases, potentially underrepresenting milder or less severe dental injuries in the community. These limitations should be considered when interpreting the results and may guide future research for a more comprehensive understanding of traumatic dental injuries in diverse populations.

Conclusion

Based on the study's objective and the results obtained, several key conclusions can be drawn:

Male predominance in dental trauma: The study revealed a higher incidence of traumatic dental injuries (TDIs) in males, with a male-to-female ratio of 1.6:1.

Falls as a primary cause: Falls were identified as the leading cause of dental trauma, contributing to more than half of the cases (52.2%).

Vulnerability of anterior teeth: The maxillary central incisors were found to be the most frequently affected teeth, both in primary (69%) and permanent dentitions (77.7%).

Types of dental injuries: The study categorized the types of dental injuries observed, with luxation injuries being the most common in primary dentition (63.1%) and complicated crown fractures predominating in permanent dentition (53.9%). These findings underscore the differences in the nature of dental injuries between primary and permanent teeth, with primary teeth being more prone to displacement without fracture and permanent teeth experiencing more severe forms of dental trauma.

Declarations

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author used OpenAI/ ChatGPT in order to check grammar and spelling. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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Conflicts of interest

The author declares no competing interests.

Data availability

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

Ethics approval

The Ethics Committee of Dr. R. Ahmed Dental College and Hospital (No. IEC/DCH/279 dated 28/9/2022) has granted approval for the study. Given that it was a retrospective study, obtaining informed consent from the subjects was not necessary. Furthermore, the identities of all subjects included in this work were anonymized.

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ORIGINAL PAPER

The influence of therapeutic education on diabetes-related distress and therapeutic adherence among patients with type 2 diabetes in Tetouan, Morocco

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ABSTRACT

Introduction and aim. Diabetes-related distress is a prevalent issue that affects many patients, leading to challenges in adhering to treatment plans and lifestyle modifications. By implementing therapeutic education programs, healthcare providers seek to enhance patients' understanding of their condition, coping mechanisms, and treatment adherence. This study aims to explore the impact of therapeutic education on diabetes-related distress and therapeutic adherence among individuals diagnosed with type 2 diabetes.

Material and methods. This quasi-experimental study involved 40 patients with type 2 diabetes receiving therapeutic education during 6 Months from March 24 to September 30, 2022. Diabetes-related distress and adherence were assessed before and after the intervention using the Diabetes Distress Scale (DDS-17) and glycated hemoglobin (HbA1c) levels.

Results. Wilcoxon signed rank tests revealed statistically significant improvements for all measures after the intervention. Patients showed improved DDS-17 scores ($p<0.001$) across all dimensions, and lower HbA1c levels ($p<0.001$). Pre- and post-intervention mean difference across all measures show that the greatest improvements were seen in emotional distress (1.37) and treatment-related distress (1.2).

Conclusion. Therapeutic education had a positive impact on therapeutic adherence and diabetes-related distress. It should be an integral part of type 2 diabetes care protocols in Morocco.

Keywords. diabetes-related distress, therapeutic education, type 2 diabetes

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Introduction

Diabetes is a metabolic disease characterized by chronic hyperglycemia resulting from the body's inability to regulate blood sugar levels effectively. It is classified into two main forms: type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM), with the latter accounting for approximately 80–90% of diabetic cases.[1] The World Health Organization (WHO) predicts that diabetes will rank as the sixth leading cause of death globally by the year 2030.[2] In 2021, diabetes caused 6.7 million deaths, with one death occurring approximately every five seconds.[3] Given its increasing prevalence and associated complications, diabetes poses a significant public health concern worldwide.[4,5]

Morocco is not exempt from this alarming global situation, as indicated by the rising prevalence of T2DM among Moroccan adults, reaching 12.4% in 2016.[6,7] Diabetes has been identified as the leading cause of end-stage chronic kidney failure and lower limb amputations in the country and is a major contributor to mortality, causing over 12,000 deaths annually and 32,000 deaths due to complications.[8,9]

Effective glycemic control, measured by maintaining a normal or near-normal glycated hemoglobin level (HbA1c<7%), is essential for reducing diabetes-related complications.[10] However, achieving this target remains challenging, with only 26.8% of Moroccan adult T2DM patients meeting the recommended HbA1c levels.[11] The key to successful management lies in patient adherence to therapeutic recommendations and active involvement in self-management.[12]

Furthermore, the biological marker for assessing glycemic control is HbA1c, which provides an estimate of blood glucose levels over the previous 60 to 90 days.[13] HbA1c is likely to be associated with adherence to medication and self-management. Several studies have shown that each 1% reduction in HbA1C is associated with a 21% reduction in the risk of diabetes-related complications.[14–17] Despite the importance of medication adherence in diabetes, it has been shown that worldwide adherence with diabetes treatment is between 38.5 to 93.1%.[18,19]

One crucial aspect affecting therapeutic adherence is diabetes-related distress (DD), encompassing concerns and anxieties associated with managing the demanding and restrictive nature of diabetes, potential complications and healthcare access.[20,21] High DD levels have been observed in approximately 36% of T2DM patients in community settings, with anxiety disorders being three times more prevalent compared to the general population.[22,23]

DD affects directly and indirectly blood sugar regulation by triggering stress hormones like cortisol and adrenaline, leading to hyperglycaemia.[24] Indirectly, DD associates with cognitive avoidance and impaired problem-solving, resulting in reduced healthy behavior adoption and compromised glycemic control.[24]

Addressing non-adherence requires an integrative approach targeting behavioral and emotional disease adaptation aspects.[24] Therapeutic education programs (TEP) have emerged as critical for equipping patients with the knowledge, skills, and behavioral changes necessary for effective diabetes management.[25] Few nursing studies have explored this, making this study relevant for nursing practice, particularly patient care. In Morocco, the national diabetes program focuses on strengthening TEP but standardized programs are yet to be established.[8]

Aim

To address this knowledge gap and enhance nursing interventions, this study assessed a TEP's impact on diabetes-related distress and glycemic control in T2DM patients in Tetouan, contributing to successful future nursing approaches in diabetes management.

Material and methods

Study design

This prospective quasi-experimental non-randomized uncontrolled study assessed a TEP's impact on diabetes-related distress and adherence among T2DM patients in Tetouan, Morocco. It was conducted at the Samsa Health Center, Medical Health Delegation of Tetouan, involving T2DM patients.

The 6-month study occurred from March 24, 2022 to September 30, 2022, with three phases:

Phase 1 – Pre-Test: Diabetes-related distress (Diabetes Distress Scale - DDS17) and glycemic control (HbA1c levels) were evaluated.

Phase 2 – Intervention: The TEP was implemented through four bimonthly group sessions (n=40) and 5 telephone follow-ups.

Phase 3 – Post-Test: Diabetes distress (DDS17) and glycemic control were reassessed.

Sampling

Convenience sampling recruited 40 participants meeting selection criteria, chosen during consultations based on appointment schedules. Eligibility included adult T2DM patients aged ≥18 years with ≥6-month diagnosis and HbA1c >7% or 53 mmol/mol attending the Samsa Health Center, reachable phone number, and ability to speak Moroccan Arabic. Exclusion criteria were type 1 diabetes, dementia, or significant communication difficulties, to focus on the population of interest and enhance validity and reliability.

Sample size calculation

Required sample size was calculated using G*Power 3.1.6. To compare data, the Wilcoxon test was used

with 0.91 power (1−β), 0.5 effect size (d), and 0.05 significance level (α). The total required sample size was 39, increased to 40 to ensure adequate power and account for potential attrition or data issues.

Data collection and assessment

Sociodemographic and medical characteristics were collected through questionnaires and medical records. The validated 17-item Diabetes Distress Scale (DDS-17), adapted and validated in Moroccan Arabic, assessed diabetes distress across four dimensions:[26,27]

Emotional distress: These items measures the emotional challenges and psychological distress associated with living with diabetes, including feelings of anxiety, depression, and the emotional impact of managing the condition.[28]

Physician-related distress: These items assesses distress related to interactions with healthcare providers, concerns about the quality of medical care, difficulties in communication and trust with healthcare professionals in the context of diabetes management.[28]

Regimen-related distress: evaluates the distress caused by the daily

management tasks associated with diabetes, such as taking medications, monitoring blood sugar levels, and following dietary and exercise recommendations.[28]

Interpersonal distress: It focuses on distress related to interactions with family, friends, and social relationships affected by diabetes, including concerns about how the condition impacts one's social life and support from loved ones.[28]

Responses were rated from 1 (not a problem) to 6 (very serious). Mean scores above 2 indicated moderate/severe distress.[29,30] The Cronbach's alpha coefficient was 0.86 for the total instrument.

Biological analysis

HbA1c levels were determined through high-performance latex immunoagglutination inhibition methodology with the Siemens DCA Vantage Analyzer of morning fasting blood samples.[31]

Educational intervention

The culturally sensitive TEP involved four weeklies 1.5-hour group sessions (N=40) and personalized 3 telephone follow-ups. It focused on patients' disease experiences, needs, and difficulties to address ambivalence and enhance self-efficacy, aiming to reduce distress and improve glycemic control. Workshops were conducted by a multidisciplinary team including a family doctor, psychologist, dietitian, psychoeducation nurse, and social worker, emphasizing active learning of cognitive and emotional aspects. Content was developed from validated international and national educational messages, expert consultations, brochures, and broadcasts. Materials were adapted to the Arabic dialect and simplified. The holistic approach aimed to help patients adapt to complex, dynamic life situations.

A psychologist guided self-discovery and problem-solving to address self-care barriers, employing:

Cognitive restructuring

involves expressing the emotions associated with diabetes, with the aim of changing harmful beliefs and behaviors.

Illness acceptance and conservation commitment

Accepting diabetes restrictions and using religion as a coping mechanism. Motivational interviewing skills (empathy, understanding ambivalence/resistance) facilitated managing negative thoughts and establishing trust.

Follow-ups enabled patient responsibility for self-care through SMART goal-setting and self-management techniques to prevent relapses.

Data analysis

Descriptive statistics characterized variables. Categorical variables were presented as frequencies and percentages, quantitative variables as means and standard deviations. Wilcoxon signed-rank tests compared pre- and post-intervention means at a 0.05 significance level. Pre- and post-intervention mean differences were calculated. In the case of our study, we opted for the Wilcoxon ranked test to compare two paired groups when the data is not assumed to be normally distributed. Jamovi statistical software, version 2.3.16, was used for all data analyses in this study (https://www.jamovi.org/).

Ethics approval

Informed consent was obtained and ethics approval received from the institutional review board (University Hospital Center, Faculty of Medicine and Pharmacy of Tangier, Morocco, reference number 06/2022). Participant confidentiality was strictly maintained through anonymization and secure data storage. Authorization was also obtained from the Tetouan medical health delegation.

Results

Participant characteristics

Among the 40 participants, most were female (80%) aged 60–69 years (47.5%), married (70%), illiterate (70%), and had social insurance (76%). Most had a ≥5-year diabetes duration (35%), comorbidities (45%), family history of diabetes (50%), not enough information (65%), and oral antidiabetic treatment (93%) (Table 1).

Therapeutic education

Pre-intervention, the DDS-17 mean score was 2.72±0.88. Dimension means ranged from 3.26 ± 1.01 (emotional) to 1.73±0.85 (physician-related). Post-intervention, the

DDS-17 mean score was 1.73±0.38. Dimension means ranged from 1.89±0.48 (emotional) to 1.37±0.4 (physician-related). Wilcoxon tests found statistically significant pre-post improvement across all dimensions (p<0.001) (Table 2).

Table 1. Description of the sample characteristics (n=40)

Variable	Frequency n (%)
Sex (%)	
Male	8 (20%)
Female	32 (80%)
Age classes (%)	
<40	2 (5%)
40–49	6 (15%)
50–59	8 (20%)
60–69	19 (47.5%)
>69	5 (12.5%)
Level of education (%)	
Illiterate	28 (70%)
Can read and write	3 (7.5%)
Primary school	9 (22.5%)
Marital status (%)	
Single	2 (5%)
Married	28 (70%)
Divorced	2 (5%)
Widowed	8 (20%)
Social insurance (%)	
No	9 (22%)
Yes	31 (78%)
Diabetes duration on years (%)	
<1	5 (12.5%)
1-5	14 (35%)
6-10	11 (27.5%)
>10	10 (25%)
Comorbidities (%)	
Yes	18 (45%)
No	22 (55%)
Treatment (%)	
Oral antidiabetics	37 (93%)
Insulin	3 (7%)
Information on diabetes (%)	
Enough information	14 (35%)
Not enough information	26 (65%)
Family history (%)	
Yes	20 (50%)
No	20 (50%)

Table 2. Assessment of (DDS17) scale pre- and post-intervention

Tools	Pre-intervention	Post-intervention	p
Diabetes-related distress (DDS17)	2.72±0.88	1.73±0.38	<0.001
Emotional distress	3.26±1.01	1.89±0.48	<0.001
Physician-related distress	1.73±0.85	1.37±0.4	<0.001
Regimen-related distress	2.88±1.02	1.68±0.37	<0.001
Interpersonal distress	2 .89±1.47	2.01±0.92	<0.001

Pre-intervention, the (HbA1c) mean was 8.6% ± 1.6. Post-intervention, the HbA1c mean was

8±1.2% Wilcoxon tests found statistically significant pre-post improvement, (p<0.001) (Figure 1).

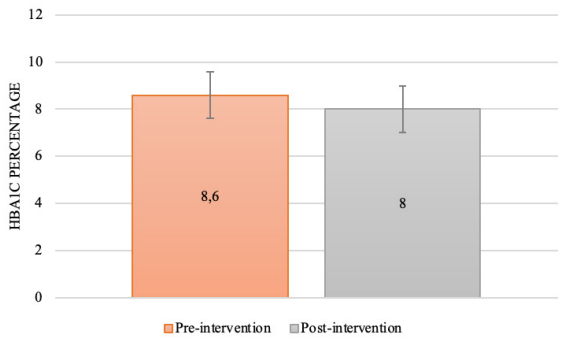

Fig. 1. Assessment of therapeutic adherence through HbA1c levels pre- and post-intervention (***p<0.001)

The pre- and post-intervention mean difference across all measures show that the most marked improvements occurred for emotional distress (1.37) and regimen-related distress (1.2) (Figure 2).

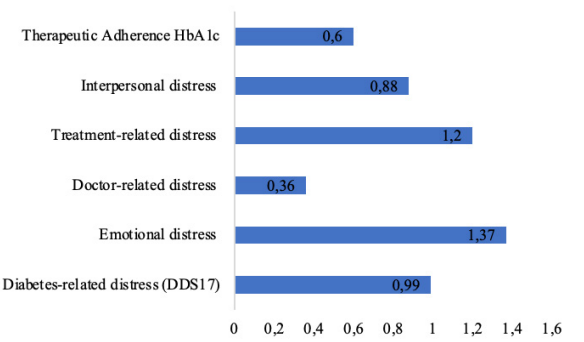

Fig. 2. Presentation of mean difference, pre and post-intervention

Discussion

In the present study, we observed a notable predominance of females, comprising 80% of the participants. This female preponderance aligns with expectations, as the study cohort was drawn from individuals with type 2 diabetes who were receiving care at the SAMSA Health Center. Previous reports have consistently shown that women tend to utilize healthcare services more frequently than men, a trend that is not unique to our setting and has been echoed in studies conducted in various other countries.[30-36] This phenomenon can likely be attributed to gender-related factors that contribute to distress.[36,37] Furthermore, women often shoulder greater responsibilities in diabetes care and face additional life and illness-related conflicts that can impact their problem-solving abilities, self-efficacy, and ultimately, glycemic control.[32,33] They may benefit from increased empowerment, gen-

der-sensitive approaches, and emotional interventions to address the distinctive challenges women encounter in managing diabetes and to mitigate distress.[32]

Post-intervention, considerable improvements occurred in emotional and regimen-related distress, consistent with other studies.[34–37] Simply discussing experiences, support, and services gave similar outcomes to TEP interventions, likely initiating sustainable behavior changes through increased self-efficacy and resilience based on personal value systems.[32,38]

Emotional and regimen-related distress improvements can be attributed to the psychological adjustment strategies employed, guided by a psychologist to enhance psychosocial adaptation and apply motivational interviewing techniques.[33,39]

Religion was highlighted as a coping strategy. Strategies aimed at improving self-esteem, self-efficacy, group membership and security. The care team helps patients to identify what is most important in their lives and to translate these values into specific, realistic and achievable goals.[40]

Nurses and patients adapt their treatments and self-management programs to achieve the identified collaborative goals to improve patient-centered outcomes.[41–43]

Glycemic control also improved significant, consistent with other studies which have confirmed that HbA1c is an essential measure to assess the level of adherence in people with diabetes.[32,38,44–46] The dynamic team-patient interaction created a supportive environment for understanding self-management strengths and challenges, which can reduce distress.[47] Nurses' health education roles contribute to resolve adherence problems and improve glycemic control.[48-50] Phone follow-ups enabled patient self-care responsibility through goal setting self-management techniques and glycaemic control.[51–53]

Conclusion

This study demonstrated a culturally sensitive, needs-based TEP improved diabetes-related distress and adherence by targeting patient autonomy, self-efficacy, problem-solving, and motivation. TEP should be integral to Moroccan T2DM care protocols. This is, to our knowledge, the first such study in Morocco showing TEP's usefulness in T2DM management. Further experimental, mixed methods studies over longer durations and with larger samples could extend these findings and provide greater insight into TEP's role in T2DM care.

Declarations

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Author contributions:

Conceptualization, H.B. and A.T.; Methodology, A.T., M.M. and H.B.; Software, D.O., and H.B Validation, H.B., H.A and M.M.; Formal Analysis, H.B. and H.A.; H.B Investigation, H.B. and A.T Resources, H.B. and A.L Data Curation, H.B. and A.L.; Writing – Original Draft Preparation, H.B.; Writing – Review & Editing, H.B.; Visualization, H.B., H.B and H.A.; Supervision, A.T., Y.S. and M.M. Project Administration, Y.S.

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 (University Hospital Centre, Faculty of Medicine and Pharmacy of Tangier, Morocco, classified under the number 06/2022).

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ORIGINAL PAPER

Caring and therapeutic services – a patient's opinion on experience and satisfaction with night nursing care

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ABSTRACT

Introduction and aim. Night nursing care ensures well-being, peace, and security allowing a patient to enter into the deep sleep phase. This study aims to analyze the relationship between an assessment of caring and therapeutic services and the opinion of in-patients on their experience and satisfaction with night nursing care.

Material and methods. A questionnaire developed by the authors and the Newcastle Satisfaction with Nursing Scale were used in this study. This study was conducted in the eastern region of Poland in the Podkarpackie province. The study included 585 patients.

Results. Tasks analyzed resulting from care and therapeutic functions, in the patient's opinion, had a significant impact on the overall assessment of nursing care. Meeting the expectations regarding night rest (experience of nursing care) and satisfaction showed the strongest correlation. Tasks resulting from therapeutic functions showed an average correlation with the exception of planned medical care.

Conclusion. The level of implemented health services by nurses at night significantly influences the overall assessment of the quality management system in health care.

Keywords. care, nursing service, patient

Introduction

A systematic review of patient satisfaction with nursing care shows that a high quality of services provided in healthcare facilities is inseparably connected with the patient's satisfaction with medical services.[1,2] Health services should meet the expectations of the patient, and the opinion of the beneficiary of medical services reflects the sensitivity of the healthcare system to their needs. A comprehensive meta-analysis of the studies available in the literature confirms that patients are satisfied with the nursing care provided during hospitalization.[3-5]

A critical review was made of papers published in Polish and foreign scientific journals between 2010 and 2018 in the category of opinion, satisfaction and satisfaction of patients with services provided by nurses at night. In the light of the review, numerous scientific publications on the nurse's work at night (the influence of shift work on the health and life of nurses, the risk of medical mistakes during night shifts) were found in the PubMed, Embase, Elsevier, Springer and Lancet databases. There are no scientific reports of patient's experiences of nursing care during the night time. In a review of published scientific papers in the context of nocturnal nursing care of a patient in a hospital, two ranges of publications can be distinguished. The first range includes publications on the specifics and consequences of night work for the health and quality and professional satisfaction of the nurse. The second range includes those publications that present selected aspects of assessment and/or opinion, satisfaction of the patient, including overall nursing care.

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Nighttime satisfaction of nursing care patients are a component of general care satisfaction surveys or completely different scopes. Analysis of the results indicates that, in many cases, the opinions of patients were divergent.[6-10]

Patients assess the hospital through the prism of conditions prevailing in the hospital ward. They pay particular attention to the conditions of sleep and rest, the cleanliness of the patients' rooms and toilets, equipment facilitating the stay in the ward (tables, furniture, hangers, cabinets, lighting, adapting bathrooms to the needs of the disabled). In the opinion of many researchers, nursing care has a key impact on the overall satisfaction of patients undergoing hospitalization.[6,11]

Professional independence of nurses, who manage patients in accordance with applicable medical standards and procedures reflects their professionalism.[12] Systematic reviews show that patients increasingly pay attention to such elements of care as availability of nurses, cordiality, empathy, as well as psychological support. Depending on how a man determines the quality of his life, their requirements regarding nursing are also variable. This assessment is performed in a subjective or objective manner. The patient, first and foremost, determines the quality as easy access to health services, receiving satisfactory information about health and treatment methods, health education aimed at gaining self-care skills, continuity of treatment process, psychological comfort, and good relations with staff.[9] Therefore, during their duty, nurses must ensure the safety of the patient and take care of his or her mental state, perform tasks resulting from professional function, as often as required by the patient's health status. The primary purpose of services provided by nurses at night is to ensure well-being, peace and security, so that the patient can enter the deep sleep phase.[9] Florence Nightingale claimed that the quality of good health care and good nursing is to avoid intentional or accidental wake-up. If a man is awakened from the first sleep, it is almost certain that he will not fall asleep again. In the opinion of Jankowiak, nurses should first of all observe the patient in terms of disturbances of sleep and wakefulness, and inform the doctor about abnormalities. The necessary condition to satisfy the need for night rest, in turn, will be ensuring appropriate conditions to meet this need. In addition, the constant presence of a patient who is suffering, sleepless and the implementation of therapeutic tasks is a guarantee of comprehensive nursing care.[9,10]

The nursing care function is based primarily on satisfying the needs of the patient and his family. The results of studies by Glińska and Lewandowska showed that nurses in their daily work most often performed tasks resulting from the therapeutic and care functions.[10]

Aim

The aim of the study is to analyze the correlation between the assessment of care and therapeutic services and the experience and satisfaction with care provided at night in the opinion of patients hospitalized in Rzeszów Clinical Hospitals.

Material and methods

Research problem

What is the variation in patient satisfaction depending on the selected scope of nursing services provided at night?

Specific goal

Analysis of the relationship between the assessment of the implementation of selected nursing services at night and the assessment made by patients and their level of satisfaction from the health services provided.

Ethics approval

The study was conducted pursuant to the approval of the Bioethics Committee at the University of Rzeszów No. 04/12/2015, dated 2 December 2015.

Study participants

In the group of respondents, there were 585 hospitalized patients of departments/clinics with the specificity of conservative and surgical treatment in clinical hospitals of the City of Rzeszów during the period between 01/01/2016 and 31/05/2016.

Patient inclusion criteria:

- patient's voluntary consent to participate in the study,
- at least two nights spent in a hospital ward,
- the need to obtain nursing assistance during night duty,
- no psychosomatic disorders in the opinion of the researcher, self-orientation and allopsychic preservation,
- age over 18 years.

A sample of 600 people was selected, among whom interviewers distributed sets of questionnaires. Five hundred and ninety-two responses were received od 585 questionnaires were complete, which constituted 97.5% of the assumed study sample.

Nurses meeting the selection criteria providing night care at the end of their night shift for 2-5 patients for whom they provided services during their duty. The examination of selected patients was carried out in the morning after the end of the shift. The described patient tools were owned by a member of the research team or a designated person who was not involved in patient care.

Admitted patients took part in the study to selected conservative and surgical departments/clinics of the Clinical hospitals of the city of Rzeszów. The study included adult respondents staying in hospital in one ward/clinic for at least two days. Additionally, during the study, the patients were only in one ward. In the opinion of the researcher, the patients were without consciousness disorders and voluntary consented to participate in

the study. The research project began on January 1, 2016 and lasted 6 months. Two interviewers were employed.

Study tool

The author's questionnaire and the standardized NSNS tool – The Newcastle Satisfaction with Nursing Scale – were used to test the patients' opinions.

The author's questionnaire contains 11 questions with the option of answering on a 5-point Likert scale and a short summary. The assessed dimensions of night nursing services include tasks resulting mainly from the caring and therapeutic function. The questions are closed-ended. The internal consistency of an 11-item original questionnaire assessing the quality of night nursing care was assessed.

The value of the a-Cronbach coefficient was 0.955, which proves the high internal consistency of the questionnaire (Table 1).

Table 1. Assessment of internal consistency of an 11-item original questionnaire assessing the quality of night-time nursing care – Cronbach's alpha

Number of item	Cronbach's alpha=0.955		
	$\bar{x}$	s	Cronbach's alpha excluding item
1	4.13	1	0.95
2	4.06	0.97	0.952
3	4.04	0.98	0.95
4	4.1	1.02	0.95
5	4.08	0.97	0.948
6	4.11	0.92	0.949
7	4.15	0.9	0.949
8	3.92	1.05	0.956
9	4	0.97	0.952
10	4.11	0.92	0.95
11	4.22	0.88	0.949

The original version of the NSNS contains a total of 50 claims in three subscales, which were rated on a growing scale from 1 to 7 or from 1 to 5.[13]

In the author's own questionnaire, 11 closed questions were included, dividing them into two areas of tasks resulting from professional functions: therapeutic and caring. The assessment was made on the Likert scale from 1 to 5. The survey was anonymous and did not allow the researcher to be identified.[14,15]

Statistical analysis

The descriptive statistics (a summary measure) were used to analyze the satisfaction of nocturnal nursing care. The numerical characteristics of the parameters examined were determined by: median, arithmetic mean and standard deviation. Statistical analysis of statistical results obtained: Mann-Whitney, Kruskal-Wallis and Spearman's rank correlation coefficient. To illustrate the obtained data from research and statistical analysis, the results were presented on the frame-mustache charts.

Calculations were performed using IBM SPSS Statistics 20 (Armonk, NY, USA). The significance level of $p<0.05$ was adopted in this study.

Results

The assessment of the quality of night nursing care was made using a standardized questionnaire the NSNS. The assessment of night nursing care with the numerical Newcastle Scale was quite high. Both the assessment of the experience of nursing care and the satisfaction with nursing care was, on average, over 60 pts. In the case of the first measure, most of the results were relatively close to the mean value, while in the second measure the discrepancy in the results was much larger (every fourth patient assessed the level of satisfaction with nursing care at less than 38 pts, therefore quite low, while one in four on at least 81 pts), (Table 2).

Table 2. The assessment of night nursing care by patients in the NSNS*

The assessment in the Newcastle Scale	$\bar{x}$	Me	s	c_{25}	c_{75}	min	max
Experience of nursing care	63.7	62.8	10	55.8	71.2	26.3	85.3
Satisfaction with nursing care	60.3	61.8	26.5	38.2	81.6	0	100

* $\bar{x}$ – mean, Me – median; s – standard deviation;
C25 – lower quartile; C75 – upper quartile
min, max – minimum and maximum values

Qualitative assessment of services performed at night and 24-hour nursing care based on the NSNS

In the scatterplots, a distribution of all assessments of nursing night care was made (based on the author's questionnaire and general nursing assessment using the Newcastle questionnaire). The graphs show the values of Spearman's correlation coefficient along with the assessment of its significance. The size of the markers in the chart was increased in proportion to the number of people corresponding to a given combination of the values of the compared measures. Auxiliary simple (so-called regression straight line) allows to better evaluate the direction of dependence. After careful analysis it was proved that the assessment of services performed at night had a significant impact on the overall assessment of nursing care. The relationship between the assessment of night care and experience and satisfaction based on the NSNS was similar and clearly correlated with a 24-hour assessment of care provided by nurses ($R = 0.51$ and $R = 0.47$), (Fig. 1).

Tasks resulting from the caring function and the overall assessment of nursing care

The selected tasks resulting from the scope of the caring function were assessed and comprehensive services in the field of nursing care. The graphs show average values of measures calculated based on the NSNS relative to the assessment of selected aspects of night care. As

a result of the conducted research, it was proved that the assessment of individual care tasks had a significant impact on the overall assessment of nursing care. These were correlations of rather average strength, but this is not surprising, because each question was about a narrow aspect of night care. The strongest with the overall assessment of the nurse's work was to correlate with wishes regarding night rest (p<0.001) and the possibility of talking with a nurse (p<0.001), (Fig. 2).

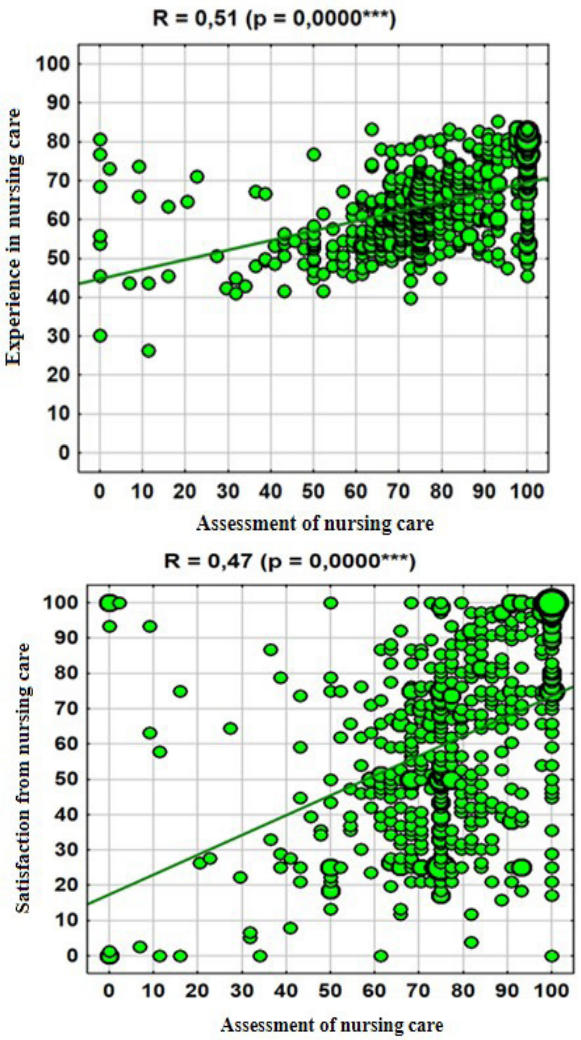

Fig. 1. Evaluation of nursing night care – experience and satisfaction with nursing care

Tasks resulting from the therapeutic function and the overall assessment of nursing care

An analysis was also made of the correlation between the quality of performed tasks resulting from the therapeutic function and overall nursing care based on the NSNS. The obtained results confirmed the average correlation with the overall assessment of nursing care (p<0.001). The only exception was the planning of delays in the implementation of tasks both in terms of experience (0.28 (p<0.001) and satisfaction (0.26 (p<0.001), (Fig. 3).

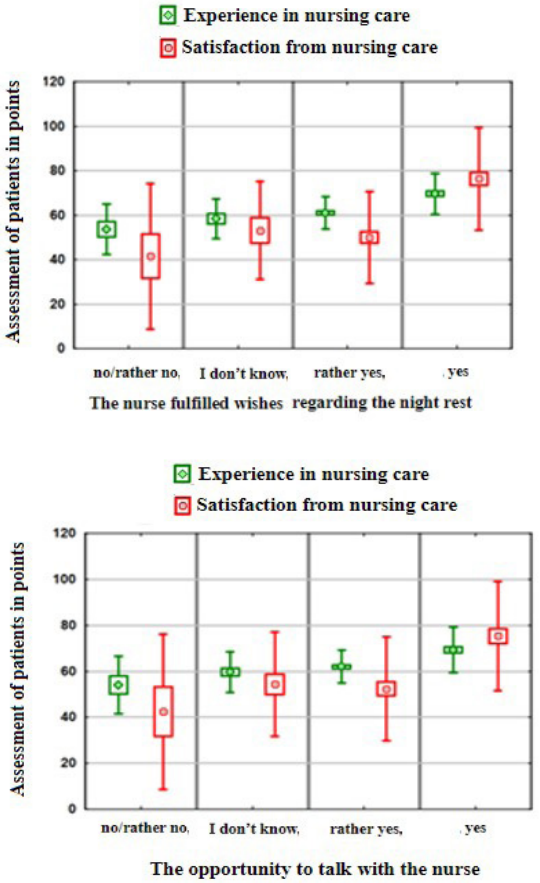

Fig. 2. Two-factor correlation between care benefits and the overall assessment of nursing care

Discussion

Nursing work at night involves mainly the implementation of tasks resulting from the caring and therapeutic functions and the activities that are inseparably connected with them. Research in Poland shows that patients increasingly pay attention to such elements of care as availability of nurses, cordiality, empathy, as well as psychological support. Depending on how a man determines the quality of his life, his requirements in terms of nursing are also different. This assessment is made in a subjective or objective manner.[13] Our research shows a statistically significant relationship between the assessment of specific care tasks and the overall assessment of nurses' work in hospitals. Similar results were obtained by other researchers.[16-18]

According to the patients, in our research, most important was the "fulfillment of wishes at night" in both the experience and satisfaction categories (p<0.001). The patients highly rated the possibility of talking with the nurse in both subscales (experiences and satisfaction with care - (p<0.001). According to Larrabee et al, the patients rated the highest the ability to provide care (79.1%), focus on the patient and his emotional needs (54.6%) and professional competences.[19] Many researchers emphasized the role and importance

of verbal emotional support with an indication of their quality.[20,21] Hussain et al. received completely different results. Eighty-four percent of respondents expressed dissatisfaction with the provided nursing care. Their nurse's task at night was not sensitive to the reported needs, nor could they establish a therapeutic conversation.[22] Mohammed and Odetola showed in the course of their research that nurses referred to patients in a disrespectful manner.[23] Respondents in Sierpinska's research pointed to the nurse's low interest in patient problems and overly loud conversations at night.[18]

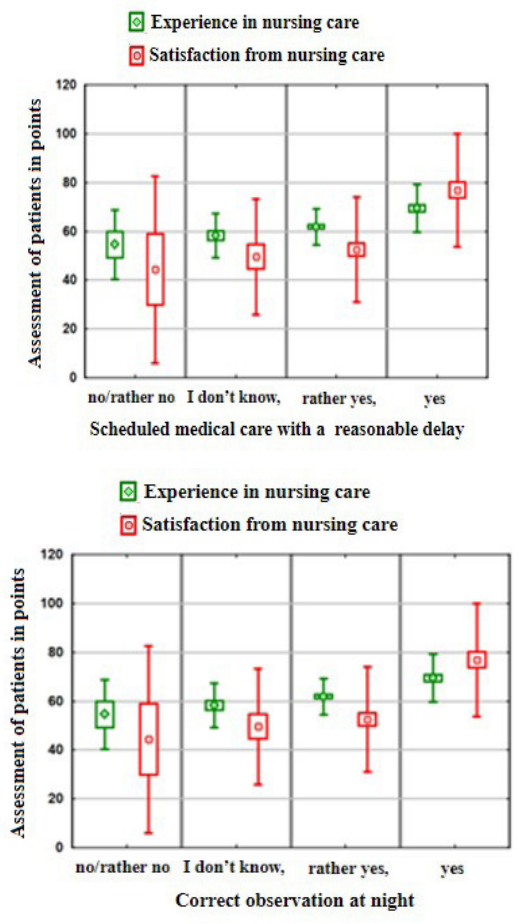

Fig. 3. Two-factor correlation between therapeutic services and the overall assessment of nursing care

According to research by Glińska and Lewandowska, nurses usually carry out tasks resulting from therapeutic functions.[10] Larrabee and Bolden distinguished the five most-valued features of "good nurturing." Respondents relied on nurses to meet their needs (caring for, checking that everything was in order, responding to requests, fighting with pain, providing information, care for the environment), polite treatment (respecting rights, positive attitude, patience), caring (showing care and interest), being competent (correct substantively performing skills, striving for excellence) and quick help (performing punctual procedures).[19] During the analysis of our research, reports of other researchers were confirmed.[10,19,24] Therapeutic benefits significantly influenced the overall assessment of nursing care. The exception was a slightly different category - planned medical care with a justified delay: experience and satisfaction with nursing care ($p<0.001$).

In an analysis of the studies of many authors, it can be stated that for the patient the most important aspect is the nurse's readiness to help, provide kindness, care and courtesy.[18,25,26] The patient's cooperation in defining the diagnosis and establishing methods as well as ways of treatment and care gives a much better chance of treatment effectiveness and satisfaction of needs.[27] Providing a sense of security and gaining trust is a very important element in assessing the quality of nursing care. Health care institutions bear responsibility for the life and health of a person in need. Patients need genuine interest in their problems and honesty and respect. Moczydłowska et al. showed in her research that nurses, while performing medical procedures, first and foremost respect the patient's dignity.[28]

Parasasuraman et al., in the course of the conducted research, indicated the hierarchy of criteria for assessing the quality of service on the part of the patient. They include not only the material components of the service process, but above all, certainty, competences, empathy, responsibility, responsiveness, solidarity and reliability.[29] Every effort should be made to ensure that one of the components, which is the provision of night nursing care, is a strong pillar of the health care system, and it can contribute to, among others, monitoring the assessment of patients' benefits. The sick pay attention to the detailed, non-specific but elementary aspects of care. These include: cordiality and empathy, availability and psychological support.

Certainly, the diversity of patients' requirements for nursing services depends on a large extent on the perception of the quality of their lives. The relative analysis made will certainly allow for quick response to irregularities resulting from system imperfections. Based on our own analyzes and other researchers based on the NSNS satisfaction rating scale, it was found that patients highly evaluate nursing care. A detailed analysis of the research results obtained indicates the possibility of adapting to the needs and expectations of healthcare service users. Currently, analyzing studies on the quality of nursing services provided at night only fragmentary studies can be found on the hospitals in Poland.[30-33]

Analyzing the collected literature on the subject and the results of scientific reports, it was observed that considering the quality of nursing services at night, both the opinion of the patient/client and the organization of the hospital were taken into account. Such a broad spectrum of issues implied the need to analyze many different aspects of services and the use of medical services. The ana-

lyzes undertaken in the paper were to prove that the tasks resulting from the caring and therapeutic functions are of the utmost importance when providing health services by the nurse at night. Hajdukiewicz emphasizes that the medical service that is "cordial, full of care and commitment", but not supported by competences does not fulfill its role. The same researcher points out that one cannot offer a dehumanized medical service, even if it was performed by a renowned specialist.[34]

Conclusion

Nursing care provided by nurses during the night time has a significant impact on the overall assessment of the nurses' work both in terms of satisfaction and the experience of the hospitalized patient. Night nursing care was focused mainly on the implementation of tasks resulting from the therapeutic and caring functions. In the patients' opinion, the most important is the professional performance of nursing procedures, openness, willingness to talk and "fulfilling wishes" related to rest at night. To improve the quality of medical services, it is advisable to include all assessments and patients' expectations regarding night-time services.

Declarations

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Author contributions

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

Conflicts of interest

The authors declare no conflict of interest.

Data availability

Data will be made available upon a justified request sent to the corresponding author an.krakowiak716@gmail.com or akrakowiak@ur.edu.pl.

Ethics approval

The study was conducted pursuant to the approval of the Bioethics Committee at the University of Rzeszów No. 04/12/2015, dated 2 December 2015.

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ORIGINAL PAPER

Antibacterial, DNA photocleavage and molecular docking studies of newly prepared Schiff-based macrocyclic complexes

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ABSTRACT

Introduction and aim. At present, several microbial diseases are prominent and of concern worldwide. The intent of this study was to examine the antibacterial potential of newly synthesized tetradentate macrocyclic complexes against different bacterial strains. The macrocyclic scaffold has gained attention as a biologically active class of supramolecular chemistry due to its unique properties and ability to target various microorganisms. Thus, the goal of the present study was to develop a series of biologically active transition metal-based macrocycles.

Material and methods. All macrocyclic compounds were synthesized by a template method and validated by molar conductivity, elemental studies, and spectral and magnetic studies. Antibacterial activities of all metal complexes were evaluated against *Escherichia coli* (MTCC 739) and *Staphylococcus aureus* (MTCC 731) bacterial strains by taking ampicillin as a standard reference drug. DNA photocleavage potential was explored using agarose gel electrophoresis.

Results. Results revealed the formation of novel macrocyclic complexes via tetra nitrogen bond trapping of metals. Copper complexes have strong potential against *S. aureus* bacteria as copper and nickel both show good DNA photocleavage potential.

Conclusion. The findings endorse the biomedical relevance of these macrocyclic scaffolds, suggesting avenues for further exploration in targeted drug delivery and potential clinical applications. The proposed octahedral geometry for the complexes enhances our understanding of their structural aspects. This research contributes substantively to the field, laying the foundation for future investigations in advanced antimicrobial design and application.

Keywords. anti-bacterial, DFT, DNA photocleavage, molecular docking, template method

Introduction

The profound significance of macrocyclic ligand complexes in physiological processes underscores their pivotal role in recent global research endeavors. These complexes are a prime example of their significance in the complex machinery of living beings, as they are vital to many crucial biological activities.[1–4] The most notable application of the macrocyclic scaffold is in photosynthesis, where chlorophyll, which contains a porphyrin, acts as a pivotal component in absorbing light energy and propelling the synthesis of carbohydrates in plants.

