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Peyronie's disease in the early phase: what to do?

Livia Fratelli[1], Camila Klotz[1], Luis Cesar Fava Spessoto[2], Fernando Nestor Facio Junior[2*]

Peyronie's disease (PD) is an abnormality characterized by fibrosis of the tunica albuginea that can be accompanied by pain, deformity, erectile dysfunction, discomfort, and/or dissatisfaction with one's self-image[1]. The prevalence ranges from 0.5 to 20.3%, but recent studies indicate underestimated data[1].

In a study of guidelines for the diagnosis and treatment of PD of the American Urological Association (AUA), International Society for Sexual Medicine (ISSM), Canadian Urological Association (CUA), and European Association of Urology (EAU), Manka et al.[2] reported that oral therapies present low level of evidence. Penile traction and intralesional injections are therapeutic options with unsatisfactory resullts[3]. There is consensus that the initial phase implies stability of the penile curvature for at least 3 months as well as a minimum period of 12 months without symptoms. Surgery should be reserved only after the stabilization of the disease.

Therefore, what should the clinical approach be during the first 12 months? This is a critical period in which many men deal not only with pain, erectile dysfunction, and deformity, the oral medicinal treatment of which is ineffective, but may also experience depression, low self-esteem, difficulty or inability having sexual relations, restrictions to intimacy, social isolation, and stigmatization[4].

Considering the lack of standardization in the available literature on PD, much information used for therapeutic counseling of patients is based on a low level of evidence[1]. Thus, physicians face an ethical dilemma. Oral therapies are indicated without adequate scientific evidence, whereas patients deal with psychological and social issues concomitantly to the disease. One can say that both the physician and patient find themselves helpless in this period, despite the advances of current medicine.

Thus, there is a need for long-term clinical trials in the early phase of treatment. In the meantime, patients should be duly counseled on the risks and benefits of current therapies, highlighting sharing in the definition of the conduct with the physician and multidisciplinary team.

AUTHORS' CONTRIBUTIONS

LF: Conceptualization, Formal Analysis, Investigation, Methodology, Project administration, Validation, Writing – original draft, Writing – review & editing. **CK:** Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Validation, Writing – original draft, Writing – review & editing. **LCFS:** Conceptualization, Investigation, Project administration, Supervision, Writing – review & editing. **FNF:** Conceptualization, Formal Analysis, Project administration, Resources, Supervision, Validation, Visualization, Writing – review & editing.

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Visually evoked potentials may be abnormal in COVID-19 patients if the infection is complicated by cerebral disease

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Dear Editor,

We read with interest Balduz and Fidancı's article about a prospective, single-centre, case-control study on the difference between pattern reversal visually evoked potentials (VEPs) and flash VEPs in 44 patients with recent SARS-CoV-2 infection compared to 40 controls[1]. Pattern reversal VEPs did not differ between COVID-19 patients and controls, but right-side P2 latency in flash VEPs was prolonged in COVID-19 patients[1]. A total of 13 patients had increased P2 latency[1]. The study is impressive, but several points require discussion.

The first point is that the conclusions drawn are unsupported[1]. The number of patients was too low and the mono-centric design was inappropriate to draw such conclusions. Since only a single parameter was abnormal (P2 latency on the right side), it is quite unlikely that VEPs are generally abnormal in COVID-19 patients in the absence of severe neurological complications.

The second point is that the cause of right P2 prolongation has not been reported[1]. We should know the results of clinical neurological examination, cerebral imaging, electroencephalography (EEG), and cerebrospinal fluid (CSF) analysis in all patients with P2 prolongation. Has a neurological cause been identified that could explain the finding?

The third point is that the cause of the headache was not specified in almost two-thirds of patients[1]. We should know whether these patients had primary or secondary headache, history of headache, or experienced headache after SARS-CoV-2 infection. The most common causes of secondary headache reported in association with SARS-CoV-2 infection include arterial hypertension, meningitis/encephalitis, intracerebral bleeding, subarachnoid bleeding, venous sinus thrombosis, reversible cerebral vasoconstriction syndrome, dissection, sleep disorders, or desiccosis, which must be thoroughly excluded.

The fourth point is that it remained unclear why P2 was prolonged on the right side but not on the left side. Assuming that P2 prolongation was due to SARS-CoV-2 infection, one would expect bilateral rather than unilateral prolongation. How do the authors explain this unusual finding?

The fifth point is that differential diagnoses, such as new-onset multiple sclerosis, new-onset neuromyelitis optica spectrum disorder (NMO-SD), MOG-associated disorder (MOG-AD), acute disseminated encephalomyelitis (ADEM), posterior reversible encephalopathy syndrome (PRES), acute, hemorrhagic necrotising encephalopathy (AHNE), acute, hemorrhagic leukoencephalitis (AHLE), and acute, necrotizing encephalopathy (ANE), were not adequately excluded. Since these conditions can be associated with unilateral P100 or P2 prolongation[2,3], it is imperative to have them off the table before attributing them to SARS-CoV-2 infection.

The sixth point is that the origin of reference limits was not described. We should know whether reference limits for VEP parameters were generated by the authors themselves or were taken from the literature or a book.

In summary, the excellent study has limitations that should be addressed before drawing final conclusions. Clarifying the weaknesses would strengthen the conclusions and improve the study. VEPs in COVID-19 patients may only be abnormal when the visual pathway is compromised by SARS-CoV-2 infection, which is the case with encephalitis, stroke, bleeding, or immunological disease triggered by COVID-19.

ETHICS

The study used only secondary data.

AUTHORS' CONTRIBUTIONS

JF: Conceptualization, Formal Analysis, Investigation, Methodology, Validation, Writing – original draft. **CAS:** Validation, Writing – review & editing. **FAS:** Validation, Writing – review & editing.

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Clinical effects of music therapy on menopausal symptoms

Maria Eduarda da Macena Tenorio[1], Williames Matheus Malaquias da Silva[1], Brenda dos Santos Teixeira[1], Wendel Aguiar Carlini[1], Mylenne Alinne Falcão de Paiva[1], Johnnatas Mikael Lopes[1*]

Dear Editor,

Over time, women begin to suffer from the interruption of their ovulatory cycles, the transition to the non-reproductive period, known as climacteric, with the last date of menstrual bleeding being defined as menopause[1]. During the climacteric period, each woman needs to be evaluated individually, so that her treatment is carried out appropriately, as the endocrine, psychological, and social factors lead to climacteric syndrome. The most common symptoms during the transitional phase are vasomotor symptoms, also known as hot flushes or hot flashes. In addition, women often experience mood changes, irritability, insomnia, urinary incontinence, as well as an increased risk of vaginal infections and osteoporosis[2].

The study by Ugurlu et al., entitled "The effect of music on menopausal symptoms, sleep quality, and depression: a randomized controlled trial"[3], presented us with a non-drug therapeutic approach for managing menopausal symptoms. However, only a statistical perspective was presented, with information on the clinical utility of the approach being absent.

Based on this gap, we developed a standardized clinical effect measure in order to understand the practical usefulness of music therapy in the management of menopause. Accepting that the measurements presented in tables 2 and 3 are means and standard deviations of the outcomes determined by the paired Student's t-test in some situations, we constructed our clinical effect measurements by estimating the unbalanced Cohen's d[4] (Table 1).

The clinical effect on the general symptomatology of music therapy is considered moderate (d=0.66). However, when observing its dimensions, it is clear that music therapy is more effective in psychological symptoms, which are of great magnitude (d=0.84), the same being evidenced by the Beck depression index (d=0.91). On the other hand, the effects on somatic symptoms are of small magnitude (d=0.46). Urogenital symptoms confirmed the absence of significant differences between the groups with a trivial clinical effect (d=0.08).

Finally, sleep quality was also affected by the music therapy approach with great clinical implications (d=0.75). This effect probably occurs due to the strong relationship between psychological state and sleep regulation. Understanding these effects in a standardized way and with a clinical perspective reveals

Table 1. Measurement of clinical effects on somatic, psychological, and urogenital outcomes based on unbalanced Cohen' d.

	x1	x2	SD1	SD2	x1-x2	SDm	N	√SD	Cohen's d
MRS total	12.40	16.00	6.51	4.12	-3.6	1738.255	59	5.427886	-0.66324
Somatic symptoms	4.97	5.87	2.13	1.69	-0.9	217.2531	59	1.918921	-0.46901
Psychological symptoms	4.57	7.16	3.41	2.71	-2.59	557.5379	59	3.074052	-0.84254
Urogenital symptoms	2.87	3.06	2.13	2.13	-0.19	267.6771	59	2.13	-0.0892
BDI	7.5	12.77	4.33	6.82	-5.27	1939.09	59	5.732882	-0.91926
PSQI	6.23	8.16	2.58	2.55	-1.93	388.1106	59	2.56479	-0.7525

x1: intervention group average; x2: mean of the control group; SD1: standard deviation of the intervention group; SD2: standard deviation of the control group; SDm: mean standard deviation; N: total participants minus the number of groups; √SD: square root of SDm; Cohen's d: (x1-x2)/√SD.

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the usefulness of the music therapy approach as a therapeutic option in the management of psychobehavioral disorders during menopause.

Furthermore, it is worth highlighting that not using more elaborate statistical strategies such as Mixed Models reduces the understanding of the effect of individual variability on experiments, as each individual evolves at a different rate over the treatment period, which we call a random effect and this becomes necessary to be taken into account when analyzing data through methods called mixed models[5]. This is valid, as a higher concentration of low education in the control group is also observed in the data.

AUTHORS' CONTRIBUTIONS

MEMT: Formal Analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **WMMS:** Formal Analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **BST:** Formal Analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **WAC:** Formal Analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **MAFP:** Formal Analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **JML:** Conceptualization, Formal Analysis, Investigation, Methodology, Project administration, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

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ORIGINAL ARTICLE

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Effects of virtual reality and nature sounds on pain and anxiety during hysterosalpingography: a randomized controlled trial

Nazlı Baltaci[1*], Sümeyye Bal[2], Emine Koç[2], Elif Keten Edis[3]

SUMMARY

OBJECTIVE: The objective of this study was to determine the effects of listening to nature sounds alone and virtual reality plus listening to nature sounds on pain and anxiety in hysterosalpingography.

METHODS: This three-arm parallel randomized controlled trial included 135 (45 in each group) women who underwent hysterosalpingography in Turkey. The virtual reality+nature sounds group viewed a nature video with virtual reality glasses and listened to nature sounds during hysterosalpingography, whereas the nature sounds group only listened to nature sounds. The control group received only routine care.

RESULTS: During hysterosalpingography, women in virtual reality+nature sounds group experienced less pain than those in control group (p=0.009). After hysterosalpingography, pain levels were lower in both virtual reality+nature sounds group and nature sounds group than in control group (p=0.000 and p=0.000, respectively), anxiety levels were lower in virtual reality+nature sounds group than in nature sounds group and control group (p=0.018 and p=0.000, respectively), and anxiety levels were lower in nature sounds group than in control group (p=0.013).

CONCLUSION: Virtual reality with nature content plus listening to nature sounds and only listening to nature sounds are effective in reducing pain and anxiety related to hysterosalpingography procedures in women. Compared with only listening to nature sounds, virtual reality plus listening to nature sounds further reduced hysterosalpingography-related pain and anxiety.

KEYWORDS: Anxiety. Hysterosalpingography. Nature. Pain. Virtual reality.