In human biology, macrocyclic derivatives play a major role in the essential movement of oxygen inside cells. The essential protein in red blood cells, he-

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moglobin, depends on macrocyclic porphyrin rings that contain iron ions in the center. Because of its structural makeup, hemoglobin may bind oxygen molecules, which makes it easier for oxygen to go from the lungs to tissues and organs. This basic mechanism is essential to the continuation of life. Furthermore, macrocyclic derivatives – especially those that incorporate iron – are essential to the process of cellular respiration. During the energy conversion process, heme groups containing iron ions are used by cytochromes, a type of protein essential to the electron transport chain for electron transfer. This emphasizes how important macrocyclic scaffolds are for supporting several vital biological processes, including respiration.[5,6]

Because of their special qualities and possible uses, heteroatom-containing macrocyclic Schiff-based complexes are a prominent family of molecules in the field of coordination chemistry. The durability and adaptability of supramolecular complexes make them significant. Their capacity to interact with metal ions and their distinct macrocyclic structure make them useful instruments for creating functional molecules with specific characteristics.[5,6]

Because macrocyclic derivatives are distinct in terms of their host-guest chemistry, structure, and behaviors, researchers have been interested in creating new macrocyclic systems. These substances have demonstrated significant promise in a wide range of applications, such as chemical sensors, catalysts, therapeutic agents, agents for magnetic resonance imaging (MRI), and numerous other biomedical uses.[7–9]

Additionally, macrocycles in medicinal chemistry have promise for improving drug development, therapeutic interventions in a range of disorders, and diagnostic procedures. Currently, the varied biological features of the metal complexes of Schiff base macrocycles (including the -C=N group) attract a lot of interest as shown in Figure 1.[10–16]

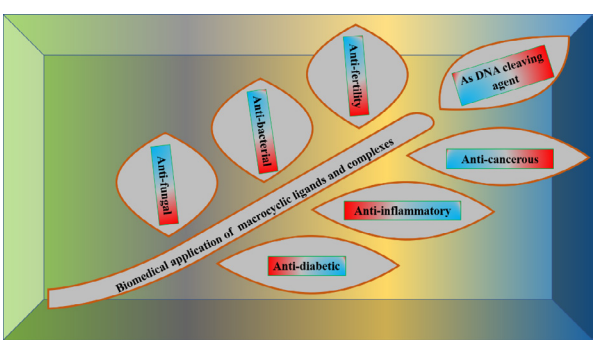

Fig. 1. Biomedical application of Schiff-based macrocyclic ligands and complexes

Aim

Prodded by all the above facts, in this research article, the biological efficacy and synthesis of macrocyclic Ni(II), Co(II), Cu(II), and Zn(II) metal complexes was carried out. Additionally, computational studies (DFT and molecular docking) have also been used to check the quantum mechanical parameters, molecular mechanisms and binding interactions between the ligands with the active sites of the receptor.

Material and methods

Materials

All solvents and chemicals utilized during synthesis were of AR grade. 1,2-phenylenediamine and maleic anhydride were obtained from SD Fine Chem Ltd, Mumbai, and Sigma-Aldrich respectively. Various metal salts of copper, nickel, cobalt and zinc were procured from Chemigens Research & Fine chemicals, CDH Chemicals, and SD Fine Chem Ltd. All the above chemicals were used as received.

Synthesis of macrocyclic complex

A novel series of tetraazamacrocyclic compounds were synthesized using template cyclization reactions. In a hot methanolic solution of 1,2-phenylenediamine (1.081 g, 10 mmol), divalent metals such as copper, nickel, cobalt, or zinc salts were added (5 millimoles) and dissolved in the least amount of MeOH (methanol). The mixture was then refluxed for nearly an hour. A minor variation in the color of the mixture was a clear indication of the reaction's progress, as it was evidence in favor of metal-amine coordination. Subsequently, to the above solution, a methanolic solution of maleic anhydride (0.9806 g, 10 mmol) was introduced, and refluxing was further continued for about 6–8 hours. The mixture obtained was kept overnight for cooling and precipitation, which was then further filtered and washed with MeOH, ethanol, and diethyl ether, then dried over vacuum (Fig. 2). The yield of the resulting complexes was ~40–60%. The synthesized compounds were soluble in DMSO, DMF, and acetonitrile and insoluble in most common solvents like methanol, ethanol, and chloroform. The proposed synthetic route for complexes has been depicted in scheme (Fig. 2).

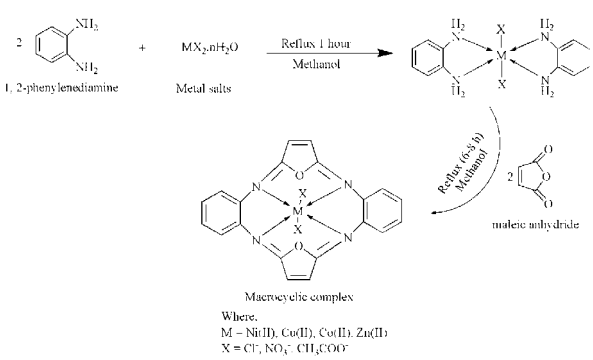

Fig. 2. Reaction procedure for the synthesizing macrocyclic compounds obtained from 1,2-phenylenediamine and maleic anhydride with divalent metal salts of copper, nickel, cobalt, and zinc

Analytical and physical measurements

The determination of the melting point (M.Pt.) was taken on the electrical M.Pt. apparatus. The molar conductivity of complexes was recorded in DMSO on an EI 181 digital conductivity meter available in the laboratory. Magnetic susceptibility measurements were determined at SAIF, IIT, and Roorkee on a vibrating sample magnetometer (Model PAR 155). The IR spectra were taken on a Shimadzu IR spectrometer in the 4000–400 cm^{-1} range and 400–180 cm^{-1} consuming KBr pellet, and the electronic spectral data were taken on a Shimadzu UV 1800 spectrophotometer at CLIR, MM(DU) Mullana. The CHNS-O-Organic elemental analyzer Flash 2000 series had been used for the elemental analysis at CIL Punjab University, Chandigarh. The NMR spectral data of the divalent zinc complex was taken on a Bruker Advance II 400 NMR spectrometer (400 MHz) in DMSO-d_6 at room temperature, and the mass spectra of the complexes were taken on a Q to F microwater LS-MS at SAIF, Punjab University Chandigarh, India. The powder XRD of the Cu(II) complex was taken on an X-ray diffractometer model X'Pert Pro at SAIF, Punjab University, Chandigarh. Gel electrophoresis cleavage experiments were performed with the help of Axygen electrophoresis supported by a Genei power supply with a potential range of 50–5000 volts.

Biological studies

All the complexes were evaluated for antimicrobial activity, and for initial testing, two of the most prevalent human pathogens were employed, namely *Escherichia coli* (MTCC 739), a fecal contaminant, and *Staphylococcus aureus* (MTCC 731), a predominant Gram-positive human pathogen. In addition, to investigate how the metal complexes exert their influence on microorganisms, an examination of their impact on the genomic DNA (extracted from the respective bacterial strains) was also carried out. In this regard, Gram-negative (*E. coli*) and Gram-positive (*S. aureus*) bacterial strains and plasmid DNA for cleaving studies were sourced from the CLIR, MM(DU) Mullana.

Methods

Anti-bacterial screening

The current screening involves the use of Gram-negative (*E. coli*) and Gram-positive (*S. aureus*) bacterial strains. The evaluation of the antibacterial properties of the metal complexes was conducted through the well diffusion method, employing various concentrations of the complexes (ranging from 100 to 500 μg/mL) dissolved in DMSO as the solvent.[8] The inhibition zone was measured after a 24-hour incubation at temperatures between 35°C and 37°C, as per the experimental conditions.

DNA cleaving studies

Concentrated solutions (100 μg/mL) of the metal complexes were prepared by dissolving them in DMSO. Subsequently, all complexes were dissolved in a suspension containing Tris, EDTA (TE) buffer, and plasmid DNA. These reaction solutions were carefully prepared in polyethylene micro-centrifuge tubes and then exposed to UV irradiation (265 nm) for a duration of 25 minutes on the surface of a transilluminator. To create the gel matrix, 1 g of agarose was dissolved in 100 ml of 1×TAE buffer. Once the temperature reached approximately 55°C, ethidium bromide (5 mg/0.5 mL) was added to the mixture. This resulting mixture was then transferred into a gel cassette equipped with a comb and allowed to solidify. Subsequently, it was placed in an electrophoresis chamber filled with TAE buffer. For the electrophoresis procedure, all samples were mixed with loading dye and carefully loaded into wells alongside untreated DNA samples. Electrophoresis was conducted for a duration of one and a half hours, after which the bands were visualized under a transilluminator.[17,18]

Computational studies

Computational methods

All calculations were made using Gaussian 09 (G 09) computational codes applying B3LYP (Becke's three-parameter hybrid exchange and Lee-Yang-Parr non-local correlation functional) using DFT (density functional theory).[19] For all computations, standard 6-31G (d,p) basis set for the lighter element such as C, H, N and O and the LanL2DZ effective core potential for metals i.e. Co, Cu, Ni and Zn apply.[20] All geometries will be optimized to zero negative vibrational-frequency to signify local minima accompanying with the positive eigen values only. To account for solvent effect, DFT calculations are coupled with CPCM (conductor-like polarizable continuum model) in a water medium.[21]

Docking methods

Lead molecules were manifest to molecular docking studies using AutoDoc 4, which covers the Lamarckian Genetic Algorithm (LGA) to calculate the binding affinities of some conformers and AutoDoc Tools (ADT) to perform operations and resulting calculations.[22] The three-dimensional structures of complexes S1, S2, S3, S4, S5, S6, S7, S8 and S9 obtained for DFT optimized geometries were converted to PDB form through Gauss View. The optimized complexes with the X-ray crystal structure of COX-2 (PDB ID: 5COX) were used for docking purposes.[22] Each atom in both case the target and the lead compounds were imbued with Gasteiger charges. Prior to docking, a grid size of 40×40×40 for the protein was assigned to the binding site, the number of points in x, y, and z were used with a spacing of 1 Å to represent all putative active site residues that most prominent are HIS886 and HIS386.[22] The functioning principle and output parameters were similar to the aforementioned DNA docking. Docked poses are visualized by PyMol and LigPlot+ molecular visual programs.[23]

Results

(S1) [$Cu(C_{20}H_{12}N_4O_2)(OAc)_2$]: Yield ~ 58%, Color=Black, M.wt.=522, M. pt.=265˚C; Analytical Calculation: M=11.11; C=53.07; H=2.85; N=9.69; O=17.34; Molar conductivity in DMSO 15 ohm^{-1} cm^2 mol^{-1}. Magnetic moment μ_{eff} (BM): 1.86. IR (cm^{-1}): $\nu_{C=N}$, 1550 (s); ν_{C-N}, 1260 (s); $\nu_{M\rightarrow N}$, 420 (s); ν_{OAc}, 1280 (m). UV-visible in DMSO (nm): ~990, ~680, ~320.

(S2) [$Cu(C_{20}H_{12}N_4O_2)Cl_2$]: Yield ~ 60%, Color=Brown, M.wt.=475, M. pt.=260˚C; Analytical Calculation: M=11.35; C=49.55; H=2.12; N=10.77; O=5.71; Molar conductivity in DMSO 13 ohm^{-1} cm^2 mol^{-1}. Magnetic moment μ_{eff} (BM): 1.90. IR (cm^{-1}): $\nu_{C=N}$, 1530 (s); ν_{C-N}, 1310 (s); $\nu_{M\rightarrow N}$, 425 (s); ν_{M-X}, 350 (m). UV-visible in DMSO (nm): ~990, ~680, ~480, ~310.

(S3) [$Cu(C_{20}H_{12}N_4O_2)(NO_3)_2$]: Yield ~ 59%, Color=Lavender blue, M.wt.=527, M. pt.=270˚C; Analytical Calculation: M=11.01; C=40.29; H=2.09; N=14.92; O=22.25; Molar conductivity in DMSO 18 ohm^{-1} cm^2 mol^{-1}. Magnetic moment μ_{eff} (BM): 1.91. IR (cm^{-1}): $\nu_{C=N}$, 1525 (s); ν_{C-N}, 1315 (s); $\nu_{M\rightarrow N}$, 450 (s); ν_{M-X}, 230 (m). UV-visible in DMSO (nm): ~1000, ~700, ~490, ~310.

(S4) [$Ni(C_{20}H_{12}N_4O_2)Cl_2$]: Yield ~ 41%, Color=White, M.wt.=470, M. pt.=243˚C; Analytical Calculation: M=11.49; C=50.12; H=2.17; N=10.92; O=5.81; Molar conductivity in DMSO 16 ohm^{-1} cm^2 mol^{-1}. Magnetic moment μ_{eff} (BM): 3.05. IR (cm^{-1}): $\nu_{C=N}$, 1515 (s); ν_{C-N}, 1280 (s); $\nu_{M\rightarrow N}$, 430 (s); ν_{M-X}, 280 (m). UV-visible in DMSO (nm): ~810, ~310, ~240.

(S5) [$Ni(C_{20}H_{12}N_4O_2)(NO_3)_2$]: Yield ~ 54%, Color=Purple, M.wt.=522, M. pt.=240˚C; Analytical Calculation: M=10.22; C=43.93; H=1.99; N=15.07; O=22.47; Molar conductivity in DMSO 18 ohm^{-1} cm^2 mol^{-1}. Magnetic moment μ_{eff} (BM): 3.10. IR (cm^{-1}): $\nu_{C=N}$, 1510 (s); ν_{C-N}, 1270 (s); $\nu_{M\rightarrow N}$, 425 (s); ν_{M-X}, 215 (m). UV-visible in DMSO (nm): ~800, ~430, ~320, ~280.

(S6) [$Co(C_{20}H_{12}N_4O_2)(OAc)_2$]: Yield ~ 45%, Color=Moss green, M.wt.=517, M. pt.=275˚C; Analytical Calculation: M=10.39; C=54.72; H=3.01; N=9.83; O=17.56; Molar conductivity in DMSO 18 ohm^{-1} cm^2 mol^{-1}. Magnetic moment μ_{eff} (BM): 4.31. IR (cm^{-1}): $\nu_{C=N}$, 1520 (s); ν_{C-N}, 1320 (s); $\nu_{M\rightarrow N}$, 425 (s); ν_{OAc}, 1230 (m). UV-visible in DMSO (nm): ~780, ~510, ~320.

(S7) [$Co(C_{20}H_{12}N_4O_2)Cl_2$]: Yield ~ 52%, Color=Dark orange, M.wt.=470, M. pt.=270˚C; Analytical Calculation: M=11.53; C=49.09; H=2.01; N=10.92; O=5.81; Molar conductivity in DMSO 15 ohm^{-1} cm^2 mol^{-1}. Magnetic moment μ_{eff} (BM): 3.99. IR (cm^{-1}): $\nu_{C=N}$, 1540 (s); ν_{C-N}, 1290 (s); $\nu_{M\rightarrow N}$, 440 (s); ν_{M-X}, 290 (m). UV-visible in DMSO (nm): ~790, ~520, ~310.

(S8) [$Co(C_{20}H_{12}N_4O_2)(NO_3)_2$]: Yield ~ 56%, Color=Dark orange, M.wt.=523, M. pt.=265˚C; Analytical Calculation: M=10.26; C=44.91; H=2.01; N=15.06; O=23.46; Molar conductivity in DMSO 19 ohm^{-1} cm^2 mol^{-1}. Magnetic moment μ_{eff} (BM): 4.15. IR (cm^{-1}): $\nu_{C=N}$, 1525 (s); ν_{C-N}, 1330 (s); $\nu_{M\rightarrow N}$, 435 (s); ν_{M-X}, 230 (m). UV-visible in DMSO (nm): ~730, ~540, ~320.

(S9) [$Zn(C_{20}H_{12}N_4O_2)(OAc)_2$]: Yield ~ 51%, Color=Moss green, M.wt.=524, M. pt.=254˚C; Analytical Calculation: M=11.48; C=53.03; H=3.02; N=9.70; O=17.33; Molar conductivity in DMSO 20 ohm^{-1} cm^2 mol^{-1}. Magnetic moment μ_{eff} (BM): 0.0. IR (cm^{-1}): $\nu_{C=N}$, 1560 (s); ν_{C-N}, 1325 (s); $\nu_{M\rightarrow N}$, 445 (s); ν_{OAc}-, 1250 (m). UV-visible in DMSO (nm): ~470, ~280. NMR (DMSO, in ppm): δ, 7.6 (m, benzene ring); 6.7 (m, furan ring); 2.50 (s, acetate ion).

ESI-MS (m/z): Mass spectral data (MS) of metal compounds were noted, as this technique provides the structural evidence of compounds under analysis. The polymeric or monomeric character of macrocyclic compounds can be interpreted with the help of MS technique.[24] The MS of some representative complexes S1, S2, S3, S5, S7, S8, and S9 showed molecular ion peaks at 521.97, 474.32, 526.08, 521.96, 469.12, 524.43, and 523.48 corresponding to $[M]^+$ ion peaks respectively. The MS of S1 exhibits peaks at 523.47 and 540.53 corresponding to $[M+1]^+$ and $[M+H_2O]^+$ ion peaks respectively. The MS of S2 complex exhibit molecular ion peak at m/z 475.01 and 473.32 corresponds to $[M+1]^+$ and $[M-H]^+$ ion respectively. $[M+DMSO]^+$ ion peak exhibited by S3 and S8 at m/z 607.39 and 601.59 respectively.

Powder X-ray diffraction

In the absence of a single crystal system, the powder X-ray diffraction methodology is a well-liked method for analyzing the structural data of inorganic and organic materials. In this study, a powder X-ray diffraction analysis of one of the Cu(II) metal complex was carried out by scanning it between Bragg's angle 2θ=5° and 90°. The $[Cu(C_{20}H_{12}N_4O_2)(NO_3)_2]$ produced shows several sharp and intense diffraction peaks at 2θ value of 8.57°, 20.83°, 22.46°, 25.92°, 30.23°, 33.69° that indicate its crystalline form in the diffractrogram of the complex. Peak positions show the value of 2θ that are corresponds to the interatomic spaces present in crystal lattice. Higher the intensity of the peak more is the number of atoms in particular arrangement in the lattice site that related to the strength of scattering.[25,26]

Antibacterial assay

All the novel macrocyclic complexes were screened for their anti-bacterial potential against *E. coli* (Gram-negative) and *S. aureus* (Gram-positive). Ampicillin is taken as standard reference drug for elucidation of bactericidal effect of synthesized macrocyclic scaffold on Gram- negative bacteria and Gram-positive bacteria. All complexes showed no activity against *E. coli* (Gram-negative) tested strains as shown in Fig. 3 (not shown in tabular form), where strong activity was observed against *S. aureus* (Gram-positive bacteria) as shown in Figures 4, 5, and Table 1.

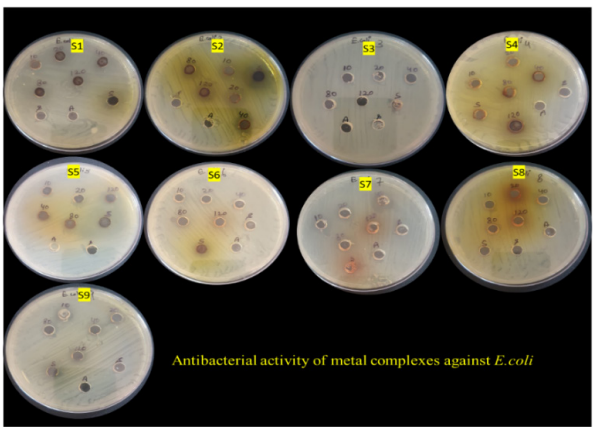

Fig. 3. Antibacterial activity of macrocyclic complexes against *E. coli* (Gram-negative bacteria)

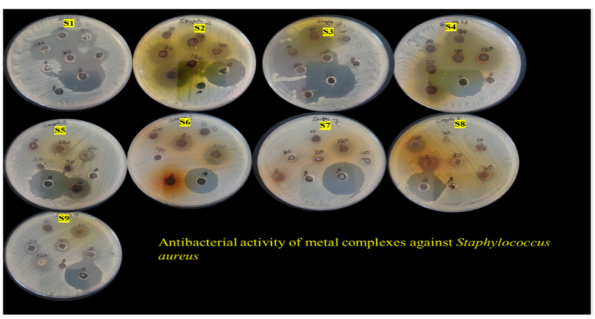

Fig. 4. Anti-bacterial activity of metal complexes against *S. aureus* (Gram-positive bacteria)

Table 1. Zone of inhibition diameter of novel metal complexes against (Gram-positive bacteria)*

	Zone diameter measurement at different concentrations of the metal complexes (in mm)					
Sample name	**100 µg/mL**	**200 µg /mL**	**300 µg/mL**	**400 µg/mL**	**500 µg/mL**	**Drug (Vancomycin)**
S1	0	12	12	17	17	30
S2	12	15	20	20	25	30
S3	12	16	20	20	25	30
S4	4	12	19	20	25	30
S5	0	0	0	6	10	30
S6	0	0	0	8	12	31
S7	0	0	0	0	10	30
S8	0	2	0	5	10	30
S9	0	3	3	9	12	35

*values – means of three replicates (including the diameter of the well)

DNA photo-cleavage studies

The most tenacious concern for humanity today is the potential conquest of cancer through the discovery of chemical nucleases. Transition metal complexes, known for their significant nuclease activities and specific DNA binding capabilities, have been investigated for their impact on DNA photocleavage.[27] The results of the DNA photocleavage analysis are depicted in Figure 6.

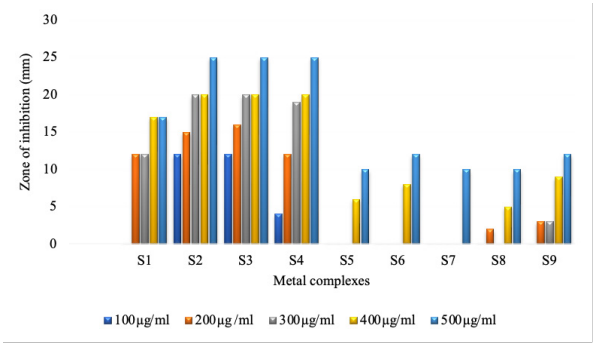

Fig. 5. Bar graph representation of zone of inhibition diameter of novel metal complexes against Gram-positive bacteria

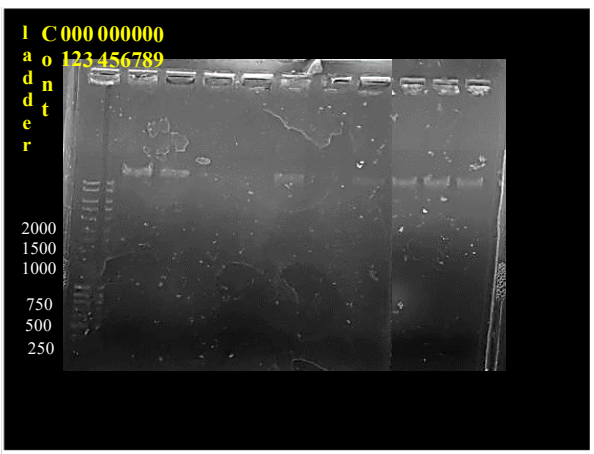

Fig. 6. DNA photo-cleavage study against metal complexes indicated S2 and S3, S5 exhibit strong DNA photo-cleavage activity, S4 and S6 showed moderate cleavage of DNA whereas S7, S8, S9 showed no DNA cleavage

DFT studies

Computational studies of synthesized *d*-metal complexes such as S1-S9 complex were carried out by using the combined DFT-B3LYP technique by means of Gaussian-09 computational codes. Various parameters of quantum-chemical were computed through B3LYP/6-31G**/LanL2DZ ECP techniques i.e. geometry optimization, ESP charges, molecular energy, the energy of FMO (frontier molecular orbitals), and bandgap etc. The square planar geometry exhibited by the macrocyclic complexes as shown in Figure 7. The ligand describes the square planar geometry with d metals (Co (II), Ni(II) Cu (II) and Zn(II)) center the corresponding key bond - lengths of the N---M and O---M was found to be 2.427, 2.506, 2.472, 2.445 Å and 1.799, 1.822, 1.760, 1.862 Å for Co(II) complexes (S6-S8), Ni(II) complexes (S4-S5), Cu(II) complexes (S1-S3), and Zn(II) complex S9 respectively (Table 2). Electrostatic potential recorded onto the surface of constant electron density for optimized geometry on the Van der Walls surface. Also, it is very beneficial in exploration of molecular structures with their photophysical properties relationship, as well

as hydrogen-bonding interaction in d-metal complexes such as S1-S9.[28] The maximum negative region that is showed by red color, is the favored site for the electrophilic attack, and the maximum positive region is shown blue color that is favored site for the nucleophilic attack (Fig. 8).

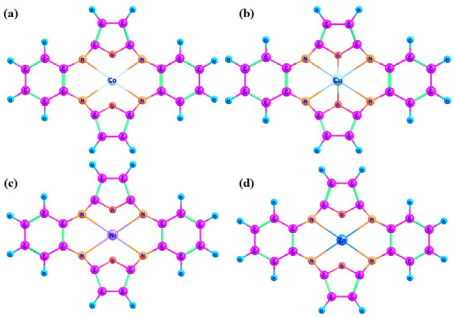

Fig. 7. Optimized molecular geometry S6-S8(a), S1-S3(b), S4-S5(c), and S9(d) complex by using the DFT/B3LYP method

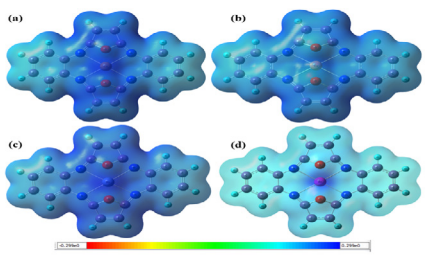

Fig. 8. Electrostatic potential map of S6-S8(a), S1-S3(b), S4-S5(c), and S9(d) complex by using the DFT/B3LYP method

Table 2. The optimized bond length of tetrahedral complexes (Å)

S. No	Complex ID	N_1---M	N_2---M	N_3---M	N_4---M	O_1---M	O_2---M
1	S1-S3	2.506	2.506	2.506	2.506	1.822	1.822
2	S4-S5	2.472	2.472	2.472	2.472	1.760	1.760
3	S6-S8	2.427	2.427	2.427	2.427	1.799	1.799
4	S9	2.445	2.445	2.445	2.445	1.862	1.862

The FMO (frontier molecular orbital) comparing the HOMO (Highest occupied molecular orbital), and the LUMO (lowest molecular orbital) with energy gap (ΔE) between the HOMO and the LUMO of all the d-complex such as S1-S9 are derivatives were calculated and displayed in Fig. 9. Energies of the FMOs (E_{HOMO} and E_{LUMO}), ΔE which describes the eventual C→T interaction within the molecule, chemical potential (μ), electronegativity (χ), global softness (S), global hardness (η) and global electrophilicity index (ω) are recorded in Table 3.[29,30] The importance of all these parameters is to calculate the molecular reactivity and stability. The electrophilicity index i.e. ω is one of the vital quantum chemical parameter, which describes the poisonousness of numerous pollutants in terms of their site selectivity and reactivity.[31] Also, the electrophilicity calculates the biological potency of drug receptor interaction.

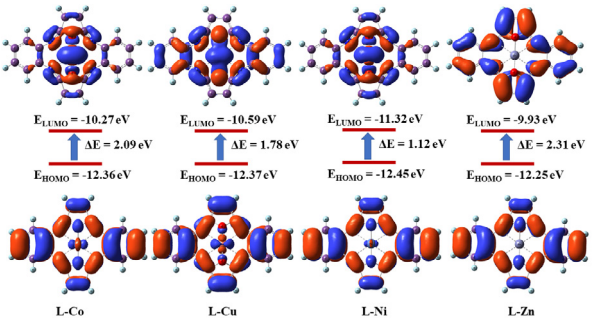

Fig. 9. FMOs of S6-S8(L-Co), S1-S3(L-Cu), S4-S5(L-Ni) and S9(L-Zn) complex by using DFT/B3LYP method

Table 3. Calculated molecular electronic parameters*

S. No	Complex	Energy (kcal/mol)	DM	HOMO (eV)	LUMO (eV)	Gap (eV)	χ (eV)	μ (eV)	η (eV)	S (eV)	ω (eV)
1	S1-S3	-840655.94	3.38	-12.37	-10.59	-1.78	11.48	-11.48	-0.89	-0.44	-58.60
2	S4-S5	-823542.95	1.84	-12.45	-11.32	-1.12	11.89	-11.89	-0.56	-0.28	-39.69
3	S6-S8	-808295.64	1.58	-12.36	-10.27	-2.09	11.31	-11.31	-1.05	-0.52	-66.95
4	S9	-758251.46	1.72	-12.25	-9.93	-2.31	11.09	-11.09	-1.16	-0.58	-71.08

* DM – dipole moment, EH – energy of HOMO, EL – energy of LUMO, ΔE – energy band gap, χ – electronegativity, μ – chemical potential, η – global hardness, S – global softness, ω – global electrophilicity index

Molecular modelling studies

A molecular docking study is significant for forecasting the molecular mechanism and binding interaction between the active site of the receptor and ligand. Molecular docking studies of macrocyclic compounds, S1-S9, in potent area of the COX-2 (PDB code is 5COX), were completed, to get a perception into the nature of the interaction between the complex and potent site of 5COX, with AutoDock4 tool. The valuation of every docked molecule was completed by assessing the scoring function as shown in Table 4, which assists as evidence of how finely the ligand-bound inside the binding pocket.

It was found that all of the complexes had fitness docking scores ranging from -7.27 to -8.08, with the medication celecoxib having a value of -5.10 (Table 4). Among all the substances, compound zinc acetate-based (S9) placed first with a docking score of -8.08 and binding energy of 8.06 kcal/mol, and Ni(II)-based (S4-S5) placed second with a docking score of -8.01 and binding energy of 8.00 kcal/mol whereas celeoxib had a docking score of -5.10 and binding energy of -6.00. Furthermore, the compounds S1, S3, and S9 exhibit hydrogen bonding interactions with the HIS207 residue, with bond lengths of 3.26, 3.09, and 3.09, respectively. It also demonstrated excellent interactions with target protein binding site residues in a fashion similar to celecoxib. Fig 10 depicts the protein-ligand interaction visualization of all compounds and the medication celecoxib.

Fig 11 depicts the 3D binding surface plot of all compounds and drugs with protein. Overall, the docking data demonstrate that the synthesized complexes outperform the conventional medication celecoxib in terms of binding interaction with COX2.

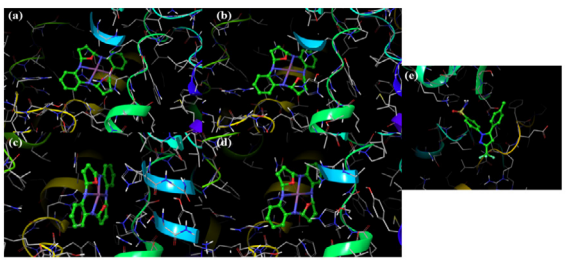

Fig. 10. Molecular docking interaction of S1-S9 complexes with COX2

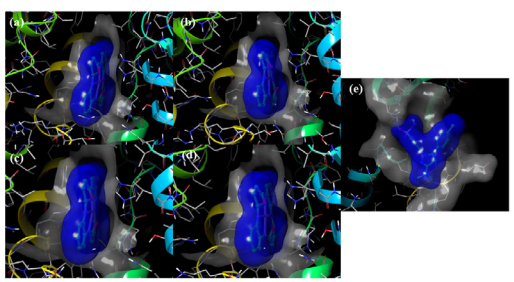

Fig. 11. Ligplot 2D residues interaction plot of S1-S9 complexes with COX2

Table 4. Molecular docking studies of S1-S9 complexes with COX2

Complex	Binding free energy ($\Delta G_{binding}$)[a]	Vdw_hb_ desolv energy ($\Delta G_{vdW+hb+desolv}$)	Electrostatic energy (ΔG_{elec})	Total internal energy (ΔG_{total})	Torsional free energy (ΔG_{tor})	H bond length (Å)	Interaction residue
S1-S3	-7.79	-7.77	-0.02	0.0	0.0	3.26	HIS207
S4-S5	-8.01	-8.00	-0.01	0.0	0.0	--	--
S6-S8	-7.27	-7.28	0.01	0.0	0.0	3.09	HIS207
S9	-8.08	-8.06	-0.02	0.0	0.0	3.09	HIS207
Cele	-5.10	-6.00	-0.3	-0.84	1.19	2.62, 2.66	ASP393, LYS405

Discussion

The analysis of metal complexes shows that the molecular formula of synthesized complexes can be $[M(C_{20}H_{12}N_4O_2)X_2]$; where M are metals i.e. divalent copper, cobalt, nickel and zinc metals and X=CH_3COO^-, Cl^-, and NO_3^-. The monomeric character of metal complexes was affirmed through molecular weight measurement and ESI-MS studies. Molar conductivity of complexes was checked in DMSO which lies in range of 12-20 ohm^{-1} cm^2 mol^{-1} that indicates the non-electrolytic nature of the complexes.[32] It was observed that the pair of bands which exist between 3,200-3,400 cm^{-1} in the spectrum of 1,2-phenylenediamine which is due to $\nu(-NH_2)$ were found missing in IR of all synthesized complexes.[8] Moreover, the absence of strong absorption band near about 1,710-1,720 cm^{-1} shows disappearance of $\nu_{(-C=O)}$ group due to maleic anhydride.[33] Furthermore, the appearance of a medium to a sharp absorption band in range 1,550-1,640 cm^{-1} provides strong evidence for condensation of carbonyl group of maleic anhydride with amine group of 1,2-phenylenediamine, and is also ascribed to the formation of azomethine (-C=N) linkage. Slight drifting in absorbance frequency was also observed due to the coordination of N of ligand with the central metal ion of synthesized metal complexes. The medium to strong intensity band existing near 1,266-1,342 cm^{-1} can be ascribed to $\nu_{(C-N)}$ vibrations. IR spectra for all nitrate complexes at 1,010- 1,030 cm^{-1}, 1,290-1,320 $cm^{-1,}$ and 1,408- 1,440 cm^{-1} suggest that the nitrate group of metal nitrate salts are coordinated in unidentate fashion with a central metal ion.

Presence of bands in regions 1,650-1,680 cm^{-1} and 1,258-1,290 cm^{-1} can be ascribed to asymmetric $\nu_{(COO-)}$ stretching vibration and symmetric $\nu_{(COO-)}$ stretching vibration of acetate ions of acetate metal salts respectively.[34] IR spectra of far infrared regions show bands at 420-450 cm^{-1} with respect to $\nu_{(M-N)}$ vibration. All synthesized macrocyclic metal complexes show bands in the 420-450 cm^{-1} region, this identifies the co-ordination of azo-methine nitrogen to copper, cobalt, nickel or zinc metal. Absorption band present in region 220-250 cm^{-1} and 300-350 cm^{-1} can be attributed to the $\nu_{(M-O)}$ stretching vibration of nitrate complexes and the $\nu_{(M-Cl)}$ vibrations respectively.[32]

Electronic spectrum of synthesized macrocyclic compounds was reported at room temperature in solution state by using DMSO as solvent. Absorbance band at ~720-790, ~500-540 and 310-320 nm can be corresponds to the $^4T_{1g} \rightarrow {}^4T_{2g}$ (F), (ν_1); $^4T_{1g} \rightarrow {}^4A_{2g}$ (F), (ν_2); and $^4T_{1g}$ (F) $\rightarrow {}^4T_{1g}$(P), (ν_3) electronic transition exhibited by Co(II) metal complexes which is similar to reported distorted octahedral geometry, whereas Ni(II) metal complexes exhibited bands at ~800-820, ~420-430, ~310-320 and ~240-280 nm which can be ascribed to the $^3A_{2g} \rightarrow {}^3T_{2g}$ (F), $^3A_{2g} \rightarrow {}^3T_{1g}$ (F), $^3A_{2g} \rightarrow {}^3T_{1g}$ (P) and n-π^* transitions respectively and show the presence of octahedral environment.[35-37] Magnetic moment (μ_{eff}) of divalent nickel complexes lies in range of 3.05-3.10 B.M. which exhibit the presence of two unpaired electrons.[8] In the case of Cu(II) metal complexes absorbance band exhibits at ~990-1010, ~660-680, ~480-490, and ~310-320 nm corresponding to various d-d transition bands while $\pi \rightarrow \pi^*$ transition band appeared at 240-290 nm and exhibit these complexes have distorted octahedral environment.[33] The effective magnetic moment of Co (II) and Cu (II) complexes was found in variation of 3.99-4.31, and 1.86-1.91 B.M. respectively.[36] The divalent zinc metal compound is

exhibiting an absorbance band near ~470-480 and ~280-300 nm, which may be easily assigned to n→π* and π→π* transition resp.[37,38] ^{1}H-NMR (nuclear magnetic resonance) spectrum of the $[Zn(C_{20}H_{12}N_4O_2)(OAc)_2]$ complex exhibited no peak with respect to the free amine ($-NH_2$) protons which indicate the complex formation.[8] Diffractogram of $[Cu(C_{20}H_{12}N_4O_2)(NO_3)_2]$ produced shows several sharp and intense diffraction peaks at 2θ value of 8.57°, 20.83°, 22.46°, 25.92°, 30.23°, 33.69° that indicate its crystalline form of the complex. The Debye Scherrer formula, $D=K\lambda/\beta\cos\theta$ (where D is normal or average crystallite size, K is Scherrer constant, λ(0.15406) is the employed for X-ray wavelength, and β is the full width half maximum), has been used to forecast the normal crystallite size from notable peaks.[39] The complex $[Cu(C_{20}H_{12}N_4O_2)(NO_3)_2]$ has a crystallite size of 28.98 nm, which denotes the coordinated compounds with nanocrystalline phase.

An urgent need exists for research into anti-infectious agents. Infectious disease outbreak rates have been seen to be rising in recent years. The WHO finds that new antibiotics that target pathogenic microorganisms may not only assist to reduce infections-related fatalities but also lessen the threat of microbes that are treatment resistant. In this regard antibacterial potential of synthesized macrocyclic complexes were also exposed, which exhibits strong activity against *S. aureus* (Gram-positive bacteria) by metal complexes S2, S3, and S4 in concentration range 100−500μg/mL. On the other hand, S1, S5, S8, and S9 showed moderate activity and showed maximum potential to inhibit bacteria at 200 μg/mL dose. There was no activity noticed in the case of complex S6 and S7. The findings of DNA photocleavage studies indicate that metal complexes containing Cu(II) metal ions, such as S2 and S3, as well as Ni(II) metal ions like S5, exhibit robust DNA photocleavage activity. In contrast, the macrocyclic complex featuring divalent nickel metal ions, S4, and divalent cobalt metal ions, S6, demonstrate a moderate level of DNA cleavage. On the other hand, S7, S8 and S9 do not exhibit any DNA cleavage activity. Previously it has been reported that macrocyclic complexes are known to possess promising antibacterial potential. For instance − Rathi et al. reported bioactive macrocyclic complexes against various gram positive and gram-negative bacterial strains in the range of 64- 512μg/mL dose. Therefore, our results are consistent with the previously reported studies.[37,38]

DFT studies describes the square planar geometry of ligand with d metals (Co (II), Ni(II) Cu (II) and Zn(II)) center. As shown in Fig. 9, the LUMO orbitals are placed on the center of metal and the HOMO orbitals are placed on the ligand system in case of Co^{2+}, Ni^{2+} and Cu^{2+}. But in the case of Zn^{2+} the LUMO orbital is placed on ligand system, showing partially filled d-metal center can act as an excited electron acceptor and filled Zn act as no involving of acceptor. Both complexes exhibit the band gap energy range from 1.12 to 2.31 eV. Furthermore, ΔE is a prime parameter for the characterization of kinetic stability and chemical reactivity of the molecule.[40] ΔE is small which designates easy charge transfer (C→T) process in it, which influences the biological potency of the molecule. The findings of molecular docking studies reveals Ni(II) complexes (S4-S5) and Zn(II) complex (S9) both were designated to be extremely active with their uppermost docking scores, i.e., -8.01 kcal/mol and -8.08 kcal/mol respectively. The Co(II) complexes (S6-S8), Cu(II) complexes (S1-S3), and Zn(II) complex (S9) show one hydrogen bonding with HIS207, and hydrogen bonds length was found to be 3.09 Å and 3.29 Å.

Conclusion

Because infection and mortality rates from microbiological diseases are rising so rapidly in the modern era, modern medicine faces enormous challenges. This work synthesized a unique series of macrocyclic compounds with the Schiff bases Ni(II), Co(II), Cu(II), and Zn(II). Analytical and spectroscopic results demonstrated the macrocyclic complex's monomeric nature. Diffractogram analyses have revealed that a complex with a dimension of 28.98 nm is nanocrystalline. The synthetic complexes' ability to cleave DNA and fight germs was assessed. At 200 μg/mL, all complexes exhibited excellent to moderate antibacterial efficacy, while macrocycles had strong DNA-cleaving potency. Due to their highest docking scores, docking studies demonstrate the extremely active behavior of both Ni(II) and Zn(II) complexes towards the active region of COX-2 through their uppermost docking score i.e. -8.01 kcal/mol and -8.08 kcal/mol respectively.

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Author contributions

Conceptualization, P.M. and P.S.; Methodology, P.M., P.S., A.K.S., D.M. and S.K.R.; Validation, P.M., P.S., D.M. and S.K.R.; Formal Analysis, P.M., P.S., D.M. and S.K.R.; Resources, P.M., P.S., A.K.S., A.T. and S.K.R.; Writing – Original Draft Preparation, P.M., P.S., D.M. and S.K.R.; Writing – Review & Editing, P.M., P.S., D.M. and S.K.R.

Conflict of interest

The authors state, there are no conflict of interest about the publication of this research work.

Data availability

The dataset generated during research work is available from corresponding author on request.

Ethics approval

Not applicable.

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REVIEW PAPER

Paroxysmal non-epileptic events vs epilepsy – what we know and where we are in medicine?

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ABSTRACT

Introduction and aim. Paroxysmal non-epileptic events (PNEEs) are neurological conditions that include behavioral changes or disturbances of consciousness. The aim of the article is to compare individual paroxysmal non-epileptic events disorders, to indicate differences in their clinical picture and to discuss their differential diagnosis.

Material and methods. A review of the most common non-epileptic paroxysmal events is presented based on the available literature of PubMed and Google Scholar databases from 2000 to 2023.

Analysis of the literature. Depending on the age of the child, the nature and type of seizures are variable. Unfortunately, epilepsy is currently overdiagnosed, which results in the inclusion of antiepileptic drugs without the need to use them. This may be related to the immaturity of the central nervous system, malfunctioning of other organs or have a psychogenic background. In most cases, they do not require pharmacological treatment.

Conclusion. Paroxysmal non-epileptic events, due to the diverse and uncharacteristic clinical picture, pose a major diagnostic challenge. Because of the current overdiagnosis of epilepsy they should always bear in mind differential diagnosis. This is important because of the differences in the treatment of these disorders.

Keywords. epilepsy, non-epileptic seizure in childhood, paroxysmal non-epileptic events

Introduction

Paroxysmal nonepileptic events (PNEEs) are a diverse range of short-lived events characterized by sudden and brief alterations in motor or behavioral activity. These manifestations can closely mimic seizures, leading to clinical confusion.[1] Non-epileptic seizure disorders, due to the diverse and uncharacteristic clinical picture, pose a major diagnostic challenge. Unfortunately, epilepsy is currently overdiagnosed, which results in the inclusion of antiepileptic drugs without the need to use them. It is estimated that about 30% of patients who see a specialist with a diagnosis of epilepsy actually suffer from other non-epileptic seizure disorders.[2,3] In most cases, when epilepsy is suspected, patients are referred for a video electroencephalography (VEEG) examination. About 43% of them are diagnosed with PNEEs.[4] An important point to emphasise is that PNEEs with a lot of sundry symptoms such us vomiting, dizziness or irregular

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breathing can resemble all types of epileptic seizures.[5] Depending on the age of the child, the nature and type of seizures are variable. They may be related to the immaturity of the central nervous system or malfunctioning of other organs. In most cases, they are mild, subside spontaneously and do not require pharmacotherapy.[2,3] Recent studies report that the background of non-epileptic disorders is a combination of neurology and psychiatry representing nuerobehavioural conditions.[6]

Aim

The aim of the article is to compare individual paroxysmal non-epileptic events to indicate differences in their clinical picture, discuss their differential diagnosis, and compare their diagnostics to epilepsy.