INTRODUCTION

Hysterosalpingography (HSG) is an uncomfortable and invasive procedure associated with fear, anxiety, and pain[1]. HSG is the radiological evaluation of the tuba uterinas, ovaries, and uterus by transcervical administration of contrast material in the investigation of the causes of infertility. This invasive and risky method is widely used for diagnosis and treatment with the help of instruments inserted into the uterus[2,3]. Various studies have reported that women experience moderate or severe pain during the HSG procedure, with an incidence of 59.3–93.7%[3-5]. It is known that women experience anxiety about the uncertainty before undergoing HSG and the possible pain, which can negatively affect the procedure, and are reluctant to undergo the procedure[1].

Different pharmacological agents and applications have been used to reduce pain among women during HSG[6]. However, to date, there is no ideal method that can be recommended to reduce pain during HSG. Some randomized controlled trials on integrated medicine have reported that during HSG, primrose and transabdominal pelvic hot water bag applications can reduce pain[7,8] and acupuncture can reduce anxiety[4].

Virtual reality (VR) is one of the recent important technological developments that can be used as a noninvasive and effective analgesic method during painful invasive procedures[9]. As a relaxation technique, VR can suppress adrenaline secretion and increase endorphin and oxytocin secretion. It can reduce pain by regulating mood and hormonal effects by diverting attention[10]. VR is used in women's health, such as colposcopy, cystoscopy, psychological problems[11], hysteroscopy[12], cesarean section[13], and labor pain[14].

The physical and mental unity of individuals with nature is closely related to their health. It has been reported that watching nature accelerates the healing process and nature calms individuals by diverting attention[15]. Nature sounds (NS) are one of the interventions used to reduce anxiety in recent years. The sounds of the sea, rain, wind, river, birds, and other animals in nature can increase parasympathetic activity and reveal

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physiological and psychological responses[15,16]. NS have been reported to reduce pain after cesarean section[17] and provide psychological and physiological relaxation[16].

To the best of our knowledge, while there are only two studies on the effectiveness of VR in women undergoing HSG[1,18], there is no study on the effectiveness of NS. Furthermore, no studies have investigated the effects of using VR with nature content plus listening to NS on pain and anxiety in women before and during HSG. Furthermore, it has been stated that there is a need for studies that investigate the effects of VR accompanied by music on pain and anxiety before and during HSG[10]. Therefore, the study aimed to determine the effects of listening to NS alone and VR plus listening to NS on pain and anxiety in women before and during HSG.

METHODS

Research type

This was a three-arm parallel randomized controlled trial with a pretest–posttest experimental design. The study was conducted in accordance with the CONSORT guidelines and was registered on ClinicalTrials.gov (registration number: NCT05192343).

Setting and sample

The women who wanted to participate in the study were recruited from the radiology center of a university hospital in northern Turkey between February and August 2022. Inclusion criteria were as follows: women who underwent HSG, those aged>18 years, and those who could read and speak Turkish. Exclusion criteria were as follows: women with psychological and mental health problems and those with hearing or visual impairments.

The sample size was calculated with a 95% confidence level ($1 - \alpha$), 95% test power ($1 - \beta$), and an effect size of 0.804 (d) by performing power analysis according to the pain level reported in a previous study[4]. Accordingly, the minimum sample size was calculated as 36 women in each group. Considering possible data loss, this study included a total of 135 women, with 45 in each group.

Randomization and blinding

Randomization into one of the three study groups was performed by a statistician blinded to the participants using the Quickcalcs GraphPad program (http://www.graphpad.com/quickcalcs/, accessed on January 20, 2022) (Figure 1). Owing to the nature of the biobehavioral interventions, participants and researchers could not be blinded.

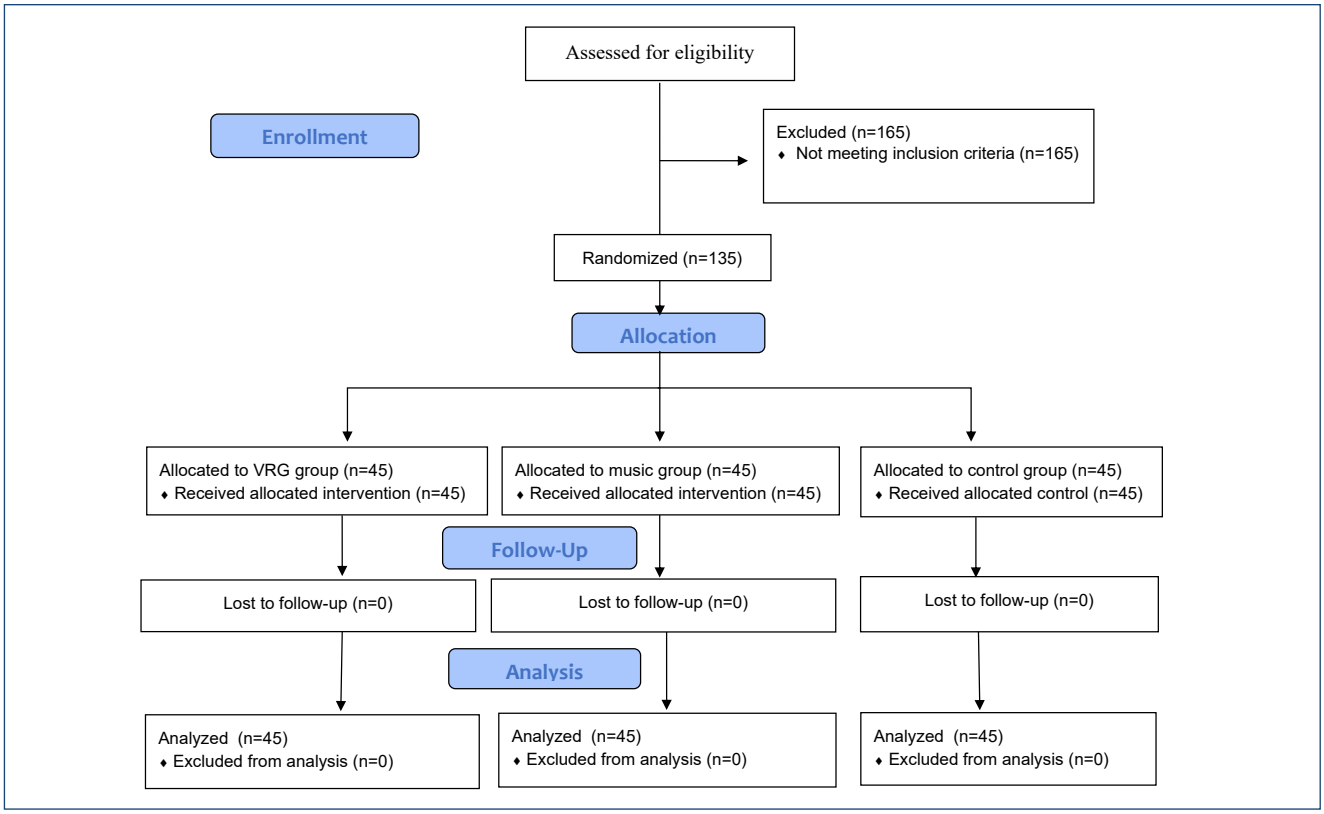

Figure 1. CONSORT flow diagram.

Interventions

The women in VR plus listening to NS group (VR+NSG) viewed a relaxing video with nature content by listening to the sounds of nature with 360° VR glasses for 30 min and 15 min before and during the HSG procedure, respectively. The use of VR glasses was explained to the women before the procedure. The women in the listening to NS group (NSG) only listened to a recording of NS for 30 min and 15 min before and during the HSG procedure, respectively. The recording included sounds of water, waves, rain, wind, dolphins, birds, and forests from Savva's (2019) CD entitled "NS for Babies" and from the CD entitled "Nature Symphonies" (2007). As suggested in the relevant literature[19], NS used in this study were relaxing, with a soft melody, a slow rhythm (ranging between 60 and 70 beats per minute, as measured using a metronome), and consistent with the normal heart rate. Before performing the HSG procedure, NS were played in a quiet room with an MP3 music player and disposable earbuds to prevent the women from being affected by ambient sounds. The control group (CG) only received routine care.

Data collection

The data were collected with the "Women Information Form" developed by the researchers in line with the relevant literature[5,7,17], "Visual Analog Scale (VAS)," and "Spielberger State–Trait Anxiety Inventory (STAI)."

Visual Analog Scale

Visual Analog Scale is a one-dimensional 0–10-cm-long scale developed by Hayes and Patterson in 1921, which is commonly used to measure pain intensity. The scale parameters range from "no pain (0)" to "intolerable pain (4)." Higher scores indicate greater pain severity[20]. In this study, pain was evaluated during and 15 min after the HSG procedure.

State–Trait Anxiety Inventory

State–Trait Anxiety Inventory was developed by Spielberger et al.[21], and the Turkish validity and reliability study of this inventory was conducted by Öner and Le Compte[22]. It includes two self-report scales: a 4-point Likert type and a 20-item state and trait anxiety inventory. Scores between 20 and 80 can be obtained from both scales. The higher the score obtained from the scales, the higher the level of anxiety. The Cronbach's alpha reliability coefficients were 0.83–0.92[22]. In this study, anxiety was evaluated immediately before and 15 min after the HSG procedure.

Ethics

This study was approved by the Clinical Research Ethics Committee of Ondokuz Mayıs University (decision dated 30.09.2021, numbered 2021/435). The study was conducted on a voluntary basis, and written informed consent of the women was obtained before the study.

Data analysis

In the IBM SPSS Statistics Version 23.0 program, analysis of variance (ANOVA) and Tukey test were used for the comparison of VAS and STAI scores between groups, while paired-sample t-test was used for the comparison within groups. Statistical significance was indicated by $p<0.05$.

RESULTS

In this study, between February and August 2022, 300 participants were assessed for eligibility, 165 of whom were excluded. Finally, 135 participants were included and randomly assigned to VR+NSG (n=45), NSG (n=45), and CG (n=45). After randomization, none of the participants discontinued intervention. Overall, 45 participants in each group completed the study and were analyzed (Figure 1).

Table 1 shows that the groups were similar, and there was no significant difference between the groups in terms of their age, duration of marriage, educational and employment status, body mass index, duration of menstruation, menstrual pattern, dysmenorrhea, having children, undergoing HSG for the first time, and receiving HSG information ($p>0.05$) (Table 1).

A significant difference was found between the groups in terms of pain levels during the HSG procedure ($p=0.013$). There was no difference between the pain levels of VR+NSG and NSG during the procedure ($p>0.05$), but the pain level of VR+NSG was significantly lower than that of CG ($p=0.009$). Moreover, a significant difference was found between the groups in terms of pain levels after the HSG procedure ($p=0.000$). After the procedure, the pain levels of both VR+NSG and NSG were significantly lower than those of CG ($p=0.000$ and $p=0.000$, respectively). Regarding within-group comparisons, there was a significant difference in the VAS scores of the intervention groups ($p=0.000$) (Table 2).

A significant difference was found between the groups after the procedure ($p=0.000$). Anxiety levels were significantly lower in VR+NSG than in NSG and CG ($p=0.018$ and $p=0.000$, respectively). Similarly, anxiety levels were significantly lower in NSG than in CG ($p=0.013$). Regarding within-group comparisons, there was a significant difference in the STAI scores after the procedure in VR+NSG ($p=0.004$) (Table 2).

Table 1. Descriptive characteristics of women.

Variables	Virtual reality+nature sounds group (n=45)	Nature sounds group (n=45)	Control group (n=45)	p-value
Age (years)	29.55 (6.09)	30.11 (5.99)	28.60 (4.15)	0.420[a]
Duration of marriage (years)	5.13 (5.19)	3.24 (2.14)	4.13 (2.89)	0.053[a]
Education status				
Secondary school	10 (22.2)	4 (8.9)	5 (11.1)	
High school	9 (20.0)	9 (20.0)	7 (15.6)	0.339[b]
University	26 (57.8)	32 (71.1)	33 (73.3)	
Employment status				
Employed	17 (37.8)	22 (48.9)	18 (40.0)	0.529[b]
Unemployed	28 (62.2)	23 (51.1)	27 (60.0)	
Body mass index				
Underweight	2 (4.4)	1 (2.2)	1 (2.2)	
Normal	22 (48.9)	19 (42.2)	23 (51.1)	0.931[b]
Overweight	9 (20.0)	13 (28.9)	11 (24.4)	
Obese and morbidly obese	12 (26.7)	12 (26.7)	10 (22.2)	
Duration of menstruation (days)	6.04 (1.67)	5.55 (1.82)	6.17(1.89)	0.230[a]
Menstruation pattern				
Regular	36 (80.0)	38 (84.4)	33 (73.3)	0.425[b]
Irregular	9 (20.0)	7 (15.6)	12 (26.7)	
Dysmenorrhea				
Yes	28 (62.2)	34 (75.6)	26 (57.8)	0.183[b]
No	17 (37.8)	11 (24.4)	19 (42.2)	
Having children				
Yes	11 (24.4)	8 (17.8)	13 (28.9)	0.459[b]
No	34 (75.6)	37 (82.2)	32 (71.1)	
Undergoing HSG for the first time				
Yes	32 (71.1)	40 (88.9)	33 (73.3)	0.087[b]
No	13 (28.9)	5 (11.1)	12 (26.7)	
Receiving hysterosalpingography information				
Yes	31 (68.9)	32 (71.1)	29 (64.4)	0.788[b]
No	14 (31.1)	13 (28.9)	16 (35.6)	

Categorical variables are presented as n (%) and continuous variables are presented as mean (SD). [a]One-way analysis of variance (ANOVA); [b]chi-square test.

Table 2. Pain and anxiety levels of women according to groups and measurement times.

VAS	Virtual reality+nature sounds group (n=45)	Nature sounds group (n=45)	Control group (n=45)	Between groups p-value[a]
During HSG	8.15 (2.28) x	8.71 (1.96) y	9.33 (1.16) z	0.013
After HSG	3.62 (1.89) x	3.46 (2.71) y	9.26 (3.03) z	0.000
Within group p-value[b]	0.000	0.000	0.183	
STAI				
Pre-HSG	90.37(12.88) x	87.77 (8.86) y	87.24 (6.95) z	0.277
Post-HSG	85.42 (8.07) x	90.02 (9.51) y	94.80 (5.64) z	0.000
Within group p-value[b]	0.004	0.125	0.000	

Data are presented as mean (SD). [a]One-way analysis of variance (ANOVA); [b]paired-sample t-test; x, y, and z: according to the Tukey test, there is no difference between data indicated by the same letter.

DISCUSSION

Hysterosalpingography is an invasive procedure that can cause anxiety and pain among women. In this study, although the pain levels in the intervention groups were similar during and after HSG, the pain level in VR+NSG was significantly lower than that in CG. In addition, the pain level decreased significantly in VR+NSG after the procedure. It has been mentioned that seeing nature views or spending time in nature can reduce anxiety or negative physical reactions[15]. It is known that the use of VR reduces pain levels and analgesic use during invasive procedures in various patient groups[9,14,23]. Similar to our result, in two randomized controlled studies, women in the VR group had lower pain levels 15 min after HSG than in the CG[1,18]. Similarly, in this study, the use of VR plus listening to NS was effective in reducing pain during and after the injection of a contrast agent into the uterus—the most painful step in HSG.

Anxiety caused by uncertainty related to invasive procedures occurs during HSG and can increase pain perception[1]. In this study, in addition to the pain level, the anxiety level in VR+NSG significantly decreased after the procedure compared with the anxiety level before the procedure and the level in CG. VR can virtually remove an individual from the medical environment and reduce negative emotions by shifting the focus to pleasant stimuli[10]. Some studies on women's health have used VR to reduce anxiety[12,13,23,24]. Different from these studies, in the randomized controlled study of Sezer et al.[1] the anxiety level of the VR group was not different from the CG during HSG. Furthermore, in this study, anxiety levels after HSG were found to be lower in VR+NSG than in NSG. This finding may be attributed to the following reasons: individuals in VR+NSG were able to focus on the sounds and images of nature; individuals were visually and spiritually removed from the foreign medical environment; and individuals could relax while using images and sounds of nature, which simultaneously affected different senses.

Nature sounds have an intercultural, pleasing, and preferred structure. Listening to NS is a physically and mentally relaxing experience[16]. In this study, we found that the postprocedure level of pain in NSG was lower than the preprocedure level and the level in CG. Similarly, some studies have reported that natural sounds are effective in reducing pain in patients[16,17]. In this study, postprocedure anxiety levels of NSG were higher than those of VR+NSG and lower than those of CG. Studies have found that NS reduce stress and provide psychological relaxation[15,16,25]. In this study, women experienced less anxiety and pain when listening to NS before and during HSG, which may be attributed to the relaxing effects of NS.

This study has two limitations. First, the findings of this study were limited to women who underwent the procedure at a single center. Second, the natural sounds used in the study were preselected by the researcher.

CONCLUSION

Virtual reality with nature content plus listening to NS and only listening to NS are effective in reducing pain and anxiety related to HSG procedures in women. Compared with only listening to NS, VR plus listening to NS further reduced HSG-related pain and anxiety. This method is an effective, easy-to-apply, noninvasive, and nonpharmacological method to reduce pain and anxiety in women undergoing HSG. The effectiveness of the combined use of VR and NS on pain and anxiety in different patient populations and invasive procedures can be investigated.

ETHICAL APPROVAL

This study adhered to the principles of the Declaration of Helsinki. This study was approved by the Clinical Research Ethics Committee of Ondokuz Mayıs University (dated 30.09.2021, numbered 2021/435). The study was conducted on a voluntary basis, and written informed consent of pregnant women was obtained before the study.

AUTHORS' CONTRIBUTIONS

NB: Conceptualization, Formal Analysis, Methodology, Supervision, Writing – original draft. **SB:** Investigation, Methodology, Validation, Writing – review & editing. **EK:** Investigation, Validation, Writing – review & editing. **EKE:** Investigation, Writing – review & editing.

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ORIGINAL ARTICLE

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NAMPT gene rs2058539 variant is a risk factor for nonalcoholic fatty liver disease

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SUMMARY

OBJECTIVE: Nonalcoholic fatty liver disease is a chronic liver disease and a growing global epidemic. The aim of this study was to investigate the association between a visfatin gene (*NAMPT*) variant and nonalcoholic fatty liver disease, owing to the connection between this disease and insulin resistance, obesity, inflammation, and oxidative stress, and the role of visfatin in these metabolic disorders.

METHODS: In the present case-control study, we enrolled 312 genetically unrelated individuals, including 154 patients with biopsy-proven nonalcoholic fatty liver disease and 158 controls. The rs2058539 polymorphism of *NAMPT* gene was genotyped using the PCR-RFLP method.

RESULTS: Genotype and allele distributions of *NAMPT* gene rs2058539 polymorphism conformed to the Hardy-Weinberg equilibrium both in the case and control groups (p>0.05). The distribution of *NAMPT* rs2058539 genotypes and alleles differed significantly between the cases with nonalcoholic fatty liver disease and controls. The "CC" genotype of the *NAMPT* rs2058539 compared with "AA" genotype was associated with a 2.5-fold increased risk of nonalcoholic fatty liver disease after adjustment for confounding factors [p=0.034; odds ratio (OR)=2.52, 95% confidence interval (CI)=1.36–4.37]. Moreover, the *NAMPT* rs2058539 "C" allele was significantly overrepresented in the nonalcoholic fatty liver disease patients than controls (p=0.022; OR=1.77, 95%CI=1.14–2.31).

CONCLUSION: Our findings indicated for the first time that the *NAMPT* rs2058539 "CC" genotype is a marker of increased nonalcoholic fatty liver disease susceptibility; however, it needs to be supported by further investigations in other populations.

KEYWORDS: NAFLD. *NAMPT*. Variant. Visfatin.

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) as the most common cause of chronic liver disease is a multifactorial disorder in which liver fat exceeds 5% of hepatocytes in the absence of the secondary factors of hepatic fat aggregation or significant alcohol consumption. A growing body of research shows that the prevalence of NAFLD which is currently around 23%–25% of adults in the world will probably increase more owing to the ongoing obesity epidemic. NAFLD patients are more predisposed to have insulin resistance (IR), type 2 diabetes (T2D), and obesity. IR also affects the rate of elevation of serum liver enzymes in NAFLD, and the rate is higher in NAFLD patients with IR than those without IR[1-3]. Interestingly, significant associations between insulin receptor (*INSR*), insulin-like growth factor 1 (*IGF1*), insulin-like growth factor-binding protein 3 (*IGFBP3*), and visfatin (*NAMPT*) gene polymorphisms, and the risk of NAFLD have been discovered[4-7].

Nicotinamide phosphoribosyltransferase (NAMPT) or visfatin which is expressed in a variety of tissues including hepatocytes and adipocytes is the product of the *NAMPT* gene. NAMPT plays a role in the production of nicotinamide adenine dinucleotide. In addition to its aforementioned intracellular enzymatic activity, visfatin also has an extracellular action as a cytokine mainly in inflammation. It appears that visfatin is involved in the development of NAFLD by modulating

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disorders, including obesity, IR, inflammation, and oxidative stress. Previous reports have indicated positive correlations between circulating visfatin level and IR[8], T2D[9], triglycerides[10], body mass index (BMI)[8,9], metabolic syndrome[9], liver enzymes[10], and NAFLD[11]. Significant associations between *NAMPT* gene variants and the expression of *NAMPT* gene[12] and circulating level of visfatin[10] have also been found. Thus, the aim of the current research was to explore the possible contribution of *NAMPT* gene to NAFLD pathogenesis. The rs2058539 variant of *NAMPT* gene was selected based on high degree of heterozygosity and its usage in previous research.

METHODS

Study population

After informed consent, 154 patients with biopsy-proven NAFLD (age range, 31–87 years) and 158 controls (age range, 31–81 years) were enrolled in the present retrospective case-control study. NAFLD patients were enrolled after fatty liver diagnosis which in turn was defined by (a) ultrasonographic confirmation of fatty liver, (b) having high circulating levels of liver enzymes, (c) excluding subjects with other causes of liver disease like Wilson's disease, alpha-1 antitrypsin deficiency, viral hepatitis, and alcohol use more than 70 g/week in women or more than 140 g/week in men, and (d) liver biopsy evidence of NAFLD by an experienced pathologist who examined the liver biopsy samples using the Brunt's criteria. Steatosis and necroinflammation grades were 0–3 and fibrosis stages were 0–4. On the contrary, the control group was recruited from the research staff of the Institute. Those who were free of elevated liver enzymes and viral hepatitis infection (examined by blood tests), had no liver steatosis (examined by abdominal ultrasonography), and were not alcoholic or on regular medications were enrolled as controls. Self-report questionnaires were used for collecting the study population characteristics. This study complied with the principles of the Declaration of Helsinki and was performed according to the Institute's Ethics Committee approval[13,14].