Material and methods

A review of the most common non-epileptic paroxysmal events is presented based on the available literature of PubMed and Google Scholar databases from 2000 to 2023.

Analysis of the literature

Breath-holding spells

Breath-holding spells are among the most common non-epileptic seizure disorders in childhood. They affect about 5% of the population. They consist of a sudden cessation of breathing, preceded by crying or a strong emotional reaction, sometimes followed by loss of consciousness, hypotonia, and even seizures. Breath-holding spells, depending on the dominant clinical symptoms, are divided into cyanotic, pale and mixed. They are called affective because of the factors that trigger them – such as stress or strong emotions. Autonomic instability is suspected in the pathomechanism of seizures. Hereditary apnea is suspected in about a quarter of patients.[7] Attacks of breath-holding spells most often begin between 6–24 months of age. They disappear spontaneously between 5 and 6 years of age, sometimes a little later.[8]

Differentiation of breath-holding spells attacks from epileptic seizures remains a major clinical challenge. An epileptic seizure, unlike breath-holding spells, is not caused by an external factor. During the breath-holding spells, the EEG shows diffuse slowing of bioelectrical activity without convulsive activity. As with seizures, a child may or may not become excessively sleepy after breath-holding spells. Always include iron deficiency anemia in the differential diagnosis.

Breath-holding spells usually do not require treatment. However, recently published studies have shown significant efficacy of piracetam. In double-blind studies, a reduction and, in some patients, cessation of attacks of breath-holding spells after treatment with piracetam was observed.[7]

Vasovagal syncope

Vasovagal syncope (VVS), also known as orthostatic syncope, accounts for approximately 60-80% of syncope in children.[9] They are characterized by a short and sudden loss of consciousness. The reason for this is a temporary decrease in blood flow to the brain.[10] The main causes of vasovagal syncope include: extreme stress, nervousness, prolonged standing in a fixed position, especially in a standing position, sudden changes in body position, the sight of blood or external factors such as high temperature, staying in crowded places.[11] Fainting can occur after very intense training or insufficient hydration. Children who develop vasogal syncope usually do not need any treatment.[12] Lifestyle modification is often recommended drinking more water and avoiding stressful situations. It is worth obtaining a thorough medical history, information from witnesses of the event, and information from the child's guardians in order to exclude cardiac causes.

Stereotypies

Stereotypies are involuntary repetitive movements that are performed in a coordinated, rhythmic manner. It is important to emphasise that every healthy person manifests stereotypical behaviour on a daily basis, e.g. when swaying to music. Anxiety should only arise when the activity is performed intensively and over a long period of time. It may also involve self-injury or interfere with daily functioning. Stereotypies are characterised by an unusually high degree of individual variability and a wide range of movement types, some of which disappear over the course of life and others persist and intensify. It is very difficult to clearly define and classify the condition, as it may be manifested by walking in circles in some people and licking the lips or picking at the skin in others. It is often confused with nervous behaviour, as it is often performed under a strong stress stimulus. Repetitive movements are often seen in infancy and in young children. They are also characteristic of people with developmental delay. It is important to emphasise that stereotypies occur in both children with normal development and those with any disorder. Initially, they may take the form of involuntary and aimless movements, only to become more conscious and deliberate as the child grows up. Usually, a properly performed differential diagnosis and a correctly made diagnosis are sufficient to provide the necessary information to parents without introducing a therapeutic process.[13]

Paroxysmal dyskinesias (PDs)

Paroxysmal dyskinesia is a condition in which there are characteristic sudden, violent, uncontrollable movements that can affect various parts of the body without impairment of consciousness. They may include involuntary movements of the face, arms, legs or other

muscles. This type of dyskinesia is considered a movement disorder and may resemble the symptoms of epilepsy. They can occur in children of all ages, but are more common in older children and teenagers, especially those aged 7 to 15 years. We distinguish primary and secondary characteristics.[14] Diagnosing paroxysmal dyskinesia in children can be difficult because the symptoms may be transient and mimic other movement disorders. The causes of paroxysmal dyskinesia in children are not fully understood. In some cases, they may be related to genetic factors, and may also be related to abnormal functioning of the nervous system. Sometimes paroxysmal dyskinesia can be caused by the use of certain drugs, chemicals. Factors causing paroxysmal dyskinesia may also be physical exertion or fatigue.[15]

The first descriptions of this condition appeared in 1977. The classification distinguishing these disorders was established 18 years later, and in 2004, the classification of paroxysmal dyskinesias, which is currently in use, was created. Currently, the following forms of the disease are distinguished: kinesigenic paroxysmal dyskinesia, paroxysmal non-kinesigenic dyskinesia, exercise-induced paroxysmal dyskinesia and paroxysmal hypnogenic dyskinesia. Typically, the diagnosis is based on patient or family reports, as these symptoms are rarely witnessed by a doctor.[16,17]

Paroxysmal kinesigenic dyskinesia (PKD)

Paroxysmal Kinesigenic Dyskinesia are among the most commonly encountered types of paroxysmal dyskinesias. They are triggered by sudden movements, changes in direction, or surprises. The most characteristic symptom is dystonia. The duration of an attack is very brief, ranging from a few seconds to a few minutes. Attacks can occur up to 100 times a day. This disorder is more commonly seen in men, occurring four times more frequently than in women. In most cases, the initial symptoms appear during childhood. Typically, the symptoms peak during adolescence, and remission is possible in adulthood.[18]

PKD diagnostic criteria:[19]

- triggered by movement
- onset between 1 and 20 years of age
- attack duration less than 1 minute
- no loss of consciousness during the attack
- good response to antiepileptic treatment
- absence of pain during the attack
- symptoms of attacks include dystonia and/or chorea

Mutations in the PRRT2 gene are the main factor causing the isolated form of paroxysmal dyskinesia. Such a mutation is present in 27% to 65% of cases.[20] It is also worth emphasizing that paroxysmal dyskinesia induced by movement may also be caused by secondary factors, such as strokes, multiple sclerosis or metabolic disorders.[16,21]

Paroxysmal non-kinesigenic dyskinesia (PKND)

Non-kinesigenic paroxysmal dyskinesiasare characterized by episodes of dystonia and/or chorea that occur without a clearly defined, immediate trigger. These episodes last longer than PKD, ranging from several minutes to even several hours.[20] Episodes of this form can be exacerbated by caffeine, alcohol, feelings of fatigue, or intense emotional stress. The frequency of episodes varies - attacks may occur several times a week but can also be sporadic, happening only a few times throughout one's lifetime. Most commonly, patients experience attacks from 1 to 3 times a day or twice a year. Similar to PKD, these attacks are more frequent in men.[18]

Diagnostic criteria for PNKD:[22]

- symptoms of attacks include dystonia and/or chorea
- no loss of consciousness during the attack
- onset of the disease typically occurs in childhood
- lack of a clear triggering factor, although precipitating factors may be present
- duration of attacks: from 10 minutes to 4 hours
- antiepileptic treatment, except for benzodiazepines, usually does not provide significant improvement.

Paroxysmal exercise-induced dyskinesia (PED)

Paroxysmal exercise-induced dyskinesiasare seizures characterized by the occurrence of dystonia and/or chorea triggered by physical exertion. The attack typically initiates in the muscles that were most heavily stressed during the physical activity.[16] The variety of attacks is wide, but most often these dystonias affect the legs. The condition usually first appears in childhood. The occurrence of seizures is observed in a male-to-female ratio of 2:3.[18]

PED diagnostic criteria:[23]

- trigger factor: exercise
- duration of attacks: from 5 minutes to 30 minutes
- dystonia and/or chorea attacks
- no loss of consciousness during the attack
- no significant response to antiepileptic treatment
- the onset of the disease typically occurs in childhood.

Paroxysmal hypnogenic dyskinesia (PHD)

Paroxysmal hypnogenic dyskinesia involves sudden episodes of dystonic and tonic movements that occur during sleep.[20] The duration of an attack ranges from 30 seconds to 50 minutes. The frequency of occurrence varies widely, from several episodes per night to several episodes per year. These attacks are more common in men than in women.[18] Currently, PHD is mostly considered a form of frontal lobe epilepsy, known as autosomal dominant nocturnal frontal lobe epilepsy.[16]

Tourette syndrome (TS)

Tourette syndrome is a neuropsychiatric disorder characterized by chronic motor, vocal or phonic tics and often accompanied by obsessive compulsive disorder and

attention deficit hyperactivity disorder. It has an incidence of 7.7 per 1,000 children, with a 4-fold prevalence in boys. The maximum severity of tics occurs between the ages of 10 and 12. Genetic, environmental and immunological factors are responsible for the development of TS. Typical motor tics include eye blinking, arm jerking, head jerking, facial grimacing, nose rubbing. Vocal tics include grunting, sniffing, clucking, squeaking. Coprolalia is characteristic of TS. Motor tics usually precede vocal tics. As many as 85% of children with TS have one or more comorbid neurodevelopmental or psychiatric conditions, such as attention deficit hyperactivity disorder and obsessive compulsive disorder. Therapy of patients with TS should be tailored to individual needs and focused on the most troublesome tics. Patient, family and school education is a key element of successful treatment. Some patients do not necessarily require specific therapy. Sometimes education about the disease is enough. If the tics are severe and cause functional impairment, pharmacological intervention may be indicated. Sometimes education about the disease is enough. If the tics are severe and cause functional impairment, pharmacological intervention may be indicated. Sometimes education about the disease is enough. If the tics are severe and cause functional impairment, pharmacological intervention may be indicated.[24-26]

Hyperekplexia

Hyperekplexia is a rare neurological disorder characterized by an exaggerated startle response, which can manifest as sudden eye blinking or body spasms in response to tactile or auditory stimuli.[27] During seizures, individuals with hyperekplexia may experience severe rigidity in their trunk, limbs, and, in some cases, even their respiratory system, which can lead to life-threatening laryngospasms. Additional symptoms may include muscle twitches and clenched fists. While the onset of hyperekplexia can occur during fetal life, it more commonly presents after birth or during childhood.

The inheritance pattern of hyperekplexia typically follows an autosomal dominant trait, although autosomal recessive or, rarely, X-linked inheritance has also been reported. Typically, hyperekplexia is caused by mutations in the alpha 1 subunit of the glycine receptor gene, known as GLRA1.[28] The exaggerated startle response observed in hyperekplexia can manifest as rapid jerks or a series of jerks, which can sometimes mimic myoclonic, tonic, or tonic-clonic seizures. VEEG plays a valuable role in aiding the diagnosis of this condition. In the full range of typical epileptic seizures in the case of hyperflexia, no changes are visible in the VEEG recording.[29]

Episodic ataxia

Episodix ataxiasare autosomally inherited disorders. A mutation in the potassium gated release KCNA1 gene is responsible for the development of coba.[30] It occurs with a frequency lower than 1/100,000, but this data may be inaccurate due to unknown genes that could be responsible for the condition.[31] Episodic ataxia causes difficulties with activities such as maintaining balance, walking, and movement. These symptoms can also be accompanied by pain, dizziness, double vision and dysarthria. This condition can occur daily or sporadically.

Muscle tremors

Muscle tremors are involuntary, rhythmic, oscillating movements of equal amplitude around a specific axis. They are divided into fine waves of high frequency (>6 Hz) and low amplitude (<3 cm) and coarse waves of low frequency and high amplitude. They can be recurrent, and then they are called tremors.[32] They occur in up to two-thirds of newborns during the first three days of life. Their intensity increases during crying. The pathogenesis of their formation is not precisely explained. One theory is that tremors are related to the immaturity of spinal inhibitory interneurons. There are also reports of the possibility of tremors occurring as a result of elevated concentrations of catecholamines in the blood. Tremors may also be a symptom of other conditions, i.e.: hypoglycemia, hypocalcaemia, sepsis, hypoxic encephalopathy, intracerebral bleeding or drug discontinuation.[33] Muscle tremors must be distinguished from epileptic seizures. Unlike seizures in epilepsy, muscle tremors can be triggered by various stimuli. They are not accompanied by forced opening of the eyes, hypertension or apnea. Muscle tremors can be stopped by gentle, passive flexion and immobilization of the trembling limb. If muscle tremors are not associated with perinatal complications, where the risk of long-term neurological complications reaches 30%, their prognosis is good. Most resolve spontaneously by 6-10 weeks of age. When diagnosing recurrent seizures in children, blood glucose and calcium levels should be checked first. Imaging tests and a thorough interview with the child's guardian are also important in order to exclude recent drug withdrawal.[33,34]

Migraine and Alice in Wonderland syndrome

According to the latest data, migraine may affect 10% of school-aged children.[35] Symptoms of the disease in the pediatric population are often less severe and diagnosis is more difficult than in adults. Migraine is most often manifested by a severeheadache, which may be accompanied by sensitivity to light, sounds and nausea. Migraines occur with the same frequency in girls and boys until late puberty. Later, the disease is more common in girls.[36] The disease can cause problems with concentration and memory, which is associated with poorer educational results at school and problems with peers. Basilar migraine also known by the name migraine

Table 1. Comparison of the characteristics of paroxysmal non-epileptic events and epileptic seizure*

Features	Onset	Ending	Duration	Trigger factors	Clinical picture	Treatment
Breath-holding spells	6–24 months of age	5–6 years of age	less than one minute	anger, frustration, fear or injury	crying with cessation of breathing, cyanosis, loss of consciousness and muscle tone, opisthotonus, jerks	not require treatment or piracetam
VVS	variable	variable	10–30 second	prolonged standing, dehydration, abnormal posture, emotional stress	visual and/or auditory progressive fading, pallor, diaphoresis, after that flaccidity, with or without brief myoclonus, opisthotonus (rare)	Avoiding triggers, discontinuing medicines that lower blood pressure, drinking plenty of fluids
Stereotypies	between 2 and 5 years	may occur in adulthood, especially in patients with intellectual disabilities	from a few seconds to a few hours	excitement	repetitive movements or sounds	cognitive behavioural therapy (CBT), self-help
PKD	6 months	33 years old, possible remission in adulthood	from a few seconds to a few minutes	sudden movements, changes in direction, or surprises	mutual dystonic movements or involuntary unilateral movements or dysarthria.	low doses of antiepileptic drugs
PNKD	childhood, about 8 years old	the severity of attacks decreases with age	from 10 min to 4 hours	alcohol, smoking, stress, coffee, tea, fatigue	attacks of dystonia and/or chorea	benzodiazepines, deep brain stimulation, antiepileptic drugs, except for benzodiazepines, typically do not produce the desired results
PED	2 years	30 years old	from 5 min to 30 min	exercise	dystonia, most often affects the legs	symptomatic treatment with antiepileptic drugs can sometimes be effective, while at other times, treatment of the underlying disease may yield results
PHD	childhood or early adulthood	around adolescence or early adulthood	about 45 seconds	occur only during sleep	attacks of dystonic and tonic movements	antiepileptic drugs
TS	childhood, especially from 4-6 years, symptoms must have occurred before the age of 18	lasts a lifetime	tics may occur individually or in series	anxiety, excitement, stressful events, fatigue, allergies, systemic diseases	chronic motor, vocal or phonic tics	cognitive behavioral therapy
Hypereklpexia	fetal life, more commonly presents after birth, childhood.	variable	loud of sudden noises, stress, anxiety	astonishment	sudden eye blinking or body spasms in response to tactile or auditory stimuli, rigidity in their trunk, limbs, and, in some cases, even their respiratory system	speach and language therapy, play-based applied behavioral analysis
EAs	childhood or adolescence	variable	from a few seconds to several days	stress, sudden movements, alcohol, caffeine, heat, fever	attacks of cerebellar ataxia, dysarthria, tremor, vertigo, nausea, diplopia, dystonia	acetazolamide
Muscle tremors	first three days of life	6–10 week of age	variable	crying	involuntary, rhythmic, oscillating movements of equal amplitude around a specific axis	can be stopped by gentle, passive flexion and immobilization of the trembling limb
Migraine	childchood or adolescence	lasts a lifetime	from 5 to 60 minutes	stress, fatigue, alcohol, drugs, smoking, weather changes, unpleasant odors	severe headache, which may be accompanied by sensitivity to light, sounds, nausea	analgesics, triptans, ergotamines, calcitonin gene-related peptideInhibitors, beta-blockers, antidepressants, antiepileptic drugs, calcium channel blockers
Epileptic seizure	variable	variable	1–2 minutes	stress, fatigue, missed medications, alcohol, drugs, lack of sleep, menstruation, nutrient deficiencies, systemic diseases	uncontrollable jerking movements of the arms and legs, loss of consciousness or awareness, fluttering eyes, staring blankly into space, stiffness, strange sensations, unusual smells or tastes, and a tingling feeling in your arms or legs	anti-epileptic drug: sodium valproate carbamazepine lamotrigine levetiracetam topiramate; epilepsy surgery, vagus nerve stimulation, deep brain stimulation, responsive neurostimulation, ketogenic diet

* VVS – vasovagal syncope; PKD – paroxysmal kinesigenic dyskinesia; PNKD –paroxysmal non-kinesigenic dyskinesia; PED – paroxysmal exercise-inducded dyskinesia; PHD – paroxysmal hypnogenic dyskinesia; TS – Tourette syndrome; EAs – episodic ataxia

with brainstem aura is a disorder that can last from 5 to 60 minutes. This form is characterized by the onset of symptoms associated with brainstem dysfunction. Dizziness, tinnitus, diplopia, visual disturbances and dysarthria may be present. Disturbances of consciousness have also been reported in children, but these symptoms were very rare. Then, after the onset of such symptoms, headache appears, which is located in the occipital region, which distinguishes this type of migraine from other types where the pain is in the frontal or temporal region.[37] The Alice in Wonderland Syndrome can be a part of the migraine aura or, more commonly, herald the onset of migraine in the future. It is a condition that was discovered in 1955. The name refers to the main character in Lewis Carroll's book 'Alice in Wonderland', who often perceived her body as smaller or larger than

it was. The syndrome is characterised by an inadequate, distorted perception of one's own body schema and that of other objects. A person affected by the syndrome may experience changes in the perception of object size, distance, shapes and proportions.[38] The pathogenesis of the syndrome is not yet fully understood. Cases have been reported in the literature where onset has been linked to infections of viral and bacterial origin.[39] It can be associated with viral infection caused by viruses such as: Epstein-Barr, varicella and Cytomegalovirus. Among the most common bacterial infections, Mycoplasma pneumoniae, Borrelia burgdorferi and Streptococcus pyogenes were distinguished. It is very important that each person with the syndrome undergoes a thorough neurological examination, performs magnetic resonance imaging, EEG and collects cerebrospinal fluid.[38]

Summary

PNEEs are neurological conditions that involve changes in behaviour or disturbances of consciousness. They represent a difficult diagnostic and therapeutic medical problem. They are often confused with epileptic seizures, which give a very similar clinical picture to the symptoms presented. Comparison of the characteristics of paroxysmal non-epileptic events and epileptic seizure presented in Table 1.[7,8,10-13,16,18-20,22-27,30-33,40-43] The list includes features, duration, time of onset and end of features, trigger factors, clinical picture and treatment.

However, they are not caused by abnormal electrical discharges in the brain. It is extremely important to take a thorough history from the parent, as a misdiagnosis may result in overly unnecessary diagnostic tests and cause damage to the child's social functioning. PNEEs encompasses conditions spanning all age groups from newborns to adults. Unlike adults, children do not experience the psychogenic nature of the disorder. Video recordings of seizures at home and provocations with a placebo test can be helpful in differentiating epileptic seizures from other disorders.[44,45] An important point to emphasise is that PNEEs often mimic non-epileptic seizures due to the similarity of clinical symptoms such as syncope, loss of consciousness, headache, vomiting, dizziness, irregular breathing or emotional and psychological problems.[1] There is no single specialised medication or treatment method specific to PNEEs that can help young patients. So far, mainly psychological and psychiatric care has been used, as well as treatment of any comorbidities (e.g. cardiac problems with vasogastric syncope).[46] It is extremely important in the treatment process to orient the patient to the reported problem. Therapy with a psychologist to help the young patient cope with daily functioning plays an important role.[47]

Conclusion

PNEEs represent a diagnostically and therapeutically difficult group of conditions specific to early childhood. Careful attention should be paid to the symptoms presented by patients, as a misdiagnosis can have a huge impact on their future. Pre-diagnosis of epilepsies is a huge problem in today's society, so special attention should be paid to the initial diagnosis.

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Author contributions

Conceptualization, B.S., A.Z. and K.S.; Validation, B.S., A.Z. and W.S.; Resources, A.Z., W.S. and M.Ż.; Data Curation, B.S., A.Z. and N.W.; Writing – Original Draft Preparation, B.S., A.Z., W.S., M.Ż. and N.W.; Writing – Review & Editing, B.S., A.Z., W.S. and N.W.; Supervision, K.S. and M.C.K.; Project Administration, B.S., K.S. and M.C.K.

Conflicts of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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REVIEW PAPER

Therapeutic advantages of omega-3 fatty acid supplementation in patients with schizophrenia – a systematic review

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ABSTRACT

Introduction and aim. In patients with schizophrenia, omega-3 (n-3) polyunsaturated fatty acids (PUFAs) treatment was found to ameliorate the cardiovascular, metabolic, and inflammatory problems caused by antipsychotic medication and even reduce the need for medication by 20%. In this study, we evaluated the potential therapeutic effects of n-3 PUFA supplementation in patients with schizophrenia.

Material and methods. The PRISMA guidelines were followed in conducting this systematic review. The Embase, MEDLINE, Web of Science, and Google Scholar databases were searched electronically. The first search yielded 50 papers in total. Subsequently, 43 publications that did not meet our eligibility requirements were removed, and seven articles were selected.

Analysis of the literature. The analysis showed that n-3 PUFA supplementation and the placebo group both decreased their psychotic (PANSS and GAF scales) and Calgary Depression Scale symptomatology and boosted their functional ability (GAF) when used as an adjuvant to antipsychotic medication. When administered as a monotherapy with a metabolic antioxidant, n-3 PUFA supplementation proved beneficial for treating schizophrenia. In patients with schizophrenia, n-3 PUFAs have therapeutic benefits as adjuvant treatments to medications, although not for different variables or patient groups.

Conclusion. In many studies, patients with chronic schizophrenia who received n-3 PUFA supplementation showed no improvement in their clinical condition.

Keywords. docosahexaenoic acid, eicosapentaenoic acid, omega-3 polyunsaturated fatty acids, schizophrenia, supplementation

Introduction

Schizophrenia is a severe, chronic mental disorder that affects approximately 1% of the worldwide population.[1] Important features include severe behavioral problems, cognitive impairments, delusions, hallucinations, anhedonia, alogia, and avolition.[2] The cause of schizophrenia remains unknown. Considering the variety of symptoms, the onset of schizophrenia is affected by hereditary and environmental factors.[3] Genetic factors only account for 50% of the risk rates in studies of identical twins; hence, genes cannot cause schizophrenia alone.[4] Numerous environmental risk factors can contribute to this disease, but a genetic abnormality may leave an individual more vulnerable to its consequences.[5]

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Schizophrenia is commonly found in late adolescence or early adulthood because myelination and brain growth occur at this time.[6] A damaged central nervous system may play a significant role in developing this condition when normal brain development is disturbed during pregnancy or the early postnatal period, leading to functional impairment of the brain.

Omega-3 (*n*-3) polyunsaturated fatty acids (PUFAs) affect several biological processes, including brain function.[7] These include neurotransmitter production, neurite formation, synaptic plasticity, membrane structure and fluidity, endothelial function, and the survival of neurons against neurodegeneration and neuroinflammation.[7] Because of their high concentration in the phospholipids that comprise the cell membranes of the neurons in the nervous system, these fatty acids are important for brain development.[8]

Several studies have examined the possible benefits of *n*-3 PUFAs in treating or preventing diseases such as diabetes, intestinal tumors, obesity, atopic dermatitis, and cardiovascular diseases because they reduce inflammation and improve immune function. Treatment with *n*-3 PUFAs improved endothelial function and microcirculation, decreased hyperlipidemia, and benefited patients with metabolic syndrome and hypertension.[9] These fatty acids partially inhibit a variety of inflammatory processes, including prostaglandin synthesis, adhesion molecule expression, leukocyte chemotaxis, and inflammatory cytokine development such as interleukin-1 and tumor necrosis factor-alpha.[10]

In patients with schizophrenia, *n*-3 PUFA treatment was found to ameliorate the cardiovascular, metabolic, and inflammatory problems caused by antipsychotic medication and even reduce the need for medication by 20%.[11] Furthermore, individuals who have a high genetic or family risk of developing this condition are less likely to develop psychosis in the future.[12]

Aim

In this study, we evaluated the potential therapeutic effects of *n*-3 PUFA supplementation in patients with schizophrenia.

Material and methods

The PRISMA guidelines were followed in conducting this systematic review.[13] The following eligibility criteria were included in the search strategy that was developed to conduct this systematic review: 1) Original articles and case reports (multicenter, randomized, double-blind, controlled trial, and randomized longitudinal study); 2) A diagnosis of schizophrenia (DSM or ICD diagnostic criteria), regardless of sex or race; 3) Type of treatment and use of *n*-3 PUFAs; 4) The intervention studied must be supplemented with *n*-3 PUFAs; and 5) Articles published in English within the last ten years until May 10, 2023.

We excluded patients in whom schizophrenia was in any of the early stages, such as those with bipolar disorder or depression. Articles with insufficient data, procedures, evaluations, or animal studies were excluded. Individuals under 15 and over 65 years of age, as well as those who were prescribed nutritional supplements other than *n*-3 PUFAs, were excluded.

The Embase, MEDLINE, Web of Science, and Google Scholar databases were searched electronically. The keywords used for this search included "schizophrenia," "nutrition," "diet," "omega-3 fatty acids," "psychotic," and "metabolic syndrome," which were obtained from the MeSH words through PubMed.

The selection criteria were based on the relevance of the topic, which was mentioned as a stage of schizophrenia, and the use of *n*-3 PUFAs. The title of the article, which was appropriate for the subject, and the abstract were read to determine the selection of articles. Following the flow diagram shown in Figure 1, articles that met these requirements were read entirely and included in the study.

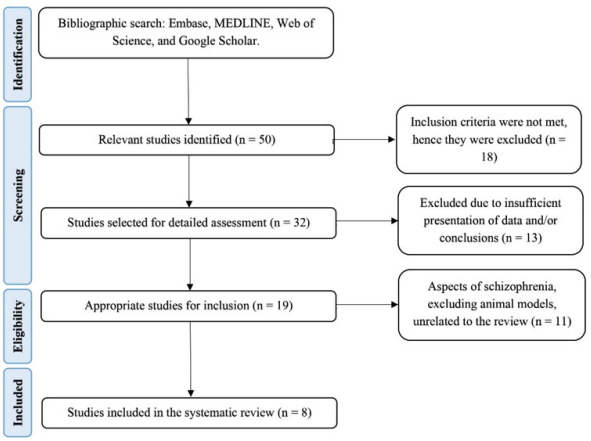

Fig. 1. Flow diagram of literature search and study of selection for systematic review (PRISMA flow chart)

Three authors (FA, HG, and AB) independently performed the search, and duplicate studies were excluded. The first search yielded 50 papers in total. Subsequently, 43 publications that did not meet our eligibility requirements were removed, and seven articles were selected. Independent reviewers conducted the data extraction procedure and used an established format to gather data from the selected publications. In the event of a difference in opinion or dispute, the writers had previously agreed to include the work and submit it for review by an unbiased advisor before deciding on its final inclusion through discussion and consensus.

Analysis of the literature

The findings of these studies varied, with slightly contradictory results (Table 1). *N*-3 PUFA supplementation had favorable outcomes.[14,15] In the Austrian trial,

Table 1. Characteristics of selected studies on the effect of omega-3 polyunsaturated fatty acid supplementation in the treatment of patients with schizophrenia*

Authors and year	Type of study	Groups and sample size	Type of patients and AP treatment	Variables and tools	Therapeutic intervention	Main results
Amminger et al., 2010	Randomized, double-blind, placebo-controlled trial.	81 individuals at high-risk for schizophrenia *n*-3 group: 41 Placebo: 40	Individuals at high-risk for schizophrenia Age range: 12–25 years Without undergoing AP therapy	Diagnosis: PANSS and DSM-IV scales Symptoms: PANSS, MADRS Global assessment of functioning: EEAF *n*-3: *n*-6 PUFA/*n*-3 PUFA ratio	Fish oil supplementation vs. placebo 12 + 40 weeks of monitoring (12 months) Evaluation: 0, 1, 2, 3, 4, 8, 12 weeks Fish oil: 700 mg EPA + 480 mg DHA Placebo: Coconut oil	**Longitudinal analysis** Two of the 41 individuals in the *n*-3 group and 11 of the 40 individuals in the placebo group had psychotic illnesses. **Intergroup analysis** When compared to the placebo group, PUFA reduced positive, negative, and overall symptoms.
Behdani et al., 2018	Randomized, double-blind, placebo-controlled trial.	56 patients with Schizophernia *n*-3 group:28 Placebo: 28	Chronic patients Age range: 18–60 years AP: Clozapine and sodium valproate (3-month dose)	Height, weight, waist circumference, serum lipid profile, fasting blood glucose, sensitivity to C-reactive protein, etc. No symptoms	Fish oil supplementation vs. placebo Duration: 8 weeks Fish oil: 180 mg EPA+120 mg DHA	Some anthropometric metrics improved after eight weeks of *n*-3 PUFA supplementation. Only differences in waist circumference continued after fasting serum TG correction.
Emsley et al., 2014	Randomized, double-blind, placebo-controlled trial.	33 patients with Schizophernia *n*-3 group:21 Placebo: 12	Chronic patients Age range: 18–48 years 2-3 years of successful AP therapy but later discontinued	Diagnosis: DSM-IV Symptoms: PANSS, CGI, Prodromal Symptom Scale: SOPS Depression: CDSS Functionality: SOFA QoL: WHOQOL-BREF Cognition: MCCB	Fish oil supplementation vs. placebo 2 years duration or until relapse. *n*-3 capsules: 2 g EPA + 1 g DHA Placebo: Olive oil	Both groups had high recurrence rates (19/21 in the *n*-3 group with just one person remaining relapse-free after two years, and 9/12 in the placebo group with no one remaining relapse-free after two years). Between the two groups, there were no significant differences in the SS severity of relapses.
Jamilian et al., 2014	Randomized, double-blind, placebo-controlled trial.	60 patients with Schizophernia *n*-3 group: 30 Placebo: 30	Chronic patients Age range: 15–55 years AP: Risperidone, clozapine, or olanzapine	Diagnosis: DSM-IV Symptoms: PANSS	Fish oil supplementation vs. placebo Duration: 8 weeks Fish oil: 1000 mg/day	**Longitudinal analysis** PANSS decreased in both groups at the end of week 8. **Intergroup analysis** When compared to placebo at weeks 4 and 6, *n*-3 group lowered both the overall and total PANSS. Efficacy *n*-3 vs AP was 0.86
Pawełczyk et al., 2016	Randomized, double-blind, placebo-controlled trial.	71 patients with Schizophernia *n*-3 group: 36 Placebo: 35	Age range for first episode patients: 16–35 years Doses of AP therapy resulted into equal doses of chlorpromazine	Symptoms: PANSS, CGI Depression: CDSS Functionality: GAF	Fish oil supplementation vs. placebo 26 weeks of intervention Fish oil: 2.2 g/day Placebo: Olive oil	**Intergroup analysis** 50% reduction in symptom severity in *n*-3 group compared to placebo. The psychopathology of the *n*-3 group improved as measured by PANSS scale scores, depression, CGI, and functional level.
Quiao et al., 2017	Randomized, double-blind; placebo-controlled trial.	50 patients with Schizophernia *n*-3 group: 28 Placebo: 22	Hospitalized patients with Schizophrenia Age range: 18–60 years MOAS>4 AP therapy	Diagnosis: ICD10 Symptoms: PANSS, CGI Aggression/Violence: MOAS DHA+EPA blood levels: gas chromatography	Fish oil supplementation vs. placebo Evaluation: 0, 4, 8 and 12 weeks Fish oil: 360 mg DHA+540 mg EPA Placebo: 10 mg vitamin E	**Longitudinal analysis** Symptoms: week 0>4>8>12 MOAS: week 0>4>8>12 **Intergroup analysis** Symptoms: No difference in *n*-3 group vs Placebo. MOAS: week 12 *n*-3 group <Placebo
Robinson et al., 2019	Randomized, double-blind; placebo-controlled trial.	46 and 4 patients with Schizophernia and bipolar disorder *n*-3 group: 25 Placebo: 25	Patients recently diagnosed with Schizophrenia Age range: 15–40 years AP: risperidone and lorazepam	Diagnosis: DSM-IV Symptoms: BRPS, SANSS, CGI General adverse effects: SAFTEE-SI Metabolic indices: Hemoglobin A1C, cholesterol, TG	Fish oil supplementation vs. placebo Evaluation: 0, 1, 2, 2, 3, 4, 6, 8, 10, 12, and 16 weeks Risperidone 16 weeks Fish oil: 740 mg EPA and 400 mg DHA. Placebo: Soy	**Longitudinal analysis** BPRS data showed that patients who took *n*-3 supplements tended to experience fewer anxious and depressed symptoms. Improvement in anxious and depressed symptoms in lorazepam group. **Intergroup analysis** Symptoms: No difference in *n*-3 group vs Placebo.

Xu et al., 2018	Randomized, placebo-controlled trial.	80 patients with Schizophernia *n*-3 group: 40 Placebo: 40	Chronic patients Patients with EZ and MeS Age group: 18–45 years AP: olanzapine	Diagnosis: DSM-IV Symptoms: PANSS Diagnosis of MeS: waist circumference, TG, high-density lipoprotein, fasting glucose.	Fish oil supplementation vs. placebo *n*-3 capsules: 720 mg EPA + 480 mg DHA (12 weeks) Placebo: 100 mg vitamin E	**Intergroup analysis** *n*-3 treatment + decrease in TG levels decreases TNF-α levels (12 weeks).

* AP – antipsychotics, BACS – Brief Assessment of Cognition in Schizophrenia, BPRS – Brief Psychiatric Rating Scale, CDSS – Calgary Depression Scale for Schizophrenia, CGI – Clinical Global Impression Scale, DHA – docosahexaenoic Acid, DSM-IV – Diagnostic and Statistical Manual of Mental Disorders, 4th Edition, EPA – eicosapentaenoic acid, GAF – Global Assessment of Functioning Scale, ICD-10 – International Classification of Diseases 10th revision, MADRS – Montgomery and Asberg Depression Rating Scale, MeS – metabolic syndrome, MCCB – MATRICS Consensus Cognitive Battery, MOAS – Modified Over Agression Scale, PANSS – Positive And Negative Syndrome Scale, SANS – scale for the Assessment of Negative Symptoms, SAFTEE-SI – Systematic Assessment for Treatment Emergent Events-Specific Inquiry, *n*-3 PUFAs – omega-3 polyunsaturated fatty acids, *n*-6 PUFA– omega-6 polyunsaturated fatty acids, PGA – Patient's Global Assessment Scale, SFS– Social Functioning Scale, SOFAS – Social and Occupational Functioning Assessment Scale, SOPS – Prodromal Symptoms Scale, TG – triglycerides, TNF-α – tumor necrosis factor alpha, WHOQOL-BREF – Quality of Life Questionnaire Short Version

out of 41 cases, there were two transitions to psychotic illness in the supplemented group compared with 11 out of 40 cases in the placebo group.[12] A statistically significant decrease in Positive and Negative Syndrome Scale (PANSS) scores and an increase in the Global Assessment of Functioning (GAF) scores were also observed in the group that received *n*-3 PUFA supplements. Similarly, the above findings are supported by studies from Iran and Poland in patients with schizophrenia.[16,17] *N*-3 PUFA supplementation and the placebo group both decreased their psychotic (PANSS and GAF scales) and Calgary Depression Scale symptomatology and boosted their functional ability (GAF) when used as an adjuvant to antipsychotic medication.

When administered as a monotherapy with a metabolic antioxidant, *n*-3 PUFA supplementation proved beneficial for treating schizophrenia.[18] The Japanese study revealed no correlation between docosahexaenoic acid (DHA) plasma levels and antipsychotic medication.[19] However, there was a correlation between the Brief Assessment of Cognition in Schizophrenia scale scores and the *n*-3 PUFA DHA and eicosapentaenoic acid (EPA) plasma levels. Iranian researchers noted significant changes in different anthropometric parameters (waist, abdomen, height, weight, etc.) after taking *n*-3 PUFA supplements for 4 weeks.[20] In addition, *n*-3 PUFA supplementation considerably reduced inflammatory (tumor necrosis factor-α) and metabolic (triglyceride) levels in patients with schizophrenia, which had been found to differ during the baseline assessment performed before therapy, according to the Chinese study.[21]

For every selected study, the Newcastle-Ottawa scale was used to assess the risk of bias.[22,23] Therefore, in 50%, 25%, and 25% of the selected articles, a significant possibility of bias was observed. A cautious analysis and interpretation of the results were required, considering the possible effects of bias, as only three of the seven articles under selection had a low risk of bias, and the remaining four had a high risk. This could have led to either an overestimation or underestimation of the results, resulting in incorrect conclusions in the current study.

Among other processes, 35% of brain lipids are PUFAs involved in cell signaling, enzymatic regulation, neuronal migration, neuroplasticity, and icosanoid synthesis. The etiology of schizophrenia is associated with alterations in phospholipid metabolism. For example, patients with schizophrenia have been found to have low levels of long-chain fatty acids in their red blood cell membranes. This can be attributed to the hyperactivity of phospholipase A2. Diet can influence the characteristics of schizophrenia symptoms. For example, a positive correlation between a good prognosis and a diet low in total and animal fats has been reported. In this case, the fats are mainly unsaturated. Patients with schizophrenia benefit from consuming doses of *n*-3 PUFAs four times higher than those recommended for the healthy population. However, patients with schizophrenia can receive adequate *n*-3 PUFAs through their diet without needing supplementation. *N*-3 PUFAs are not naturally isolated but are part of larger molecules such as triglycerides and phospholipids. Therefore, the formulation of *n*-3 PUFA capsules involves hydrolysis, purification, and stabilization, resulting in a chemical product with lower bioavailability than that observed when ingesting *n*-3 PUFAs in their natural state.

Supplementation showed no positive effect on psychotic symptoms, according to the GAF results and PANSS scales of the Chinese study.[14] The PANSS scores (negative symptomatology) in the American clinical trial showed no improvement in supplemented individuals despite worsening outcomes at weeks 4, 8, and 12. This also occurred in the placebo group, indicating that the use of antipsychotics, which are major medications for these patients.[15] Reliability is increased because the patients in both studies were in the acute phase of the

disease following hospitalization. The two samples had comparable age ranges (18–60 and 15–40 years, respectively), with average sample sizes of 50 and 46.

However, in the Austrian study, young people (13–25 years old) at high risk for psychosis and who already showed clinical and functional abnormalities were examined.[12] The psychotic symptoms of the disease had improved, according to the PANSS scale. The potential role of *n*-3 PUFAs in delaying the onset of schizophrenia in people at high risk for the disease must be noted.

In the Iranian study, the symptomatological results of the PANSS scale decreased on both the general and total scales, indicating an improvement in the psychopathology of schizophrenia.[16] The factors and features of patients with schizophrenia were compared with those of the Chinese study and the American clinical trial (patient age, EPA and DHA dose, schizophrenia diagnosis, sample size, medication used, etc.).[14,15] Patients in this situation were not included in the study after an acute episode and undergoing therapy with stable medication for the previous 8 weeks. This suggests that *n*-3 PUFA supplementation in combination with antipsychotic dosages and therapy with stable medications provides beneficial results.

In the Polish study, first-episode young patients, compared with placebo, had a 50% reduction in overall PANSS symptoms because of the supplementation.[17] Patient ages were lower because they were first-episode cases, and the intervention length (26 weeks), amount of supplemental *n*-3 PUFAs (2.2 g), and stage of illness were early. These latest findings suggest that young age and recent disease onset may be important factors in determining the efficacy of *n*-3 PUFA supplementation.

In the South African study where patients did not receive pharmacological therapy, most patients experienced relapses and intensified psychotic symptoms, as evidenced by a 25% increase in the total PANSS scale scores.[18] *N*-3 PUFAs should not be used as monotherapy in patients with chronic schizophrenia owing to their poor efficacy. Because of the small sample size (n = 33), additional research is recommended.

In the Austrian study of supplemented patients, GAF scale scores increased following a 12-month intervention, and it was the only secondary variable positively correlated with changes in the *n*-6/*n*-3 PUFA ratio.[12] These findings somewhat support the findings of the Polish study, which found that changes occur after an intervention of 6 months but are not especially significant.[17]

This may be attributable to the early diagnosis and treatment, similar to improving psychotic symptoms. A relapse in psychotic symptoms was found in all patients in the South African study, leading to a significant decrease in the ability to function in patients.[18] The patients did not receive pharmacological treatment, had the condition, and received *n*-3 PUFA treatment, unlike the group in the Austrian study, which could account for the differences in outcomes.[12]

The physiological responses of the body to variations in blood *n*-3 PUFAs can be understood using metabolic measures. The DHA and EPA compositions in the blood were measured using gas chromatography in three separate tests. In the Chinese study,[14] after 4 weeks of intervention in the supplemented group compared with the placebo group, a negative association was identified between blood *n*-3 levels and the Modified Overt Aggression Scale scores. However, after week 12, this correlation disappeared. These findings are consistent with those of the Austrian study,[12] which found that the blood *n*-6/*n*-3 PUFA ratio was weakly linked with the functioning scale.