Genotyping

Blood samples were collected in EDTA vials, and genomic DNA was purified from the white blood cells using phenol chloroform extraction and ethanol precipitation protocol and then stored at –20°C until use. The genotypes of the *NAMPT* gene rs2058539 variant were determined using PCR-RFLP. Genomic DNA was amplified using the primers: 5'-ATTAACTTTGGTATTCTTGCC-3' and 5'-TAGCCAGTTTTACCTTGAAGAC-3' to detect the genotypes of the *NAMPT* rs2058539 polymorphism. PCR was carried out with an initial denaturation at 95°C for 10 min, followed by 35 cycles of denaturation at 95°C for 45 s, annealing at 61°C for 45 s, and extension at 72°C for 45 s. The final extension was at 72°C for 10 min. The PCR products were digested with HphI (Fermentas, Leon-Rot, Germany) in a water bath at 37°C overnight, subjected to agarose gel electrophoresis, and visualized through ethidium bromide (0.5 μg/mL) staining using a UV transilluminator[15]. The "C" allele of the *NAMPT* rs2058539 had bands of 216 bp and 134 bp, whereas its "A" allele had a band of 350 bp, thus an individual with band(s) at 216 and 134 bp, at 350 bp only, and at 350, 216, and 134 bp was defined as CC homozygotic genotype, AA homozygotic genotype, and AC heterozygotic genotype, respectively. Genotyping of 20% of all the samples was performed twice by different laboratory personnel; the reproducibility was 100%.

Statistical analysis

To perform statistical analyses, the SPSS software package for Windows, version 25.0 (SPSS Inc. Chicago, IL, USA), was used. To examine the normality of distribution of continuous variables, the Kolmogorov-Smirnov goodness-of-fit test was used. The demographic, anthropometric, clinical, and biochemical features of the patients with NAFLD were compared with those found in the controls by Student's unpaired t-test or chi-square (χ^2) test as appropriate. Hardy-Weinberg equilibrium (HWE) for the *NAMPT* gene rs2058539 polymorphism in the control and case groups was also separately analyzed by χ^2 test with 1 degree of freedom, comparing the observed genotype frequencies with those expected. The differences in allele frequencies between the NAFLD and control groups were also assessed using χ^2 test. To appraise the association between the genotype frequencies and NAFLD and to adjust confounding factors, we employed logistic regression analysis. The odds ratios (OR) and their respective 95% confidence intervals (95%CI) were used as the measure of strength for the associations. Differences in biochemical parameters among the different *NAMPT* genotypes were tested by analysis of variance (ANOVA) and analysis of covariance (ANCOVA) when appropriate. A value of $p<0.05$ was taken as a statistically significant difference.

RESULTS

Table 1 presents the demographic, anthropometric, clinical, and biochemical data of the NAFLD patients and controls. The cases were more likely to be male ($p<0.001$) and smoker

(p=0.011) and had higher age (P<0.001), BMI (P<0.001), systolic blood pressure (p<0.001), diastolic blood pressure (p<0.001), AST (p<0.001), ALT (p<0.001), and GGT (p<0.001) than the controls.

Genotype and allele distributions of the *NAMPT* rs2058539 polymorphism conformed to HWE test both in the case and control populations (p>0.05). This implies that a representative sample population was used in this study. Analysis of this SNP revealed a significant difference between the cases and the controls (Table 2). The "CC" genotype of the *NAMPT* rs2058539 compared with "AA" genotype was associated with a 2.5-fold increased risk of NAFLD after adjustment for confounding factors (p=0.034; OR=2.52, 95%CI=1.36–4.37). In addition, the *NAMPT* rs2058539 "C" allele was significantly overrepresented

Table 1. General characteristics of the study groups.

Variables[a]	Controls (n=158)	Cases with nonalcoholic fatty liver disease (n=154)	p-value
Age (years)	28.9 (7.7)	39.1 (9.1)	<0.001
BMI (kg/m^2)	23.3 (3.5)	29.5 (5.2)	<0.001
Sex			
Male	83 (52.5)	114 (74.0)	
Female	75 (47.5)	40 (26.0)	<0.001
Smoking history			
No	143 (90.5)	115 (74.7)	
Former	10 (6.3)	20 (13.0)	
Current	5 (3.2)	19 (12.3)	0.011
SBP (mmHg)	114.8 (13.2)	123.7 (15.2)	<0.001
DBP (mmHg)	69.4 (8.3)	75.0 (9.6)	<0.001
AST (IU/L)	19.6 (7.3)	39.8 (17.4)	<0.001
ALT (IU/L)	19.4 (10.6)	72.2 (41.3)	<0.001
GGT (IU/L)	18.3 (8.5)	58.8 (31.7)	<0.001
Steatosis			
Grade 0		-	
Grade 1		40 (26.0)	
Grade 2		83 (53.9)	
Grade 3		31 (20.1)	
Necroinflammation			
Grade 0		48 (31.2)	
Grade 1		58 (37.7)	
Grade 2		46 (29.8)	
Grade 3		2 (1.3)	
Fibrosis			
Stage 0		88 (57.2)	
Stage 1		59 (38.3)	
Stage 2		7 (4.5)	
Stage 3		-	
Stage 4		-	

[a]Variables presented as mean (SD) or number (%); BMI: body mass index, SBP: systolic blood pressure; DBP: diastolic blood pressure, AST: aspartate aminotransferase; ALT, alanine aminotransferase; GGT: gamma glutamyl transferase.

Table 2. Distribution of the visfatin gene (*NAMPT*) rs2058539 variant in the cases with biopsy-proven nonalcoholic fatty liver and the controls[a].

Gene (Variant)	Controls (n=156)	Cases (n=152)	OR (95% CI) p-value[b]
NAMPT (rs2058539)[c]			
Genotype-wise comparison			
AA	81 (51.9)	61 (40.1)	1.0 (reference)
AC	62 (39.8)	64 (42.1)	1.92 (0.67–5.51)0.225
CC	13 (8.3)	27 (17.8)	2.52 (1.36–4.37)0.034
AC and CC	75 (48.1)	91 (59.9)	2.29 (0.84–6.25)0.107
CC versus others	13 (8.3)	27 (17.8)	3.82 (0.73–8.09)0.081
Allele-wise comparison			
A	224 (71.8)	186 (61.2)	1.0 (reference)
C	88 (28.2)	118 (38.8)	1.77 (1.14–2.31)0.022

[a]Variables presented as number (%). [b]Adjusted for age, body mass index (BMI), sex, smoking status, systolic blood pressure (SBP), and diastolic blood pressure (DBP) in genotype-wise comparisons. [c]Distribution of the ***NAMPT*** gene rs2058539 variant in 156 controls and 152 patients.

Table 3. Association between *NAMPT* gene rs2058539 polymorphism and different variables in 152 patients with biopsy-proven nonalcoholic fatty liver.

Variables[a]	AA+AC	CC	p-value
Number	125	27	
BMI (kg/m^2)	29.8 (5.3)	27.9 (5.1)	0.754
AST (IU/L)	39.6 (17.3)	41.0 (16.8)	0.572
ALT (IU/L)	72.5 (41.8)	70.9 (40.2)	0.629
GGT (IU/L)	57.0 (30.2)	67.1 (33.5)	0.197
SBP (mmHg)	122.8 (14.3)	127.6 (15.9)	0.466
DBP (mmHg)	74.5 (9.2)	77.3 (9.8)	0.523
Hypertension	30 (24.0)	8 (29.6)	0.206
Diabetes	26 (20.8)	6 (22.2)	0.549
Steatosis, Grade 3	24 (19.2)	7 (25.9)	0.294
Necroinflammation, Grades 2, 3	40 (32.0)	8 (29.6)	0.601
Fibrosis, Stages 1, 2	52 (42.4)	12 (44.4)	0.739

[a]Variables presented as mean (SD) or number (%); BMI: body mass index, SBP: systolic blood pressure; DBP: diastolic blood pressure, AST: aspartate aminotransferase; ALT: alanine aminotransferase, GGT: gamma glutamyl transferase.

in the NAFLD patients than the controls (p=0.022; OR=1.77, 95%CI=1.14–2.31).

Table 3 shows the association between the rs2058539 variant of *NAMPT* gene and anthropometric, biochemical, and pathological parameters in 152 patients with biopsy-proven NAFLD; no significant association was found.

DISCUSSION

Genes involved in glucose metabolism, IR, fatty acid metabolism, obesity, oxidative stress, inflammation, and fibrosis development are among the candidate genes for NAFLD. Interestingly, visfatin participates in many of these pathways, hence it is not unreasonable to presume that visfatin and its gene (*NAMPT*) play a role in NAFLD pathogenesis. The human *NAMPT* gene that consists of 11 exons is a highly polymorphic gene and has a wide variety of biological functions, so any defects in it may lead to IR, obesity, and inflammation that are implicated in the pathogenesis of NAFLD[16]. In this research, we found that the *NAMPT* rs2058539 "CC" genotype in comparison to the "AA" genotype increases the risk of NAFLD more than 2.5-fold. Consistently, the *NAMPT* rs2058539 "C" allele was more frequent in the cases with NAFLD too. Of note, RNA splicing and protein expression may be influenced by alterations in the intronic sequences. Perhaps, the *NAMPT* rs2058539 variant (located in intron 1) per se not to be functional; instead, it could be in linkage disequilibrium with another functional variant of the *NAMPT* gene. Consistently, Chang et al. have shown that *NAMPT* rs2058539 and *NAMPT* rs61330082 are in a linkage disequilibrium block, and the latter SNP is associated with the transcription rate of *NAMPT* gene, and serum levels of NAMPT, total cholesterol, triglyceride, and C-reactive protein, as well as cardiovascular diseases[8]. The other possibility is that the rs2058539 "C" allele might be more stable and translates more efficiently into visfatin, which in turn finally increases the risk of NAFLD. Prior investigations have also shown significant associations between *NAMPT* gene variants and its transcription rate[12], and circulating levels of visfatin[10], triglyceride[10], glucose, and insulin[10]. There have been associations between *NAMPT* gene polymorphisms and risk of obesity[16] and T2DM[17] too. Visfatin, an insulin-mimetic adipokine, can link IR with obesity. NAMPT is involved in glucose homeostasis and the regulation of glucose-stimulated insulin secretion in pancreatic β cells through NAD biosynthesis, and it induces IR. Interestingly, glucose and insulin regulate the release of visfatin[8,9,18]. Consistently, NAMPT levels were positively associated with triglyceride[10], BMI[8,9], IR[8], T2D[9], metabolic syndrome[9], liver enzymes[10], and NAFLD[11]. There is also more evidence that supports the hypothesis that visfatin plays a role in NAFLD. Visfatin is a proinflammatory cytokine and its level is positively associated with portal inflammation[9]. NAMPT induces the expression of pro-inflammatory cytokines, including TNF-α, IL-1β, and IL-6 via the STAT3 and NF-κB signaling pathways[18]. The NF-κB pathway has a key role in inflammation, and visfatin induces its activation. The injection of visfatin in mice aggravates inflammation, hepatic steatosis, and fibrosis, and increases oxidative stress and plasma levels of liver enzymes[19]. Visfatin also regulates the expression of some important microRNAs (miRNAs), including miR-146a, miR-155, and miR-181a that participate in inflammation and the activation of the immune system cells. MiRNAs are non-coding and single-stranded RNA molecules containing 22–25 nucleotides, which act in post-transcriptional regulation of gene expression[20,21]. From the above accumulating evidence, it appears plausible to assume that visfatin and its gene, *NAMPT*, may be involved in the development of NAFLD.

This study had strengths and limitations. First, the sample size of our study was modest due to using liver biopsy. Hence, performing sub-analyses was unreasonable. Second, owing to the fund limitations, we were unable to evaluate serum levels of visfatin. However, the design of this study was good and, more importantly, we employed liver biopsy as the gold standard method for confirming NAFLD diagnosis. Moreover, our report presented some novel and interesting findings.

CONCLUSION

Our findings revealed a role for the *NAMPT* rs2058539 gene variant in the pathogenesis of NAFLD: the carriers of *NAMPT* rs2058539 "CC" genotype had a 2.5-fold increased risk for NAFLD. This observation is made for the first time and it supports previous literature; however, further research is needed to confirm it.

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INSTITUTE WHERE THE RESEARCH WAS CONDUCTED

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AUTHORS' CONTRIBUTIONS

SN: Data curation, Writing – original draft. MN: Data curation, Writing – original draft. **FZ:** Data curation, Writing – original draft. **MR:** Data curation, Writing – original draft. **TM:** Conceptualization, Supervision, Formal Analysis, Writing – original draft. **GR:** Data curation, Writing – original draft. **AA:** Data curation, Writing – original draft. **HN:** Data curation, Writing – original draft. **RD:** Data curation, Writing – original draft. **SPT:** Data curation, Formal Analysis, Writing – original draft.

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ORIGINAL ARTICLE

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The association of *CYP11A1* gene polymorphisms with the polycystic ovary syndrome patients

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SUMMARY

OBJECTIVE: The objective of this study was to investigate the allele frequencies of polymorphisms in genes *CYP11A1* rs4886595 and *CYP11A1* rs4887139 that are responsible for the steroidogenesis mechanism in polycystic ovary syndrome patients and control females.

METHODS: Samples were obtained from the Department of Obstetrics and Gynecology in the Near East University Hospital from September 2019 to December 2019. Only the nonobese patients between the ages of 18–40 years were included in this study following informed consent. Obese patients and patients more than 40 years of age were excluded from the study. Nonobese women and normal ovulation were included in the control group. DNA was isolated from blood samples. Real-time polymerase chain reaction (PCR) was used to analyze single nucleotide polymorphisms (SNPs) in various genes linked to polycystic ovary syndrome. The studies were carried out using the samples obtained from 120 women, of whom 55 were nonobese and had normal ovulation, and 65 were polycystic ovary syndrome patients. The allelic frequencies of SNPs in genes linked to polycystic ovary syndrome were calculated using real-time PCR outcomes.

RESULTS: The variation of the *CYP11A1* rs4887139 G>A did not show any significance, while the variation of *CYP11A1* rs4886595 C>A showed significant differences between the patient and the control groups (p=0.01), respectively.

CONCLUSION: Future research ought to focus on elucidating the susceptible causes of polycystic ovary syndrome with a wide range of SNPs and more sample size. The genome-wide association studies in polycystic ovary syndrome patients of different origin will be important to identify candidate genes as well as proteins that are implied in polycystic ovary syndrome risk.

KEYWORDS: PCOS. Polymorphism. *CYP11A1*. SNP.

INTRODUCTION

Polycystic ovary syndrome (PCOS) is a complicated and common issue that affects 5–20% of women of reproductive age. PCOS has been diagnosed in around 105 million women aged approximately 15–49 years worldwide[1]. PCOS continues to be one of the most difficult medical conditions to treat because of its intricate nature, characteristics of development, and effects on women's lives from the teenage years until the postmenopausal phase[2]. The most common outcomes of PCOS are female anovulation, marked by hyper-androgen and insulin resistance, and it is among the most common sources of irregularities in menstruation, amenorrhea, and oligo-menorrhea, which is the most common reason for female infertility[3]. PCOS is marked by gonadotropin deficiencies, elevated androgen levels, insulin resistance, chronic anovulation, and irregular menstrual cycle. Reproductive anomalies are the habitual feature of PCOS. Hormones play a dominant role in the ovary's function and the menstrual cycle's management, which preserves fertility. If there is a chronic hormone level disruption in females, it can disrupt the activity of the ovary, contributing to the development of a cyst within the ovary, whereas androgen has risen in females affected by PCOS beyond its normal range[4]. Pregnant women with PCOS are more likely to undergo pregnancy difficulties and unfavorable outcomes in their offspring, which may be associated with factors related to the pathology of the syndrome and its related comorbidities[5]. Furthermore, there is an elevated risk of developing metabolic disorder, cardiovascular diseases, and type II diabetes. The diagnosis of PCOS includes the presence of a biochemical and/or clinical androgen surplus, polycystic ovarian morphology (PCOM) on ultrasound, and anovulation or oligo-ovulation[6]. Diabetes mellitus is 5–10 times more common in PCOS women[7]. The genetic background is suspected in approximately 79% of PCOS patients, while the environment and lifestyle account for 21%[8].

Polycystic ovary syndrome is a multigenic disease in which the heterogeneous, physiological, and biochemical phenotype

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is associated with both genetic and environmental influences. Approximately 40% of degree-relatives of PCOS females are also expected to develop PCOS compared with women in the general population (with an incidence of 4–6%)[9]. Several genes are predicted to have a contribution to PCOS etiology, and the recent analyses of the genome-wide studies have discovered a number of candidate genes[10]. Epigenetic and environmental influences, such as an unhealthy diet or low to absence of physical exercise, probably complicate any underlying genetic predisposition[11]. It has been suggested that metabolic syndrome in PCOS is a progressive condition; however, there are limited confirming follow-up data to support this theory. Over a 20-year period, 193 patients had follow-up visits every 5 years as part of a research conducted by Carmina et al. Although the patients' oligo/anovulation and hyperandrogenism improved, metabolic problems continued, particularly in relation to an increase in abdomen circumference. Therefore, fat tissue could be involved in PCOS's metabolic problems. A recent study has established that the fourth decade of life is associated with a decrease in PCOS characteristics[12]. Moreover, it is thought that problems with carbohydrate metabolism and a chronic inflammatory process associated with PCOS might impede the contact between the mother and the embryo and trophoblast invasion, which may result in miscarriage[13].

The *CYP11A1* (cytochrome P-450 11A1) gene belongs to the cytochrome P450, family 11. This gene is located on chromosome 15q23-q24. *CYP11A1* regulates the synthesis of pregnenolone from cholesterol in the inner membrane of the human mitochondria is one of the most promising candidate genes for PCOS enzyme codes, which cultivate the first and the most important step in the production of steroid hormones[14].

The objective of this study was to investigate the allele frequencies of polymorphisms in genes *CYP11A1* rs4886595 and *CYP11A1* rs4887139 that are involved in the steroidogenesis mechanism in PCOS patients and control females. We hypothesize that the allelic frequencies of single nucleotide polymorphisms (SNPs) would be different in the two groups.

METHODS

Ethical approval

Ethical approval was granted by the Near East University, Health Sciences Ethical Committee (YDU/2019/67-784).

Sampling and processing

Whole blood samples were obtained from September to December of 2019 from 120 patients undergoing routine checkup at the Department of Obstetrics and Gynecology in the Near East University Hospital. Each patient signed an informed consent form. The patients' clinical information was obtained. The inclusion criteria for the study included nonobese women between the ages of 18 and 40 years. The exclusion criteria for the study were obese women and nonobese women of more than 40 years of age. Samples were divided into two groups for analysis. Nonobese women with normal ovulation and considered not to have PCOS were included in the control group, and non-obese PCOS patients were included in the patient group. The study group included a total of 120 women, of whom 55 were nonobese and had normal ovulation, and 65 were PCOS patients.

DNA extraction and real-time PCR

DNA was extracted from each sample following the manufacturer's protocol (Invitrogen pure link genomic DNA mini kit, USA). NanoDrop (Nanodrop ND200, Thermo Scientific, Pittsburgh, USA) was used to measure the concentration and purity of the DNA at the 260:280 ratio. Allelic frequencies at specific SNP locations within the genes *CYP11A1* rs4886595 and *CYP11A1* rs4887139 were determined using real-time polymerase chain reaction (PCR) (LightCycler 480 Sybr Green, Roche Life Science) following the manufacturer's protocol. A final concentration of 25 μM forward and reverse primers was used in the reaction mixture. Each reaction contained 2 μL of DNA. All of the PCRs were performed in a laminar flow hood to avoid contamination. The high-resolution melting method analysis was used to investigate the allelic frequencies of the two SNPs within *CYP11A1*, and the thermal cycler software was used to calculate the cycle of threshold (Ct) and melting temperature (Tm) values. The PCR conditions for amplification were followed at 56°C annealing for 30 s according to the manufacturer's instructions.

Statistical Packages for the Social Sciences (SPSS version 10, Chicago, USA) were used in this study. Descriptive statistics and Mann-Whitney test were applied. Both Kolmogorov-Smirnov test of normality and the Mann-Whitney U test were implemented where applicable because the data did not support parametric assumptions. A p-value of 0.05 was chosen as the degree of significance.

RESULTS

The heterozygosity status of the SNPs was investigated using the real-time PCR analysis by evaluating the cycle of threshold (Ct) for each amplification. The whole number of cycles required for the fluorescent signal to cross the threshold is shown by the Ct values. Melting temperature (Tm) values were also recorded for each amplification. When the DNA is 50% double-stranded and 50% single-stranded, Tm shows the melt curve. In HRM

analysis, following PCR amplification, the amplicons produced melted gradually. This allows fluorescence to be emitted, which is detected using real-time PCR equipment. Due to the variances in Tm values, these melt curves have various shapes.

The results showed that, for the PCOS group, *CYP11A1* rs4887139 polymorphism, higher percentage of patients were found to be homozygous (89.2%), while the heterozygotes (3.0%) were found in low percentage of the patients (Table 1). Among the control group for the *CYP11A1* rs4887139 polymorphism, the results show a higher percentage (90.9%) of patients who were homozygous, while the heterozygous was found in a lower percentage of the patients of about 3.6%.

Table 1. The percentage of *CYP11A1* rs4887139 and *CYP11A1* rs4886595 heterozygosity in the polycystic ovary syndrome and control groups.

CYP11A1 rs4887139	Number of samples in patients	Percentage	Number of samples in the control group	Percentage
Homozygous	58	89.2	50	90.9
Heterozygous	2	3	2	3.6
Total	60	92.3	52	94.6
No result	5	7.7	3	5.4
Total	65	100	55	100
CYP11A1 rs4886595				
Homozygous	45	69.2	42	76.4
Heterozygous	14	21.5	10	18.2
Total	59	90.7	52	94.6
No result	6	9.3	3	5.4
Total	65	100	55	100

The homozygosity of *CYP11A1* rs4486595 was high of about 69.2%, while the heterozygosity was low of about 21.5%. Referred to the control group for the *CYP11A1* rs4886595 polymorphism, a higher percentage (76.4%) of patients were found to be homozygous, while the heterozygous was found to be in a lower percentage of about 18.2% (Table 1).

Mann-Whitney U test was conducted on the results (Table 2) to investigate the differences between homozygosity and heterozygosity of the patients using the melting temperature (Tm) analysis. The *CYP11A1* rs4487139 Tm in the patient group showed that the heterozygosity was significantly higher than the homozygosity (mean±SD; 90.70±0.28; mean±SD; 88.29±0.95; $p<0.05$, respectively). In contrast, Tm of *CYP11A1* rs4487139 for the control group showed that the heterozygosity was significantly higher than the mean homozygosity (91.50±1.97; 88.15±1.07; $p<0.05$, respectively).