In the American clinical trial, there was no improvement in anthropometric parameters (body mass index and weight) after *n*-3 PUFA supplementation,[15] while the Chinese study was feasible because of the amount of *n*-3 PUFAs administered.[21] However, there were minor improvements in the waist circumference in the Iranian study, a parameter linked to many diseases associated with schizophrenia, including cardiovascular disease, metabolic syndrome, and type 2 diabetes mellitus.[20]

The symptoms of schizophrenia are more severe in patients with low *n*-3 PUFA levels than in those with high levels,[24] and these patients respond better to *n*-3 PUFA supplementation. Supplementation with *n*-3 PUFAs is more likely to be beneficial for patients whose baseline levels of EPA and alpha-lipoic acid are greater.[25,26] Changes in the levels of different fatty acids following *n*-3 PUFA supplementation have been linked to clinical improvement, including a rise in arachidonic acid and elevations in *n*-3 PUFA and *n*-6 PUFA levels in erythrocyte membranes.[27,28] In patients with schizophrenia using antipsychotics, metabolic changes are observed, and abnormally high blood triglyceride levels and low serum high-density lipoprotein levels have been found.[29] EPA has been found to reduce arachidonic acid levels in membranes and prevent the synthesis of pro-inflammatory mediators, both of which have been shown to reduce inflammatory responses.[30,31]

Supplementation with *n*-3 PUFAs may also have positive effects on the gut flora. Poor dietary habits, higher saturated fats, and lower *n*-3 PUFAs, especially *n*-3 PUFAs, are common in patients with schizophrenia.[32,33] The microbiota-gut-brain axis may be modulated by this difference, which may also impact symptoms.[34] Supplementation with *n*-3 PUFAs changes the gut flora, which in turn affects neuropsychological and cognitive behaviors.[35,36]

Although antipsychotic drugs frequently cause drug-induced movement disorders, they may also enhance brain function and reduce symptoms. Taking *n*-3 PUFA supplements may also reduce the number of antipsychotic medications needed to treat symptoms, mak-

ing it easier for the body to absorb antipsychotics, reduce drug-related movement disorders, and improve cognitive function. Identifying the group that will benefit from *n*-3 PUFA intervention can be facilitated by measuring *n*-3 PUFA levels at an earlier stage of the disease.

Conclusion

In patients with schizophrenia, *n*-3 PUFAs have therapeutic benefits as adjuvant treatments to medications, although not for different variables or patient groups. In many studies, patients with chronic schizophrenia who received *n*-3 PUFA supplementation showed no improvement in their clinical condition.

Younger patients, and therefore, those with first-episode schizophrenia, benefit more from *n*-3 PUFA supplementation in terms of psychopathological, functional, and metabolic effects. Monotherapy with *n*-3 PUFAs is not recommended, and combined medication is essential because these fatty acids affect disease prognosis in patients with schizophrenia. Further research on how antipsychotics interact with *n*-3 PUFAs is required to determine whether they are more effective at improving the efficacy of nutritional adjuvant treatment for schizophrenia.

Declarations

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Author contributions

Conceptualization, S.V., K.P.K., Y.V., T.T. and U.D.; Formal Analysis, Y.V., T.T. and U.D.; Writing – Review & Editing, S.V., K.P.K., Y.V., T.T. and U.D.

Conflict of interest

The authors declare no conflicts of interest.

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REVIEW PAPER

Consensus Committee of experts on Kawasaki Disease and *Chinese Journal of Contemporary Pediatrics* – the expert consensuses on intravenous immunoglobulin, aspirin, and glucocorticoid

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ABSTRACT

Introduction and aim. Kawasaki disease (KD) is an acute vasculitis with unknown etiology, usually occurring in children under 5 years old. This article will summarize the three consensuses formulated in China about KD.

Material and methods. English databases for consensus search include UpToDate, BMJ Clinical Evidence, National Guideline Clearinghouse, Joanna Briggs Institute Library, Cochrane Library, and PubMed, etc.; Chinese databases include China Biomedical Literature Service, China Knowledge Network, Wanfang database, etc. All literature searches ended on February 28, 2022.

Analysis of the literature. KD is a common acquired heart disease in children and can lead to severe complications such as coronary injury. However, intravenous immunoglobulin (IVIG) combined with oral aspirin (Asp) is currently recognized as the most effective treatment in KD acute stage and the first-line treatment to prevent cardiovascular complications. Glucocorticoid (GC) is mainly used for KD patients with a high risk of coronary artery aneurysm (CAA), no immunoglobulin response, and confirmed CAA. There are already consensus guidelines on diagnosing and treating KD in different countries. This article summarizes the relevant expert consensus on aspirin, glucocorticoids and IVIG for the treatment of Kawasaki disease in China.

Conclusion. Still, there are inconsistent opinions in the literature on the mechanism, optimal timing, and dosage of medication for KD.

Keywords. aspirin, children, glucocorticoid, intravenous immunoglobulin, Kawasaki disease

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The list of abbreviations

Asp – aspirin, **CAA** – coronary artery aneurysm, **CAL** – coronary artery lesion, **Fc** – fragment crystallizable, **GC** – glucocorticoid, **GRADE** – grading of recommendations assessment, development, and evaluation of evidence, **IVIG** – intravenous immunoglobulin, **KDSS** – Kawasaki disease shock syndrome, **MAS** – macrophage activation syndrome, **KD** – Kawasaki disease

Introduction

Kawasaki disease (KD), also known as cutaneous mucosal lymph node syndrome, is a common febrile disorder in children, commonly seen in children under 5. The main pathological feature is systemic vasculitis, and the clinical features include terminal changes in the extremities, bilateral bulbar conjunctival congestion, lip and oral changes, non-purulent enlargement of the cervical lymph nodes, and fever. KD is mainly complicated by damage to the cardiovascular system, such as coronary artery dilation and thrombosis. In addition, KD can also cause multi-system complications such as pulmonary nodules, arthritis, hepatitis, urethritis, Kawasaki disease shock syndrome (KDSS), etc.[1-2]

The prevalence of KD varies widely among countries. The prevalence of KD is 10-30 times higher in East Asian countries, including Japan, Korea, and China than in the United States or Europe, and the prevalence is increasing year by year.[3] In 2015, the prevalence of KD among children under five years old was 19.1/100,000 in the United States and 19.6/100,000 in Canada in 2014.[4] The countries of Japan, Korea, and China have the highest KD prevalence rates in the world (>50/100,000 among children under five years old) and are increasing gradually.[5-7] Japan is reported to have the highest KD mortality rate in the world, estimated at approximately 264/100,000 deaths in children under five years old; the recurrence rate of KD is 3.5%, the mortality rate is <0.02% and 17.0% of children develop resistance to IVIG.[7] In China, the incidence of KD is on the rise, with a prevalence of approximately 7.06-55.1/100,000 children <5 years of age, and in Taiwan was 82.8/100,000 in 2010.[8-10] Hong Kong has the highest prevalence of KD in China (74/100,000 among children <5 years of age).[11]

Epidemiological studies in some regions of China have shown that the incidence of KD combined with coronary artery lesion (CAL) is as high as 15.9%, and the incidence of combined coronary artery aneurysm (CAA) is 1.8%.[12] Standardized treatment with intravenous immunoglobulin (IVIG) can reduce the risk of CAL occurrence from 15–20% to 3–5%.[13,14] Medications are currently the main treatment options for KD and its complications, among which the preferred treatment option IVIG combined with aspirin (Asp) has been widely used, and GC is used as a complementary treatment for IVIG non-response and KD combined with CAA.

Current studies suggest that KD pathogenesis may involve pathogenic infections, environmental factors, immune dysregulation, and genetic predisposition. However, definitive conclusions are still deficient, making individualized treatment for different etiologies particularly important.[15,16] Studies on the dosage, duration, and timing of drug treatment for KD have been inconsistently reported in many countries.2021 The KD Treatment Center in Shaanxi, China, the Shaanxi Clinical Medical Research Center for Pediatric Internal Diseases, the Children's Hospital of Shaanxi Provincial People's Hospital, the Pediatric Capacity Building Committee of the National Society for Research on Maternal and Child Health, and the General Pediatrics (General Practice) Group of the Pediatricians Branch of the Chinese Medical Association, formed a KD expert group, including more than 100 scholars. Through several online video conferences, they discussed the mechanism, treatment dose, course, optimal timing, and safety of IVIG, Asp, and GC for KD. Finally, they formed three consensuses, all published in the Chinese Journal of Contemporary Pediatrics.[17-19] The consensus aims to provide a basis for the standardized clinical management of KD in China, ultimately achieving effective prevention of complications and sequelae in children with KD and reducing the risk of cardiovascular events and death in children with KD.[20,21]

Aim

This article will summarize the three consensuses formulated in China about KD.

Material and methods

These consensuses apply to children under 18 years old with all types of initial and retreatment KD, except those with a history of allergy to IVIG, GC, or Asp or with drug contraindications.[17] The population of use includes all pediatric rheumatologists, pediatric cardiovascular physicians, and general practitioners. All these consensus items have been registered on the International Practice Guideline Registrable Platform (http://www.guidelines-registry.cn) under the registration numbers IPGRP-2021CN183, IPGRP-2021CN183, and IPGRP-2021CN321, respectively.

English databases for consensus search include UpToDate, BMJ Clinical Evidence, National Guideline Clearinghouse, Joanna Briggs Institute Library, Cochrane Library, and PubMed, etc.; Chinese databases include China Biomedical Literature Service, China Knowledge Network, Wanfang database, etc. All literature searches ended on February 28, 2022. Nearly 200 papers were finally included, including seven guidelines, nine expert consensus and standards, 2 BMJ Best Practices, 12 UpToDate, 41 Meta-analyses and systematic reviews, 18 randomized controlled trials, and 102 observational studies.

The development of these consensuses is based on the current research progress and relevant research data on the medication of KD in children, as well as concerning domestic and international guidelines and experience in diagnosing and treating KD, and was developed after many discussions. The consensus follows the following principles:

(1) Participation of professionals from multiple centers, including pediatric specialty physicians, pediatric cardiovascular physicians, and experts in evidence-based medicine.

(2) Adopting the Grading of Recommendations Assessment, Development, and evaluation method, GRADE while guided by the GRADE manual, the recommendation level of a specific clinical issue in this consensus is determined based on the credibility level of the literature or data (guideline recommendation intensity are shown in Table 1, grade quality of evidence and strength of recommendation are shown in Table 2).[22]

Table 1. Guideline recommendation intensity

Grade	Content
Strongly recommended (1)	Effective measures that are clinically accepted and supported by curative cases
Weak recommendation (2)	Treatment with conflicting effectiveness and usefulness

Table 2. Grading quality of evidence and strength of recommendations in clinical practice guidelines[22]

Rank	Explanation	Examples
High	Further research is improbable to change our confidence in estimating the effect.	Randomized trials without severe limitations Well-performed observational studies with substantial effects (or other qualifying factors)
Moderate	Further research will likely significantly impact our confidence in the effect estimate and may change the assessment.	Randomized trials with severe limitations Well-performed observational studies yielding significant effects
Low	Further research will likely significantly impact our confidence in the effect estimate and may change the assessment.	Randomized trials with severe limitations Observational studies without particular strengths or significant limitations
Very low	Any estimate of the effect is very uncertain	Randomized trials with severe limitations and inconsistent results Observational studies with severe limitations Unsystematic clinical observations (e.g., case series or case reports)

Analysis of the literature

Chinese expert consensus on IVIG for KD

Mechanism of IVIG for the treatment of KD

The main objectives of treatment in the acute phase of KD are to control and terminate the inflammatory response, reduce the incidence of CAA, and prevent coronary thrombosis.[23] IVIG is an immunoglobulin preparation isolated from the blood of healthy people, of which IgG is the most abundant immunoglobulin, accounting for more than 95%. The IgG molecule is hydrolyzed to obtain a Fragment crystallizable (Fc); IgG Fc can bind to harmful complement components in the body and block their deposition in target tissues, thus avoiding immune damage, while IgG Fc can bind to Fc receptors and regulate immune function by activating intrinsic immunity.[24,25] Although the therapeutic regimen of IVIG applied to Kawasaki disease has been gradually refined and matured, its specific mechanism has not been elucidated in detail, and it is currently believed that IVIG treatment of Kawasaki disease may be through the following pathways:

(1) Modulates macrophage activity by inhibiting autoantibodies that bind to Fc receptors; inhibits endothelial cell activation, adhesion molecule expression, and secretion of soluble mediators; neutralizes antibodies to cytokines, chemokines, and activated complement proteins that activate inhibitory Fc receptors on macrophages; and blocks the transport of adhesion molecules critical for inflammatory cells to vascular endothelial cells; produces anti-liposomes to reduce inflammation and attenuate endothelial cell injury.[26]

(2) Immunoglobulins stimulate an adaptive immune response that can bind to bacteria or viruses and their toxins, and interact with unique type determinant clusters on pathogenic autoantibodies (and autoantibody-producing B cells), allowing direct neutralization of pathogens and thus their clearance; IVIG may also affect the number and function of regulatory T cells that help control inflammation.[27]

(3) IVIG can also bind to the Fc receptor. Still, the Fc receptor is not directly involved in regulating immune cell activation, but acts as a protective receptor by preventing the catabolism of immunoglobulins.

(4) Analysis of serum cytokine levels in children with Kawasaki disease treated with IVIG revealed that the levels of interferons-γ and IL-10 decreased rapidly. In contrast, IVIG treatment enhanced the Treg transcription factor FoxP3 expression. In IVIG, IgG monomers accounted for more than 95%, with the remainder being dimeric or multimeric IgG. Clinically, large doses of IVIG are often more effective in treatment, suggesting a better anti-inflammatory effect of IgG dimers or multimers. The specific mechanism is unclear, and it is speculated that the IgG dimer structure may enhance the binding ability of Fc to Fc receptors, thus effectively inhibiting the activation of intrinsic immune cells and reducing autoimmune damage.[28]

Summary of expert consensus recommendations for IVIG

Application in Kawasaki disease

The recommendations of IVIG for the treatment of KD are shown in Table 3.

Table 3. The recommendations of IVIG for the treatment of KD

Items	Recommendations	Recommendation strength and evidence level
Timing of IVIG application	1. The best time is 5-10 d after the onset of the disease, and the best within 7 d	1A
	2. The use within 5 d after onset may lead to an increased incidence of IVIG resistance (1B); in severe cases, such as combined hypertension, shock, hemodynamically unstable myocarditis, paralytic intestinal obstruction, etc., should still be applied promptly (1A)	1B; 1A
	3. Children with an onset of more than 10 d, excluding other causes of persistent fever with elevated ESR or CRP or elevated inflammatory markers combined with CAL, still need to be treated with sub-IVIG	2B
IVIG application dose and rate	A single dose of IVIG (2g/kg) is usually administered intravenously by drip over 12-24 hours.The recommended initial infusion rate is 0.01mL/(kg.min) [5% IVIG 30mg/(kg.h)] for 15-30min, then increase the dose to 0.02mL/(kg.min), if well tolerated, adjust to 0.04mL/(kg.min), and finally adjust to the maximum rate of 0.08mL/(kg.min)	1B
IVIG application protocol	1. Complete Kawasaki disease, incomplete Kawasaki disease, recurrent Kawasaki disease: IVIG dose is 2g/kg, single intravenous infusion in 12~24h, with oral aspirin	1A
	2. Non-responsive Kawasaki disease (IVIG-resistant Kawasaki disease): early reapplication of IVIG at a dose of 2g/kg, single intravenous infusion over 12 to 24h is recommended. For those who still have a fever, glucocorticoids can be used in combination with IVIG	1B
IVIG application safety	1. Infants and children with fluid restriction need to avoid low-concentration preparations	1A
	2. Infants and children with cardiovascular disease should be careful to avoid IVIG with high sodium content	1B
	3. Preparations using maltose or glucose as stabilizers are not recommended for use in patients with diabetes and risk of renal injury	1B
	4. Amino acid-containing preparations need to be used with caution in patients with specific genetic metabolic abnormalities	2A
IVIG adverse reaction management	1. Headache is a common adverse reaction, usually occurring during or 2-3d after infusion, and mild cases can be treated with NSAIDs for pain relief	1A
	2. Transient asymptomatic neutropenia after IVIG treatment usually occurs 2-4 d after infusion and recovers within two weeks; generally, no treatment is needed, but some scholars believe that glucocorticoids can prevent it	2B
	3. IgG subclass deficiency and high IgM syndrome are not contraindications to IVIG. For patients with severe allergic reactions, anti-IgA antibodies can be detected, and if the anti-IgA antibody titer is high (>1/1000), 1gG replacement therapy should be applied with caution.	2A
	4. Renal impairment is firstly manifested by elevated blood urea nitrogen or creatinine, followed by oliguria and renal failure, which peaks 5-7 d after high-dose infusion. In patients with existing renal impairment, IVIG should be infused slowly and hydrated appropriately, and IVIG products containing sucrose should be avoided	1B
	5. The estimated incidence of thrombotic events ranges from 1% to 16.9%, with risk factors including first high-dose IVIG, previous/current thrombosis, previous atherosclerotic disease, hyperviscosity syndrome, hereditary hypercoagulability, rapid infusion rate, pre-hydration, a rate less than 50 mg/(kg. h), hypotonic IVIG products (3% to 6%) and prophylactic use of aspirin or Low-molecular-weight heparin and other measures to reduce the incidence of thrombosis in high-risk patients, and patients with thrombotic complications need to receive antithrombotic therapy	2B

Chinese expert consensus on Asp for KD

Mechanism of Asp for the treatment of KD

Asp can act on the hypothalamic thermoregulation center and cause peripheral vascular dilation, increasing skin blood flow, sweating, heat dissipation, and other cooling effects in children. In addition, Asp can cause the acetylation of serine at position 530 of a polypeptide chain, the active site of cyc-1 in children, to completely inactivate cyc-1, block the conversion of arachidonic acid to thromboxane A_2 and achieve the effect of anti-platelet aggregation, to effectively avoid embolism in children and affect blood pressure circulation. Therefore, Asp in treating KD children will play a role in antipyretic analgesia and preventing thrombosis.[16,29]

Summary of expert consensus recommendations for Asp application in KD

The recommendations of Asp for the treatment of KD are shown in Table 4.

Chinese expert consensus on GC for KD

Mechanism of GC for the treatment of KD

Vascular endothelial injury is a critical link in the pathogenesis of KD. Neutrophils, $CD8^+$ T lymphocytes, and mononuclear macrophages accumulate in the coronary artery mesothelium during the acute phase of KD, causing vascular endothelial injury is a critical link in the pathogenesis of KD. Disruption of the vascular barrier releases cytokines and adhesion molecules that diffuse into the vessel wall, leading to vessel wall edema, elastic fiber fracture, and destruction of the flexible layer, causing vascular remodeling and coronary artery dilation or CAA. GC can reduce the transcription of inflammatory mediators and decrease fever and inflammation in KD patients, thus reducing the incidence of coronary artery damage and future cardiovascular sequelae.[37,38]

Indications for GC application for KD

Indications for GC application for KD include the following :

(1) IVIG unresponsive KD remedial therapy;

(2) Children with combined CAA with persistently elevated inflammatory markers;

(3) KDSS;

(4) KD combined with macrophage activation syndrome (MAS);

(5) Children at high risk of IVIG unresponsiveness, including those with an age of onset less than 0.5 years, high levels of inflammatory markers, and a Kobayashi

Table 4. The recommendations of Asp for the treatment of KD

Items	Recommendations	Recommendation strength and level of evidence
Asp suitable dosage form	1. Enteric-coated tablets or enteric-coated capsules are recommended for long-term use (swallowed whole)	1A
	2. Infant preferred drops and syrup agent, 2~5 years old can use solution agent, syrup agent, suspension agent, foaming agent, etc	1B
	3. Effervescent tablets are convenient for precise dosage and easy to take (but there are problems such as preservation and waste when taking them at different times)	2B
Asp dose and course of treatment	1. In the acute stage of KD children, as was given 30~50mg/(kg.d) orally 2~3 times and changed to 3 5mg/(kg.d) in 48-72h or 14 days after the onset of fever, and maintained in one dose.[30-33] Continued oral administration for 6-8 weeks; children with CAL need normal oral coronary arteries.	1A
	2. Children with undiagnosed KD and atypical KD before IVIG can usually receive Asp 3~5mg/(kg.d) in one dose at a time for 6~8 weeks.	2A
	3. Children with CAL must take it orally until their coronary arteries are normal.	2B
Application of Asp in KDSS	According to the dosage and usage of Asp in KD treatment.	2A
Asp adverse reactions and prevention	Common adverse reactions include nasal bleeding, gastrointestinal bleeding, gastrointestinal ulcer, subcutaneous bleeding, intracranial hemorrhage, asthma, liver and kidney failure, rash, loss of appetite, Rehmannia syndrome, tinnitus, hearing loss, toxic epidermal necrolysis/mucosa-ocular syndrome, etc.	1A
	If the above adverse reactions occur, the dose of Asp should be reduced, or the Asp should be discontinued.[34] Gastric mucosal protectants are also recommended during oral Asp treatment.	2A
Precautions for Asp use	1. Contraindication: allergy to Asp, active bleeding, liver, and kidney failure, digestive ulcer and frequent recurrence, hemophilia, other coagulation disorders, etc. 2. Caution: abnormal liver function, minor bleeding of subcutaneous mucosa, transient nosebleed, asthma, glucose-6-phosphate dehydrogenase deficiency, Reay's syndrome, genetic metabolic diseases similar to Reay's syndrome, ASP-related rash, gastrointestinal disorders, etc. 3. Other precautions: If liver transaminase increases in KD subacute or recovery stage, the Asp dose should be reduced and discontinued. As KD's acute phase often appears to be a persistent high fever, clinical use of ibuprofen can reduce fever. Ibuprofen combined with ibuprofen can counteract the irreversible platelet inhibition induced by Asp, so ibuprofen should be avoided for fever reduction in children with CAL, and acetaminophen can be used for fever reduction.[35] In the KD recovery period, it is still recommended that children taking low doses of Asp can be inoculated, but the relevant clinical symptoms need to be strictly observed.[36]	

warning score greater than or equal to 5 (Kobayashi score are shown in Table 5), or children with high-risk KD as judged by the IVIG high-risk warning score at each hospital.[39,40]

Table 5. Kobayashi score of high-risk Kawasaki disease[41]

Indicator	Critical value	Score
Serum sodium levels	≤133 mmol/L	2
Aspartate transaminase	≥100 IU/L	2
Start time of treatment	Day 4 or earlier	2
Percentage of neutrophils	≥80%	2
C-reactive protein	≥ 100mg/L	1
Blood platelet count	≤ 300×10^9/L	1
Age	≤12 months	1

Different kinds and methods of GC are applied to KD

Recommendation: The type of GC treatment for KD patients is methylprednisolone intravenous shock followed by oral prednisone sequential therapy (1A).[38]

Dose and course of GC applied to KD

Kobayashi early warning score suggests first-line treatment for children with IVIG non-responsive KD or persistently elevated inflammatory markers combined with CAA or peripheral vascular tumors inflammatory index

TNF-α is involved in the occurrence and development of KD inflammatory/immune response as a significant pro-inflammatory cytokine. It is positively correlated with CAL, so it has an essential clinical value in the prediction and prognosis evaluation of KD induced.

Table 6. The recommendations of GC for the treatment of KD

Items	Recommendations	Recommendation strength and level of evidence
The dose and course of GC applied to KD	Prednisone [1-2 mg/(kg.d)], taken in the morning, total dose <60 mg/d, or methylprednisolone [1-2 mg/(kg.d)], intravenously, once or twice a day, starting to be reduced when body temperature and CRP return to normal. After 15 days, it gradually decreased [1-2 mg/(kg.d)] for five days. 0.5 to 1 mg/(kg.d) for 5 days. 0.25-0.5 mg/(kg.d), 5 d].	1A
Different kinds and methods of GC are applied to KD	The type of GC treatment for KD patients is methylprednisolone intravenous shock followed by oral prednisone sequential therapy	1A

Recommendation: Prednisone [1-2 mg/(kg.d)], taken in the morning, total dose <60 mg/d or methylprednisolone [1-2 mg/(kg.d)], intravenously, once or twice a day, starting to be reduced when body temperature and CRP return to normal. After 15 days, it gradually decreased [1-2 mg/(kg.d)] for five days. 0.5 to 1 mg/(kg.d) for 5 days, 0.25-0.5 mg/(kg.d), 5 d (1A).[42-46]

Second-line treatment of IVIG non-responsive KD

Optional 2nd dose infusion of IVIG combined with prednisone (methylprednisolone).

Recommendation: Prednisone [1-2 mg/(kg.d)], taken in the morning, total dose <60 mg/d or methylprednisolone [1-2 mg/(kg.d)], intravenous drip, 1-2 times a day,

after body temperature and CRP returned to normal, the dose began to be reduced, and gradually stopped within 15 days [1-2 mg/(kg.d)], five days. 0.5-1 mg/(kg.d) for 5 days. 0.25-0.5 mg/(kg.d), 5 d] (1A).[42-46]

First-line treatment of KDSS

Recommendation: Methylprednisolone 10-30 mg/(kg.d) for 1 to 3 d with 2 to 3 h of each intravenous infusion. Heparin anticoagulation [10 U/(kg.d) of heparin concurrently two h before the start of methylprednisolone] for 24 h is recommended, or low-molecular heparin anticoagulation with coagulation, echocardiography, and blood pressure monitoring (2A).[47-50]

First-line treatment of KD combined with MAS

Recommendation: Methylprednisolone 10-30 mg/(kg.d) for 3 d, with each IV infusion for 2-3 h. Sequential prednisone orally [1-2 mg/(kg.d)] until complete control and remission of MAS with gradual dose reduction and discontinuation (2A)[51-53]

GC is not recommended as routine first-line therapy for KD. GC alone is unsafe and contraindicated as a first-line treatment for KD, as studies have shown that GC alone used as an initial treatment for KD can significantly increase coronary artery damage.[37,54]

Prevention of adverse reactions

During treatment with GC in children with KD, special attention should be paid to the prevention of Cushing's syndrome, infection, thrombosis, osteoporosis, aseptic necrosis of the femoral head, diabetes mellitus, hypertension, hormonal glaucoma, cataract, bradycardia, secondary adrenocortical insufficiency, and growth retardation. To prevent and treat osteoporosis, it is recommended to supplement vitamin D 600-800 U/d and calcium 1000-1200 mg/d while applying GC. Various infections, such as tuberculosis, fungus, and chickenpox, should be entirely excluded before high-dose methylprednisolone shock therapy, and blood pressure and blood glucose should be closely observed and tested to detect any of the above complications in time and deal with them actively. While applying CC, strive to minimize the adverse effects to improve the prognosis of children with KD.

Precautions

(1) Contraindicated: hypersensitivity to GC drugs, epilepsy, fractures, uncontrolled infections (e.g., chickenpox, fungal infections), active tuberculosis, etc.

(2) Caution: Cushing's syndrome, myasthenia gravis, hypertension, diabetes mellitus, intestinal disease or chronic malnutrition, infectious diseases, etc., must be combined with effective antibiotics.

(3) Other precautions: 1. Prevent cross-allergy; those who are allergic to one GC drug may also be allergic to other GCs. 2. When using GC, adopt low sodium, high potassium, high protein diet, supplement calcium, and vitamin D, and add drugs to prevent peptic ulcer and bleeding and other adverse reactions. 3. If there is an infection, antibiotics should be applied simultaneously to prevent the spread and aggravation of the disease. 4. The interaction between GC and other drugs should be noted; for example, excessive potassium loss can be caused when GC is combined with potassium-removing diuretics (e.g., thiazide or tab diuretics), and the incidence of gastrointestinal bleeding and ulcers increases when GC is combined with NSAIDs.[38,42,55-57]

Conclusion

After more than thirty years of clinical validation, concerning half a century of research results on KD, and combined with the treatment experience of hundreds of pediatric KD clinicians and experts in China, these consensuses standardize the use of IVIG, Asp, and GC in pediatric KD medication, which has important clinical significance in effectively reducing the incidence of complications in all systems of KD and preventing cardiovascular sequelae caused by KD. The limitations of consensuses include relatively few high-quality randomized controlled studies, fewer and more foreign references, and insufficient consideration of ethnic differences. Because the pathogenesis of KD is not fully understood, the medication for KD is constantly updated and researched. It is necessary to continuously update the consensus of KD medication according to the latest international studies and supplement the dosage and regimen of other complications in various medicines for myocarditis, acute inflammatory response syndrome, MAS, and other diseases.

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Author contributions

Conceptualization, R.S., X.L. and J.F.; Methodology, R.S. and X.L.; Validation, D.F., W.H. and J.F.; Formal Analysis, D.F.; Investigation, D.F.; Resources, D.Z.; Data Curation, D.Z.; Writing – Original Draft Preparation, D.Z.; Writing – Review & Editing, Y.X.; Supervision, Y.X. and X.L.

Conflicts of interest

The authors declare that the research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

Consent to publish

The authors affirm that the Chinese Journal of Current Pediatrics provided informed consent for the publication of this paper.

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REVIEW PAPER

Comparison of algorithms for detection of active inflammatory lesions in sacroiliitis

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ABSTRACT

Introduction. Artificial intelligence is increasingly being used in the medicine, particularly in radiological diagnosis of diseases such as an axial spondyloarthritis (axSpA). The aim of this study is to compare the available algorithms designed to detect active sacroiliitis in patients with axSpA.

Material and methods. Four algorithms, two semi-automated and two full-automated for the assessment of bone marrow edema (BME) on magnetic resonance imaging (MRI) of the sacroiliac joints (SIJs) were included in the study. They were described and compared in terms of specificity, sensitivity, and correlation of BME detection findings between AI and experts.

Analysis of the literature. Among all automated algorithms, the one created by Bressem et al. had the highest number of examinations analyzed in the study, involving 593 MRIs of SIJs. The sensitivity and specificity, as well as the correlation between the AI's detection of BME versus manual, were not calculated for each algorithm. Rzecki's algorithm had the greatest sensitivity and specificity for BME detection reaching 0.95 and 0.96, respectively. In addition, its Speraman's coefficient of correlation between manual and automated measurements was 0.866.

Conclusion. Each of described algorithms is certainly useful in assessing BME in the MRI examinations of SIJs.

Keywords. artificial intelligence, axial spondyloarthritis, bone marrow edema

Introduction

Artificial intelligence in medical imaging

Artificial intelligence (AI) can be loosely defined as the ability of a computer system to execute a task that typically or conventionally requires human intelligence.[1] AI encompasses systems that can perform tasks without the need for learning. In the field of medical imaging, for instance, AI can be employed to identify anatomical structures using predesigned algorithms that embody the concepts of software engineers. Conversely, a subset of AI techniques known as "machine learning" (ML) has the ability to automatically learn from presented data, often using ground truth data as training sets (i.e., supervised learning). This range of methods includes various algorithms for automatic pattern recognition, many of which have been developed over the past decades. "Deep learning" is a subcategory of machine learning that relies on artificial neural networks, mimicking human learning by employing mathematical representations of neurons and their connections. Within both of these AI categories, there is a wide spectrum of applications in the field of medical imaging diagnosis.[1]

Sacroiliitis

Sacroiliitis, which is characterized by inflammation of the sacroiliac joint (SI), typically results in pain. The sacroiliac joint, one of the largest joints in the body, frequently contributes to discomfort in the lower back

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and buttocks region. It connects the ilium bone to the sacrum. Diagnosing sacroiliitis can be challenging as its symptoms resemble those of other common causes of back pain, often leading to it being overlooked as a source of discomfort. Pain in this condition is often associated with chronic degenerative factors, although it is relatively uncommon. Sacroiliitis can be related to rheumatic, infectious, drug-related, or oncologic sources. Some specific nondegenerative conditions that can lead to sacroiliitis include ankylosing spondylitis, psoriatic arthropathy, Bechet disease, hyperparathyroidism, and various pyogenic sources.[2-4]

Axial spondyloarthritis

Axial spondyloarthritis (axSpA) results in persistent inflammation of the sacroiliac joints (SIJs), leading to chronic back pain, stiffness, and changes in skeletal structure and posture. This condition hampers the ability to carry out daily activities, contributing to a negative impact on the health-related quality of life (HRQoL) of individuals.[5] The development of new criteria for classifying and screening patients with axSpA has been crucial for early identification and treatment of such patients, with MRI being the most critical imaging method available.[6] This type of examination enables the assessment of various aspects, including bone marrow edema (BME), erosions, fat lesions, sclerosis, or ankylosis (bone formation).[7] While the utilization of magnetic resonance imaging and its integration into diagnostic standards has improved the recognition of initial axSpA, a consistent enhancement in early diagnosis has not been consistently documented in all research. Insufficient understanding of the full spectrum of axSpA symptoms and the failure to identify inflammatory back pain (IBP) in primary healthcare settings might be contributing factors to this issue.[8] The implementation of appropriate algorithms for recognizing axSpA through MRI imaging analysis may lead to earlier diagnoses, which in turn could be associated with the potential for improving patient treatment outcomes. According to the ASAS classification criteria, MRI is used to define active sacroiliitis through the following parameters:

1. Bone marrow edema, which is the accumulation of fluid within bone marrow cells due to inflammation, is observable in the "fluid-sensitive" STIR sequence (Short Tau Inversion Recovery) (also known as the T2-weighted sensitive to water sequence) as regions hyperintense to the sacral interforaminal bone marrow.
2. Bone marrow enhancement (osteitis), which can be detected in the T1-weighted sequence after contrast media administration.[9-11]

Algorithms for sacroiliitis diagnosis

It is possible to use algorithms for bone marrow edema. So far, only two algorithms for the semi-automated detection and measurement of sacroiliitis related to axSpA have been created. The first one, known as SCAISS, was developed by Zarco et al. in 2018, and the second one was introduced by Kucybała et al. in 2020.[12,7] In the research conducted by Rzecki et al., a fully automated algorithm for evaluating BME was outlined in 2021, and it was subsequently compared with alternative approaches in another study, affirming its potential for clinical application.[13,14] The subsequent year saw the development of another algorithm for analyzing MR images of sacroiliac joints in axial spondyloarthritis, as presented by Bressem et al.[15] Moreover, in 2023 in the study of Ożga et al. the algorithm created by Kucybala et al. and Rzecki et al. had been further developed and proven to handle a range of various conditions.[16] In contrast the one proposed by Bressem et al. is primarily focused on classifying entire MR images as either normal or abnormal.[15] Consequently, it does not determine the lesion's location or volume using the algorithm.[15]

Aim

In the following sections of this paper, each of the existing algorithms is described and compared in relation to one another.

Material and methods

The algorithms described in the following article were found in the PubMed database as those used to assess BME and thus detect active sacroiliitis.

Analysis of the literature

SCAISS algorithm by Zarco et al.

The method, known as SCAISS (Spanish abbreviation for "herramienta eSpañola para la Cuantificación semi-Automática de Inflamación de Sacroilíacas en resonancia magnética en eSpondiloartritis"), requires an MRI image in the STIR sequence saved in DICOM format.

It focuses only on two specific planes: semi-axial and semicoronal, which are oriented perpendicular to the sacroiliac joint, specifically within the periarticular region exhibiting hyperintense signals. Using the computer screen image, the physician identifies areas with visible bone marrow edema (BME) that appear hyperintense, one by one, with a mouse click. The software automatically selects adjacent areas with intensity falling within a predefined tolerance range centered around the pointer-click. Once the area is outlined, the software proceeds to calculate its size, perimeter length, and the mean signal intensity (brightness) within that region. It is essential to note that the lesions identified by mouse clicks should be situated in the periarticular area, as defined by the ASAS consensus regarding the anatomical characteristics of sacroiliitis.[12,17] The primary advantage of SCAISS compared to other methods is its simplicity – the reader only needs to choose ROI with a mouse

click – supported by demonstrated validity and reliability. Additionally, the selected images can be saved (as ROI), not just the score, which implies improved tracking of the measurement process, allowing for reevaluation if necessary and easier monitoring of the same or new areas. Further benefits of the approach include the requirement of only STIR sequences and the capability to reliably interpret both coronal and axial slices, whereas other techniques can only be evaluated in coronal images. Nevertheless, it is important to consider certain limitations when interpreting these findings. It is crucial to note the small sample size, necessitating further confirmation in subsequent validation studies. Furthermore, the sensitivity to changes in the SCAISS scale has not yet been evaluated, which precludes recommending the use of this method in clinical trials.[12] On the other hand, the main disadvantage of this approach is its reliance on manual selection of lesions (as the software only identifies their outlines) and the inability to detect lesions overlooked by the observer.[7]

Algorithm by Kucybała et al.

This algorithm's development was grounded in the systematic approach outlined by Maksymowych et al. for evaluating active inflammatory changes in sacroiliac joints.[18] The semi-automated procedure for detecting bone marrow edema comprised the subsequent stages:

1. The sacral bone and visible portions of both iliac bones were manually delineated on T1-weighted sequence images using the Segmentation Editor plugin for ImageJ (National Institutes of Health, Bethesda, MD, USA). Each bone was assigned a distinct label.
2. Identification of the reference signal area: The algorithm determined the central axis of the sacral bone and identified all pixels within the sacrum that were closer to this central axis than a user-defined distance threshold, REFTH. Then these marked pixels were classified as part of the reference signal region.
3. Detecting central lines in the sacroiliac joint: Firstly, the algorithm calculated the distance from each non-bone pixel to both the iliac and sacral bones. Next, it assigned an absolute value representing the difference between these two distances to each non-bone pixel. Pixels positioned at the central line of the joint, where the distances to the sacral and iliac bones were equal, received zero values. Finally, the algorithm utilized Dijkstra's shortest path algorithm to identify the central joint lines.
4. Identifying regions of interest (ROIs): ROIs were defined as bone areas located near joint surfaces at a user-defined distance, where the algorithm aimed to detect inflammatory changes. Initially, the algorithm established the bony boundaries of the joint surfaces by projecting the central lines of the sacroiliac joints onto the surfaces of both the sacral and iliac bones. Following this, for each bone individually, the algorithm computed the distances from the pixels within the bone to its corresponding joint surface. Any pixel with a distance less than 10 mm was categorized as part of the ROI for that particular bone.
5. The partitioning of ROIs into quadrants: First, the central line of each sacroiliac joint was identified, and its midpoint was established. Then, a straight line, perpendicular to the central line and passing through its midpoint, was defined to separate the ROIs into upper and lower quadrants.
6. Identification of inflammatory changes: Since the patient's position remained consistent during the acquisition of both T1-weighted and STIR sequence images, the reference region and quadrants initially determined on T1-weighted sequence images were transferred to STIR sequence images to identify bone marrow edema.
7. within STIR sequence images, each pixel within the ROI was matched with a set of R reference pixels from the reference region. Subsequently, the mean and standard deviation of the signal intensity for this reference set were calculated. Following that, the test statistics were computed, which represented the difference in signal intensity between the tested pixel and the mean intensity of the reference set, divided by the standard deviation of the reference set. If these test statistics surpassed a user-defined threshold, it indicated the presence of bone marrow edema within the tested pixel.

The manual process was only required for the first step; steps 2 to 6 were completely automated.[7] The main advantage of this method is that by concentrating the detection on specific pixels, it becomes possible to identify and emphasize regions where the presence of bone marrow edema is suspected. Consequently, the radiologist can confirm the actual significance of the identified alterations and readily elucidate the findings of the examination. Nonetheless, the primary constraint of our approach is the prerequisite for manual preparation of bone segmentations forming the sacroiliac joints before the automated detection of inflammatory changes. This currently impedes the integration of this method into routine clinical practice.[7]

Algorithm by Rzecki et al.

Back then, only two algorithms had been developed for the semi-automated detection of sacroiliitis related to axSpA on MRI. The first one was created by Zarco et al., which allowed for the detection of inflammatory change boundaries as chosen by the observer, but it couldn't identify missed lesions.[12] On the other hand, the semi-automated algorithm by Kucybała, Rzecki

et al. provided reliable identification of bone marrow edema lesions.[7] However, it required a labor-intensive manual segmentation of the sacroiliac joint bones before lesion detection. At that time, no fully automated method had been developed to aid in the diagnosis of axSpA through MRI. Subsequently, the study of Rzecki et al. significantly enhances the previously published algorithm with regard to bone and inflammatory change segmentation.[7] Firstly, Rzecki et al. replaced the manual bone segmentation, used in the algorithm by Kucybała et al. with a fully automated segmentation method based on deep learning. Secondly, Rzecki et al. substantially improved the precision of determining the volume of marrow edema lesions.

The automated algorithm developed by Rzecki et al. for bone marrow edema detection involved the following procedures:

1. Segmentation of the sacrum and the left and right iliac bones on 2D slices from a 3D T1-weighted sequence.
2. Identification and extraction of ROIs where the algorithm detects inflammatory changes.
3. Segmentation of the inflammatory lesions within the identified ROIs.

Subsequently, the algorithm was further tested on a larger number of MRI examinations by the team of Ożga et al.[16] The research team validated the algorithm's performance depending on the technical correctness of the MRI scan.

The project consisted of the following steps:

1. Assessment of the correctness of the alignment of the MRI section of the sacroiliac joints. The deviation angle of each examination was measured to validate the method of determining the technical correctness of the MRI examination.
2. Enhancement of the pre-existing algorithm in the form of post-processing adjustments. The algorithm by Rzecki et al. was updated by introducing the rule that BME is to be located up to 1 cm from the joint space.[7]
3. The following manual and automatic segmentation of the sacrum and iliac bones in T1-weighted images were performed at each examination.
4. Evaluation of inflammatory lesions present on the included examinations using the SPARCC scale.
5. Manual and automatic segmentation of bone marrow edema present on the sacrum and iliac bones in STIR images.[16]
6. The results of bone and BME segmentations performed by the algorithm and by experienced researchers were compared.

The results of the study revealed that the evaluated algorithm performs satisfactorily regardless of the angle of deviation and, consequently, the technical correctness of the examination. It is worth mentioning that the key advantage of this algorithm is its full automation, which eliminates the time-consuming manual segmentation, and has achieved significantly higher sensitivity and specificity compared to other algorithms. However, the sample size remains insufficient to take significant steps towards the implementation of this algorithm for routine use in clinics

Algorithm by Bressem et al.