Mann-Whitney U test was implemented (Table 3) to investigate the difference between homozygosity and heterozygosity in the patient group using the melting temperature (Tm) of *CYP11A1* rs4886595 gene polymorphism. The Tm of *CYP11A1* rs4886595 for the patient group showed that the heterozygosity was significantly higher than the mean homozygosity (87.80±1.39; 86.04±1.41; $p<0.05$), respectively. While the Tm of *CYP11A1* rs4486595 for the control group showed the heterozygosity was significantly higher than the mean homozygosity (87.34±2.49) (85.38±1.59) ($p<0.05$), respectively.

DISCUSSION

The results of this study showed that the patient and control groups have no significant difference in the Tm of *CYP11A1* rs4887139 ($p=0.203$), while the mean±SD of Tm in the patients

Table 2. Descriptive statistics for heterozygosity evaluation of *CYP11A1* rs4887139 using Tm values in the patient and control groups.

Patient group						Control group						p-value between the patient and control groups
	Mean	SD	Median	Min	Max		Mean	SD	Median	Min	Max	
Homozygous	88.29	0.95	88.8	86.0	89.30	Homozygous	88.15	1.07	88.70	86.20	90.70	p=0.203
Heterozygous	90.70	0.28	90.70	90.50	90.70	Heterozygous	91.50	1.97	91.50	90.10	92.90	

Table 3. Descriptive statistics for heterozygosity evaluation of *CYP11A1* rs4886595 using Tm values in the patient and control groups.

Patient group						Control group						p-values between the patient and control groups
	Mean	SD	Median	Min	Max		Mean	SD	Median	Min	Max	
Homozygous	86.04	1.41	86.10	80.10	89.60	Homozygous	85.38	1.59	85.95	82.10	88.60	p=0.01
Heterozygous	87.80	1.39	87.70	84.60	90.30	Heterozygous	87.34	2.49	87.60	82.70	90.60	

group was remarkably higher than the mean±SD of Tm in the control group in *CYP11A1* rs4886595 (p=0.01), respectively. However, when further analysis was done, it was shown that there was a significant difference in the Tm rs4886595 among the patient and control groups, while no significant difference was observed for the Tm of rs4887139.

The ovary is considered a major organ in the female reproductive system, and its interruption due to endocrine anomalies may lead to female infertility. PCOS is a metabolic and hormonal disorder that devastates women throughout their reproductive years[15]. Nonetheless, regarding its heterogeneity and complex structure, PCOS remains unclear in commonly recognized clinical significance. Moreover, the existence of PCOM in these patients happens to be usual occurrences in most PCOS patients[16]. Approximately 95% of females with this syndrome have decreased degree of follicle-stimulating hormonal and polycystic ovaries at the initial follicular stage, which prompt antral-follicle development and elevate LH expression[17]. In this study, two SNPs of the *CYP11A1* (rs4887139 and rs4886595 variants) were chosen to examine the possible association with the disorder. The global allelic frequency of *CYP11A1* rs4887139 is 0.8902, whereas the global allelic frequency of *CYP11A1* is 0.8291. Neither of the variants are considered pathogenic since neither had been reported in ClinVar. These variants are located in the noncoding regions of the gene. The chemical transformation of cholesterol to pregnenolone that is facilitated by the cytochrome enzyme (P450scc) is the most important step in the biosynthesis of steroid hormones in the ovary. Genetic alterations in the regulatory region of *CYP11A1* and *CYP17A1* genes are associated with the pathogenesis of PCOS. Common polymorphisms of *CYP17A1* and *CYP11A1* genes are hypothesized to predict the individual's susceptibility to PCOS[18]. Similar studies were performed previously in such seven SNPs within *CYP11A1* (rs12917295, rs11632698, rs1484215, rs6495096, rs4887139, rs9806234, and rs4886595) in PCOS patients that were genotyped. The results show that the genotype "GG" of rs4887139 was associated with a high PCOS risk with odds ratio (OR)=1.79, 95% CI=1.04–3.10, p=0.035, and the genotype "CC" of rs4886595 with increased PCOS risk with OR=4.29, 95% CI=0.90–20.36, p=0.04[19]. Similar results were also reported previously in such genotypic distributions of the SNP *CYP11A1* rs4077582 in PCOS patients that were substantially different from the controls in 106 Egyptian females between the ages of 18 and 45 years. Thus, they concluded that *CYP111A1* rs4077582 is associated with the pathophysiology of PCOS, implying that *CYP11A1* rs4077582 may alter the P450scc compound activity, and, as a result, androgen production[20]. In contrast, another study showed insignificant differences in Tm among the patient and control groups in rs4887139[21].

This study has a number of limitations, one of which was the sample size. The small sample size in both the case and control groups might impair the reproducibility of the findings. Although a few research studies aimed to investigate the heterozygosity at these two polymorphisms (rs4887139 G>A and rs4886595 C>A), further analysis is required to establish the correlation between these polymorphisms and PCOS. Another limitation was the number of SNPs investigated. Future studies should include more SNP genotyping.

CONCLUSION

In this study, the differences in the heterozygosity status of the alleles at the rs4886595 C>A within *CYP11A1* involved in PCOS recorded significant differences in the patient and control groups, while the heterozygosity of rs4887139 G>A within *CYP11A1* was shown to be insignificant in both groups. Therefore, future research ought to focus on elucidating the susceptible causes of PCOS with a wide range of SNPs and more sample size. More genome-wide association studies in PCOS patients of different origin will be important to recognize prospective genes as well as proteins that are implied in PCOS risk.

AUTHORS' CONTRIBUTIONS

SSHA: Formal Analysis, Investigation, Methodology, Software, Validation, Visualization, Writing – original draft, Writing – review & editing. **BO:** Data curation, Investigation, Writing – original draft, Writing – review & editing. **ACO:** Data curation, Investigation, Writing – original draft, Writing – review & editing. **PT:** Conceptualization, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing

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ORIGINAL ARTICLE

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Long-term oxygen therapy to reduce length of hospital stay in COVID-19

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SUMMARY

OBJECTIVE: The aim of this study was to evaluate the efficacy of long-term oxygen therapy as a strategy to reduce hospitalization time in patients affected by COVID-19.

METHODS: Between April and December 2021, COVID-19 patients with stable clinical conditions needing supplementary oxygen therapy during hospitalization were oriented to have hospital discharge with long-term oxygen therapy and reassessment after 15 days.

RESULTS: A total of 62 patients were evaluated and, 15 days after discharge, 69% of patients had suspended long-term oxygen therapy, with no difference between the groups admitted to the intensive care unit or the ward (p=0.319). Among the individuals who needed to maintain long-term oxygen therapy, in addition to worse P/F ratio (265±57 vs. 345±51; p<0.001) and lower partial pressure of oxygen (55±12 vs. 72±11 mmHg; p<0.001), were those more obese (37±8 vs. 30±6 kg/m2; p=0.032), needed more time for invasive mechanical ventilation (46±27 vs. 20±16 days; p=0.029), had greater persistence of symptoms (p<0.001), and shorter time between the onset of symptoms and the need for hospitalization (7 [2–9] vs. 10 [6–12] days; p=0.039).

CONCLUSION: Long-term oxygen therapy is an effective strategy for reducing hospitalization time in COVID-19 patients, regardless of gravity. Additionally, more obese patients with persistence of respiratory symptoms, faster disease evolution, and more days of invasive mechanical ventilation needed to maintain the long-term oxygen therapy longer.

KEYWORDS: COVID-19. SARS-CoV-2. Hospitalization. Hypoxemia. Oxygen inhalation therapy.

INTRODUCTION

Despite its high mortality rate, most individuals affected by COVID-19 overcome the acute phase and recover. However, for those who survive, the disease can be associated with varying degrees of functional impairment. One of the challenges faced is the care of these patients after the acute phase, especially those subjected to more invasive treatments[1,2].

A study conducted in Italy in 2020 identified that, among patients who had recovered from the acute phase of the disease, 87.4% reported the persistence of at least one symptom, the most prevalent being fatigue, followed by shortness of breath[3]. These were also the two main complaints identified in another study conducted in the United Kingdom in the same year[4]. Several factors are associated with the persistence of these manifestations, such as persistent chronic inflammation, organic changes related to the disease, prolonged hospitalization, and associated intensive care[3,5].

In this sense, fatigue and shortness of breath are the two main symptoms that lead to post-COVID-19 functional impairment, and therapeutic and pulmonary rehabilitation measures should be started early, with the aim of mitigating the consequences of the disease. Due to the persistence of refractory hypoxemia, which is considered an important severity factor, oxygen therapy has become one of the mainstays of treatment and can be instituted at home. Thus, before hospital discharge, the need and possibility of indicating it to the patient must be evaluated[6].

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In addition to ensuring tissue oxygenation in patients with some degree of pulmonary impairment, long-term oxygen therapy (LTOT) is a strategy that allows patients who are already recovering and in the final phase of treatment to be discharged from the hospital, which reduces the length of hospital stay (and its associated complications) and makes room for patients with more serious conditions. Not all patients have an indication for the use of home oxygen; therefore, it is necessary to investigate and understand the characteristics and clinical conditions associated with those who need it after hospital discharge.

Therefore, the authors designed the present study to evaluate the effectiveness of prolonged home oxygen therapy as a strategy to reduce hospitalization time in patients hospitalized for COVID-19 as well as the clinical characteristics of these patients after hospital discharge.

METHODS

This study included patients hospitalized at the Clinical Hospital of Botucatu Medical School (HCFMB) between 2020 and 2021, affected moderately or severely by COVID-19 who met the criteria for indication of home oxygen therapy (stable for over 48 h with $SpO_2 \leq 92\%$ on room air, oxygen flow ≤ 3 L/min, and either $PaO_2 \leq 60$ mmHg or $SaO_2 \leq 92\%$ in arterial blood). They were evaluated after 15 days of home oxygen therapy.

After approval by the Research Ethics Committee of the Botucatu Medical School (approval number: 4999534) and acceptance of the informed consent form, each patient was analyzed and classified, taking into account sociodemographic data and clinical characteristics during hospitalization such as respiratory symptoms and presence of comorbidities, in addition to the drugs used.

Patients over 18 years of age, of both sexes, diagnosed with COVID-19 and admitted to the HCFMB, who had an indication for home oxygen therapy after hospital discharge were included in the study. Patients who died before reassessment, were lost to follow-up, or did not sign the informed consent form were excluded from the study.

Aspects related to hospitalization, such as pulmonary impairment shown on tomography, maximum oxygen flow during hospitalization, need for intensive care, dependence on invasive ventilatory support, and renal replacement therapy, were also evaluated. The period, in days, from the onset of symptoms to the need for hospitalization, total length of hospital stay, time in the intensive care unit (ICU), and use of invasive mechanical ventilation (IMV) were also measured if intensive care was required.

Additionally, the possibility of suspending LTOT was evaluated, investigating how many patients needed to remain on oxygen therapy and why as well as checking the parameters such as body composition, handgrip strength, arterial blood gas, blood pressure, and the 4-min step test that were taken into account in this decision.

Descriptive statistics were used to describe the characteristics of all participants. Variables with normal distribution were expressed as mean values, standard deviations, medians, and 25–75% percentiles for nonparametric variables. Student's t-test compared normally distributed variables, while the Mann-Whitney test assessed non-normally distributed ones. The chi-square test examined binary qualitative variables with frequencies >5, and the McNemar test compared proportions within the same group. Relevant correlations were investigated using Pearson's and Spearman's correlation. A 5% significance level was used for all tests. The Jamovi version 2.3 statistical package (The Jamovi project, Sydney, Australia) was used.

RESULTS

Between April and December 2021, 62 COVID-19 patients who had an indication for the use of LTOT after hospitalization were evaluated. Of these, 51% (n=32) required intensive care, with a median length of stay of 11 (6–33.5) days, and of these, 50% (n=16) required invasive ventilatory support for approximately 21.5 (9.5–32) days and 19% (n=6) had an indication for acute renal support. Bacterial co-infection requiring antibiotic therapy throughout hospitalization was present in approximately 79% (n=49) of the patients.

The general characteristics of the patients included in the sample according to the severity of COVID-19 (whether or not intensive care is needed) are shown in Table 1, and impairment on chest CT is presented in Table 2.

After 15 days of hospital discharge, approximately 31% (n=19) met the criteria for continuing therapeutic oxygen use. Among these individuals, there was no distinction in maintaining LTOT between those who required critical care or exhibited lung involvement on chest CT scans. However, in addition to poorer P/F ratio and lower partial pressure of oxygen, those who needed to continue LTOT were more obese, required a longer duration of invasive mechanical ventilation, experienced greater persistence of symptoms, and had a shorter interval between symptom onset and the need for hospitalization (Table 3).

Furthermore, of the total number of patients, seven needed to maintain LTOT for more than 3 months, and, among them, it was observed that cardiovascular disorders patients showed a greater need for the maintenance of LTOT (p=0.006).

No significant correlations were found among the other studied variables.

Table 1. Characteristics of individuals according to the severity of COVID-19.

Variables	ICU (n=32)	Ward (n=30)	p
Age (years)	56±13.5	68±15	0.001[a]
Sex, F/M	16/16	14/16	0.793[b]
BMI, kg/m²	33±8.5	30±4.6	0.267[a]
Smoking, yes/no	16/16	17/13	0.599[b]
Smoking history, pack years	0 (0–41.5)	9 (0–31.5)w	0.438[c]
Evolution of symptoms, days[1]	8.5 (6.75–11.3)	7 (4–11)	0.533[c]
Length of hospital stay, days	25.5 (15–43)	15 (12–24)	0.001[c]
Persistence of symptoms[2], yes/no	12/20	13/17	0.640[c]
PaO_2 (admission), mmHg	63 (55–69)	65 (58–74)	0.187[c]
PaO_2 (reevaluation), mmHg	69±13	66±14	0.404[a]
PaO_2/FiO_2 (admission)	110 (79–219)	220 (185–255)	0.002[c]
PaO_2/FiO_2 (reevaluation)	327±64	313±68	0.402[a]
CT ≥50%, yes/no	24/8	14/15	0.031[b]
Previous lung disease, yes/no	6/26	7/23	0.658[b]
Multimorbidity, yes/no	23/9	23/7	0.667[b]

Values expressed as mean ± standard deviation or median (25–75%). PaO_2: partial pressure of oxygen; FiO_2: fraction of inspired oxygen; CT: computed tomography (chest). [1]Time between symptoms onset and hospital admission. [2]Symptoms of dyspnea and fatigue after hospital discharge. [a]Student's t-test; [b]chi-square or Fisher's exact test; [c]Mann-Whitney U test.

Table 2. Previous comorbidities according to the pulmonary injury.

Previous comorbidities	CT <50% (n=23)	CT ≥50% (n=38)	p
Cardiovascular, yes/no	17/6	26/12	0.649
Endocrinology, yes/no	8/15	15/23	0.714
Kidney, yes/no	3/20	3/35	0.513
Neurological, yes/no	4/19	4/34	0.461
Lung, yes/no	7/16	6/32	0.208
mental, yes/no	2/21	1/37	0.551
Multimorbidity, yes/no	19/4	26/12	0.222

Chi-square or Fisher's exact test.

Table 3. Characteristics of the individuals according to the need to maintain the long-term oxygen therapy.

Characteristics	No LTOT (n=43)	LTOT (n=19)	p
Age, years	60±15	64±15	0.365[a]
Sex (M/F), n	22/22	10/9	0.934[b]
BMI, kg/m²	30±6	37±8	0.032[a]
Smoking, yes/no	18/26	11/8	0.334[b]
Smoking history, pack years	0 (0–35)	15 (0–30)	0.813[c]
Evolution of symptoms, days[1]	10 (6–12)	7 (2–9)	0.039[c]
Persistence of symptoms[2], yes/no	11/32	14/5	<0.001[b]
PaO_2 (admission), mmHg	64 (58–71)	63 (56–71)	0.561[c]
PaO_2 (reevaluation), mmHg	72±11	56±12	<0.001[a]
PaO_2/FiO_2 (admission)	207 (136–261)	185 (84–228)	0.089[c]
PaO_2/FiO_2 (reevaluation)	345±51	265±57	<0.001[a]
CT ≥50%, yes/no	27/15	11/8	0.633[c]
Previous lung disease, yes/no	9/34	4/15	1.000[c]
Multimorbidity, yes/no	29/14	17/2	0.114[b]
Length of hospital stay, days	21 (14–30)	21 (12–36)	0.945[c]
Length of ICU stay, days	11 (6–30)	14 (6–65)	0.498[c]
IMV time, days	20±16	46.5±27	0.029[a]

Values expressed as mean ± standard deviation or median (25–75%). PaO_2: partial pressure of oxygen; FiO_2: fraction of inspired oxygen; ICU: intensive care unit; IMV: invasive mechanical ventilation. [1]Time between symptoms onset and hospital admission. [2]Symptoms of dyspnea and fatigue after hospital discharge. [a]Student's t-test; [b]chi-square or Fisher's exact test; [c]Mann-Whitney U test.

DISCUSSION

While it was anticipated that ICU individuals with COVID-19 would display more severe symptoms and prolonged hospitalizations, it is important to note that these patients were younger and showed no disparities in comorbidities or previous multimorbidities. Typically, advanced age is linked with higher mortality rates and a greater need for critical care. Our sample, however, comprised of younger patients, prompting further investigation into unanalyzed factors like inflammatory markers or specific laboratory parameters that may account for these differences[4].

Unlike chronic pulmonary conditions, which have well-established criteria and protocols in the literature on the indications and benefits of LTOT, the use of oxygen after hospital discharge does not have specific guidelines defined for patients with sequelae of COVID-19[7]. In contrast, a study by Weerahandi et al.[8]. demonstrated that about 13.5% of approximately 160 COVID-19 patients who needed home oxygen continued LTOT 30–40 days after discharge. Additionally, Loerinc et al.'s study with 310 COVID-19 patients found that 13% required LTOT, but post-discharge duration was unspecified[9].

COVID-19 itself can lead to chronic hypoxemia, resulting from damage to both the parenchyma and pulmonary vasculature. However, the precise mechanism of chronic hypoxia requires further exploration[10]. In our sample, pulmonary impairment on chest CT during hospitalization was not associated with the need for LTOT. Nevertheless, patients requiring oxygen for > 15 days exhibited poorer oxygenation, experienced ongoing symptoms of dyspnea and fatigue, had higher obesity rates, longer mechanical ventilation periods, and a quicker progression from symptom onset to hospital admission.

Obese patients, especially those with obesity hypoventilation, generally benefit from oxygen therapy when noninvasive ventilation (NIV) alone cannot correct hypoxemia, and in such cases, it can be used in conjunction with oxygen[11]. An observational study by Priou et al.[12] found that supplemental oxygen therapy was an independent risk factor for mortality in 130 patients with obesity hypoventilation. However, there is no consensus on its benefits, particularly due to potential adverse effects like exacerbating respiratory acidosis or inducing compensatory metabolic alkalosis[13,14].

We hypothesized that hypoxemia might be attributed to obesity hypoventilation since chest tomography did not indicate significant impairment in the group requiring ongoing oxygen therapy. This could potentially eliminate pulmonary sequelae of COVID-19. However, confirming this hypothesis requires additional post-hospital discharge chest CT analysis and possibly pulmonary function testing, including diffusing capacity for carbon monoxide[15].

Furthermore, extended periods of mechanical ventilation in these patients likely contributed to increased muscle weakness, potentially leading to hypoventilation and hypoxemia, particularly in those who were more obese. Prolonged mechanical ventilation is known to result in respiratory muscle weakness. In obese individuals, alterations in respiratory mechanics ultimately lead to reduced ventilation in the lower lobes, affecting the ventilation–perfusion ratio and causing hypoxemia[16].

Patients requiring LTOT had a shorter time from symptom onset to hospitalization, and our findings align with literature reporting similar timelines for hospitalization and ICU admission in Brazil's initial COVID-19 cases. This underscores rapid clinical deterioration. While symptom onset is a valuable baseline for prognostic models, studies on worse outcomes due to swift disease progression remain limited[17].

The high rate of individuals who have persistent impairment after acute infection by COVID-19 has impacted both the quality of life and the health systems of those affected. The persistence of symptoms for weeks or months after the initial manifestation of the disease is called long COVID or post-COVID syndrome, and the most frequent symptoms are related to the presence of fatigue, dyspnea, cough, muscle pain, arthralgia, palpitations, chest pain, anosmia, dysgeusia, brain clouding, insomnia, anxiety, and depression[18,19].

The exact mechanism behind the long COVID remains unclear due to a lack of understanding about sustaining factors. However, indications suggest persistent symptoms could be linked to acute phase organic damage, its extent, and system recovery time. Other factors may include ongoing inflammation, immune response, autoantibody generation, virus persistence, nonspecific effects of hospitalization, post-ICU syndrome, SARS-CoV-2-related complications, comorbidities, and acute-phase drug side effects. Additionally, physical deconditioning, psychological issues, post-traumatic stress, and social/financial impacts may contribute to prolonged symptom persistence[18-23].

Despite differences in our sample, it is crucial to note that patients requiring LTOT after 15 days post-hospital discharge did not necessarily have a greater COVID-19 severity during hospitalization. In other words, there was no significant difference in terms of worse tomographic compromise or critical care necessitating LTOT maintenance. This underscores that discharging individuals who only require oxygen therapy, irrespective of initial severity, and reevaluating the need for LTOT after 15 days, is an effective therapeutic and administrative strategy. This approach not only frees up hospital beds but also led to 69% of the sample being discharged from LTOT after reassessment, highlighting its dual benefits.

Finally, our study has some limitations. Data that could explain findings, like the influence of initial lab parameters on critical care need, were not analyzed. Moreover, post-LTOT discharge reassessment with chest CT and pulmonary function tests could clarify hypoxemia due to hypoventilation in those requiring extended oxygen therapy. These unanswered questions serve as a stimulus for further research in this population.

CONCLUSION

The indication of LTOT for patients affected by severe COVID-19 has proven to be an effective strategy for reducing the hospitalization time of patients with persistent hypoxemia. In addition, factors related to worse P/F ratio, length of stay, and greater lung impairment may influence the severity of the disease; and aspects that go beyond poor oxygenation such as maintenance of respiratory symptoms, obesity, duration of mechanical ventilation, and shorter time between the onset of symptoms and hospital admission may influence the need to maintain LTOT.