A deep learning tool created by Bressem et al. was employed to identify signs of active inflammation and structural abnormalities associated with axSpA in sacroiliac joint MRI scans. Its primary function involves categorizing entire MR images as either normal or abnormal, without specifying the lesion's location or volume through the algorithm.[15] One of the strong points of this research is the incorporation of MRI scans obtained from various machines with diverse settings, the centralized standardized assessment of images by professionals, and the utilization of an external test set. This study has also several limitations. Firstly, the low axSpA prevalence in the test set might introduce performance uncertainty. Secondly, in GESPIC-Uveitis and OptiRef, MRI was conducted only in a subset of patients, potentially causing selection bias. Thirdly, the models were exclusively trained with semicoronal images, potentially leading to model failure with different orientations. Fourthly, the choice of global labels for model training and the absence of a quadrant analysis of the sacroiliac joints hindered a spatially accurate assessment of various joint regions. Finally, the variety of scanners and protocols used made it impossible to provide imaging parameters for all MRI scans, thus limiting the reproducibility of the data.[15]

Algorithm comparison

AI algorithms are a technological accomplishment enabling the development of many branches of science, including medicine.[19] Since the accurate diagnosis of axSpA depends on experience, the discussed algorithms could be particularly helpful for doctors without much experience in evaluating MRI of SIJs. The use of artificial intelligence in the clinical practice of doctors can contribute to reducing the time, increasing the accuracy and precision of their performance.[20] This results in reducing health care costs and a more efficient use of specialists' time, as well as enabling them to make the correct diagnosis in a larger number of patients, while recognizing a disease at an earlier advanced stage.[21]

It is crucial to choose the right algorithm to maximize the sensitivity and specificity of detecting inflammatory lesions in the sacroiliac joints using AI. Among the algorithms compared, Bressem et al. evaluated the largest number of images of patients (593), at the same time it is one of the two fully-automated algo-

Table 1. Comparison of algorithms created for the BME detection

	Zarco et al. (SCAISS)	Kucybała et al.	Rzecki et al.	Bressem et al.
Algorithm's automation	Semi-automated	Semi-automated	Fully-automated	Fully-automated
Algorithm's outcomes	The area, perimeter length, and mean of signal intensity (brightness) in bone marrow edema	The volume of marrow edema lesions	The volume of marrow edema lesions	Detection of active inflammatory changes (BME) or structural changes indicative of axSpA
Number of SIJs MRI included in the study	23	22	30	593
Number of patients with BME presence	23	22	30	222
Sensitivity in BME detection	Not calculated	Not calculated	0.95	0.88
Specificity in BME detection	Not calculated	Not calculated	0.96	0.71
Correlation between automatic and manual detection of BME	The three-phase Speraman's coefficient of correlation was 0.747, 0.729 and 0.74 compared to the Berlin method and to the SPARCC was 0.772, 0.840 and 0.793 for the first, second and third evaluators, respectively.	The correlation coefficient between semi-automated and manual detections was 0.87 for pixel-wise comparison and 0.83 for quadrant-wise analysis.	The Speraman's coefficient of correlation between verified ground truth and automated measurements was equal to 0.866 while the intraclass coefficient of correlation ICC (1,1) is equal to 0.947.	Not calculated
The time to analyze the whole MRI examination of one patient	28 s	Up to 10 s	Not mentioned	18.9 s

rithms described (the other was described by Rzecki et al.). Comparing the values achieved by these algorithms for sensitivity and specificity, significantly better results are obtained by the Rzecki's et al. algorithm than by the Bressem's one. Regrettably, in the case of semi-automated algorithms, these parameters were not calculated. Fully-automated algorithms provide a much broader range of actions carried out by AI than semi-automated algorithms, which makes them more widely used in clinical practice. The algorithm by Zarco et al. is the only one that requires a physician to manually mark the area with visible marrow edema to obtain its size, perimeter length, and the mean brightness calculated by software, which demonstrates its limited applicability in practice. However, none of the discussed algorithms considered the impact of the technical correctness of performing the MRI examination on its efficiency – only Ożga et al. evaluated the impact of this parameter on the performance of fully-automated algorithm previously created by Rzecki et al.

It is difficult to determine which algorithm is the best, because each of them performs in a slightly different manner. The algorithm by Bressem et al. additionally detects structural changes in axSpA, and the one by Zarco et al. measures more parameters, but it is semi-automated. The creation of algorithms by different research teams encourages each group to improve their algorithms. Possibly, with the cooperation of all researchers involved in the development of AI in diagnostic imaging of axSpA, an algorithm combining the advantages of all will be created in the future.

Conclusion

The development of artificial intelligence in diagnostic imaging axSpA is incredibly important and will help minimize costs and increase clinicians' productivity. Each of the algorithms presented in the paper has advantages and disadvantages. The algorithm created by Bressem et al. was trained on the largest number of examinations. The algorithm created by Rzecki et al. has the greatest sensitivity and specificity. The algorithm created by Kucybała et al. has the shortest time to analyze the whole MRI examination of one patient. However, it is impossible to determine the ultimate algorithm.

Declarations

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Author contributions

Conceptualization, I.G. and J.O.; Methodology, I.G.; Software, I.G.; Validation, J.O. and A.R.; Formal Analysis, I.G.; Investigation, I.G.; Resources, J.O.; Data Curation, I.G.; Project Preparation, I.G., A.R. and J.O.; Writing – Review and Editing, I.G.; Visualization, J.O.; Supervision, I.G.; Project Administration, I.G.

Conflicts of interest

The authors declare no conflict of interest.

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REVIEW PAPER

The benefits and limitations of using blue-blocking filters – a review of results of clinical trials

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ABSTRACT

Introduction and aim. Blue light is part of the natural light spectrum and plays a role in regulating the circadian rhythm. However, with the increasing use of electronic devices and energy-efficient lighting emitting high levels of artificial blue light, concerns are raised regarding its potential effect on human health. Blue-blocking filters have been developed and are advertised as a solution to be used in spectacles or intraocular lenses. This review aims to provide an in-depth analysis of the use of blue-blocking filters based on the results of clinical trials.

Material and methods. This review included relevant original papers reporting on clinical trial results from PubMed, Science Direct, and Google Scholar databases using specified keywords.

Analysis of the literature. Trials conducted with patients reveal mixed results, with some showing no significant changes in vision and reading abilities, while others indicating potential limitations such as reduced contrast vision. However, blue-blocking filters have demonstrated potential benefits in improving sleep quality and mood, particularly in patients with sleep disturbances or psychiatric disorders. The efficacy of blue-blocking filters in mitigating symptoms of digital eye strain remains inconclusive.

Conclusion. Overall, this paper provides a comprehensive assessment of the benefits and limitations associated with the use of blue-blocking filters, highlighting the need for further investigation in certain areas.

Keywords. blue light, blue-blocking filters, clinical trials

Introduction

Blue light is an essential part of the natural light spectrum emitted by the sun, which falls within the wavelength range of approximately 400 to 490 nanometers and has high energy. During the day, exposure to natural blue light helps regulate our internal body clock, or circadian rhythm, by signaling wakefulness and alertness.[1] However, the widespread use of electronic devices and energy-efficient lighting has led to increased exposure to artificial sources of blue light. This has raised concerns about its potential effects on human health.

Artificial sources of blue light

Artificial sources of blue light include electronic devices such as smartphones, tablets, computer monitors, televisions, and light-emitting diodes (LEDs).[2] With the ever-increasing reliance on technology, the average person spends a significant amount of time engaging with digital screens. These screens emit blue light at higher intensities compared to other wavelengths, potentially leading to prolonged exposure that exceeds the levels encountered in natural environments.[3] Energy-efficient lighting, such as compact fluorescent lamps and LEDs, has gained pop-

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ularity due to its reduced energy consumption. However, these lighting technologies emit higher levels of blue light compared to traditional incandescent bulbs.[4] The shift towards energy-efficient lighting in residential, commercial, and public spaces has increased overall blue light exposure, particularly during nighttime hours. However, along with the benefits of these technological advancements comes a growing concern regarding the potential health effects of blue light exposure.

The biological impact of blue light

The biological impact of blue light on humans influences the circadian rhythm. Research suggests that excessive blue light exposure can disrupt sleep patterns and lead to sleep disturbances, such as difficulty falling asleep or maintaining deep sleep. Blue light exposure in the evening can suppress the production of melatonin, a hormone that promotes sleep. Consequently, individuals exposed to blue light before bedtime may experience delayed sleep onset and reduced sleep quality.[5-7]

Moreover, blue light has implications for eye health. Even though the research results are not unanimous, prolonged exposure to blue light, especially at close proximity through digital screens, is proposed as a possible risk factor for eye strain, visual discomfort, and eye fatigue.[8,9] There are also concerns about potential long-term effects on retinal health, as studies suggest a possible link between blue light exposure and retinal damage, including macular degeneration. This can be caused by the release of reactive oxygen species in response to blue light absorption.[10] LEDs have been associated with causing damage to photoreceptors and necrosis of the retina.[11]

In addition to sleep and eye-related effects, blue light exposure has been investigated for its impact on mood disorders, such as depression, mania, or bipolar disorder. Even though the results are vague, excessive blue light exposure, particularly during nighttime hours, may disrupt mood regulation and contribute to symptoms of mood disorders.[12-14]

Blue-blocking filters

To address the concerns related to the adverse impact of blue light (which possibly include: sleep disturbances, mood disorders, eye strain, visual discomfort, eye fatigue, and retinal damage), blue-blocking (BB) filters have been developed and incorporated into spectacles and intraocular lenses (IOLs) as a potential solution to reduce the transmission of blue light and mitigate its potential adverse effects.[15] BB filters aim to selectively absorb or reflect short-wavelength light (blue light and shorter wavelengths of 440-500 nm), thereby reducing the amount reaching the retina.[10] However, the efficacy of BB filters in spectacles and IOLs in reducing eye fatigue symptoms when using digital devices, improving sleep quality, and protecting from retinal phototoxicity remains a topic of debate. While some studies suggest potential benefits, others have found limited or inconsistent effects on its supposed potential influence on ocular disorders.[16]

Aim

This review aims to analyze the results of clinical trials investigating the use of BB filters in spectacles and IOLs and their effectiveness in mitigating undesirable symptoms associated with excessive exposure to blue light. By critically evaluating the available evidence, we seek to provide a comprehensive assessment of the benefits and limitations associated with their use.

Material and methods

This narrative review was performed by researching the PubMed, Science Direct, and Google Scholar databases with the keywords "blue light", "blue-blocking filters", "blue light filters", "blue light filters spectacles", "blue light filters lenses" and "clinical trial". The search was performed from May 19th, 2023, through June 8th, 2023. Considered were only original papers that reported on the results of clinical trials, written in English or Polish, and included were those deemed relevant to the analysis by the authors. The exclusion criteria included: preclinical trials, and manuscripts unrelated to the topic. All articles that fit the aim of this review paper were included in the "result" section of the article. The narrative review has been constructed with the use of the scale for the quality assessment of narrative review articles (SANRA).

Analysis of the literature

The results of the reviewed clinical trials were not consistent in all aspects, and some of them concluded that BB filters are not favorable for patients. Different aspects were taken under consideration and the results either supported or disproved the thesis of the trial. The clinical trials also used various outcome measures. The details of each study design can be found in the referenced literature, while this review summarizes the most important conclusions drawn from the studies. The most important advantages and disadvantages of using BB filters are summarized in Table 1.

Table 1. Summary of the advantages and disadvantages of BB filters in IOLs and spectacles*

Advantages	Disadvantages
- may be beneficial as part of the treatment of amblyopia,[26]	- no influence on contrast vision or visual acuity,[17-20]
-improve mood and sleep quality,[28]	- do not reduce symptoms of DES,[36,37]
- may be used as an additive treatment for patients with sleep disturbances in the course of psychiatric disorders,[13,30]	- might dysregulate the circadian rhythm,[36]
- may lead to better reading capacity in children with reading difficulties.[27]	- do not influence mental symptoms and psychiatric disorders in patients after cataract surgeries,[38,39]
	- do not reduce number of injuries in patients after cataract surgeries,[40]
	- do not prevent development of AMD.[45-48]

* DES – digital eye strain, AMD – age-related macular degeneration.

The impact of BB filters on vision and reading

Many studies investigated the influence of BB filters on visual function. Up to date, no effect on contrast vision, or visual acuity has been observed.[17-20] Wirtitsch et al. conducted a clinical trial that revealed that the contrast acuity in patients with BB IOLs was lower in comparison to users of ultraviolet-filtering IOLs. The biggest difference in contrast vision was observed in the setting of low-mesopic light (a range of human vision, where both rods and cones are active), where the contrast acuity was tested at low illumination levels (0.5 lux) with different levels of contrast.[21] Also in another work, the researchers found that contrast vision among the patients was worse under lower illumination using the IOLs with BB filters compared to photochromic or clear IOLs.[22] On the other hand, some studies concluded that there is no clinically significant change in contrast vision among patients with BB IOLs, and no difference between BB IOLs in comparison to clear ones regarding mesopic contrast sensitivity nor subjective visual perception.[17,19,20,23,24] Yellow or orange-colored BB IOLs and clear IOLs have similar results in photopic and mesopic contrast sensitivity.[23]

Another important aspect assessed in the clinical trials was color vision reduction. Under mesopic conditions, patients with yellow-tinted IOLs had the least favorable outcomes during the assessment of color perception and made more mistakes during tests.[19,22] Especially, the perception of the color blue has been affected both under mesopic and photopic conditions (a range of human vision, where only cones are active).[20] In another research, there was no significant difference in color sensitivity among the tested groups, but the subjective perception of the colors has been changed and reported by some patients.[18] On the other hand, one clinical trial conducted by Stopyra et al. revealed a possible positive influence of BB filters on color perception.[25]

Another clinical trial assessed the use of BB filters as part of the treatment of amblyopia. Metzler et al. showed, that a BB filter treatment protocol gave better results for treating amblyopia than conventional classic occlusion treatment.[26]

Regarding the influence of BB filters on reading abilities, a clinical trial reported that children with reading difficulties, who wore glasses with blue or yellow filters for 3 months, had a better reading capacity than they did before the start of the clinical trial.[27] A detailed summary of chosen representative clinical trials is shown in Table 2.

The impact of BB filters on sleep disturbances

Many studies suggested that BB filters may have a positive effect on patients with sleep disturbances. Wearing glasses with a BB filter 3 hours before sleep was shown to significantly improve mood and sleep quality in comparison to patients who wore glasses blocking ultraviolet only.[28] In a similar study, after wearing BB glasses 90 min before bedtime a substantial reduction of subjective sleep latency and an increase in subjective total sleep time was observed.[29] Many studies indicated that patients with psychiatric disorders may benefit from BB filters applied in ocular lenses. In one study, patients with bipolar disorder and coexisting circadian rhythm abnormalities who used BB filters in ocular lenses in the evenings presented an improvement in sleeping efficiency.[13] Their use may also be an additive treatment for manic patients in a hospital environment,[30] but no significant changes were seen in patients with depressive symptoms and coexisting sleep onset insomnia.[14] Another group that can be positively influenced is non-sleep-deprived recreational athletes. A clinical trial reported that wearing BB filters in the evening improved the subjective sleep onset latency, sleep quality, and alertness in the morning.[31] Another study stated that the use of amber lenses before bedtime leads to delayed wake time and higher mean subjective total sleep time, overall quality, and soundness of sleep.[32] On the other hand, studies state that long-term blue light reduction might negatively influence mental health.[16] Blue light filtration may negatively affect normal circadian rhythm and sleep, but there is conflicting evidence.[33] Moreover, in a randomized controlled trial BB IOLs lowered nocturnal melatonin secretion in comparison to neutral IOLs, but surprisingly BB IOLs increased sleep efficiency in patients one year after cataract surgery.[34] Another clinical trial also indicated that BB IOLs implantation can be beneficial for patients after cataract surgery in improving the quality of sleep. In the referenced group of patients, blocking the blue light during the day has not had adverse effects, because the amount of light transmitted overall through the lens rises after cataract surgery. The BB IOLs can partially filter purple and blue visible light between 400 and 500 nm, but a residual blue light transmission is still enough to prevent melatonin production during the day. Inhibiting the release of melatonin throughout the day enhances the quality of sleep at night.[35]

The impact of BB filters on the symptoms of digital eye strain

In general opinion, BB lenses are viewed as a tool to prevent computer users from digital eye strain (DES). However, the reviewed clinical trials showed that using BB filters did not reduce the symptoms of DES.[36,37] Additionally, the authors agreed that currently, there is no evidence for using BB filters as a remedy for DES.[36] Furthermore, the usage of BB filters might disintegrate the circadian rhythm. However, it is difficult to conduct a credible clinical trial that would prove that the reduction in the absorption of light impacts the human's sleep schedule.[38]

Table 2. Summary of chosen representative clinical trials that assessed the benefits of using BB filters in glasses and IOLs*

Group of patients	Title of the study	Main objective of the study	Outcome measures	Main outcomes of the trial	References
Positive effects of BB filters					
Patients with bipolar disorder and insomnia	"A double-blind, randomized, placebo-controlled trial of adjunctive blue-blocking glasses for the treatment of sleep and circadian rhythm in patients with bipolar disorder"	To assess the influence of BB glasses on the change in sleep quality and circadian rhythm	Overall quality of the sleep experience, subjective and objective sleep, circadian rhythm	No difference in sleep quality, but visible changes in actigraphic sleep efficiency	13
Strabismic children with amblyopia aged 3 to 7 years old without previous treatment and a visual acuity up to 0.3	"Blue filter amblyopia treatment protocol for strabismic amblyopia: a prospective comparative study of 50 cases"	To assess the effectiveness of BB IOLs as a treatment method for amblyopia	Visual acuity, fixation behavior, visual evoked potentials	Patients presented with better visual acuity and eye-fixation behavior outcomes	26
Children with reading difficulties aged 7 to 10 years old	"A comparison of two-coloured filter systems for treating visual reading difficulties"	To assess the effectiveness of BB IOLs in treating reading difficulties	Reading and spelling scores, irregular and non-word reading capacity	Patients with visual stress who wore colored filters had better reading and spelling outcomes	27
Patients with insomnia aged 18 to 68 years old	"Amber lenses to block blue light and improve sleep: a randomized trial"	To assess the influence of blocking blue light on improving sleep	Changes in sleep quality	Glasses with BB filters led to improvement of sleep quality and benefited patients' mood	28
Patients with bipolar disorder in a manic state aged 18-70 years old	"Blue-blocking glasses as additive treatment for mania: Effects on actigraphy-derived sleep parameters"	To assess the effectiveness of BB filters on sleep parameters in patients with mania	Actigraphy-derived sleep parameters	The efficiency of sleep was higher, and sleep was more consolidated among patients using BB glasses	30
Negative effects of BB filters					
Patients with bilateral cataract	"Effects of blue light-filtering intraocular lenses on the macula, contrast sensitivity, and color vision after a long-term follow-up"	To assess side effects and potential protection after implantation of BB IOLs	Contrast sensitivity, color vision, macular findings	No significant influence on color perception, scotopic contrast sensitivity or photopic contrast sensitivity	17
Patients after phacoemulsification and primary posterior curvilinear capsulorhexis	"Intraindividual comparison of color contrast sensitivity in patients with clear and blue-light-filtering intraocular lenses"	To assess the effect of BB IOLs on color contrast sensitivity	Best distance-corrected visual acuity	No change in color contrast sensitivity	18
Patients scheduled for cataract surgery without history of ocular surgery or ocular pathologies	"Comparison of visual performance with blue light-filtering and ultraviolet light-filtering intraocular lenses"	To assess contrast sensitivity, glare, color perception, and visual acuity at different light intensities	Contrast sensitivity, glare, color perception, visual acuity, color discrimination	No significant influence on visual performance	19
Patients with bilateral cataract scheduled for phacoemulsification and posterior IOL implantation in both eyes	"Intraindividual comparison of a blue-light filter on visual function: AF-1 (UY) versus AF-1 (UV) intraocular lens"	To assess the effect of BB IOLs on visual function	Uncorrected and best corrected visual acuities, pupil size, contrast vision, color discrimination	No change in contrast vision and visual acuity, and worse perception of blue color	20
Patients aged between 16 and 27 years old and best corrected visual acuity of at least 20/20	"A double-blind test of blue-blocking filters on symptoms of digital eye strain"	To assess the influence of BB filters on DES symptoms	Pre- and post-reading task symptom scores of DES	No significant change in symptom scores before and after task	36
Healthy young patients	"Blue-blocking filters do not alleviate signs and symptoms of digital eye strain"	To assess whether BB filters are affective in reducing symptoms of DES	Orbicularis oculi muscle activity, visual discomfort	No influence of BB filters on reducing symptoms of DES	37
Cognitively healthy patients scheduled for bilateral cataract surgery	"Blue light-filtering intraocular lenses and post-operative mood: a pilot clinical study"	To assess influence of BB IOLs on post-operative mood and symptoms of depression	Symptoms of depression assessed using geriatric depression scale	BB IOLs did not affect mood differently than untinted IOLs	38
Patients after bilateral cataract surgery	"Association of clear vs blue-light filtering intraocular lenses with mental and behavioral disorders and diseases of the nervous system among patients receiving bilateral cataract surgery"	To assess the development of mental and behavioral disorders and nervous system diseases in patients after cataract surgery	Appearance of mental, behavioral, and nervous system diseases subcategorized by the ICD codes	BB IOLs do not influence the development of mental, behavioral, and nervous system diseases	39
Patients after bilateral cataract surgery	"Association of Blue Light-Filtering Intraocular Lenses With All-Cause and Traffic Accident-Related Injuries Among Patients Undergoing Bilateral Cataract Surgery in Finland"	To assess the influence of BB IOLs on risk of injuries and quality of driving	Kaplan-Meier and Cox regression analyses for the risk of injuries after surgery	BB IOLs did not reduce the risk of injuries and led to worse glare during driving in the dark	40
Patients with bilateral cataract and AMD	"Comparative assessment of the course of age-related macular degeneration in patients after phacoemulsification cataract surgery with implantation of AcrySof Natural SN 60 at and AcrySof SA 60 at lenses"	To assess the influence of BB IOLs on progression of AMD	Ophthalmic examination, fluorescein angiography, multifocal ERG	BB IOLs have no protecting influence on AMD progression	45
Patients who underwent uneventful cataract surgery	"The Effect of Blue-Light Filtering Intraocular Lenses on the Development and Progression of Neovascular Age-Related Macular Degeneration"	To assess the ability of BB IOLs to prevent neovascular AMD after cataract surgery	Kaplan-Meier and Cox regression analyses for overall risk of neovascular AMD development	Study showed no significant benefit of using BB IOLs	46
Patients previously treated for wet AMD and were implanted with BB IOL at least 3 years before	"The Influence of Blue-Filtering Intraocular Lenses Implant on Exudative Age-Related Macular Degeneration: A Case-Control Study"	To assess whether BB IOLs prevent the onset of wet AMD	Monitoring -follow ups	No beneficial change in the occurrence of wet AMD in patients with BB IOLs	47
Patients who underwent cataract surgery in both eyes	"Effect of Blue Light-Filtering Intraocular Lenses on Age-Related Macular Degeneration: A Nationwide Cohort Study With 10-Year Follow-up"	To assess the incidence of AMD after cataract surgery and the incidence of AMD after implantation of BB IOLs and non-BB IOLs	Monitoring – follow ups	No positive influence on the incidence of AMD	48

* BB – blue-blocking, IOLs – intraocular lens, DES – digital eye strain, ICD – International Classification of Diseases, AMD – age-related macular degeneration, ERG – electroretinogram

The impact of BB filters on patients after cataract surgeries

Clinical trials have been conducted to assess the relationship between using BB filters and the outcomes of cataract surgeries. In a study performed by Leruez et al., patients were observed post-surgery to detect mood changes assessed based on the geriatric depression scale, with regard to the type of IOLs implanted. It has been discovered that there is no significant difference in post-operative depression symptoms after implantation of clear IOLs, or BB IOLs.[38] Furthermore, patients who were implanted a BB IOLs after bilateral cataract surgery did not develop any mental and behavioral disorders or diseases of the nervous system.[39] Another study proved that the use of BB IOLs did not reduce the number of injuries and patients with BB IOLs had a worse glare during driving at nighttime, but that did worsen the comfort of driving.[40] Another assessed factor was the influence of BB IOLs on the thickness of the retinal nerve fiber layer (RNFL), and a clinical trial by Kim et al. proved, that BB IOLs did not influence the RNFL after cataract surgery, so there is no additional benefit of using these types of lenses.[41] Also, the type of used lenses does not affect the development of inflammation connected to cataract surgery.[42]

The impact of BB filters on the progression of age-related macular degeneration

Blue light can cause damage to the retina and lead to age-related macular degeneration (AMD).[43] It was suggested that patients with a high risk for the development of AMD could benefit from wearing IOLs with BB filters.[44] However, opposite results were presented by Łak et al., who showed that the implantation of BB IOLs did not stop the progression of AMD, and did not protect the maintenance of contrast sensitivity.[45] In a cohort study, the implementation of BB IOLs did not show any positive effect on the incidence of neovascular AMD and its progression over a non-BB IOLs use in patients after cataract surgery.[46] Moreover, another study found that there is no evidence that BB IOLs prevent the onset of wet AMD.[47] Furthermore, even the use for up to 10 years of BB IOLs had no significant advantage over a non BB IOLs in the incidence of AMD.[48] Another study, which included patients with AMD, assessed the reading rates among patients, and no advantage of using BB filters was proven. Additionally, BB filters users had worse reading rates compared to users of other color filters.[49]

Conclusion

In conclusion, the use of BB filters in spectacles and IOLs has shown mixed results in clinical trials. The impact of BB filters on vision and reading abilities is still a topic of debate, with some studies reporting no significant changes and others indicating potential limitations, such as reduced contrast acuity and color perception. However, BB filters have demonstrated potential benefits in improving sleep quality and mood, particularly in patients with sleep disturbances or psychiatric disorders. The efficacy of BB filters in mitigating symptoms of digital eye strain remains inconclusive, and further research is needed to establish their effectiveness in this area.

Declarations

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Author contributions

Conceptualization, A.P. and E.S.; Methodology, M.D.; Software, P.S.; Validation, A.P., and P.S.; Resources, M.D.; Data Curation, E.S.; Writing – Original Draft Preparation, A.P., E.S., P.S. and M.D.; Writing – Review & Editing, A.P., M.D., P.S., and E.S.; Visualization, M.D.; Supervision, E.S.; Project Administration, P.S.

Conflict of interest

The authors declare no conflict of interest.

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REVIEW PAPER

PET and SPECT imaging as a solid guide to detect and discriminate atypical phenotypes of neurodegenerative disorders

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ABSTRACT

Introduction and aim. Atypical or mixed presentations of neurodegenerative disorders may postpone or confound the final diagnosis. Molecular imaging with positron emission tomography (PET) and single photon emission computed tomography (SPECT) radioligands provide target-specific information and may anticipate the diagnosis by "in vivo" detection of the neuropathological substrate, as Aβ deposition, nigrostriatal dopaminergic depletion or tau inclusions. This concise review will discuss the potential of PET and SPECT imaging as a solid guide to better characterize atypical phenotypes of neurodegeneration in the clinical routine, with the potential to drive personalized interventions, improve cohort uniformity for clinical trials, and serve as biomarkers for targeted molecular therapies.

Material and methods. Literature search was performed focusing on the role of PET and SPECT imaging in assessing atypical phenotypes of neurodegeneration, using the electronic source of database PubMed/MEDLINE and the web-based search engines Google, Google Scholar.

Analysis of the literature. New disease-modifying drugs may increase their effect with early initiation, which is especially important in working persons and younger subjects presenting atypical symptoms. In older individuals, the coexistence of neurodegeneration, age-related changes, cerebrovascular lesions, or depression makes challenging a definitive diagnosis.
Quantitative tools able to measure tracer distribution increase the accuracy of molecular neuroimaging creating topographic maps that compare subject's data with healthy controls databases.

Conclusion. Atypical phenotypes may be associated with quantitative key-pattern allowing a more precise and early diagnosis of the neurodegenerative disorder. Finally, quantitative assessment of the pathological substrates allows us to track the disease process and measure treatment response.

Keywords. atypical phenotypes, neurodegenerative diseases, positron emission tomography, single photon emission computed tomography

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Introduction

Neurodegeneration is the leading cause of cognitive and physical disability across the globe with an increasing economic burden for patient families and healthcare systems.

According to the latest report by the World Health Organization, the global prevalence of dementia stands at over 55 million individuals, with a yearly increase of nearly 10 million cases.[1]

In the prodromal stage neurodegenerative disorders (Nds) can debut with a continuum of non-specific symptoms and signs postponing a correct diagnosis.[2]

Overlapping symptoms and comorbidities in different diseases may be confounding, especially at an early stage, and makes critical the time-opportunity for new disease-modifying treatments.[3-8]

The clinical phenotype can be the result of multiple different neuropathologies that synergically explain their detrimental role, as it happens in Alzheimer's disease (AD), dementia with Lewy bodies (DLB), and Parkinson-dementia, typically defined by specific complex protein abnormalities, as amyloidoses, α-synucleinopathies, tauopathies, and transactivation response DNA binding protein 43 (TDP-43) proteinopathies. Their presence, conformation and anatomical distribution represent the major hallmark of histopathological diagnosis.[9,10]

The spreading of pathological protein deposition along disease-specific vulnerable neural networks can explain progression and may be associated with specific cognitive phenotype.[11]

Therefore, improving the pathophysiological understanding of the neurodegenerative process allows the development of targeted treatments and disease prevention strategies, while non-pharmacological interventions, such as brain training and physical rehabilitation techniques, may represent potential add-on treatments.

Much effort is currently spent in translational research to develop disease biomarkers that enable early diagnosis, identify subclinical progression, and monitor treatment.

Additionally, studies on the mechanism underlying neurodegeneration move from clinicopathological data to connectome disruption, even suggesting that brain functional connectivity abnormalities might provide "in vivo" signature of molecular pathology.[12]

In a context of such great heterogeneity, the need for precise biological biomarkers is continuously growing and molecular imaging is playing a progressively leading role in the "in vivo" investigation of neurodegeneration. Indeed, positron emission tomography (PET) and single-photon emission computed tomography (SPECT) can visualize and measure the pathophysiological processes in the living brain using selective radioligands as imaging probes. PET and SPECT provide target-specific information that can identify distinct patterns related to neuropathological substrates and quantify the rates of the biological processes.

Aim

This concise review will discuss the potential of PET and SPECT imaging as a solid guide for improved detection of atypical phenotypes of neurodegenerative disorders in the clinical routine, including speech difficulties, visual abnormalities, executive, behavioral, and motor functions.

The accuracy of clinical diagnosis remains insufficient and highly dependent on the clinician's experience and level of expertise and the follow-up duration, despite many efforts of experts in determining detailed clinical criteria for a correct diagnosis.[13]

Material and methods

Literature search was performed focusing on the role of PET and SPECT imaging in assessing atypical phenotypes of neurodegeneration, using the electronic source of database PubMed/MEDLINE and the web-based search engines Google, Google Scholar.

The following search algorithm was employed: (A) "atypical phenotypes" AND (B) " Alzheimer's disease " OR "Parkinson' disease" OR "dementia with Lewy bodies" OR "multiple system atrophy" OR "parkinsonism" OR "primary progressive aphasia" OR "corticobasal syndrome/degeneration" OR "progressive supranuclear palsy" OR "posterior cortical atrophy " OR "frontotemporal dementia" OR " amyotrophic lateral sclerosis" AND (C) "PET" OR "SPECT" OR "molecular imaging" OR "DaTscan" OR "FP-CIT" OR "MIBG" OR "amyloid-PET" OR "tau-PET" AND (D) "autopsy validation" OR "neuropathological correlation". The authors did not apply any restriction concerning the publication date. Moreover, the authors screened the bibliography of the included studies searching for additional suitable articles to improve the research. The literature search was lastly updated on September 2023.

Analysis of the literature

PET and SPECT images as quantitative biomarkers of neurodegeneration

PET is the most used technique for the characterization of neurodegeneration profiles, being able to assess neuron glucose consumption, beta-amyloid (Aβ) deposition, and dopamine neurotransmission.[14-16]

Most recently, tracers targeting tau inclusions in the brain have been entering the diagnostic roadmap providing better comprehension of neurodegenerative processes as well as radioligands for neuroinflammation and microglial activation.[17-19]

Dopamine system imaging has become a standard approach in patients with symptoms of dopaminergic neurodegeneration with SPECT radioligands assessing presynaptic (e.g. dopamine synthesis and storage, transporter density) or postsynaptic terminals (i.e. D2 receptors availability).[20]

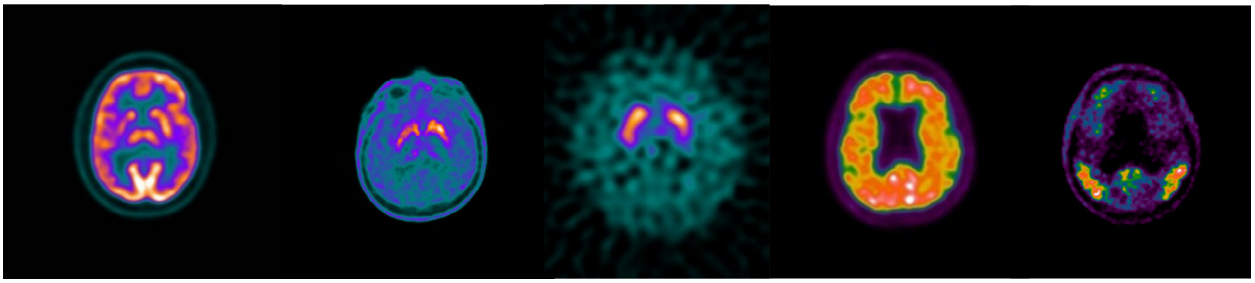


Fig. 1. Representative images of the most used molecular imaging techniques targeting the pathological substrates of neurodegeneration; from the left to the right: glucose consumption (PET with glucose analog [18F]FDG); presynaptic dopamine transporters function (SPECT with [123I]ioflupane also known as DaTscan); nigro-striate synthesis of dopamine (PET with [18F]DOPA); β-amyloid deposition (PET with [18F]flutemetamol); tau protein accumulation (PET with [18F]GTP1)

Highly specific imaging biomarkers and their multimodal combination (Fig. 1) increase diagnostic accuracy and may allow a better patient management, even more when additional symptoms, such as autonomic, pyramidal or cortical sensory disturbances, are present.[21,22]

The diagnosis of atypical variants with high sensitivity and specificity remains a challenge in the differential diagnosis of different neuropathologies. In the context of Alzheimer's disease it's critical to recognize patients with less common syndromes such as the logopenic variant of primary progressive aphasia (PPA) or corticobasal syndrome, because patients phenotypically similar have non-Alzheimer's pathology. On the other hand, the considerable overlap of signs and symptoms for parkinsonian syndromes makes clinical diagnosis challenging.

Extraction of quantifiable features from PET and SPECT images may provide a more precise selection of patients to be included in clinical trials for neurodegenerative diseases with a more aggressive course, as atypical parkinsonian syndromes, with the aim to enrich treatment trial eligibility for disease-specific therapies, such as anti-tau drugs for progressive supranuclear palsy (PSP) and corticobasal syndrome (CBS).

The imaging departments are even more frequently asked to assist neurologists and geriatricians in defining the underlying neuropathology of atypical symptoms in clinical and research settings.

Standardization of image acquisition and validation of the used radiotracers represent an ongoing critical challenge to obtain homogeneous and comparable data. The correlation of imaging measures with neuropathology may also improve the identification of at-risk patients and the detection of possible changes resulting from therapy. Therefore, relationships of PET and SPECT results with post-mortem measurements are critical for validating the sensitivity and specificity of imaging biomarkers across clinical phenotypes of neurodegeneration. In Table 1 autopsy validation studies of PET and SPECT imaging biomarkers are reported.[23-59]

To standardize imaging reporting, validated scoring systems have been implemented and visual assessment of PET images with amyloid tracers was the first step to stage amyloid deposition.[60,61] The regulatory authorities require a certified reader training specific for each radiotracer targeting β-amyloid.[60-62]

However, the need for a more precise analysis of regional tracer uptake, especially in the context of atypical patterns, pushed the development and clinical application of quantitative tools to assess biodistribution.

A commonly used approach is the region of interest (ROI)-based analysis with the standardized uptake value ratio (SUVR) calculation between the target regions and the reference region. Pons, whole cerebellum, cerebellar cortex, or cerebral white matter are used as reference regions as they are considered free from abnormal Aβ deposition.[63,64]

More recent methods include the Centiloid (CL) scale and the *z*-scores, both based on SUVR calculation, and magnetic resonance imaging (MRI)-independent indexes have been proposed for quantifying amyloid load across different tracers.[65-67]

Comparison of the subject's data with a database of healthy controls can be used to highlight areas with statistically significant alterations[68,69] and assessment of Z-scores defines the deviation of a sample with respect to the mean of a distribution. Thurfjell et al. demonstrated high concordance of amyloid imaging and an autopsy cohort using a threshold of z=2.0.[63] Z-scores may be calculated for composite cortical regions, individual regions, and at the voxel level obtaining maps due to the underlying statistical calculations (Z-maps) that improve pattern recognition accuracy and facilitate differential diagnosis.[69]

The growing use of quantitative evaluation of PET and SPECT scans in the clinical context increases the probability of reaching a conclusive diagnosis providing information on the extent and regional burden of the neuropathologic features.[70,71]

Objective data from quantification also enable an objective monitoring of the disease process and the biological mechanisms driving tracer accumulation.[64,72,73]

Finally, quantitative measures mainly support the nuclear medicine physician by increasing specificity and

Table 1. Autopsy validation studies of PET and SPECT imaging biomarkers of neurodegeneration*

Imaging biomarker	Tracer	Clinical spectrum	Reference list
Glucose consumption	[18F]FDG	AD/non-AD dementias	23
	[18F]FDG	AD vs FTD	24
	[18F]FDG	AD/non-AD dementias	25
	[18F]FDG	AD	26
	[18F]FDG	AD	27
	[18F]FDG	DLB vs AD	28
	[18F]FDG	DLB, AD, FTD	29
Aβ deposition	[18F]florbetapir	AD	30
	[18F]florbetapir	AD	31
	[18F]florbetaben	AD	32
	[18F]flutemetamol	AD	33
	[18F]flutemetamol	AD/non-AD dementias	34
	[18F]flutemetamol	AD	35
	[11C]-PIB	AD	36
	[11C]-PIB) vs [18F]FDG vs	AD/non-AD dementias	37
	[11C]-PIB	FTD	38
DAT binding	[123I]FP-CIT	DLB	39
	[123I]FP-CIT	DLB	40
	[123I]FP-CIT	DLB/AD	41
	[123I]FP-CIT + [1F]FDG	DLB	42
	[11C]Altropane + [11C]-PIB	DLB	43
	[123I]FP-CIT	CBD	44
	[123I]FP-CIT	DLB/other dementias	45
	[123I]FP-CIT	Parkinsonism (differential diagnosis)	46
	[123I]FP-CIT	MSA/PD	47
Postganglionic cardiac sympathetic denervation	[123I]MIBG	DLB	48
	[123I]MIBG	DLB	49
	[123I]MIBG	DLB	50
Tau accumulation	[18F]flortaucipir	AD	51
	[18F]flortaucipir + [18F] florbetapir	PPA	52
	[18F]flortaucipir	AD, CAA, PiD, PSP, CBD, FTLD-TDP-43, DLB, MSA, HC	53
	[18F]flortaucipir	AD/non-AD, primary tauopathies	54
	[18F]flortaucipir + 11C-PIB	FTD	55
	[18F]flortaucipir	AD/non-AD	56
	[18F]flortaucipir	AD/non-AD dementias	57
	[18F]flortaucipir	AD	58
	[18F]flortaucipir	AD	59

* AD – Alzheimer's disease, DLB – dementia with Lewy bodies, FTD – frontotemporal dementia, MSA – multiple system atrophy, PPA – primary progressive aphasia, PSP – progressive supranuclear palsy, CBD – corticobasal degeneration, PiD – Pick's disease, CAA – cerebral amyloid angiopathy, TDP-43 – frontotemporal lobe degeneration (FTLD)-transactive response DNA binding protein-43, HC – healthy control

diagnostic confidence in reading and interpreting brain scans.[74]

Development and validation of quantitative methods for brain molecular imaging is continuously ongoing even with the support of machine learning and deep learning algorithms.[75-79]

Clinical use of molecular imaging for atypical neurodegeneration

Precise discrimination of neurodegenerative diseases presenting with atypical phenotypes is still challenging in daily clinical practice, especially at the early stages of the disease, but accurate diagnosis is fundamental, because treatment and prognosis vary. Therefore, establishing imaging biomarkers is necessary for early detection and stratification of patients according to the underlying disease. A summary of distinct PET and SPECT imaging patterns of atypical phenotypes of neurodegeneration is reported in Table 2 and 3.[80-121]

The neurodegenerative cascade that accompanies amyloid deposition has been associated with multiple cerebral dysfunctions, mainly affecting executive, behavioral and motor abilities language and visual perception.

In a cause-and-effect relationship, it has been reported that amyloid burden precedes and induces metabolic changes, which could be highlighted by PET with the glucose analog [18F]Fluorodeoxyglucose (FDG) in the early stages of neurodegenerative diseases.[122] Moreover, a temporal ordering of amyloid β and tau lesions spread throughout the brain has been described in Alzheimer's disease, confirming that early accurate diagnosis may provide a window of opportunity for new treatments.[123]

In primary progressive aphasia amyloid-PET may help to predict the underlying neuropathology facilitating differential diagnosis of PPA subtypes, as in the case of the logopenic variant (lvPPA) most commonly associated with AD (Fig. 2a-b).

A recent systematic review of the literature has shown amyloid-PET positivity in 84.9% of lvPPA.[124] Interestingly, in the same study amyloid-PET showed positivity in 54.5% of unclassified PPA suggesting underlying Alzheimer's pathology.[124] On the other hand, PPA can remain isolated for years before the development of impairments in other domains suggesting neurodegeneration and, in these cases, classification of PPA variants may be challenging.

Compared to the amyloid-PET imaging, in which the site of deposition does not correlate with aphasic deficits in terms of topographic correspondence, uptake patterns of tau-PET differ across the PPA variants,[93] allowing differentiation of overlapping clinical profiles.[86]

Moreover, tau-PET burden provides a spatial relationship with cortical regional thickness, showing a greater engagement of the left hemisphere in the majority of patients due to the more common left-lateralized language networks.[87]

Clustering analysis of metabolic images from FDG-PET has been recently used to classify more PPA subtypes than the current recognized ones (non-fluent, semantic, and logopenic PPA) with distinct neuroimaging characteristics and more predictive of clinical course, splitting non-fluent variant into three subtypes, and lvPPA into two subtypes.[91]

Table 2. Summary of distinct patterns in PET imaging of atypical phenotypes of neurodegeneration*

Clinical spectrum	FDG PET	Amyloid PET	TAU PET
Typical AD	Posterior cingulate, precuneus and temporal-parietal associative cortex hypometabolism[80]	Diffuse tracer accumulation across the cerebral cortex, according to Thal's stages[81]	Intense tracer retention in the parietal lobes,(especially precuneus, and posterior cingulate) and mesial basal temporal structures[82]
Frontal AD	Greater medial and orbital frontal hypometabolism compare to typical AD[83]	Diffuse tracer accumulation across the cerebral cortex indistinguishable from typical AD[81]	Classic temporo-parietal tracer retention with potential involvement of frontal areas (>lateral)[84]
Logopenic PPA	Hypometabolism in left, middle. superior temporal areas with less involvement of right medial temporal area and posterior cingulate[85]	Diffuse tracer accumulation across in the cerebral cortex without a clear topographic correspondence[89]	Asymmetric, left greater than right temporoparietal language regions tracer retention[87]
PCA	Bilateral occipitoparietal hypometabolism[88]	Diffuse tracer accumulation across the cerebral cortex[88]	Parieto-occipital tracer retention with less retention compared to typical AD in the hippocampus[88]
bvFTD + ALS	Hypometabolism in frontal association cortex and anterior temporal lobe, usually asymmetric[89] Frontotemporal and insular cortex hypometabolism with the basal ganglia and the thalamus involvement[135]	Until 38% percent of positivity with different grading of binding, increasing with age of the patients. GRN mutation > C90RF72 expansion. Not reported for MAPT mutation[138]	Increased tracer retention in the temporal lobes, temporal white matter, and basal ganglia[90]
Nonfluent PPA	Two main subtypes of hypometabolism: -1: more confined to dominant lobe with involvement of superior temporal and inferior frontal gyri (more related to TDP-43 type A proteinopathy, can evolve to dementia) -2: more medial bilateral frontal lobe involvement (possible evolution to PSP)[91]	10% of positivity (similar or slightly lower than normal individuals)[92]	Accrual in white matter of the prefrontal lobe, including orbitofrontal, inferior, middle and superior regions, and temporal lobe, with greater uptake in the left hemisphere. Involvement even of subcortical grey matter structures, including bilateral thalamus, putamen and globus pallidus[93]
Semantic PPA	Hypometabolism in the whole left temporal lobe, right temporal pole, left thalamus[94]	15% of positivity (similar or slightly lower than normal individuals)[92]	Accrual mainly in temporal pole, inferior and middle temporal gyri, fusiform gyrus, amygdala, parahippocampal gyrus and entorhinal cortex, with left prevalence. TAU Positivity might also be present in TDP-43 proteinopathy (focal TAU or off-target binding?)[93]
CBS	Asimmetric hypometabolism in frontoparietal lobe and striatum[95] -In CBD pathology: > basal ganglia -In AD pathology: > lateral parietal, temporal lobe, posterior cingulate -in CBS-PSP pathology: > medial frontal regions and the anterior cingulate[96]	Percentage of AD pathology ranges from 13% to 24%[97]	Binding in precentral lobe, midbrain, putamen, globus pallidus, thalamus, corticospinal tract with asymmetric feature in CBS-CBD differently from CBS AD and CBS-PSP[95]
PSP	Hypometabolism in medial frontal cortex, striatum and brainstem[95]	Positivity until 40% in patients with clinical features suspicious for PSP[98]	Engagement of subthalamic areas, midbrain, and cerebellar white matter. Involvement of the neocortex in the advanced stages of the disease[95]
MSA	Hypometabolism in cerebellum, putamen and brainstem[95]	Not reported amyloid accrual	Not reported TAU binding, except for retention in posterior putamen perhaps related to interaction with iron deposition[93]
DLB	Hypometabolism in parieto-occipital cortex, temporal lobes, substantia nigra and thalamus. Compared to AD, preservation of medial temporal areas and posterior cingulate metabolism ("cingulate island sign")[95]	High Aβ values are observed until 60% of the DLB patients, often reflecting mixed pathology. The amount of B-amyloid uptake is lower in "pure" AD cases compared to the patients affected by an AD/DLB pathology[99]	In "pure" DLB not differences of accrual compared to controls[100]

* AD – Alzheimer's disease, PPA – primary progressive aphasia, PCA – posterior cortical atrophy, FTD – frontotemporal dementia, ALS – amyotrophic lateral sclerosis, CBS – corticobasal syndrome, PSP – progressive supranuclear palsy, MSA – multiple-system atrophy, DLB – dementia with Lewy bodies

Speech difficulties may also represent an early marker of motor abnormalities in Parkinson's disease (PD).[125]

Recent data have shown that uptake of [123I]FP-CIT (DaTscan), a radioligand with high binding affinity for presynaptic dopamine transporters (DATs), is lower in the striatum ($p<0.001$), caudate ($p=0.003$) and putamen ($p=0.003$) in Parkinson's disease patients with speech difficulties than in patients without speech abnormalities.[126]

Figure 3 shows the DAT-SPECT of a subject with speech abnormalities, akinetic phenotype and autonomic dysfunction.

A challenging common presentation in the spectrum of Lewy body disorders including PD and DLB is autonomic dysfunction. In patients with PD autonomic dysfunction is associated with a more rapid disease progression and shorter survival and may include orthostatic hypotension, bladder disturbances, gastrointestinal malfunction, cardiovascular dysregulation and sexual dysfunction.[127]

Severe cardiac sympathetic degeneration occurs in DLB, but not AD, offering a potential target for molecular imaging. Scintigraphy with [123I]meta-iodobenzylguanidine (MIBG), an analogue of norepinephrine that

Table 3. Critical outcomes of SPECT imaging biomarkers in differentiating clinical presentations of neurodegeneration*

Clinical spectrum	[123I]FP-CIT (DAT imaging)	[123I]MIBG (sympathetic innervation)	[123I]FP-CIT + [123I]MIBG (combined imaging)
PD	Differential diagnosis from Parkinsonism with authopsy validation[46] Differentiation of PD and MSA[47]	Differentiating PD from other neurodegenerative parkinsonism[50,112] Differentiation of PD and MSA[118]	Differentiating PD from other neurodegenerative parkinsonian syndromes[121]
DLB	Specificity of 90.4% for excluding non-DLB dementia (101) Loss of striatal DAT binding more intense in the putamen than in the caudate (43,101) Class I evidence that [123I]-FP-CIT accurately identifies patients with DLB (40,102,103) Authopsy validation[39,40,41,43,46]	High sensitivity and specificity of MIBG myocardial scintigraphy for differentiating PD from other neurodegenerative parkinsonism in both early and delayed imaging phases[112] Class II evidence that reduced cardiac uptake of 123I-MIBG accurately identifies patients with DLB and cardiac sympathetic denervation[113] Early and delayed H/M ratio strongly correlate with residual cardiac sympathetic nerve fibers[114,115] 3-year follow-up of 133 patients confirms high correlation between abnormal cardiac MIBG and clnical diagnosis of DLB with early and delayed H/M ratio 2.51 and 2.20[116,117]	Sensitivity and specificity of combined techniques in differentiating DLB from AD 96.1 and 90.7 %, respectively[120,121]
MSA	Severe decrease DAT binding and higher asymmetry in MSA-P than in MSA-C[104-107] Higher striatal uptake in MSA-C variant (probably due to predominant degeneration of ponto-cerebellar rather than nigrostriatal pathways)[106,108]	MIBG scintigraphy distinguish between PD and MSA, and between AD and DLB (H/M ratio 1.77 with 94% sensitivity and 91% specificity)[116] Most MSA patients show a normal myocardial MIBG uptake (118) MSA-P patients show a mild cardiac sympathetic dysfunction without any correlation to disease duration[119]	
PSP	More intense decreased DAT binding compared to PD and MSA-P in both caudate and putamen (higher putamen/caudate ratio)[104,105,109,110]		
CBS	Mild-to-moderate reduction of striatal presynaptic dopamine uptake with greater uptake asymmetry compared to PD[111,44,110]		

* PD – Parkinson disease, DLB – dementia with Lewy bodies, MSA – multiple-system atrophy, MSA-P – MSA with predominantly parkinsonian signs, MSA-C – MSA with cerebellar features, PSP – progressive supranuclear palsy, CBS – corticobasal syndrome, DAT – dopamine transporte, [123I]FP-CIT – [123I]*N*-ω-fluoropropyl-2β-carbomethoxy-3β-(4-iodophenyl)nortropane), [123I]MIBG – [123I]metaiodobenzylguanidine

assesses the post-ganglion peripheral autonomic nervous system, has been used as a non-invasive method to assess myocardial sympathetic nerve damage. The DLB Consortium consensus report recommends MIBG scanning as a biomarker of DLB.[128] Calculation of MIBG uptake using the heart-to-mediastinum (H/M) ratio provides a semiquantitative diagnostic index for distinguishing DLB from AD with high specificityand an autopsy study has validated the diagnostic accuracy of MIBG cardiac scintigraphy for DLB revealing that residual cardiac sympathetic fibers strongly correlate with H/M ratios.[114,116,129] A recent paper has provided a Class I evidence that cardiac MIBG scintigraphy using the H/M indicator may also distinguish mild cognitive impairment with Lewy bodies from mild cognitive impairment due to AD.[130]

In Figure 4, the assessment of cardiac autonomic innervation with MIBG-SPECT in subjects with DLB and AD is represented.

Low MIBG uptake associated with autonomic dysfunction (mild memory disorder, constipation/postural hypotension, depression/anxiety, visual hallucination/psychosis, REM sleep disorder) may detect PD very early in the pre-motor phase according to the multiple Braak stages on the pathological accrual of α-Synuclein.[131,132]

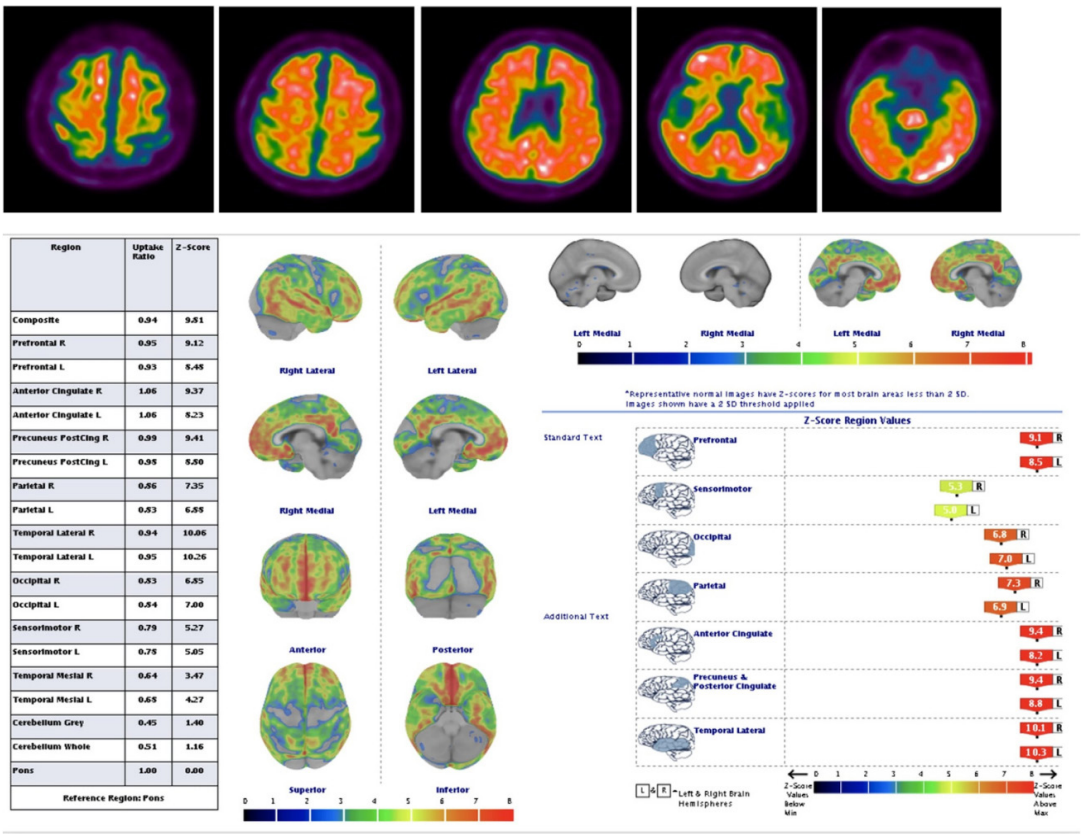
Accumulating evidence shows that Lewy body disorders affect central and peripheral autonomic nervous systems requiring the combination of both [123I]FP-CIT and MIBG imaging to provide early and accurate diagnosis and appropriate treatment.[120,121]

An additional topic in the diagnostic work-up of patients with DLB is distinguishing them from those with AD or mixed pathology. The role of amyloid-PET imaging in this clinical context is established and lower amyloid tracer uptake accurately distinguishes cases with DLB.[96] However, high Aβ values are observed in up to 60% of the DLB patients, often reflecting mixed pathology. Interestingly, the amount of β-amyloid uptake is lower in "pure" AD cases compared to the patients affected by an AD/DLB pathology, with lesser involvement of the occipital regions in the former.[133]

Challenging fields are continuously emerging in the world of neurodegeneration with a high need for reliable imaging biomarkers supporting clinical features to reach a correct diagnosis and prognostic assessment (Table 2, 3), especially for disorders with more aggressive courses as atypical parkinsonism and amyotrophic lateral sclerosis (ALS). PET and SPECT imaging are increasingly used in these settings with earlier onset, faster progression, and poor response to treatment, aiming to resolve the initial diagnostic uncertainty.

In Multiple System Atrophy (MSA) FDG-PET shows a reduced metabolism in the cerebellum,

A



B

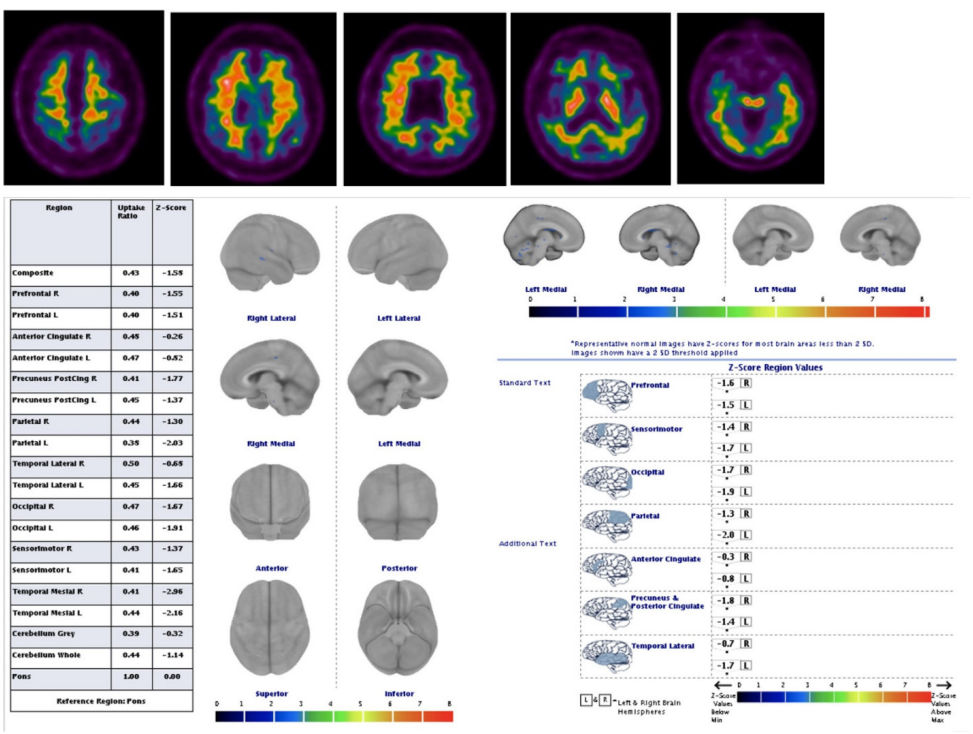


Fig. 2. Amyloid-PET with [18F]flutemetamol in subjects with primary progressive aphasia (PPA) – A): A case of semantic variant of PPA with negative amyloid-PET: representative axial sections show low retention of the tracer in the cortical grey matter confirmed by the Z-score images obtained from quantitative analysis, B): A case of logopenic PPA with positive amyloid-PET suggesting underlying Alzheimer's pathology: representative axial sections show diffuse increased retention of [18F]flutemetamol in the cortical gray matter and Z-score images show all pixels with a deviation above the mean of the normal controls in number of standard deviations (cut-off value +2 SD); quantitative analysis was performed using CortexID Suite, GE Healthcare®, pons as reference region

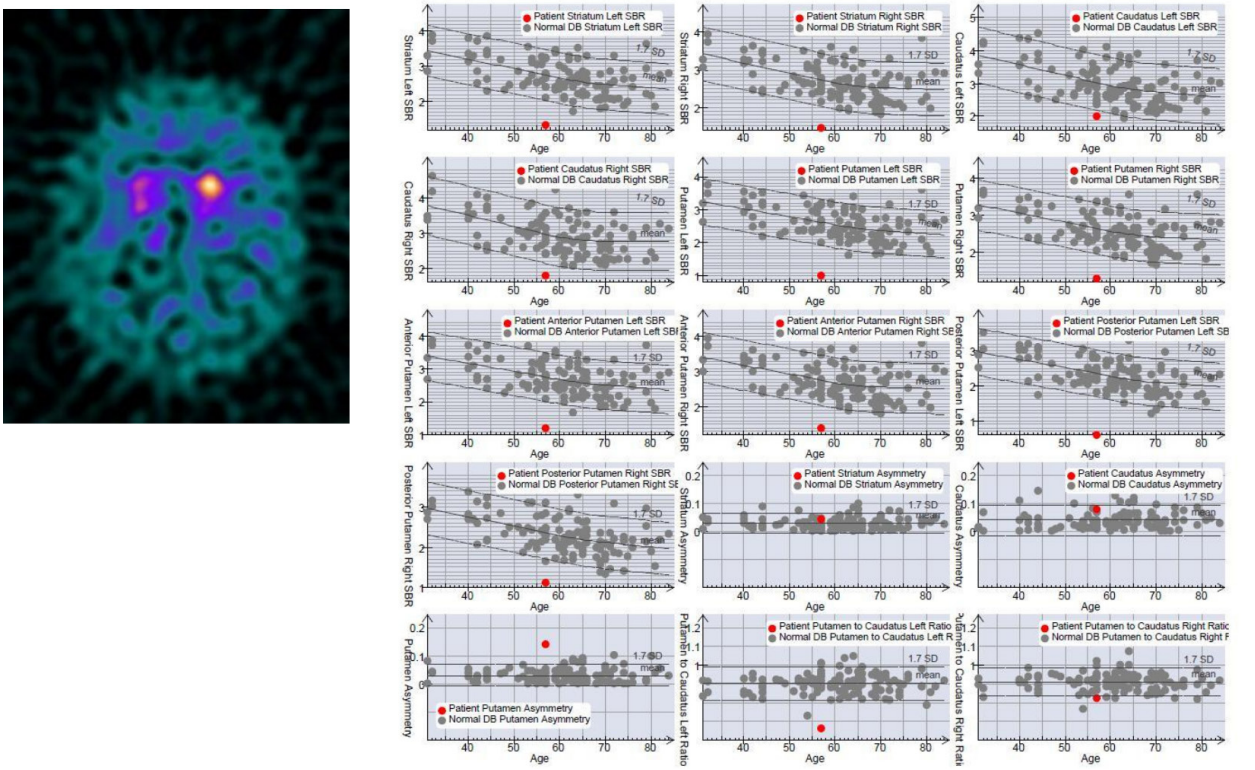


Fig. 3. Visual and quantitative analyses of DaTscan SPECT in a patient with Parkinson disease associated with speech abnormalities, akinetic phenotype and autonomic dysfunction, axial section shows reduced [123I]FP-CIT uptake in the right caudate and in both the putamen, quantitative assessment of tracer uptake (DaTQUANT software, GE Healthcare®) confirms the visual findings, but also indicates reduced uptake in the left caudate compared to normal controls

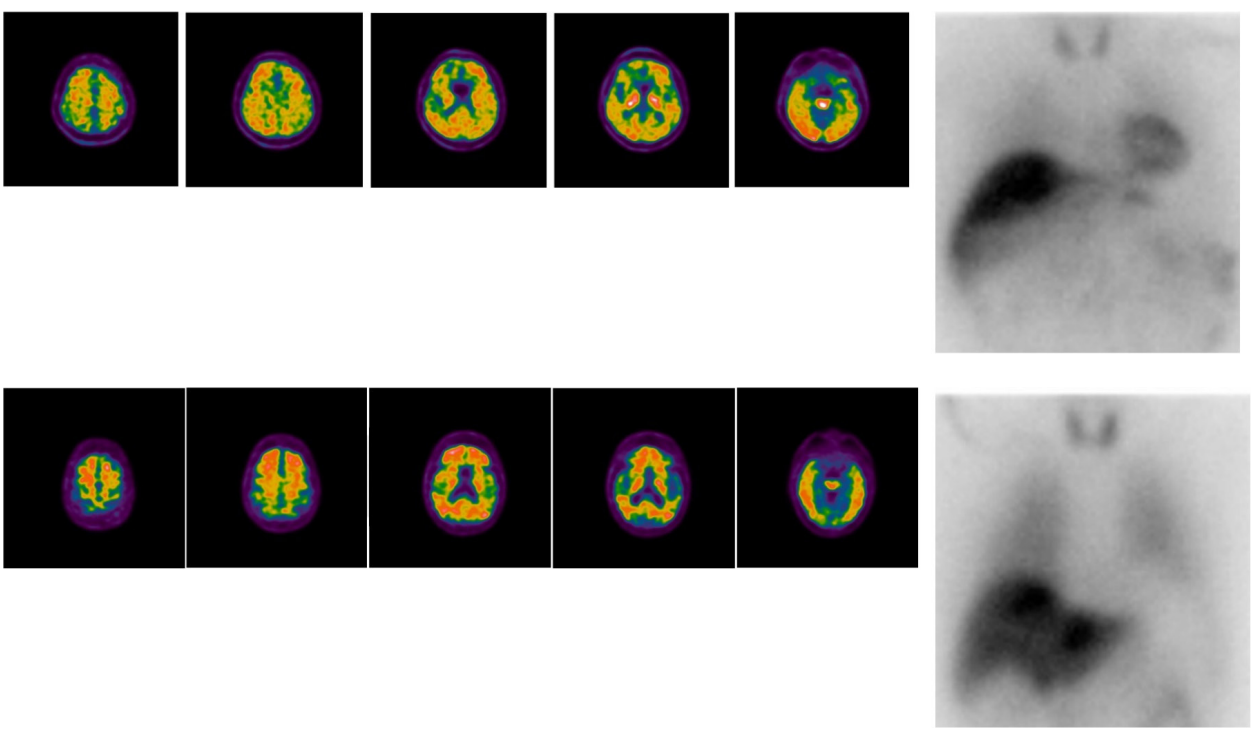


Fig. 4. Assessment of autonomic dysfunction in the differential diagnosis of DLB from Alzheimer's disease. Amyloid deposition, evaluated with 18F-Flutemetamol PET, is increased in both cases with a global z-score +5.30 SD in the AD subject (upper axial PET images) and +5.16 SD in the DLB case (lower axial PET images), cardiac MIBG uptake is intense in the AD case (upper static image) with H/M uptake ratio 2.4, obtained 15–20 min after tracer administration, while in the DLB case (lower static image) MIBG uptake is dramatically reduced with H/M uptake ratio 1.2, reflecting the myocardial sympathetic nerve damage, quantitative software for amyloid-PET analysis: CortexID Suite, GE Healthcare®, cut-off value +2 SD, pons as reference region

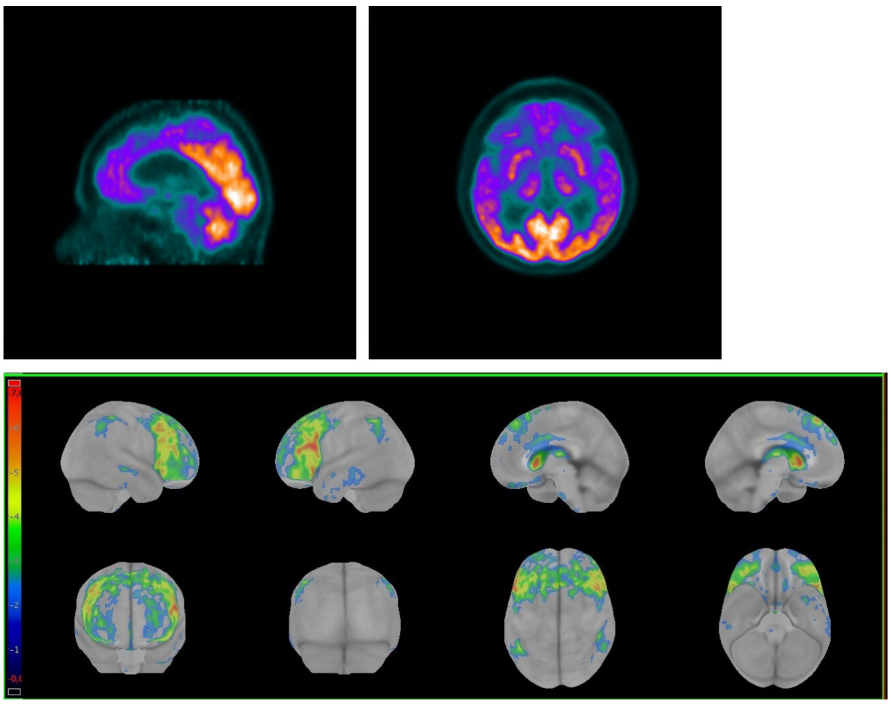


Fig. 5. Metabolic pattern of amyotrophic lateral sclerosis on FDG-PET. Representative sagittal and axial PET images (upper pictures) show hypometabolism in the prefrontal and premotor cortex associated with symmetric relative hypermetabolism in the occipital cortical pole and in the cerebellum, z-score images (lower picture) confirm significant hypometabolism in the prefrontal and premotor cortex showing all pixels with a deviation below the mean of the normal controls in number of standard deviations (cut-off value -2 SD), quantitative software: CortexID Suite GE Healthcare®, pons as reference region

putamen, and brainstem regions compared to PSP, in which the medial frontal cortex, prefrontal areas, striatum, and brainstem are preferentially involved.[95,133] In Corticobasal Syndrome, the critical element is the asymmetric decrease of brain metabolism, engaging mainly the frontoparietal lobe and striatum.[95,96] In PSP patients, the tau-PET may show the engagement of the subthalamic areas, midbrain, and cerebellar white matter with the further involvement of the neocortex in the advanced stages of the disease.[95] Moreover, FDG-PET may be used as a gatekeeper method to select patients candidates to the second level or more expensive imaging as tau-PET.[133]

Recent studies have also shown the diagnostic value of FDG-PET in identifying ALS from controls with most discriminating hypometabolism in the prefrontal and premotor cortex and relative hypermetabolism in the occipital cortex, cerebellum, upper brain stem, and medial temporal cortex).[135-138] Metabolic pattern of ALS on FDG-PET imaging is presented in Figure 5.

A higher mortality rate has been revealed in the presence of extensive frontotemporal hypometabolism.[136,137] A precise definition of neurodegeneration pathophysiology could shorten the period from symptom onset to diagnosis and allow earlier interventions.

An additional source of diagnostic uncertainty that patients with neurodegenerative disease frequently experience are visual alterations and neuropsychiatric symptoms (NPS) that may be mistaken for a psychiatric disorder.

Visual symptoms can present as independent and early signs of neurodegenerative disease and they may determine a challenge in the patient's life including repeated appointments with eye specialists, eventual unnecessary surgeries (e.g., cataract removal) and diagnosis delay.

A recent survey among neuro-ophthalmologists demonstrated that at least 5–10% of new patients referred to them had a previous diagnosis of a neurodegenerative disease. For new patients without a diagnosis of neurodegeneration, visual complaints were attributed to undiagnosed neurodegenerative disease in more than 5% of cases.[139]

Interestingly, 40% of the interviewed neuro-ophthalmologists indicated the lack of tools to assess visual dysfunction due to neurodegeneration as a barrier to a specific diagnosis.[139]

In these cases the quantitative assessment of glucose metabolism, amyloid deposition or tau accumulation may guide accurate diagnosis and patient management providing information about regional, particularly occipital, involvement in FDG, amyloid- or tau-PET.

Recently, dysfunction of visual contrast sensitivity has been strongly associated with cerebral deposition of amyloid plaque and tau protein, primarily in temporal, parietal and occipital brain regions.[140] In Figure 6, am-

Fig. 6. Alterations in visual contrast sensitivity and cerebral deposition of β amyloid, representative left sagittal images of amyloid-PET with 18F-flutemetamol in two subjects with dysfunction of visual contrast sensitivity; in one case (left picture), Aβ accumulation was detected by PET in the occipital lobes (right z-score +7.61 SD, left +9.76 SD) suggesting Alzheimer's disease-related pathophysiology; in the second case (right picture), amyloid-PET showed a normal tracer retention in the occipital lobes (right z-score +1.70 SD, left +1.81 SD), quantitative software: CortexID Suite GE Healthcare®, z-score cut-off value +2 SD, pons as reference region

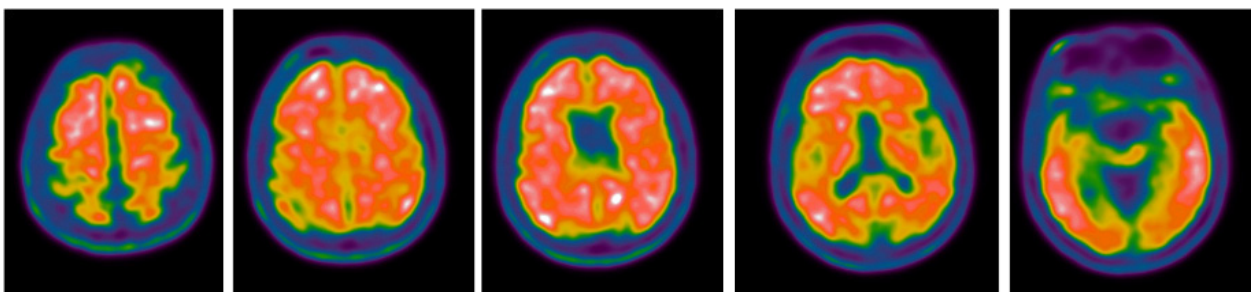


Fig. 7. Neuropsychiatric symptoms associated with elevated Aβ deposition and cognitive decline as early markers of Alzheimer's disease, representative axial sections of amyloid-PET with [18F]florbetaben in a patient with anxiety, depression and cognitive impairment show diffuse elevated Aβ deposition in the examined cortical regions, visual assessment of brain amyloid-β plaque load (BAPL) was graded as score 3, according to the method described in Barthel et al.[57]

yloid-PET images are presented in subjects with visual contrast sensitivity alterations.

In posterior cortical atrophy (PCA), the paradigmatic neurodegenerative disease impairing visuospatial perceptions and mainly due to Alzheimer's disease pathology, FDG-PET hypometabolism in occipital regions correlates with a highly regional concordance with hyper-fosforilated tau accrual. In contrast, the amyloid burden is more diffuse along the neocortex, although a possible link between amyloid deposition in the primary visual cortex and the onset of visuospatial impairment has been suggested.[88,141]

Furthermore, the phenotypic PCA heterogeneity can be disentangled, through the assessment of glucose metabolism, highlighting the primary involvement of either the right or left hemisphere and the ventral or dorsal visual streams.[142]

The presence of NPS is an independent risk factor for cognitive impairment, faster decline and poorer outcomes in functional status and quality of life.[143,144] Among NPS, delusions and delirium are the most associated with worse cognitive and functional outcomes.[145] Neuropsychiatric symptoms and cognitive decline are both signs of similar brain pathologies; thus it is crucial to investigate the underlying pathway linking NPS to neurodegeneration.

Assessment of amyloid deposition with PET might help in selected cases of NPS with slight cognitive deficits. In cases of major depression with episodes of transient amnesia, a normal amyloid-PET might contribute to confirming a psychiatric disorder, especially when the clinical history is suggestive of depression, but neuropsychological assessment has shown some cognitive deficits.

Recently, great attention has been paid to the relationship between depressive symptoms and neuroimaging biomarkers, such as glucose metabolism or amyloid deposition, that appear distinctly related.[146]

Touron et al. have shown that preclinical depressive symptoms are associated with glucose hypometabolism in the brain areas particularly susceptible to AD, such as the hippocampus, amygdala, the precuneus/posterior cingulate cortex, the medial and dorsolateral prefrontal cortex, insula, and the temporoparietal cortex, and in-

dependent of amyloid-PET results as shown in the previous studies.[147-149]

Older adults with cognitive impairment are at risk of having or developing NPS and even slight levels of depressive symptoms are associated with the increased risk of cognitive decline.[150,151]

The Mayo Clinic Study of Aging has demonstrated that subjects with regional glucose hypometabolism (measured with FDG-PET) and depression (Beck Depression Inventory-II ≥13) had a more than threefold increased risk of incident MCI.[152]

The risk was also significantly elevated for participants with anxiety (Beck Anxiety Inventory ≥10) and glucose hypometabolism.

On the other side, a recent systematic review has shown that NPS, particularly depressive and anxiety symptoms, are associated with higher Aβ deposition, as presented in Figure 7.[153]

Longitudinal studies have shown that baseline Aβ deposition and NPS have a synergistic interaction in the very early stages of AD[154]: greater baseline cortical amyloid and increased depressive symptoms are associated with more significant cognitive decline over time.[155,156]

No association was revealed between NPS and tau pathology.[153]

A small percentage of people with dementia also experiment with early behavioral changes – such as a disregard for social norms or loss of empathy – that can lead to a mistaken diagnosis (i.e. behavioral variant of frontotemporal dementia instead of a variant of AD), also due to the lack of standardized clinical criteria for this AD phenotype.[157] Assessment of brain glucose consumption with FDG-PET may identify the metabolic pattern of the behavioral subtype of AD (bvAD).

Hypometabolism in the frontal regions distinguishes frontal variant (bvAD) from typical AD, while it largely matches typical AD in the posterior cingulate cortex, precuneus, and lateral temporoparietal regions.[158]

The frontal-hypometabolism pattern in bv-AD can be highly comparable to behavioral frontotemporal dementia (bv-FTD), leading to a significant risk of misdiagnosis considering the clinical features' resemblance. To obtain the correct diagnosis, the amyloid-PET takes a lead role for its high negative predictive values since the absence of pathological β-amyloid rules out AD diagnosis with great sensibility and specificity.[159] A large series of pathologically confirmed FTD reported Aβ deposition in 38% of patients with bvFTD and increased progression with age, suggesting a role for amyloid imaging in clinical assessments of FTD syndromes.[81]

In a high percentage of cases, estimated at around 12.5%, an overlap between FTD and motoneuronal disease occurs, and a more significant number of FTD patients also show subtle motor system involvement.[38,160] Recently, in such overlap conditions, an increase in glucose metabolism has been observed along the brainstem with a shorter survival if this occurs in the medulla oblongata.[161]

Mild behavioral impairment (MBI) is emerging as a novel marker of preclinical Alzheimer's disease.[162] Higher β-amyloid tracer uptake resulted strongly associated withMBI in normal elderly individuals, specifically in the neocortex, including the frontal cortex, followed by the striatum, according to the sequential stages of hierarchical amyloidosis in AD.[163,164]

No significant associations have been demonstrated with tau-PET uptake, suggesting that in cognitively normal elderly MBI is not associated with tau-PET signal, according to the observation that considerable tau aggregation is rarely observed in cognitively normal older individuals, but it is present in dementia due to AD.[163-165]

At different stages of neurodegenerative disorders, motor symptoms may be present, including bradykinesia, extrapyramidal rigidity or spasticity. Less severe motor disorders such as gait slowing may occur at an early stage of dementia and alterations in dual-task performance (walking while simultaneously performing another task) is often present in elderly people with MCI.[16,166]

Motor and cognitive disorders may coexist in PD, ALS, PSP or CBS, and motor impairments are often associated with worse cognitive decline. Data from the DEMPARK/LANDSCAPE study have demonstrated that less severe cognitive deficits are present in tremor-dominant PD rather than in the akinetic variant.[168]

In genetic FTD motor severity appears strictly related to time course and the affected gene.[169]

Multimodal molecular imaging may improve diagnostic accuracy in the motor-cognitive phenotype setting, detecting the disease's key neuropathological correlates. Tau imaging can detect tau aggregation in PSP and corticobasal degeneration (95) as well as DAT imaging is the most accurate marker for PD.[170] Cardiac adrenergic imaging with MIBG-SPECT may provide the diagnosis of pre-motor PD in patients presenting with mild memory impairments and other non-specific symptoms as autonomic dysfunction, sleep disorder, depression or anxiety, visual hallucinations.[112]

A systematic meta-analysis including 74 studies, 2323 patients with PD and 1767 healthy controls, has shown glucose hypometabolism on FDG-PET in the bilateral inferior parietal cortex and left caudate nucleus, respectively related to cognitive deficits (inferior parietal cortex) and motor symptoms (caudate nucleus).[171] In the same study, FDG-PET hypometabolism outperformed results of structural MRI in identifying functional brain abnormalities in PD.

Actual challenges for molecular imaging of neurodegeneration

Pattern overlaps

Throughout recent years, pattern overlaps between ND's phenotypes have gained increasing attention. A recent study on 895 autopsy cases from patients with neurodegenerative disease measured regional aggregation of β-amyloid, tau, α-synuclein, and TDP-43.[172] Authors identified 6 disease clusters reflecting primary tauopathies, AD typical coexistence of amyloid-β and tau pathology, TDP-43 proteinopathies, synucleinopathies, tau-α-synuclein copathology, and minimal cerebral pathology.

The same proteins can represent risk factors for different NDs implying an overlap between them at a subcellular level. On the other side, the cells and the fold of protein aggregates involved in the disease process can also overlap between multiple diseases.

Molecular neuroimaging allows us to detect and monitor *in vivo* the ND hallmarks, their anatomical distribution and the interrelationships of the underlying molecular and cellular processes, disentangling pattern overlaps of neurodegeneration.

Co-pathologies suggest that NDs might share common pathogenic pathways as shown by the genome-wide association studies (GWASs).[173,174] Genetic overlap between neurodegenerative diseases is more frequently studied in pairwise investigations, and more recently across multiple neurodegenerative disorders.[174-176]

The case of tau-directed imaging

The composition of tau aggregates and their geometric arrangements may vary in disease subtypes, while it is constant in patients with the same disease.[177-180,10] Tauopathy is classified by the type of tau isoforms present in the neurofibrillary tangles, differing in the number of carboxy-terminal repeating domains (3R or 4R).[181] While AD is associated with both 3R and 4R forms, 4R tau is abundant in CBD and PSP, 3R in Pick's disease and three subtypes (3R, 4R or 3R/4R) are present in FTD.[182,181,177]

In addition to tau isoforms, distinctive folds in the tau fibrils characterize AD (paired helical filaments » straight filaments) and non-AD tauopathies as PSP (straight filaments; rare twisted filament), CBD and Pick's disease (straight filaments >> twisted filament).[183]

Therefore, awareness of these differences is relevant in molecular imaging with tau-directed radioligands.

Among the first-generation of tau PET radiotracers, the pyridoindole derivative[18F]-flortaucipir was the first radioligand approved for clinical use by the FDA on May 2020.[184]

However, off-target binding to white matter or other neural structures (i.e. in the striatum and choroid plexus) and low affinity for tau fibrils in non-AD tauopathies such as PSP and CBD,[185] pushed the development of second-generation tau PET tracers, including [18F]-RO-948, [18F]-MK-6240, [18F]-PI-2620, [18F]-JNJ-311, [18F]PM-PBB3, and [18F]-GTP1.[186]

A post-mortem radioligand binding study on second-generation tau PET tracers PI2620, MK6240 and RO948 in AD, CBD and PSP has shown different binding properties of the different tracers, suggesting the potential for development of pure selective 4R tau PET tracers.[187]

A recent in-depth analysis of the binding mechanism across 10 first- and second-generations PET tracers using multiple approaches (i.e. molecular dynamics, docking, and metadynamics simulations) has demonstrated that MK6240 binds better to tau aggregates in AD than in CBD and PSP, and that CBD2115, PI2620, and PMPBB3 represent 4R tau binders.[188]

Fluid and imaging biomarkers for neurodegeneration

Currently, several fluid biomarkers including beta-amyloid, tau protein, neurofilament light chain, alpha-synuclein and glial fibrillary protein, can differentiate different neurodegenerative diseases. The best-validated fluid biomarkers derive from CSF, but blood-based tests may are improving in accuracy and predictive value especially for the ratio of amyloid-beta 42/40 (Aβ 42/40), pTau, and NfL.[189-191] Serum biomarkers may enable much broader accessibility of testing, in light of lower costs and less invasive collection. However, as of yet no single biomarker allows for definitive diagnoses.[192] Integrating information from imaging and fluid biomarkers in a "composite tool" may increase sensitivity and specificity of diagnosis, especially in screening at-risk subjects.[193] Considering that false positive results are expected using blood tests for AD in general population, a positive result is likely to require a definitive confirmation through PET imaging able to detect region specific findings differentiating similar disease phenotypes in a non-invasive fashion. Moreover, in prodromal disease stages, molecular imaging may allow to assess not only the presence but also the location and the stage of the pathological and therapeutic target. In the case of tau imaging, the regional PET signal may allow to identify the different tauopathies.

Finally, a unique advantage of molecular imaging is the quantitative capability which allows to estimate the specific disease hallmark that may be monitored during treatment intervention also in the early phases.

When conducting molecular imaging in atypical phenotypes

Suggesting a standardized sequence for the utilization of PET and SPECT techniques in the context of atypical neurodegenerative disorders is a complex task.

We propose that within the spectrum of disorders potentially linked to AD-related pathology (such as pri-

mary progressive aphasias, behavioral disorders, and posterior cortical atrophy, as discussed in our review), the assessment with PET-amyloid should be consistently performed following the initial diagnostic workup, including clinical and neuropsychological evaluation, and structural imaging. The PET analysis enables a precise diagnosis, ensuring access to clinical trials and specific pharmacological treatments. Moreover, recent evidence suggests that early acquisition-phase PET-amyloid acquisition resembles the corresponding FDG-PET images, allowing the assessment of neuropathology and brain metabolism in a single PET scan.[194] However, it is important to consider the potential decrease in specificity of the PET-amyloid for individuals above 75 years and the presence of amyloid pathology as incidental or co-pathology.[195]

In this regard, FDG-PET remains a key tool in differentiating neurodegenerative dementias from psychiatric disorders and maintains a relevant role in the early stages of neurodegenerative disorders, especially in conditions where atrophy is not yet significant and cannot be detected by conventional neuroimaging methods.[196,197]

The anticipated widespread integration of quantitative analyses in routine clinical practice shortly (i.e., DaTscan SPECT and tau PET) will enable the assessment of prognosis for various pathologies at the time of diagnosis. This consideration is crucial, given that the high costs associated with these methods do not allow for their repeated application throughout the progression of the pathology on a large scale.

Conclusion

This concise review summarizes the current use and potential role of Molecular Imaging techniques such as PET and SPECT in discriminating atypical phenotypes of neurodegeneration and it may represent a quick guide to choosing the best imaging method in this heterogeneous clinical setting.

PET and SPECT radioligands targeting the key neuropathological substrate of neurodegenerative disorders could anticipate the time for a correct diagnosis when atypical symptoms or signs may be confounding. This issue is crucial in working persons and younger subjects with an early-onset of the disease, especially if they have the chance for the effect of new modifying-disease drugs. Delaying disease progression and symptoms by even a few years can highly impact the quality of life of patients, as well as their families and caregivers.

The application of new treatments requires patient screening in the prodromal phase to provide neuropathological target detection, such as cerebral Aβ deposition, tau inclusions, or α-synuclein accumulation, and to monitor treatment effects especially at the subclinical level. On the other hand, precise staging and diagnosis of neurodegenerative diseases may assist patient care and management in daily clinical practice. Moreover, the utilization of objective imaging techniques providing an "in vivo" quantitative assessment of specific disease targets, provides an accurate diagnosis in older individuals where the coexistence of cerebral age-related changes, cerebrovascular lesions, depression and neurodegenerative diseases may increase the complexity of the diagnostic process. In this heterogeneous context, a multilevel approach is needed and a strong cooperation between primary care physicians and specialized centers for personalized patient care is needed.

One potential limitation of our review is the need for more detailed technical description of the radioligands used for PET and SPECT imaging, but it was outside of our goal as well as an in-depth analysis of quantitave methods to process acquired images.

An additional limitation could be the narrative approach of this review. However, our purpose was to deepen the understanding in the research area of molecular imaging of atypical presentation of neurodegenerative disorders focusing on existing debates, previous studies conducted on the topic, and latest applications available, summarizing their results so that they are easily translatable into clinical practice.

A big effort should be made in the future to provide an "imaging continuum" able to assess and integrate all the aspects of neurodegeneration, from pathology substrates to functional connectivity, facing the challenge to stratify patients for an appropriate allocation of new arriving treatments.

Radioligand landscape will be probably enriched by tracers of neuroinflammation and synaptic density, while the diffusion of hybrid PET/MRI scanners, as well as advanced imaging protocols could install a precision medicine approach for a comprehensive workup of neurodegenerative disorders with atypical presentation.

Declarations

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Author contributions

Conceptualization, L.R. and M.S.; Methodology, L.R. and A.Z.; Software, F.M.; Validation, M.S., M.S., F.L.; M.M.; Formal Analysis, M.S., S.M.; Investigation, V.C.; Data Curation, S.M., F.M; Writing – Original Draft Preparation, L.R. And A.Z.; Writing – Review & Editing, L.R., M.S.; Visualization, G.B. T.G. C.C..; Supervision, L.R. and M.S.

Conflicts of interest

No conflicts of interest to disclose.

Data availability

No new data were created or analyzed in this study. Data sharing is not applicable to this article.

Ethics approval

The review figures are derived from scans of patients included in the following protocols approved by the Ethical Committee of our institution (345/2019/OSS*/AOUPR and 666/2021/FARM/AOUPR).

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REVIEW PAPER

Prolonged screen-time as the cause of ocular disorders: what can we do with the problem? – a review

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ABSTRACT

Introduction and aim. Recently, an increased use of video display terminals has been observed in workplace environments, as a result of the evolution of communication technologies and new information-sharing strategies. It has led to an increased prevalence of computer-related ocular disorders, such as computer vision syndrome, dry eye disease, refractive errors and convergence insufficiency. In this review we describe problems associated with these disorders and propose preventive methods.
Material and methods. With the use of specific keywords, the databases of the PubMed, Science Direct, and Google Scholar were searched for relevant original papers.
Analysis of the literature. The listed disorders might have similar symptoms, such as eye burning, itching, blurred vision, and tearing, and their severity correlates with the time of exposure to video display units. However, there are preventive measures, which can help in decreasing the negative effects of computers on our vision, such as adequate viewing distance, proper workspace lighting, eyeglasses with anti-glare coating, taking 5-minutes breaks after every 30 minutes, or following the 20-20-20 rule.
Conclusion. Prolonged usage of the video display terminals is connected to many ocular disorders, and in today's world, it is very important to remember actions that can be undertaken to minimize the risk.
Keywords. computer vision syndrome, convergence insufficiency, eye strain, prolonged screen time, refractive errors, video display terminals

Introduction

Recently, an increased use of video display terminals (VDTs) has been observed in workplace environments as a result of the evolution of communication technologies and new information-sharing strategies.[1] According to the Sixth European Working Conditions Survey, more than half of the citizens of Europe use VDTs at work. It is mostly used in financial services and public administration, but the number of workers using VDTs in other sectors, e.g. health, construction, or agriculture, is still rising.[2] Nowadays, it is required to use smartphones, computers, tablet computers as well as electronic book readers in any location, not only at home or in the workplace.[3] Especially during the SARS-CoV-2 pandemic, many employees had been forced to start teleworking.[4] The actual time spent using electronic devices during the lockdown

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increased by 3.1±2.2 h/d or even 4.8 ± 2.8 h/d.[5,5] In addition, more than 60% of people reported a prolongation of screen time due to work or leisure.[6] Computer usage for relaxation is likely to occur during nighttime and thus can be associated with sleep problems.[7] Using social media is a major activity in the current world for emerging adults – over half of young adults spend 61-70 min/day on social networking sites and access social media multiple times a day.[9,8] Moreover, the pandemic has imposed other lifestyle changes, e.g., increased sedentary time and decreased physical activity.[9] Dietary problems and reduced exposure to sunlight can also be observed, predisposing to vitamin deficiency.[10] All of the listed changes in behavior, especially the prolonged screen time, are associated with visual and ocular discomfort.[11] The most common transformations connected to the increased use of VDTs are summed beneath (Table 1). In this review, we would like to present the most popular ocular issues related to computer work and emphasize the meaning of appropriate vision hygiene in the modern day.

Table 1. The main changes in people working with VDTs

Human body system	Change
External ocular	redness[12] increased tears evaporation[13]
Internal ocular	inflammatory changes of the eye[14] changed shape of the eye[15]
Visual	double vision[16] blurred vision[12] convergence change[17]
Oculomotor	reduced blink rate[14]
Musculoskeletal	posture change[18] muscle pain in the neck and shoulders[16]

Aim

This review aims to emphasize the problem of eye disorders connected to prolonged usage of screen devices by analyzing the results of clinical trials. In addition, through a critical assessment of available data, we present an analysis of the possible methods to prevent these changes.

Material and methods

For this narrative review, the keywords "computer vision syndrome," "uncorrected refractive errors," "convergence insufficiency," "dry eye disease," and "visual display terminal" were used to search PubMed, Science Direct, and Google Scholar databases. The search was conducted from December 21st, 2021, through June 13th, 2022. Only original papers, published in Polish or English that discussed the outcomes of clinical trials were taken into consideration. Papers deemed pertinent by the authors were included in the analysis. Manuscripts unrelated to the subject were excluded. All articles that met the review paper's criteria were included in the "result" segment. The quality assessment scale for narrative review articles (SANRA) has been applied to the construction of this narrative review.

Analysis of the literature

Computer vision syndrome

Computer vision syndrome (CVS) is becoming a growing public health issue.[19] Especially recently, in the times of online work and education, the scale of the problem grew to a much larger extent.[20] Computers and other electronic devices have become an integral part of everyday life.[21] People who are using computers and handheld electronic devices not only at work but also for leisure activities are more likely to develop CVS compared with people who use electronics in the work area only.[22] The other names for CVS are Digital Eye Strain or Visual Fatigue, which refer to a wider range of devices that can cause eye problems linked to the described condition.[23] Computer vision syndrome is a collection of visual, ocular, and musculoskeletal symptoms caused by prolonged use of computer displays or other electronic devices.[16] Symptoms are classified into internal ocular symptoms (strain, ache), external ocular symptoms (dryness, irritation, and burning), visual (blurred, double vision), and musculoskeletal (neck, shoulder, and back pain).[16] Eye burning, itching, blurred vision, and tearing are the most often reported symptoms.[24] Besides eye symptoms, one of the most common problems in people with CVS is headaches.[18,25] The severity and specific type of symptom depend on the time of exposure, environmental factors, and individual visual abilities.

Words and images on computer screens are created by combinations of tiny points called pixels, which makes it difficult for the human eye to maintain focus.[19] The use of computers causes tear evaporation, which leads to a reduction in the number of blinks and an incomplete eyelid closure.[13] The discomfort caused by the use of electronic devices has not yet been known to cause permanent damage, but it may negatively influence work productivity and the quality of life.[26] It is reported that nearly 60 million people suffer from CVS globally and that there are a million new cases each year.[27]

Many risk factors may be associated with the occurrence of symptoms of CVS. They can be divided into modifiable and unmodifiable risk factors, presented in table 2 (Table 2).

Table 2. CVS risk factors

Unmodifiable risk factors	Modifiable risk factors
age of the patient[24]	time spent on the near work[27]
sex[26]	size of the written text[28]
occurrence of the refractive error[29]	contrast of a bad quality[28]
dysfunction of the Meibomian gland[30]	distance from the monitor[31]
	lighting in the workplace[16]
	using contact lenses[32]
	air humidity in the workplace[33]
	using anti-glare screens[25]
	nicotine addiction[34]

Unmodifiable risk factors

The prevalence of computer dry eye differs with age.[35] There is a higher incidence of CVS in older patients,[27] although, children and adolescents might be at higher risk of developing computer vision syndrome in the COVID era due to excessive use of electronic devices.[36]

The prevalence of dry eye is higher in women than in men.[37] The difference might be caused by differences in the way that hormonal profiles changes with age between men and women.[38] A study showed that older women working with computers during the workday may be more vulnerable to computer eye dryness compared to nonuser women.[39] Significantly higher Ocular Surface Disease Index scores were observed in females.[40]

Uncorrected or under-corrected refractive error and binocular vision can predispose to CVS.[29] Even a small amount of refractive error of myopia, hyperopia, or astigmatism can increase discomfort with computer use.[41] Presbyopia has been identified with a high incidence of asthenopia.[23] Therefore, appropriate refractive correction is crucial to reduce symptoms of CVS. Moreover, Meibomian gland dysfunction and long screen exposure may influence the severity of symptoms.[30]

Modifiable risk factors

Risks that can be modified include the amount of time spent in front of the computer, using a humidifier, wearing contact lenses, increased psychological stress, and ergonomic practices.

Time spent in front of a computer plays a crucial role in developing symptoms of CVS.[27] A recent study concluded that the prevalence of CVS among students was much higher during the COVID-19 pandemic due to online classes.[42] The most common reported symptoms were: eye strain, headaches, blurred vision, heaviness, and redness of the eyes.[12] Long-term use of computers causes instability in the distribution of tears on the ocular surface which leads to increased evaporation of tears.[43] Tear film break-up time (TBUT) is the main measurement showing the effects of long computer use and TBUT values are significantly lower in groups of people using a computer and in the morning hours.[43]

Studies have shown that poor quality of the text, small font size, and low contrast can all contribute to the greater severity of dry eye.[28] Closer viewing distances strain the accommodation and vergence systems.[31] The height and tilt of the visual display, which can lead to musculoskeletal symptoms, should be optimized in the workplace.[44] Improper lighting such as the presence of glares and reflections on the screen can also lead to visual discomfort.[16] Studies prove that a natural source of light in the workplace lowers the risk of developing CVS compared to using a fluorescent bulb.[45] One study showed that workers who wear contact lenses and are exposed to the computer for more than 6 hours a day are more likely to suffer from computer dry eye than non-wearers working the computer for the same amount of time.[32] Contact lens wear is a major cause of tear film instability and dry-eye symptoms leading to end-of-the-day discomfort. Symptoms of CVS are exacerbated by longer wearing time, demanding visual tasks, and by dry environments. Ineffective cleaning of lenses may also play part in the occurrence of symptoms.[32] Low humidity conditions in the room have been shown to significantly affect tear stability in wearers of soft contact lenses which leads to a shortening of non-invasive tear break up time and thinner tear film.[46] Low-humidity rooms are recognized to exacerbate the severity of dry eye, especially in places where air-conditioning and central heating are used. A USB-powered desktop humidifier is proven to cause significant improvements in tear-film stability and can increase comfort during work in front of a monitor.[33] Some studies also suggest that symptoms of dry eye may be connected with increased psychological stress.[47] Nicotine use has also been found as a risk factor because cigarette smoke contains toxic substances and particles which can destabilize the tear film.[34]

Management of CVS

Ergonomic practices carry a significant impact on the occurrence of CVS. Taking frequent short breaks during work with a computer is considered the most effective preventive method that lowers the risk of developing computer dry eye.[48] Therapies that have been proposed to treat CVS include the use of lubricating drops which reduce tiredness, dryness, and difficulty focusing during computer use.[49] Taking Omega-3 supplements can alleviate dry eye symptoms and decrease tear evaporation in patients with CVS.[50] Blink training to increase the blink rate and adjustment of the humidity in the room are also worth considering.[3] Optimizing the workplace that uses computer displays is also crucial. Distance from the screen should be around 50 centimeters (40–75 cm) for large displays, e.g. laptops, computers, and for smartphones it is required to be at least 30 centimeters.[51,52] The top of the screen should be 10-20 degrees below eye level.[27] The use of higher frequency and higher resolution LED monitors, screen filters, and adjusting the ergonomic placement of the monitor can lower the symptoms of CVS.[41]

Uncorrected refractive errors

Uncorrected refractive error (URE) is a failure in focusing images on the retina, associated with the changed shape of the eye, which results in blurred vision.[53] It is the main reason for vision impairment (VI) and the second leading cause of blindness.[15,54] By 2050, the amount of people with VI or blindness (as a result of uncorrected refractive error) is expected to increase.[54] Three main types of URE are distinguished and involve: hyperopia and myopia which stand for spherical errors, and astigmatism, which is related to optical asymmetry.[55] The

prevalence of each type of URE is different in various world regions and it is in relation to age.[56] There might be an association between genetic factors and ethnic differences, but also the shared environments may differ with ethnicity.[55] The risk factors related to URE are female sex, family history, computer and smartphone usage, and near work e.g. reading, writing.[57-59] The studies show that myopia is the most common refractive error associated with screen time.[58-64] Regular use of a computer has 4.5 times higher impact on children than irregular use.[65] Furthermore, using VDTs for more than 6 hours/day increases the odds of myopia two times compared to using these devices for less than 2 hours/day.[66] There are also differences in age and the correlation between increased screen time and myopia – in children of 5 and 6 years old there is no association, but there is in children of 7 years old.[60] On the other hand, some of the studies do not show a statistically notable relation between computer work and myopia progression.[67,68] In addition, the bond between computer usage and myopia may not be meaningful when characterizing myopia as ≤−1.00 D and it indicates that the total amount of time spent on near work may not be as important as reading intensity.[66] It has also been shown that outdoor time decreases the prevalence.[56,58-60,62-64,69] The optimal time of outdoor activities requires more studies, but 2 hours per day may reduce the progression of myopia.[62] In addition, besides near computer work, reading may also increase the risk, even more than computer screens, but the effect can be limited by outdoor exercises.[59] Nowadays, even children are exposed to higher computer usage, due to homeschooling during the pandemic and the studies have shown a great impact of prolonged screen time on children's vision.[57-61,63,65] Moreover, some of the studies have demonstrated that watching television is not highly associated with expanded refractive error, while other ones have shown the relation.[70,71,57,62,64] To prevent changes, time spent on digital devices should be decreased to the minimum, there should be a rest for 10 minutes after 30–40 minutes of working and more time should be spent on outdoor activities.[62] Also, early screening for refractive error by eye examination has a significant impact.[54,69] The possibilities of treatment are eyeglasses, validated auto-refractors, and refractive surgery [laser *in situ* keratomileusis (LASIK)].[72]

Convergence insufficiency

Convergence insufficiency (CI) is a vision dysfunction in both eyes, represented by the inability to correctly converge or maintain precise convergence during concentrating on nearby objects.[17] Symptoms of this disorder contain diplopia, asthenopia, headaches, dizziness, and blurry vision.[73] The prevalence of CI is estimated around the range from 2.7% to 19.6% in children and adolescents.[74-77] It is a wide range of diversity and it is caused by different diagnostic criteria in the studies.[76] The main method to measure symptoms of CI is the convergence insufficiency symptom survey, which was designed in 1999.[78] The survey contains 15 questions about concentration during reading, words "jumping," pulling around eyes, etc., and the frequency of appearance.[79] It should not be used as a single tool to diagnose, but it is useful in controlling the therapy.[80] Another type of test to identify CI is the near point of convergence break (NPC) with a cut-point ≥6cm and it means the distance where there is reported diplopia or eye drifting out.[73,81] The leading issue connected to CI is near vision activity, which includes reading, drawing, video gaming, or using a computer, smartphone, etc.[17,78,82-84] Between digital devices, handheld electronics (smartphones, tablets) have the highest impact on CI, equivalent to reading.[82] Additionally, there are more negative symptoms during reading from smartphones and it might be associated with scrolling the text, which causes temporary loss of sharpness.[85] The studies show that after 60 minutes of reading on a smartphone the eyestrain symptoms escalate and it is correlated with closer viewing distance.[86] The eye disorders may also get worse during reading from VDTs in a dark room, particularly when the display brightness is high.[85] The impact of increased use of these utensils on CI is expressed as a change of NPC – after 4 hours of continuous computer gaming, it significantly rises.[84] However, even a short period of time (1h) of using the VDTs leads to an increased level of NPC.[87] The studies indicate that the time needed to return to the baseline may depend on the strength of VDTs work.[84] Nowadays, it may cause problems with performing duties in the office and also with learning for children.[88] Children with CI are more likely to declare struggle with schoolwork, and avoid reading due to disturbance.[89] The treatment of CI involves office-based vergence/accommodative therapy (the most effective), home-based pencil push-ups, home-based computer vergence/accommodative therapy, and prism glasses.[90,91] Vision therapy has a substantial role in improving binocular vision in the population of children with learning difficulties.[92] Moreover, conducting the screenings has a great impact on detecting people with this disorder and implementing the treatment.[81]

Dry eye disease

Dry eye disease (DED) is a chronic disorder of the ocular surface associated with a disturbance of tear producing and/or evaporation, which leads to the manifestation of discomfort and tear film instability, linked to increased tear osmolarity.[93] The main symptoms of DED are eye fatigue, sore eyes, irritation and itching of the eye, and a burning sensation.[11] Symptoms of DED are very similar to CVS symptoms – dry eye is the core manifestation of digital eye strain, but the CVS represents a wider spectrum

of vision-related and musculoskeletal dysfunctions.[14] There are two validated tests to assess the occurrence of dry eye, including the Dry Eye Questionnaire and the ocular surface disease index questionnaire.[94] The prevalence depends on the age and gender and rates from 2.7% in young people (18–45 years) up to 75% among older adults, especially women.[96,95] The risk factors connected to DED are female sex, autoimmune disorders, ophthalmic disorders, allergy and atopy, use of contact lenses, low physical activity, or increased use of electronic devices (smartphones, tablets).[95-101] There are several causes of the dry eye among VDTs users, including Meibomian gland dysfunction, decreased blink rate, and ocular surface inflammation.[102-104] The reduced blink incidence and insufficient blinks may cause increased tear evaporation.[105] Especially effort when reading influences the blink rate – it has been shown that reading in the extended display has a higher percentage of incomplete blinks than reading in the normal display.[105] Some of the studies also show the association between DED and blue light exposure, but none have been demonstrated *in vivo*.[14,109,110] Additionally, other work has presented that using blue-blocking filters has no validation.[106] There is no consensus on the impact on lacrimal gland functioning – some works suggest that increased time of using electronic devices might lead to aqueous deficiency, while others reveal normal lacrimal functioning.[100] Another risk factor for DED – low total physical activity is inversely related to the occurrence of dry eye, an extended time of sitting or using VDTs leads to increased DED prevalence.[98] All the listed factors may disrupt the homeostatic balance of the eye, which leads to tear instability and experiencing undesirable symptoms.[101] The treatment includes educational counseling, which helps with reducing modifiable risk factors, specialist anti-inflammatory medications, and controlling a diet high in Omega-3 fats, antioxidants and carotenoids.[94] Moreover, blinking exercises may increase the number of complete blinks, which improves the hydration of the ocular surface.[107]

Possible preventive measures for computer-related ocular disorders

As VDTs become an indispensable part of life, people search for ways to alleviate the impact of computers on our vision. Here, we assemble some recommendations with the potential to minimize the prevalence of computer-related ocular disorders (Table 3.).

The viewing distance for smartphone use should amount to over 30 centimeters and for larger display devices, such as computers or laptops, it should be extended to 40–75 centimeters. The angular size of the image resulting on the retina will be around 20 degrees, being consistent with the recommendations of the International Organization for Standardization and their requirements for electronic visual displays.[52,110] Some researchers, however, postulate that the distance for near work should be even greater, as reading from <30 cm is associated with the risk of myopia progression.[111]

Table 3. Preventive modifications

Unhealthy habits modifications	Environmental modifications
reduction of the time spent in front of the VDTs[62]	increasing the distance from the VDTs – around 30 cm for smartphones and 40–75 cm for computers[51]
adequate resting time – 5 minutes for every 30 minutes in front of the screen[108]	using the correct lightning during work[39]
increasing the time spent on outdoor activities[62]	putting the monitor below the eye level (15–20 degrees)[19]
blinking exercises[3]	placing VDTs on the side of the window, not in front[109]
using eye-drops[18]	
conducting screenings in the population[54]	

Proper lighting of the workspace should be ensured. Working with natural light has been shown to lower the risk of developing CVS, compared to workers using fluorescent bulbs.[45] When using artificial light, adjustable task lighting as an additional source of light was shown to increase musculoskeletal and visual comfort, as well as posture.[109,112]

Wearing computer eyeglasses or spectacles can lower the risk of CVS, probably due to the protective anti-glare coatings of the lens.[45] Blue-light filters, however, have been shown to decrease neither the symptoms of digital eye strain, nor eye dryness.[106,113,114] Screens of VDTs should emit circularly polarized light. In contrast to linear polarization, circular has been proven to decrease symptoms of asthenopia, eye dryness, and visual discomfort.[115]

Taking breaks during working with VDTs has a proven protective effect against eyestrain and muscle pain.[116] A proposed schedule to follow includes 30 minutes of work and 5 minutes of rest.[108] A different recommendation includes the 20-20-20 rule, which requires taking breaks after every 20 minutes of near-work and looking into a distance of at least 20 feet (6 meters) for 20 seconds.[117] Moreover, another study recommend a modified rule, 20-20-2, which to the classic one adds the encouragement to spend 2 hours/day outside.[118]

Conclusion

In an era where it is difficult to function without access to VDTs, it is very important to remember about actions we can undertake to minimize the negative effects of computers on our vision. Signs and symptoms of computer-related ocular disorders should not be ignored. Furthermore, patients ought to be educated on how to eliminate the risk factors for CVS development.

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Conflicts of interest

The authors declare no conflict of interest.

Data availability

Data supporting the results of this study shall, upon appropriate request, be available from the corresponding author.

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REVIEW PAPER

Water-related diseases following flooding in South Asian countries – a healthcare crisis

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ABSTRACT

Introduction and aim. Industrialization and elevated greenhouse gas emissions pose significant threats to the environment, raising atmospheric carbon dioxide levels and leading to climate change. Climate change may impact human health either directly via increasing extreme weather frequency and altering disease patterns or indirectly via social institutions and disrupted global supply chain resulting in consequences like undernutrition

Material and methods. This review conducted a comprehensive literature search on PubMed, Google Scholar, and Cochrane Library, from inception to September 2022, using relevant keywords.

Analysis of the literature. Massive flooding in South Asia is leading to a surge in water-related diseases. *Cholera* outbreaks have occurred in countries like Pakistan, India, and Bangladesh following floods, and waterborne diseases like typhoid, rotavirus, hepatitis A, and leptospirosis are prevalent in flood-affected regions. Additionally, *Cryptosporidium*, *Campylobacter*, *Shigella*, and *Polio* outbreaks are reported. Water-scarce diseases, including scabies, impetigo, and cellulitis, are also on the rise in flood-affected areas. Water-based diseases, such as dracunculiasis, schistosomiasis, and Leishmaniasis, pose significant risks. Vector-borne diseases, including malaria, dengue, and Leishmaniasis, are becoming more prevalent due to flooded areas providing breeding grounds for disease vectors like mosquitoes and sandflies. These diseases are now more common in flood-affected regions, affecting millions of people.

Conclusion. Urgent measures are needed, including early warning systems, resilient infrastructure, drainage maintenance, and stricter land-use regulations, to reduce the impact of these natural disasters. International cooperation and immediate action at national and global levels are essential to mitigate the health crises caused by flooding and other natural disasters.

Keywords. floods, South Asia, vector-borne disease, water-based disease, water-borne disease, water-washed disease

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Introduction

Increasing industrialization and the resultant elevated greenhouse gases (GHG) emission unequivocally pose great threats to our environment. The economy-centered human initiatives have pushed the environmental carbon dioxide levels to a striking 412 parts per million (ppm), an almost fifty percent escalation since the industrial revolution. Figure 1 illustrates the increasing CO_2 levels since 2015. These atmospheric changes and the consequent temperature rise results in changes in precipitation patterns, rapid snow melting, and several environmental deteriorations.[1] Climate change may impact human health in two ways: (a) Directly via increasing extreme weather frequency and altering disease patterns; (b) Indirectly via social institutions and disrupted global supply chain resulting in consequences like undernutrition.[1,2]

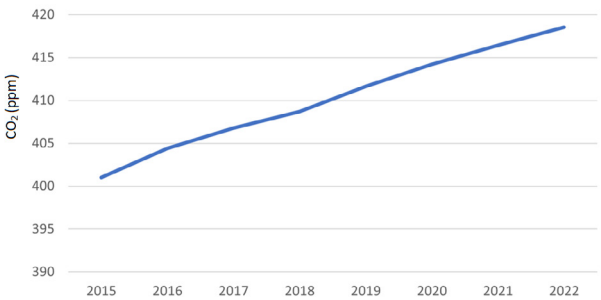

Fig. 1. CO_2 levels from 2015-2023[1]

Floods, the most recurrent natural disaster, occur when a water overflow submerges dry land. Several factors including heavy rainfalls, glacier melting, and storms like the tsunami in coastal areas may contribute, all of which may be attributed to climate change. Floods depending upon their cause and severity can be classified into three categories: (a) flash floods are usually caused by excessive rains which significantly increase water levels and results in rapidly flowing flood water; (b) river floods usually result from persistent and slow rain or snow-melting which gradually raises river levels; (c) coastal floods which are generally associated with storms like cyclone and tsunami.[3]

According to the World Health Organization (WHO), floods impacted over 2 billion individuals worldwide between 1998 and 2017.[3] South Asia is amongst one of the most vulnerable and prone regions to floods and natural disasters. One such example is of Pakistan, which has experienced several floods during the previous few decades. Recently it was affected by the worst of all, due to the unprecedented torrential rainfall with the country receiving almost 60% of the record-breaking monsoon rains in just three weeks. The recent flooding has impacted the entire country with prominent damage in Baluchistan, Khyber Pakhtunkhwa, and Sindh. Following massive flooding, a state of emergency has been declared with the government urging local and foreign organizations for optimum humanitarian support.[4]

Alongside financial and economic crises, the overburdened healthcare infrastructure and disrupted water supply further exacerbates communicable and non-communicable water-related diseases burden. These can be classified into four broad types, as depicted in figure 2. In this review, we have highlighted the potential water-related disease outbreaks that have followed massive flooding in South Asia and discuss the potential way forward.

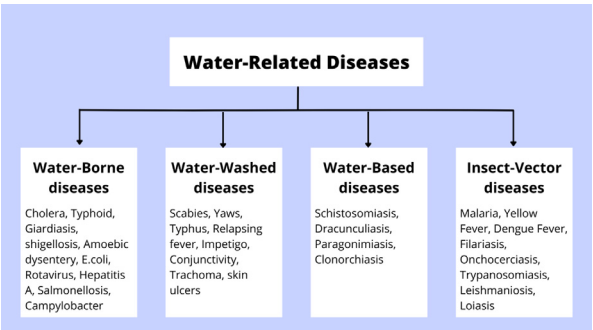

Fig. 2. Classification of water-related diseases

With contaminated flood water covering more than one-third of the country and disrupting clean water access, water-related diseases are expected to rise steeply, and may potentially cause more grievous consequences than the floods themselves. In an interview, the secretary general of the Pakistan Medical Association (PMA) highlighted that water-related diseases are capable of causing more deaths than the flood itself.[5]

Aim

This paper aims to determine the impact of flooding in South Asia on water related diseases.

Material and methods

Two independent authors (SHA, SW) conducted a thorough literature search over PubMed, Google Scholar and Cochrane Library from inception till September 2022 using the following key words: "Floods", "flooding", "flash flood", "natural disaster", "waterborne disease", "water washed diseases", "water-based disease", "insect vector disease", "Asia", "South Asia" and "South East Asia". The results of the literature search were screened for their adherence to the eligibility criteria, which encompassed all original studies (cross-sectional, cohort, longitudinal) reporting the healthcare consequences of foods in South-east Asia. Following a thorough literature search, relevant data was extracted by two authors (TGS, MZ) independently.

Analysis of the literature

Water-borne diseases

According to WHO, infectious WBD are one of the leading causes of morbidity and mortality in humans worldwide accounting for 1.5 million deaths annually.[6] Water-borne outbreaks are among the most well-known, immediate effects of flood catastrophes promoted by the failure of sewage disposal and purification systems. However, additional impacts of flooding, such as displacement of large numbers of people into over-crowded shelters and subsequent fecal-oral dissemination of gastrointestinal pathogens, may contribute to the acute aftermath of the flood.[7] Since 1980, there has been an increase in water-borne infections worldwide, which has been correlated with an increase in flood events.[8]

Low-resource nations like Pakistan are further taxed in times of floods secondary to accelerated, urban sprawl, which is driving people into regions with deteriorating drainage systems and poor hygiene standards.[9] The outbreak of water-borne diseases following the devastating floods of 2010 and 2013 are particularly relevant in this regard. Reports from the 2010 flooding in Pakistan revealed that from the start of the floods until the August 12th, 86,671 cases of diarrhea were reported. Similarly, the 2013 flooding in Pakistan showed that from 6th to 21st Baluchistan alone reported a total of 10,431 patient consultations from flood-affected districts of Baluchistan oof which 2,031 (20%) cases were of acute diarrhea.[10] There are several bacterial, viral, and parasitic species that can cause acute watery diarrheal disease, listed below.

Vibrio cholerae, a gram-negative bacteria causing cholera, has wreaked havoc in impoverished nations and thrives in environmental upheavals like floods.[11] Countries like India and Bangladesh are at high risk for cholera due to their dense population, lack of development, and poverty.[12] In August 2010, Pakistan experienced major floods and a subsequent *cholera* epidemic ensued. WHO reported 164 laboratory-confirmed sporadic *cholera* cases from a wide geographic area in the flood-affected provinces of Sindh, Punjab, and KPK.[13] Similarly, in Dhaka, Bangladesh, *cholera* was the most frequently identified pathogen in flood-associated diarrheal epidemics.[14] The impact of flooding can be judged by the fact that the *V. cholera* O139 re-emerged during April and May 2017 following a massive flooding in Odisha, India.[15]

Typhoid fever is caused by a bacterium *Salmonella enterica serotype Typhi* and to a lesser extent, serotypes *Paratyphi* A, B, and C. With 493.5 cases per 100,000 people reported in 2018, Pakistan has the highest rate of typhoid among the South Asian nations. Cases in Pakistan sharply surged in 2016, following the introduction of a highly drug-resistant (XDR) typhi outbreak in Hyderabad.[16] Typhoid appears to have a seasonal pattern, peaking from July to October after rain, notably in the majority of South Asian and South East Asian countries, perhaps due to floods brought on by heavy rains that contaminate drinking water sources with sewage.[17] Per this, a study conducted in Karachi showed a prevalence of serologically positive patients at 20.13% in the summer and 17% in the winter.[18]

Rotaviruses are members of the double-stranded RNA family *Reoviridae*. With a mortality incidence of 67.6 deaths per 100,000 children in Pakistan, it is one of the most common causes of fatal dehydrating diarrhea in children under five.[19] High precipitation and flooding events can induce water logging, which eventually paves pathways for pathogen transmission.[20] In 2014, flooding in the Solomon Islands led to a *rotavirus* outbreak that spread across the country. During the 2007 floods, Dhaka witnessed one of the most severe rotavirus outbreaks.[14] The history of rotavirus in Bangladesh is not new. In 1988, the nationwide floods the prevalence of *Rotavirus* mixed infections from 8.1% to 22.7%. In 1994, an estimated one death per 111-203 children under five years of age was attributed to this deadly virus.[21]

Hepatitis A is an enterically transmitted viral disease caused by the virus, *hepatitis A virus* (HAV).[22] It has been acknowledged that hepatitis A outbreaks could occur when flood-related sewage contaminates sources of drinkable water.[8] Children in a flood relief camp in Uttarakhand State, India, had an outbreak of acute viral hepatitis in 2013 that was brought on by the HAV.[22]

Leptospirosis is one of the most pervasive zoonotic illnesses in the world.[23] The disease is typically reported post-flooding in impoverished nations that struggle with disaster management.[24] Excessive precipitation has also been linked to Leptospirosis outbreaks in flood zones in Australia, the United States, Germany, India, Malaysia, and Thailand.[25] Investigations conducted in populations affected by flood disasters in 2000 in India and Thailand reported Leptospirosis epidemics to be associated with bathing and drinking contaminated water.[24] A cross-survey study of Pakistan showed significant differences in leptospiral seroprevalence in different geographic locations, with the highest in the humid sub-tropical climatic region. Further analysis revealed a positive correlation with exposure to flooding water.[26]

Cryptosporidium, a water-borne zoonotic parasite is the second leading cause of diarrhea and deaths in children after rotavirus.[27,28] In a study of 300 water samples from the flood-affected district, Nowshera, the overall prevalence of *Cryptosporidium* was found to be 30.33%. The highest prevalence was recorded in the summer (June-September).[27] According to studies from India, a rise in temperature and humidity increases the frequency of infections. This may be attributed to the dissemination of infectious oocysts into the surface water post heavy rainfall between March and July.[28]

Campylobacter infections in children commonly occur under the age of two years. South Asian countries have a significant burden of *Campylobacter* infection and a study revealed it to be one of the most frequently occurring organisms that cause gastroenteritis in Bangladesh, India, and Pakistan.[29] According to Cann et al., the most common water-borne pathogens associated with heavy rainfall were *Campylobacter*, followed by *vibrio spp*.[8] According to the available data, campylobacter and cryptosporidium were amongst the most common pathogens in outbreaks associated with extreme water related weather events.[30]

Shigellosis is a highly contagious disease in poor and crowded communities.[31] *Shigella* breed and thrive better in humid environments during floods due to its increased fecal-oral transmission. According to a prospective multi-center study, the *Shigella* is prevalent in impoverished Asian population and over a period of time antibiotic resistant strains have developed and the conditions become worse during floods.[32]

Poliomyelitis is a viral disease that primarily affects children under the age of five causing acute paralysis, muscle weakness, and autonomic dysfunction. The fecal-oral mode of transmission is most prevalent in areas with poor sanitation and health issues. In 2022, Pakistan had 15 cases of wild poliovirus recorded. In the previous year, only one case was reported.[33] Due to the significant population dislocation caused by the floods in 2010, a nationwide polio vaccination program was impeded. The trend is predicted to continue given the ongoing, enormous flooding and it would be very challenging to keep up the pace needed for a successful polio vaccine program.[34]

Water-washed diseases

Water-washed diseases, or water-scarce diseases, intuitively result from inadequately available clean washing water. Their incidence is primarily determined by the quantity, hence adequate availability and hygienic conditions are intrinsic to controlling their spread. Diseases classified in this category include dermatologic and ophthalmologic conditions like scabies, impetigo, skin ulcers, trachoma, typhus, relapsing fever, yaws, and conjunctivitis.[35]

Several factors contribute to its increased incidence in flood-affected areas. The water along with its flow brings contaminants, allergens, and sewage discharge into the environment. Ambient temperature further enhances microbial growth.[36] Moreover, massive flood-associated destruction disrupts clean water supply, washing areas, and sanitation facilities, hence causing a water outage, and further predisposing individuals to greater risk.[37] Often flooding results in trauma compromising skin's continuity, leaving individuals susceptible to developing infections.

Scabies, a parasitic infestation caused by *Sarcoptes scabiei*, infects almost 200 million individuals globally with a higher prevalence in resource-limited regions. In Pakistan, scabies accounts for 38.15% of dermatological conditions.[38] It is characterized by itchy lesions comprising of papules, nodules, and vesicles predominantly affecting the skin between fingers, axillae, wrists, genital area, palm, soles, and head but may involve any other region. The diagnosis is usually clinical via examination using a dermatoscope, which allows visualization of the characteristic scaly curved burrows.[39] Several studies report an association between scabies and natural disasters like flooding.[2,36] Following the 2010 flooding in Pakistan, scabies was among the most common causes of morbidity in Baluchistan accounting for 2,070 cases between 29th July and 11th August.[40] Similarly, reports from the 2013 flooding in Pakistan revealed scabies accounting for the highest proportion of consultations in the affected regions of Punjab with 2,708 consultations between 6th to 16th August[41] and 9,943 between 12th and 21st August 2013.[10]

Impetigo, a superficial bacterial skin infection, commonly affects children between the age of two and five years. It has a global prevalence of 140 million and is usually caused by either Staphylococcus aureus or Streptococcus pyogenes. Clinically, it is characterized by honey-crusted lesions that may involve the face, trunk, extremities, and intertriginous regions. It may manifest either as a bullous or non-bullous form of the disease. The diagnosis is based on clinical examination while culture and sensitivity assist in detecting the causative agent and treatment regimen.[42]

Inflammation of the deep skin and the subcutaneous tissue is referred to as cellulitis. Cellulitis classically manifests as erythema, edema, tenderness, and warmth, but the diagnosis may be challenging due to its resemblance with certain other conditions such as stasis dermatitis, ulcers and deep vein thrombosis.[43] Literature also reports increased consultations for lower limb cellulitis in flood-affected areas, which may be due to lower limb trauma disrupting the physical skin barrier.[36]

Multiple studies report skin infections following natural disasters like flooding.[36,44] During the 2010 flooding in Pakistan, 40,100 skin infections were reported from Khyber Pakhtunkhwa (KPK) between 31st July and 12th August, making it a more significant cause of morbidity than watery diarrhea, which accounted for 34,373 cases.[40] Skin infections also remained a significant contributor to consultations in other flood-affected areas of Punjab and Sindh.[40] Similarly, in 2015 the devastating floods in Chennai and South Indian coast following extreme rain. A study conducted in the medical camp of Thiruvallur, one of the worst affected districts of the region observed that 14.41% of the people in those camps had skin diseases.[45]

Along with causing dermatologic conditions such as impetigo, cellulitis and scabies, contaminated flood water and the associated clean water inadequacy may escalate eye infections. Trachoma, a leading cause of infectious blindness worldwide, refers to chronic conjunctivitis caused by Chlamydia trachomatis. It may lead to scarring of the tarsal conjunctiva, trichiasis, and corneal opacity. Poor hygiene, inadequate water supply, and overcrowded settings increase its risk. Although it is typical in children, scarring occurs more frequently in adults.[46] Increased exposure to the underlying risk factors during flood events can increase its incidence and potentially lead to life-long visual disabilities.[36,47] Similarly, studies suggest an increased incidence of bacterial conjunctivitis during flooding events.[48] *Toxoplasma gondii* infection-associated cases of focal retinal whitening and necrotizing retinochoroiditis were also observed during flooding episodes in Brazil.[49] During the 2010 flooding in Pakistan, 337 eye infections were reported between 29th July to 12th August in Baluchistan.[50] Similarly, it remained among the top contributors to consultations in Sindh, alongside skin and respiratory problems.[51] Again, ocular infections remained a major health problem during the 2013 flooding in Pakistan.[52]

Several factors may account for these associations including water outages, poor hygienic conditions, shared resources like towels, clothes, and beds in refugee camps, overcrowded camps, and malnutrition. Therefore, the government and authorities working in flood-affected regions must promote good hygiene practices among the affected and ensure adequate medicines availability alongside isolation facilities to prevent outbreaks as in previous floodings.

Water-based diseases

The term refers to the diseases caused by parasites in the intermediate organisms living in contaminated fresh water. It most commonly includes dracunculiasis, paragonimiasis, schistosomiasis, and Clonorchis's.

The fight against dracunculiasis, also known as guinea worm disease, was initiated in Pakistan in the year 1986 as a collaborative effort between Pakistan's National Institute of Health, the Global 2000 Project of the Carter Center, and the CDC. Rapid detection, thorough investigation, intensive surveillance, and case-containment measures resulted in the complete eradication of dracunculiasis by 1994.[53] A further follow-up on the eradication of dracunculiasis in the district Dera Ismail Khan, KPK, reported no active cases in the area.[54]

Another water-based disease, schistosomiasis, is caused by blood flukes (trematode worms) of the genus *Schistosoma*. The infectious form of parasite, cercariae, is acquired by skin contact with freshwater snails, which serve as an intermediate host, and is transmitted when the excretory material of the infected person contaminates the freshwater resource.[55] According to the WHO, schistosomiasis is prevalent in tropical and subtropical regions, especially in poor communities without access to safe water and inadequate sanitation. There are two major forms of schistosomiasis, intestinal and urogenital, caused by five species of blood fluke worm. The Intestinal form usually causes abdominal pain, diarrhea, and hematochezia. In severe cases, the liver might be enlarged. The urogenital form mostly manifests as hematuria.[56] There are reports of isolated cases of schistosomiasis mostly due to traveling history in endemic areas, but according to Rollinson D et al., the prevalence of schistosomiasis in Pakistan was less than 10% based on the data from WHO's weekly epidemiological record.[57,58] Therefore, human schistosomiasis is less likely to become endemic in Pakistan owing to the continuous efforts of the government in providing safe drinking water and proper sanitation. According to the official data of 2015, 91.4% of the population had access to improved drinking water and 63.5% of the population had access to improved sanitation.[59] But the recent catastrophic flooding in Pakistan has severely affected the under-developed areas with already prevailing resource inequities. Lack of safe drinking water and improper sanitation conditions possess a great risk of waterborne and vector-borne diseases including schistosomiasis.[60] Although most of the cases of Schistosomiasis in Pakistan are secondary to travel in endemic areas, the presence of *Biomphalaria* species of snails, carriers of *Schistosomiasis mansoni*, which is already present in Pakistan, may trigger the disease in the flood-affected areas.[55]

Vector-borne diseases

These diseases are caused by insect vectors that require water for breeding. Several species of mosquitoes and flies may serve as vectors and are responsible for multitudinous life-endangering diseases like malaria, dengue fever, kala-azar, yellow fever, and multiple others.[61] Their incidence is majorly determined by the environmental aptness involving climate, altitude, vegetation, and application of control measures; as a result, poverty, natural disasters, and wars are intrinsically tied to their increased prevalence.[62] Natural calamities like flooding provide additional breeding grounds to these vectors and increase their densities, hence placing the populations at a higher risk. The risk is further exacerbated by the increased exposure and absence of control facilities in relief camps.

Malaria, caused by the *Plasmodium* species, is transmitted to humans by the female anopheles mosquito. Although over 120 species of *plasmodium* have been recognized, only six are responsible for morbidity in humans: *Plasmodium falciparum, Plasmodium vivax, Plasmodium malariae, Plasmodium knowlesi, Plasmodium*

ovale curtisi and *Plasmodium ovale wallikeri*.[62] According to the recent world malaria report by WHO, approximately 241 million cases and 627,000 deaths were reported in 85 malaria-endemic countries in 2020, with the African region being affected the most.[63] About 98 percent of Pakistan's population resides in malaria-endemic regions, with almost 1 million new cases and 50,000 deaths reported annually.[64] With the recent nationwide flooding, the numbers are expected to rise, and if prompt action is not taken, the repercussions may be irreparable. According to a Ugandan study, individuals were about 30% more likely to test positive for malaria in flood-affected river-bordered settlements, with a striking 47 percent rise in positive malaria tests observed in the post-flood era compared to the pre-flood span.[65] Similarly, malaria-related hospital admissions were significantly higher during the post-flood period, with almost 50 percent more admissions reported.[65] According to data from the 2010 flooding in Pakistan, almost 182,762 consultations between 29th July and 17th September in 36 flood-affected malaria-endemic regions, were conducted for suspected malaria. Similarly, a malarial surge was witnessed during the 2013 flooding in Pakistan, with 17 percent of consultations conducted for suspected malaria in Baluchistan between the 6th and 21st of August.[10] The current literature suggests an almost two to three months delay between flooding and post-flood peak malaria epidemic.[65–67] This delay may be accountable to rapid flood-water flushing away the existing breeding grounds and vectors setting up new breeding sites in the affected regions.[65] The situation in Nepal and Bangladesh was not so different. According to several media reports there had been thousands of cases of malaria following flooding in 2017 which is considered the worst flood in South Asia in the last decade. In Nepal alone thousands of cases related to malaria had been reported.[68]

Dengue and yellow fever belong to a family of single-stranded RNA, positive sense, *flavivirus* transmitted through Aedes aegypti mosquito in endemic and epidemic regions. According to the WHO, there could be 390 million dengue cases globally of which 70% could be in Asia alone.[69,70] Pakistan's first dengue outbreak was reported in 1994.[71] The annual epidemic trend was first reported in Karachi, Pakistan in the year 2005 potentially due to irregular monsoon patterns and rising temperatures, making the climate favorable for mosquito breeding.[69,71] The year 2022 is no different at least in terms of dengue cases. It's been more than two months since the calamitous floods and heavy rain spells began and yet across Pakistan, many villages are submerged in water, leaving hundreds and thousands of families displaced. According to a local news report published on 14th September 2022, the Sindh province reported at least 1,098 dengue cases during the current month and nine people lost their lives.[72] Similarly, the cases of dengue are on a continuous rise in the federal capital, Islamabad, where the total number of dengue patients has reached 871.[73] In 2010, Pakistan was severely affected by massive flooding and extreme rainfalls which impacted 14-20 million people. The floods were followed by an enormous spike in dengue cases and according to data, the country recorded the highest dengue cases during that year.[74] The numbers can be significantly higher this year since the unofficial data suggests that the flooding this year has directly impacted 33 million people so far and the numbers are expected to rise further.

The situation across the border is not very different too. The recent heavy rain and flooding in Delhi and other states such as Punjab, Assam, and Haryana, the cases of dengue and other vector-borne infections are at high year high. Karnataka alone has reported over 4500 cases of dengue from January2023 to July 2023.[75]

With over one million cases reported annually, Leishmaniasis is a parasitic protozoan infection caused by over 20 species of *Leishmania*.[76] Preponderantly transmitted via sandfly phlebotomine, Leishmania may manifest in three distinct ways: (i) Visceral Leishmaniasis (VL), also known as Kala Azar or Black fever, (ii) Cutaneous Leishmaniasis (CL), and (iii) Mucocutaneous Leishmaniasis (ML). While CL is the most prevalent one, the deadliest is the visceral form of Leishmania. Depending on the type, the disease may emerge with a myriad of signs and symptoms, with VL typically inducing fever and hepatic enlargement while the cutaneous variant causes sores on the skin.[77]

While Leishmaniasis occurs worldwide, its highest frequency is associated with illiteracy, malnutrition, lack of resources, and poor housing facilities. Moreover, it is a fact that climate changes affect the vector reservoir, thereby directly affecting areas pounded by a natural calamity.[78] Flood-affected regions have traditionally provided an enormous breeding ground for the parasite. In addition, flood-affected regions in Bangladesh and India recorded higher instances than others in history.[79,80] Bihar, a region in India is endemic to floods have seen a remarkable surge in leishmaniasis since 1977.[7] Pakistan has been no different. Record-breaking cases were identified during the mass displacement at the Afghan-Pakistan border. Similarly, during the flood of 2010, a Leishmania surge was observed throughout the afflicted areas in Pakistan.[7]

The way forward

Much of the associated destruction secondary to natural disasters is human induced, aggravated by our dwelling patterns, uncontrolled deforestation, poor infrastructural quality, and the geographical, economical, and social marginalization of our impoverished population. Floods and other natural disasters have been socially construct-

ed due to years of weak governance, inaction, and negligence. One such example is of Pakistan. Despite having large bodies at both the federal and provincial levels, meager progress has been achieved. The lack of district-level disaster management systems has further exacerbated the situation. According to the Federal Flood Commission, Pakistan has witnessed 28 riverine floods since its independence in 1947, which have affected 616,558 square kilometers of land, caused a loss of over 39 billion rupees to the economy, and snatched at least 13,262 lives. The situation at provincial level is equally worse. The provincial governments of Sindh province in Pakistan since partition have been unable to act, despite it having a long history of floods. The lack of proactiveness can be judged from the fact that on July 22, 2010, the flood started in Baluchistan, and though the governments of Sindh and Punjab had ample amount of preparation time to arrange resources, several areas of Sindh were still inundated with flood water.[81]

There is a long history of international agencies such as the WHO and the United States Agency of International Aid (USAID) helping in relief operations. Additionally, civilian volunteers, independent teams of doctors, non-governmental organizations, and various other people always come forward for help with their limited resources. There is a widespread mistrust to donate for the governmental funds in South Asian countries and even in 2022, this perception has not changed, which is hindering people from cooperating with governmental initiatives.

Since the beginning of these devastating floods, several countries have come forward and have provided aid, including the UN, to the flood effected countries.[82] But the solution to natural disasters like floods cannot be resolved with financial assistance alone. Pakistan contributes less than 1% to the global greenhouse gas emissions but according to the German Watch index, it is constantly on the list of top 10 climate vulnerable countries and is bearing the brunt of global industrialization.[83]

There are several scientific based actions which developing South Asian countries can take to reduce the effect and improve its ability to deal with the floods. One such example is the use of nationwide early warning systems that can alert the authorities of an expected flood and give them sufficient time for evacuation operations. In addition to this, immediate attention should be given to have modified construction plan in flood susceptible areas to reduce structural damage. Along with this, flood prone areas should be regularly monitored and any construction on such land should be banned, and any violation should be dealt with accordingly. There is also a need for regular drainage system maintenance, especially in urban cities and drains should be cleaned before every monsoon season to prevent incidents of flash flooding.

There is an urgent need for global acknowledgment that despite contributing less, several countries are on the worst to receive the repercussions of climate change, including flash floods, water scarcity, unbearable heatwaves, rising sea levels, food shortages, and the displacement of its people. International cooperation, immediate measures at the national level, and individual contributions can halt this climate change.

The concerned international and national stakeholders need to act now and implement the necessary measures, including:

a. Issuing well-defined guidelines, demarcating roles and responsibilities of the provincial and the federal entities.
b. At the district level, there should be proper guidance tools to speed up the rescue process with full transparency and competitiveness.
c. A proper financing mechanism should be established for disaster management and a small portion of the budget should be allotted to combat future natural disasters.

Conclusion

This manuscript underscores the urgent need for comprehensive policies to address the escalating impact of climate change-induced flooding on public health in South Asia. The increasing frequency and severity of floods expose vulnerable populations to a surge in water-borne, water-washed, water-based, and vector-borne diseases. As exemplified by recent flooding in Pakistan, the aftermath extends beyond immediate damage to infrastructure, exacerbating health crises and overburdening healthcare systems. Key findings reveal heightened risks of cholera, typhoid, rotavirus, hepatitis A, leptospirosis, cryptosporidium, campylobacter, shigellosis, and polio. In addition, inadequate clean water access during floods leads to a surge in dermatologic and ophthalmologic conditions, including scabies, impetigo, cellulitis, and eye infections. Contaminated freshwater fosters diseases like schistosomiasis, with potential resurgence in flood-affected regions. Furthermore, floods create breeding grounds for disease vectors, increasing the risk of malaria, dengue, leishmaniasis, and other life-threatening illnesses. Policy recommendations include the implementation of early warning systems, modification of construction plans, international cooperation, comprehensive data collection, transparency, and the establishment of financial mechanisms for disaster management. Immediate and coordinated action is imperative to mitigate the adverse health consequences of climate change-induced flooding. Policymakers must prioritize the development and implementation

of robust policies addressing both the immediate aftermath and long-term resilience against the compounding challenges posed by these natural disasters.

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The authors declare no conflict of interest.

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This paper uses publicly available data.

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CASUISTIC PAPER

Total resection of foramen magnum meningioma via a far-lateral approach – a case report

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ABSTRACT

Introduction and aim. The foramen magnum is a rare location of meningioma development, accounting for 1.8 to 3.2% of all reported tumors of this type. Microsurgical resection, representing a gold standard in foramen magnum meningioma treatment, may result in various neurological deficits or incomplete resection due to challenging anatomical conditions. Currently, even surgical resections of foramen magnum meningioma conducted by experienced neurosurgeons are burdened by a relatively high complication rate of 17.2%

Description of the case. We report the case of a 25-year-old male who presented increasing headaches and decreasing activity for 5 months. In his medical history, the patient had been diagnosed with cerebral palsy, autism spectrum disorder, and suffered partial seizures. Magnetic resonance imaging revealed anterolateral foramen magnum meningioma. The tumor was resected via a far-lateral approach. After the surgery, the patient maintained a preoperative neurological state without additional neurological deficits. The post-operative magnetic resonance imaging demonstrated complete tumor removal. Histopathological examination revealed transitional meningioma (WHO grade I).

Conclusion. Our case demonstrates that the far-lateral approach can be efficient for the resection of anterolateral foramen magnum meningioma. In such cases, Simpson grade 1 can be achieved without complications, providing immediate relief of symptoms and minimizing the risk of recurrence.

Keywords. case report, far lateral approach, foramen magnum meningioma, meningioma, transitional meningioma

Introduction

Meningiomas constitute the most frequently reported primary central nervous system (CNS) tumors (39% of all CNS tumors), with an incidence rate of about nine cases per 100,000 individuals.[1] The foramen magnum is a rare location of meningioma development, accounting for 1.8 to 3.2% of all reported tumors of this type.[2] Foramen magnum menigiomas (FMM) are defined as me-

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ningiomas arising from the arachnoid cells in the dura mater in the anatomical area described by George et al. – bounded anteriorly from the superior edge of the C2 vertebral body to the lower third of the clivus, posteriorly between the C2 spinous process and the anterior border of the occipital bone and laterally from the upper margin of C2 laminae to the jugular tubercle.[3] This region is abundant in multiple vital neural and vascular structures such as lower cranial nerves (IX-XII), upper cervical nerves (C1 and C2), cerebellar tonsils, brainstem, V3 and V4 segments of the vertebral artery, and posterior inferior cerebellar artery (PICA).[3] Therefore, FMM may result in various neurological deficits and technically challenging surgical resection, which represents the golden standard in FMM treatment.[4] Currently, even surgical resections of FMM conducted by experienced neurosurgeons are burdened by a relatively high complication rate of 17.2%.[2]

Aim

The present article, prepared according to Surgical CAse REport (SCARE) 2020 guidelines (Supplementary Material No. 1), describes a case of a 25-year-old man with a large FMM treated at the Department of Neurosurgery of the Independent Public Teaching Hospital No. 4 in Lublin, Poland.[5] The main aim of this paper was to present an overview of the clinical presentation of FMM in the young patient and provide an example of effective surgical management of FMM via a far-lateral approach.

Description of the case

Patient information

A 25-year-old Caucasian male with increasing headaches and decreasing activity for 5 months, was presented to the emergency department on 18th September 2020 because of a grand mal seizure. The patient had been diagnosed with pediatric cerebral palsy and autism spectrum disorder. Since the age of 17, the patient was treated for focal seizures. He had a penicillin allergy. In 2014, FMM was diagnosed in the patient based on a performed MRI scan. Since then, the patient remained under regular clinical and radiological follow-up. Smoking, alcohol, or other stimulant abuse were absent. The patient's social, family, and occupational history were unremarkable.

Clinical findings

At admission (18.09.2020), the patient was in a general average condition, conscious, and complying with simple commands. The neurological examination revealed hypoglossal nerve palsy. Apart from that, the patient preserved arbitrary limb movements without paresis. The verbal contact with the patient was hampered due to his autism spectrum disorder.

Diagnostic assessment

The patient was assessed by physical and neurological examination, laboratory examinations, and radiological imaging (computed tomography (CT) and MRI). The initial laboratory examinations revealed abnormalities including leukocytosis, increased RBC, HGB, and HCT levels, and increased level of C-reactive protein (CRP). CT scan of the head performed on the day of admission revealed a tumor in the area of the craniocervical junction, measuring 38×27×29 mm, with significant compression of the medulla oblongata. Radiologically, the tumor was diagnosed as anterolateral FMM. Compared with radiological examinations performed at diagnosis, the volume of the tumor significantly increased. Cerebellar atrophy and enlargement of cerebrospinal fluid spaces of the posterior cranial fossa were comparable. Magnetic resonance imaging (MRI) of the head performed on 3rd September 2020 revealed similar findings (Figure 1a, 1b). Based on these results, the patient was qualified for neurosurgical treatment.

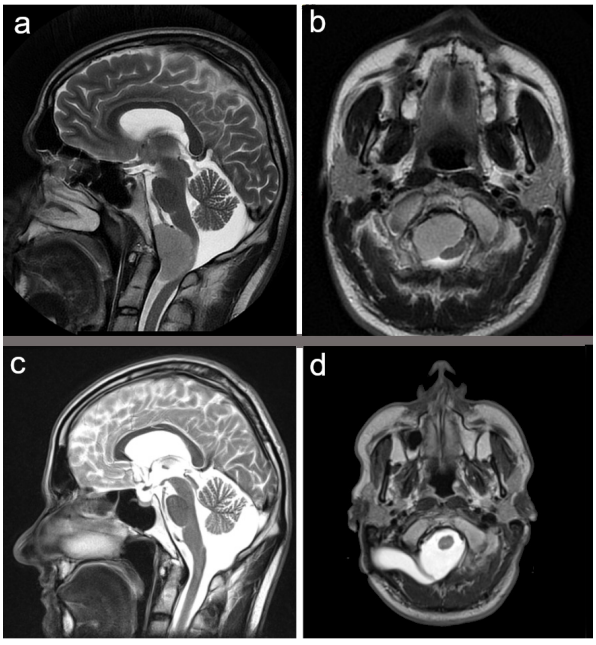

Fig. 1. Foramen magnum meningioma tumor in a 25-year-old man visualized on MRI (T_2 sequence): sagittal section (a), transverse section (b), post-operative MRI (T_2 sequence) performed 2 years after surgery, demonstrating no lesion at the foramen magnum: sagittal section (c), transverse section (d)

Surgical intervention

After obtaining informed consent from the patient, surgical resection of FMM was planned. The surgical procedure was conducted on 29.09.2020 by an experienced neurosurgeon.

The surgery was performed via the right far-lateral approach with the use of microscopic magnification, neuronavigation, lower cranial nerves electroneuro-

myography, and motor and sensory evoked potentials. The patient was placed in the lateral park bench position with the head fixed in the Mayfield frame. The skin incision was S-shaped extending from above the right auricle to the C3 level in the midline. After the muscle retraction, a suboccipital craniotomy was performed, and a free bone flap was created. Subsequently, the opening was extended to the foramen magnum with partial mastoidectomy and removal of one-third of the right occipital condyle. The dura mater was opened in a hockey stick fashion. After the cerebrospinal fluid aspiration from the cerebellomedullar cistern, the gray-pearlescent tumor was visualized.

The tumor capsule was incised and a fragmented specimen of 2.5 cm in diameter collectively was obtained for histopathological examination. After the localizations of IX, X, and XI cranial nerves tensioned on the tumor surface were confirmed, the tumor mass was totally removed with the use of an ultrasonic aspirator. Thereafter, lower cranial nerves were separated from the tumor capsule, and the capsule was removed in a few fragments.

The vertebral artery and lower cranial nerves were preserved without any damage. The Simpson Grade I had been achieved.

Follow-up and outcomes

After the surgery routine clinical observation and follow-up were implemented. The patient maintained their preoperative neurological state and was in good general condition, without additional neurological deficits. The control post-operative head MRI demonstrated an image corresponding to complete tumor removal. At 8 days post-surgery, the patient was discharged from the neurosurgical department with no severe complications, appropriate surgical wound adaptation, and minor subaponeurotic fluid collection at the surgical site.

Histopathological examination revealed a transitional meningioma (WHO Grade I).

Post-operative control MRI examinations were performed every 6 months after surgery, and none revealed tumor recurrence. At 2 years post-surgery, the clinical and neurological examination did not indicate the presence of a recurrent tumor and the MRI demonstrated no lesion at the foramen magnum (Figure 1c and 1d).

The order of events in the patient's history are presented in Figure 2.

Discussion

Despite the development of neurosurgical operative techniques in recent decades, obtaining total resection of a FMM with minimal morbidity remains a challenge, even for experienced skull base surgeons.

Several surgical approaches can be considered when the FMM resection is planned including the midline suboccipital approach, far-lateral approach, extreme-lateral approach, and anterior approach.[6] It is generally accepted that the extreme-lateral approach and far-lateral approach are used for anteriorly located FMMs, whereas posterior FMMs are feasible for the standard midline suboccipital approach.[7] That statement is caused by the fact that resection of anteriorly or anterolaterally localized FMMs via posterior midline approach resulted in a lower extent of resection and high morbidity due to significant brainstem retraction.[8] Moreover, in the suboccipital approach, safe visualization of VA is limited.[9] On the other hand, anterior approaches are not generally accepted due to the difficulty in achieving total resection and the high risk of complications such as infection and CSF leakage.[10] Currently, the posterior-lateral approaches including the far-lateral approach and extreme-lateral approach provide the best surgical access for anterior and anterolateral FMMs.[11] However, complications such as lower cranial nerve deficits, CSF leak, VA injury, or hydrocephalus are still common in FMM resections via these approaches.[10]

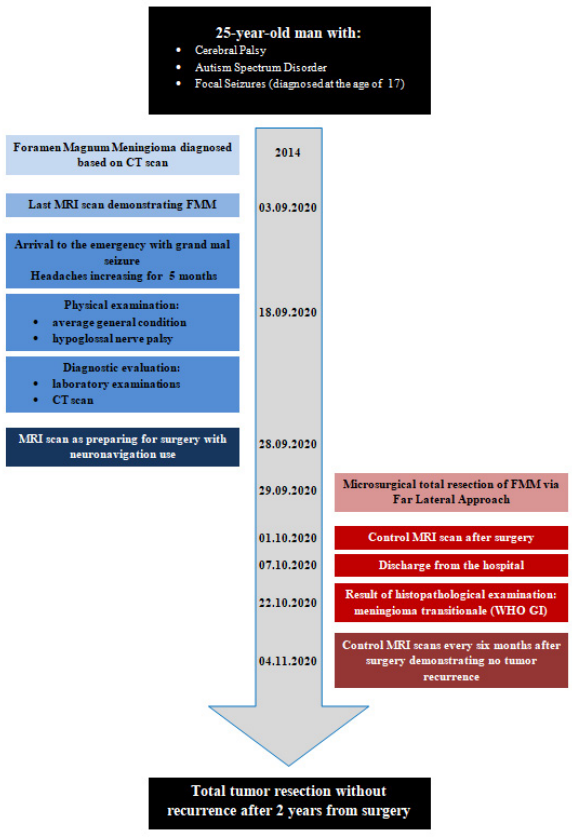

Fig. 2. The timeline representing the sequence of events in the patient's history

Although the choice between the far-lateral and extreme-lateral approach should depend on the location of the lesion in relation to the clivus, contralateral foramen magnum part, and jugular foramen, the latter technique is burdened with more limitations. Compared with the

far-lateral approach, the extreme-lateral approach requires more time and more extensive condylectomy, which increases the risk of craniocervical junction instability.[12] Moreover, postoperative complications such as CSF leakage and lower cranial nerve damage were more commonly observed in the extreme-lateral approach than in the far-lateral approach.[12] On the other hand, the extreme-lateral approach provides an extended surgical corridor and increased exposure compared with the far-lateral approach.[2] In our case, the far-lateral approach was implemented due to anterolateral localization of the tumor with achieving Simpson grade 1 and recurrence-free survival. Furthermore, we did not observe post-operative complications such as vascular damage, neurological deficits, or infections.

During the far-lateral approach, condylectomy significantly reduces the working distance and expands the lateral angle of exposure, which contributes to better access to the FM lesions.[13] However, the higher extent of occipital condyle removal results in a higher risk of occipitocervical joint instability.[14] There is still no consensus concerning the safe extent of condylectomy to date and available evidence suggests that the removal of more than 50% of occipital condyle results in major instability.[15] In these cases, stabilization of the occipitocervical joint is highly recommended. In our case, one-third of the occipital condyle was removed. Thus, according to the literature, the risk of craniovertebral junction instability was low and occipitocervical fusion was not necessary. Furthermore, postoperative instability of the craniovertebral junction was not observed.

In the reported patient, histopathological examination revealed transitional meningioma, which is classified as Grade I by the 2021 WHO Classification of CNS Tumors.[16] The histological image of transitional meningioma combines the characteristics of fibrous meningioma and endothelial meningioma.[17] Since this histological type of meningioma is relatively rare, evidence about their risk of recurrence is scarce. A recent study on 298 cases of transitional meningiomas reported recurrent tumors in 8.6% of patients.[18] For better estimation of the recurrence risk, p53, and Ki67 proliferation index may be helpful tools.[19] However, in our patient, these parameters were not evaluated.

The patient described in our case presented a constellation of characteristic symptoms and diseases, including spontaneous development of generalized seizures, autism spectrum disorder, and the presence of large, infratentorial meningioma. Considering additionally the young age, the genetic background could be suspected as the cause of the patient's complaints. However, genetic testing has not been conducted in the case of our patient and should be considered to elucidate the above disorders. Given the clinical image of the patient, genetic syndromes such as NF2, Cowden syndrome, BAP1 predisposition syndrome, or Rubinstein-Taybi syndrome could be considered in further differential diagnosis.[20]

One of the limitations of our case report was relatively short follow-up length. According to a long-term clinical study on meningioma patients, the recurrence rate was 13% and 38% for 10-year and 25-year follow-ups, respectively for total tumor resection (1-2 Simpson grades).[21] Therefore, the patient should remain under continuous radiological and clinical follow-up. Furthermore, we were not able to present the pictures from the surgical procedure, which would have been valuable for the presentation of our case. Nevertheless, this paper provides an excellent example of effective surgical treatment of large FMM with 2-year recurrence free-survival. Moreover, we did not observe post-operative complications such as vascular damage, neurological deficits, or infections.

Conclusion

Our case demonstrates that the far-lateral approach can be efficient for the resection of anterolateral FMMs. In such cases, Simpson grade 1 can be achieved without complications, providing immediate relief of symptoms and minimizing the risk of recurrence.

Declarations

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Author contributions

Conceptualization: R.R.; Methodology: M.S.; Software: Ł.D.; Investigation: L.S.; Resources: R.R., D.S.; Data Curation: A.D.; Writing – Original Draft: M.S., L.S., Ł.D., J.K.G, Z.H.; Writing – Review & Editing: W.C., R.R. Visualization: A.D.; Supervision: R.R., D.S., K.T.; Project administration: R.R.

Conflicts of interest

The authors declare that they have no competing interests.

Data availability

Not applicable.

Ethics approval

Patient signed informed consent regarding publishing their data and photographs.

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— **Each author** is expected to have made substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data; or the creation of new software used in the work; or have drafted the work or substantively revised it
— **AND** to have approved the submitted version (and any substantially modified version that involves the author's contribution to the study);
— **AND** to have agreed both to be personally accountable for the author's own contributions and to ensure that questions related to the accuracy or integrity of any part of the work, even ones in which the author was not personally involved, are appropriately investigated, resolved, and the resolution documented in the literature.

The Eur J Clin Exp Med does not require all authors of a research paper to sign the cover letter upon submission, nor do they impose an order on the list of authors. Submission to the Eur J Clin Exp Med is taken by the publication to mean that all the listed authors have agreed to all of the contents. The corresponding (submitting) author is responsible for having ensured that this agreement has been reached, and for managing all communication between the publication and all co-authors, before and after publication.

Author contributions statements

Authors are required to include a statement of responsibility in the manuscript (at the end of the main text, before the 'References' section) that specifies the contribution of every author. For articles with several authors, a short paragraph specifying their individual contributions must be provided. The following statements should be used "Conceptualization, X.X. and Y.Y.; Methodology, X.X.; Software, X.X.; Validation, X.X., Y.Y. and Z.Z.; Formal Analysis, X.X.; Investigation, X.X.; Resources, X.X.; Data Curation, X.X.; Writing – Original Draft Preparation, X.X.; Writing – Review & Editing, X.X.; Visualization, X.X.; Supervision, X.X.; Project Administration, X.X.; Funding Acquisition, Y.Y.".

Corresponding author – responsibilities

The corresponding (submitting) author is solely responsible for communicating with the Eur J Clin Exp Med and for managing communication between co-authors. Before submission, the corresponding author ensures that all authors are included in the author list, its order has been agreed by all authors, and that all authors are aware that the paper was submitted.

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The Eur J Clin Exp Med treats the submitted manuscript and all communication with authors and referees as confidential. Authors must also treat communication with the Eur J Clin Exp Med as confidential: correspondence with the Eur J Clin Exp Med, referee reports and other confidential material must not be posted on any website or otherwise publicized without prior permission from the Eur J Clin Exp Med publishing team, regardless of whether or not the submission is eventually published. Our policies about posting preprints and post prints, and about previous communication of the work at conferences or as part of a personal blog or of an academic thesis, are described in the Confidentiality section.

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During the submission process, please suggest three potential reviewers (names and institutional e-mail addresses) with the appropriate expertise to review the manuscript, but please keep in mind that we are not obliged to follow these recommendations. The proposed referees should neither be current collaborators of the co-authors nor have published with any of the co-authors of the manuscript within the last five years. Proposed reviewers should be from different institutions to the authors. You may suggest reviewers from among the authors that you frequently cite in your paper. You may also name a limited number of scientists who should not review your paper (up to 3 named individuals or laboratories); these exclusions will be honored. The decision of the Editorial Board Member on the choice of referees is final.

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For articles in the Eur J Clin Exp Med reporting experiments on live vertebrates and/or higher invertebrates, the methods section must include a statement: (i) identifying the institutional and/or licensing committee approving the experiments, including any relevant details; (ii) confirming that all experiments were performed in accordance with relevant guidelines and regulations.

For research involving human participants, authors must identify the committee that approved the research, confirm that all research was performed in accordance with relevant guidelines/regulations, and include in their manuscript a statement confirming that informed consent was obtained from all participants and/or their legal guardians.

Authors may be required to submit, on request, a statement from the research ethics committee or institutional review board indicating approval of the research.

Competing interests policy

In the interests of transparency and to help readers to form their own judgements of potential bias, authors must declare any competing financial and/or non-financial interests in relation to the work described. For the purposes of this policy, competing interests are defined as financial and non-financial interests that could directly undermine, or be perceived to undermine, the objectivity, integrity and value of a publication, through a potential influence on the judgements and actions of authors with regard to objective data presentation, analysis and interpretation. Examples of potential competing interests include employment, consultancies, stock ownership, honoraria, paid expert testimony, patent applications/registrations, and grants or other funding.

Competing interests statement format guidelines

The statement included in the article file must be explicit and unambiguous, describing any potential competing interest (or lack thereof) for EACH contributing author.

Examples of declarations are:

— Competing interests: The author(s) declare no competing interests.
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Availability of materials and data

In order to maintain the integrity, transparency and reproducibility of research records, authors are encouraged to make their experimental and research data openly available either by depositing into data repositories or by publishing the data and files as supplementary information in this journal.

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Data availability statement format guidelines

The statement should be provided as a separate section (titled 'Data Availability') at the end of the main text, before the 'References' section. Data availability statements should include, where applicable, accession codes, other unique identifiers and associated web links for publicly available datasets, and any conditions for access of non-publicly available datasets. Where figure source data are provided, statements confirming this should be included in data availability statements. Depending on the data described in the manuscript, data availability statements commonly take one of the following forms, or can be a composite of the statements below:

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— The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.
— All data generated or analyzed during this study are included in this published article (and its Supplementary Information files).
— The datasets generated during and/or analyzed during the current study are not publicly available due to [REASON(S) WHY DATA ARE NOT PUBLIC] but are available from the corresponding author on reasonable request.
— No datasets were generated or analyzed during the current study.
— The data that support the findings of this study are available from [THIRD PARTY NAME] but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. Data are however available from the authors upon reasonable request and with permission of [THIRD PARTY NAME].

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Publishable amendments must be represented by a formal online notice because they affect the publication record and/or the scientific accuracy of published information. Where these amendments concern peer-reviewed material, they fall

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Publisher Correction (formerly Erratum). Notification of an important error made by the journal that affects the publication record or the scientific integrity of the paper or the reputation of the authors or the journal.

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Once submitted, your manuscript will be assigned to a member of our Editorial Board, who will read the paper and decide whether it is appropriate for the journal. Manuscripts that are within scope and seem, on initial assessment, to be technically sound and scientifically valid, will be sent to external reviewers. Copies of any papers containing similar or related work under consideration or in press at other journals must be included with the submission.

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Peer review

Once a manuscript passes the initial checks, it will be assigned to at least two independent experts for peer-review. Reviewers will be able to access your manuscript securely using our online system, whilst maintaining referee anonymity. A double-blind review is applied, where authors' identities are unknown to reviewers and vice versa. Peer review comments are confidential and will only be disclosed with the express agreement of the reviewer.

Editorial decision

After considering the reviewer reports the Editorial Board Member will make one of the following decisions:

— Accept outright,
— Request a minor revision, where authors revise their manuscript to address specific concerns,
— Request a major revision, where authors revise their manuscript to address significant concerns and perhaps undertake additional work,
— Reject outright.

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In cases where the referees or Editorial Board Member has requested changes to the manuscript, you will be invited to prepare a revision. The decision letter will specify a deadline for submission of a revised manuscript. Once resubmitted, the manuscript may then be sent back to the original referees or to new referees, at the Editorial Board Member's discretion.

A revised manuscript should be submitted via the revision link provided in the decision letter, and not as a new manuscript. Authors should attach a cover letter to explain, *point by point*, the details of the revisions to the manuscript and responses to the referees' comments. Cover letters should not contain information that could identify the authors. The destination of the cover letter file in the submission system is 'Supplementary File for Review'. Please ensure that all issues raised have been addressed in the first round of revision. Where the authors disagree with a reviewer, they must provide a clear response.

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When all editorial issues are resolved, your paper will be formally accepted for publication. Once accepted, the manuscript will undergo professional copy-editing, English editing, final corrections, pagination, and, publication on the http://www.ejcem.ur.edu.pl/. The Eur J Clin Exp Med reserves the right to make the final decision about matters of style and the size of figures.

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Even in cases where the Eur J Clin Exp Med does not invite resubmission of a manuscript, some authors may ask the Editorial Board to reconsider a rejection decision. These are considered appeals, which, by policy, must take second place to the normal workload. In practice, this means that decisions on appeals often take several weeks. Only one appeal is permitted for each manuscript, and appeals can only take place after peer review. Final decisions on appeals will be made by the Editorial Board Member handling the paper.

Decisions are reversed on appeal only if the relevant Editorial Board Member is convinced that the original decision was a serious mistake. Consideration of an appeal is merited if a referee made substantial errors of fact or showed evidence of bias, but only if a reversal of that referee's opinion would have changed the original decision.

Similarly, disputes on factual issues need not be resolved unless they were critical to the outcome.

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A cover letter must be included with each manuscript submission. It should be concise and explain why the content of the paper is significant, placing the findings in the context of existing work and why it fits the scope of the journal. Confirm that neither the manuscript nor any parts of its content are currently under consideration or published in another journal. The names of proposed and excluded reviewers should be provided in the submission system, not in the cover letter.

Accepted file formats

Authors must use Microsoft Word to prepare their manuscript. Please insert your tables, graphics (schemes, figures, etc.) in the main text after the paragraph of its first citation.

In most cases, we do not impose strict limits on word count or page number. However, we strongly recommend that you write concisely and stick to the following guidelines:

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— The main text should be no more than 4,500 words (not including Abstract, Methods, References and figure legends).
— The title should be no more than 20 words.
— The abstract should be no more than 250 words.
— Recommended font: Times New Roman, 12 points.
— Manuscript text should be double-spaced. Do not format text in multiple columns.

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Manuscripts submitted to the Eur J Clin Exp Med should neither be published previously nor be under consideration for publication in another journal. The main article types are as follows:

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The Eur J Clin Exp Med accepts also the following types of submissions: case reports, letters to the editor, commentaries, book reviews, and reports from scientific meetings and conferences.

Reporting guidelines

The guidelines listed below should be followed where appropriate. Please use these guidelines to structure your article. Completed applicable checklists, structured abstracts and flow diagrams should be uploaded with your submission; these will be published alongside the final version of your paper.

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— ARRIVE guidelines for *in vivo* animal studies
— CARE guidelines for clinical case reports
— CONSORT guidelines for clinical trials
— PRISMA guidelines for systematic reviews and meta-analyses
— SPIRIT for clinical trials
— STARD guidelines for studies of diagnostic accuracy
— STROBE guidelines for observational studies

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Your paper should consist of the following parts. Title page should be supplied as a **separate** file.

Research manuscripts should comprise:

— Title page: Title, Author list, Affiliations, Abstract, Keywords.
— Research manuscript sections: Introduction, Aim, Materials and Methods, Results, Discussion, Conclusions.
— Back matter: Supplementary Materials, Acknowledgments, Funding Statement, Author Contributions,

Conflicts of Interest, Data Availability, Ethics Approval, References.

Research manuscript sections:

— *Introduction*

State the objectives of the work and provide an adequate background, avoiding a detailed literature survey or a summary of the results.

— *Material and methods*

Provide sufficient details to allow the work to be reproduced by an independent researcher. Methods that are already published should be summarized, and indicated by a reference. If quoting directly from a previously published method, use quotation marks and also cite the source. Any modifications to existing methods should also be described.

— *Results*

Results should be clear and concise. The section may be divided into subsections, each with a concise subheading. Tables and figures central to the study should be included in the main paper. Do not use the term "significant" unless p-values are provided. Show p-values to 2 or 3 decimal places. The Results section should be written in past tense.

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This should explore the significance of the results of the work, not repeat them. Avoid extensive citations and discussion of published literature.

— *Conclusions*

Summarize the work's findings, state their importance, and possibly recommend further research.

Review manuscripts should comprise:

— Title page: Title, Author list, Affiliations.
— Abstract, Keywords, Literature review sections.
— Back matter: Supplementary Materials, Acknowledgments, Funding Statement, Author Contributions, Conflicts of Interest, Data Availability, References.

Structured reviews and meta-analyses should use the same structure as research articles and ensure they conform to the PRISMA guidelines.

Case reports should comprise:

— Title page: Title, Author list, Affiliations.
— Abstract, Keywords. Case reports should include a succinct introduction about the general medical condition or relevant symptoms that will be discussed in the case report; the case presentation including all of the relevant de-identified demographic and descriptive information about the patient(s), and a description of the symptoms, diagnosis, treatment, and outcome; a discussion providing context and any necessary explanation of specific treatment decisions; a conclusion briefly outlining the take-home message and the lessons learned.
— Back matter: Supplementary Materials, Acknowledgments, Funding Statement, Author Contributions, Conflicts of Interest, Data Availability, Ethics Approval, References.

Requirements for case reports submitted to Eur J Clin Exp Med:

— Patient ethnicity must be included in the Abstract under the Case Presentation section.
— Consent for publication is a mandatory journal requirement for all case reports. Written informed consent for publication must be obtained from the patient (or their parent or legal guardian in the case of children under 18, or from the next of kin if the patient has died).

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Manuscripts must be submitted in English (American or British usage is accepted, but not a mixture of these).

Title page

These sections should appear in all manuscript types:

Title: The title of your manuscript should be concise and informative. It should identify if the study reports (human or animal) trial data, or is a systematic review, meta-analysis or replication study. When gene or protein names are included, the abbreviated name rather than full name should be used.

Author list and affiliations: Authors' full first and last names must be provided. For each affiliation provide the details in the following order: department, institution, city, country. If available, the e-mail address of each author should also be provided. At least one author should be designated as *corresponding author*, and his or her email address and other details should be included at the end of the affiliation section.

Abstract: The abstract should be a total of about 250 words maximum. The abstract should be a single paragraph and should follow the style of structured abstracts: *Introduction and aim:* Place the question addressed in a broad context and highlight the purpose of the study; *Material and methods*: Describe briefly the main methods or treatments applied. Include any relevant preregistration numbers, and species and strains of any animals used. *Results:* Summarize the article's main findings; and *Conclusion*: Indicate the main conclusions or interpretations.

Keywords: Three to six pertinent keywords need to be added after the abstract in alphabetical order. We recommend that the keywords are specific to the article, yet reasonably common within the subject discipline.

Back matter

Supplementary materials: Describe any supplementary material published online alongside the manuscript (figure, tables, video, spreadsheets, etc.). Please indicate the name and title of each element as follows Figure S1: title, Table S1: title, etc.

Acknowledgments: Thank all of the people who helped with the research but did not qualify for authorship. Acknowledge anyone who provided intellectual assistance, technical help, or special equipment or materials.

Funding statement: All sources of funding of the study should be disclosed.

Author contributions: Authors must supply an Author Contribution Statement as described in the *Author contributions statements* section.

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Data availability: Authors must include a Data Availability Statement in all submitted manuscripts; see *Availability of materials and data* section for more information.

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References: References must be numbered in order of appearance in the text (including table captions and figure legends) and listed individually at the end of the manuscript. We recommend preparing the references with a bibliography software package, such as EndNote, Reference Manager or Zotero to avoid typing mistakes and duplicated references.

References style

In-text citations and references should be prepared according to the American Medical Association (AMA) style. Each item should be listed in numerical order.

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Each reference should be cited in the text using superscript arabic numerals. These superscript numbers should be outside periods. If you are citing sequential references, these should be indicated with a hyphen. Nonsequential references should be separated with commas. There should not be a space between numbers. For example: The degree of respiratory muscles fatigue depends on the applied exercise protocol and the research group's fitness level.[1,2] The greatest load with which a patient continues breathing for at least one minute is a measure of inspiratory muscles strength.[3] Diabetes mellitus is associated with a high risk of foot ulcers.[4-6]

Sample Reference

In listed references, the names of all authors should be given unless there are more than 6, in which case the names of the first 3 authors are used, followed by "et al.". If the source does not have any authors, the citation should begin with the title.

To find the proper abbreviation of journal go to the National Library of Medicine PubMed Journals Database at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=Journals.

Page number(s) should be inserted in full (for example: use 111–112, not 111–2).

The following are examples of individual citations made according to the required rules of editing and punctuation:

— **Article from a journal, number of authors from 1 to 6**

Author AA, Author BB, Author CC. Title of article. *Accepted Abbreviated Journal Title*. Year;Volume(Issue):Page-Page. doi (if available)

Lee JC, Seo HG, Lee WH, Kim HC, Han TR, Oh BM. Computer-assisted detection of swallowing difficulty. *Comput Methods Programs Biomed*. 2016;134(2):72-78. doi: 10.1016/j.cmpb.2016.07.010

Morris A. New test for diabetes insipidus. *Nat Rev Endocrinol*. 2019;15(10):564-565. doi: 10.1038/s41574-019-0247-x

— ***Article from a journal, number of authors* more *than 6***

Author AA, Author BB, Author CC, et al. Title of article. *Accepted Abbreviated Journal Title*. Year;Volume(Issue):Page-Page. doi (if available)

Gonzalez ME, Martin EE, Anwar T, et al. Mesenchymal stem cell-induced DDR2 mediates stromal-breast cancer interactions and metastasis growth. *Cell Rep*. 2017;18:1215-1228. doi: 10.1016/j.celrep.2016.12.079

Jordan J, Toplak H, Grassi G, et al. Joint statement of the European Association for the Study of Obesity and the European Society of Hypertension: obesity and heart failure. *J Hypertens*. 2016;34:1678-1688. doi: 10.1097/HJH.0000000000001013

— **Websites**

Author AA (if indicated). Webpage title. Name of Website. URL. Published or Updated date. Accessed date.

Cholera in Haiti. Centers for Disease Control and Prevention Web site. http://www.cdc.gov/haiticholera/. Published October 22, 2010. Updated January 9, 2012. Accessed February 1, 2012.

Address double burden of malnutrition: WHO. World Health Organization site. http://www.searo.who.int/mediacentre/releases/2016/1636/en/. Accessed February 2, 2017.

— **Book**

Author AA, Author BB. *Title of Work*. Location: Publisher; Year:Page-Page

Doane GH, Varcoe C. *Family Nursing as Relational Inquiry: Developing Health– Promoting Practice*. Philadelphia, PA: Lippincott Williams & Wilkins; 2005:25-28.
London ML, Ladewig PW, Ball JW, et al. *Maternal & Child Nursing Care*. Upper Saddle River, NJ: Pearson Education; c2011:101-103.
— Chapter in a book
Chapter Author AA. Title of chapter. In: *Name of Book*. Edition Number. Editor AA, ed. Location: Name of Publisher; Year:Page-Page.
Grimsey E. An overview of the breast and breast cancer. In: *Breast Cancer Nursing Care and Management*. 2nd ed. Harmer V, ed. Chichester, UK: Wiley-Blackwell; 2011:35-42.
NOTE: The Editorial Board requires consistent and carefully made references prepared according to the above-mentioned AMA standards. Otherwise, the work will be sent back to the authors.

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File for Figures and Schemes must be provided during submission and at a sufficiently high resolution (minimum 1000 pixels width/height, or a resolution of 300 dpi or higher). Common formats are accepted, however, TIFF, JPEG, EPS and PDF are preferred.

Please ensure the figures and the tables included in the single file are placed next to the relevant text in the manuscript, rather than at the bottom or the top of the file. The corresponding caption should be placed directly below the figure (not on the figure itself) or above the table. All figures, schemes, and tables should be numbered following their number of appearance (Figure 1, Scheme 1, Figure 2, Scheme 2, Table 1, etc.).

Tables should present new information rather than duplicating what is in the text. Readers should be able to interpret the table without reference to the text.

All table columns should have an explanatory heading. To facilitate the copy-editing of larger tables, smaller fonts may be used, but no less than 8 pt. in size. Tables must be provided in an editable format in appropriate place in the main text. Tables provided as jpeg/tiff files will not be accepted. Do not submit your tables in separate files.

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The journal requires using only standard abbreviations. Abbreviations should be defined in parentheses the first time they appear in the abstract, main text and in figure or table captions and used consistently thereafter. Ensure consistency of abbreviations throughout the article. Keep abbreviations to a minimum.

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SI Units (International System of Units) should be used. Imperial, US customary and other units should be converted to SI units whenever possible.