AUTHORS' CONTRIBUTIONS

DICS: Writing – original draft, Writing – review & editing. **LYI:** Writing – original draft, Writing – review & editing. **EATF:** Data curation, Writing – review & editing. **MSCS:** Writing – review & editing. **LFPB:** Data curation, Writing – review & editing. **CAC:** Data curation, Writing – review & editing. **EGS:** Data curation, Writing – review & editing. **LHSM:** Data curation, Writing – review & editing. **SET:** Supervision, Writing – review & editing. **RP:** Formal Analysis, Supervision, Writing – review & editing.

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ORIGINAL ARTICLE

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Association between nonalcoholic steatohepatitis and high serum ferritin levels in type 2 diabetes mellitus

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SUMMARY

OBJECTIVE: The aim of this study was to assess the role of elevated serum ferritin levels in the onset, pathological progression and prognosis of nonalcoholic fatty liver disease. Nonalcoholic fatty liver disease has been rapidly increasing worldwide. Despite extensive research on the pathogenesis of nonalcoholic fatty liver disease, a lack of sufficient clinical research on the relationship between nonalcoholic fatty liver disease and serum ferritin levels remains.

METHODS: We analysed 968 patients with type 2 diabetes mellitus who underwent liver ultrasound examination and had their serum ferritin levels measured. The presence of nonalcoholic fatty liver disease and advanced liver fibrosis was determined through abdominal ultrasound examination and the nonalcoholic fatty liver disease fibrosis score.

RESULTS: Compared to that in the non-nonalcoholic fatty liver disease group, the presence of hyperferritinemia was significantly more common in the nonalcoholic fatty liver disease group (83.3 vs. 56.3%, p=0.005). When patients with nonalcoholic fatty liver disease were stratified by the nonalcoholic fatty liver disease fibrosis score, those with advanced liver fibrosis exhibited a higher prevalence of hyperferritinemia (56.3, 78.9, and 88.9% for none, simple steatosis, and advanced fibrosis, respectively; p for trend=0.002). In multivariate logistic regression, liver fibrosis was independently associated with hyperferritinemia (odds ratio [OR] 1.45; 95% confidence interval [CI] 1.18–2.02; p=0.014), and this association remained significant in male patients after adjusting for other risk factors (OR 2.66; 95% CI 1.43–5.48; p=0.026).

CONCLUSION: Identifying nonalcoholic fatty liver disease patients at a risk of developing nonalcoholic steatohepatitis and advanced fibrosis is crucial for implementing timely interventions and improving patient outcomes. This study highlights the potential utility of serum ferritin levels as a serum biomarker for identifying nonalcoholic steatohepatitis patients and those at a risk of late-stage fibrosis, particularly in male patients with nonalcoholic fatty liver disease.

KEYWORDS: Ferritin. Nonalcoholic fatty liver disease. Fibrosis. Male. Steatosis.

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is the most common chronic liver disease, affecting approximately 100 million people worldwide[1,2]. The spectrum of the disease ranges from hepatic steatosis to nonalcoholic steatohepatitis (NASH), which can progress to advanced liver fibrosis, cirrhosis, or hepatocellular carcinoma[3]. In addition, a significant proportion of NAFLD patients have metabolic comorbidities, which significantly increase the risk of cardiovascular disease and extrahepatic malignancies[4,5]. Notably, the prevalence of NAFLD in Asian countries is similar to and, in some cases, higher than that in Western countries, approaching 30%[6]. By 2030, the total number of NAFLD cases in China is expected to increase to 1.3158 billion, making it the country with the highest growth rate in NAFLD incidence in the world[7].

Ferritin is a major intracellular iron storage protein[8]. The maintenance of iron homeostasis in the body is achieved through the regulation of iron ion transport and storage. Studies suggest that ferritin can enter the circulation via the classical endoplasmic reticulum/Golgi-dependent secretion pathway in hepatocytes[9]. In addition, another possible mechanism of ferritin secretion involves leakage from damaged cells[10,11]. Ferroptosis is a recently discovered iron-dependent form of cell death with distinct cellular morphology and biological mechanisms[12]. Therefore, the disruption of iron metabolism induced by ferroptosis may explain the strong association between serum ferritin (SF) and markers of hepatocellular damage. However, the relationship between SF and liver disease remains controversial. Previous reports have suggested that higher levels of SF are associated with a higher incidence of NAFLD, severe steatosis, or advanced fibrosis in patients with NAFLD[13]. Ferritin has

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also been shown to be a significant factor in the progression of hepatitis C to advanced liver fibrosis or even cirrhosis[14,15]. Conversely, others argue that SF levels are not very accurate in diagnosing liver fibrosis caused by NAFLD[16]. In addition, the prevalence and incidence of NAFLD are higher in China than in Western countries, although the overweight and obesity rates in the Chinese population are much lower[17]. Therefore, there is an urgent need to identify serum biomarkers for the rapid and accurate detection of NAFLD[18].

The aim of this clinical study was to assess the role of elevated SF levels in the onset, pathological progression and prognosis of NAFLD. In addition, we would perform further stratification to investigate other potential factors that may influence SF levels to gain a deeper understanding of the relationship between NAFLD and elevated SF levels.

METHODS

Study population

We included a total of 1872 type 2 diabetes mellitus (T2DM) patients aged ≥18 years who underwent liver ultrasound examination and SF level measurement at Tianjin Huanghe Hospital between 2020 and 2021. Individuals with a history of coronary artery disease or ischaemic stroke, individuals with an alcohol intake greater than 140 g/week, and individuals with a history of malignancy, chronic kidney disease, thyroid dysfunction, or hepatitis B or hepatitis C infection were excluded (n=904). The analysis was conducted on the remaining 968 participants. This study was performed according to the Declaration of Helsinki and with permission from the local ethics committee (No. 2021KY-032-01).

Anthropometric and biochemical measurements

Data on demographic characteristics, medical history, and social habits, including alcohol consumption, were obtained through self-report questionnaires at the first visit. Obesity was defined based on the Asia-Pacific criteria (BMI ≥ 25 kg/m^2). Waist circumference was measured at the midpoint between the lower rib margin and the iliac crest during normal expiration. Blood pressure was measured using a mercury sphygmomanometer after resting for at least 5 min. Hypertension was defined as a blood pressure ≥ 140/90 mmHg or the use of antihypertensive medications. Plasma glucose concentrations were measured using the Beckman Glucose Analyser II (Beckman Instruments, Fullerton, California, USA). HbA1c was quantified using high-performance liquid chromatography (Variant II; Bio-Rad, Hercules, California, USA).

Abdominal ultrasound examination

Abdominal ultrasound was performed by a radiologist who was blinded to laboratory and clinical data using a high-resolution ultrasound system (VISION 900; HI, Tokyo, Japan). NAFLD was defined as the presence of hepatic steatosis on ultrasound examination. In NAFLD patients, the presence of advanced liver fibrosis was determined using the NAFLD Fibrosis Score (NFS), which was calculated as follows: -1.675+0.037 × age+0.094 × BMI+1.13 × (impaired fasting glucose or diabetes)+0.99 × AST/ALT - 0.013 × platelet count - 0.66 × albumin ≥ -1.445. Simple steatosis was defined as the detection of hepatic steatosis on ultrasound examination without fibrosis predicted by the NFS.

Serum ferritin and transferrin saturation

Serum ferritin levels were measured using an immunoradiometric assay.

Statistical analysis

Normally distributed continuous variables are reported as the mean±standard deviation. Categorical variables are reported as frequencies. Between-group comparisons for continuous variables were performed using Student's t-test or analysis of variance (ANOVA), while the chi-square test was used for categorical variables. The odds ratio (OR) and 95% confidence interval (CI) were calculated using multivariate logistic regression. A p-value<0.05 was considered statistically significant. All statistical analyses were performed using Stata Version 17.0.

RESULTS

Clinical and serum ferritin levels

Clinical characteristics and SF levels were stratified according to the presence of NAFLD, as summarized in Table 1. The mean age was 55.8±10.2 years, and 654 participants (67.56%) were male. The mean duration of T2DM was 7.8±5.6 years. NAFLD was present in 533 participants (55.1%). Compared to those in the non-NAFLD group, participants in the NAFLD group had significantly higher BMIs and waist circumferences and were more likely to have metabolic syndrome (all p<0.001). Fasting glucose and HbA1c levels were higher in patients in the NAFLD group (p<0.001). Serum cholesterol, triglyceride, and liver enzyme (AST and ALT) levels were significantly elevated in the NAFLD group (all p<0.001). The NAFLD group had higher SF levels than the non-NAFLD group (p<0.001). Thus, 689 participants (71.2%) had elevated SF levels, and the prevalence was significantly higher

Table 1. Clinical and echocardiographic parameters.

	Non-NAFLD (n=435)	NAFLD (n=533)	p-value
Age (years)	55.60±13.66	56.01±12.86	0.3483
Gender (%)			
Male	65.75	69.04	0.128
Female	34.25	30.96	
Age (years)			
≥50	59.78±8.27	59.04±7.38	0.0779
<50	37.13±8.06	37.70±7.67	0.0831
Duration of diabetes mellitus (years)	7.60±5.12	8.67±3.21	0.6827
Systolic BP (mmHg)	125.26±19.02	132.57±17.85	<0.0001
Diastolic BP (mmHg)	78.81±19.64	83.03±9.82	<0.0001
BMI (kg/m^2)	24.09±3.53	27.92±3.42	<0.0001
Waist circumference (cm)	88.00±10.17	99.80±8.07	<0.0001
Laboratory features			
Total cholesterol (mmol/L)	5.05±0.98	5.89±0.99	<0.0001
HDL-cholesterol (mmol/L)	1.40±0.28	1.28±0.25	<0.0001
LDL-cholesterol (mmol/L)	3.01±0.77	3.05±0.76	0.0988
Triglyceride (mmol/L)	1.61±1.04	2.15±1.18	<0.0001
Glucose (mmol/L)	5.12±1.17	5.82±1.91	<0.0001
HbA1c (%)	5.37±0.87	5.94±1.01	<0.0001
ALT (IU/L)	24.64±9.88	28.02±11.67	<0.0001
AST (IU/L)	28.95±21.42	41.79±30.68	<0.0001
γGT (IU/L)	27.70±26.06	36.89±26.89	<0.0001
Serum albumin (g/L)	46.92±2.21	46.61±2.11	<0.0001
Platelet count (10^9/L)	234.52±55.91	229.34±49.76	0.0341
Serum creatinine (μmol/L)	72.15±15.68	76.09±17.17	<0.0001
serum urea nitrogen	5.15±1.33	5.35±1.30	<0.0001
Uric acid (μmol/L)	338.85±79.43	381.70±80.05	<0.0001
Ferritin (ng/mL)	124.38±152.01	325.90±157.03	<0.0001

Values are presented as mean±standard deviation or number (%). NAFLD: nonalcoholic fatty liver disease; BMI: body mass index; BP: blood pressure; HbA1c: glycosylated hemoglobin; HDL-C: high density lipoprotein cholesterol; LDL-C: low density lipoprotein cholesterol; ALT: alanine aminotransferase; AST: aspartate transaminase; γGT: gamma glutamyl transpeptidase.

in the NAFLD group than in the non-NAFLD group (83.3 vs. 56.3%, p=0.005).

Association between type 2 diabetes mellitus-associated hepatic steatosis and elevated serum ferritin levels

The prevalence of elevated SF levels increased gradually with the severity of hepatic steatosis (56.3, 76.0, and 88.2% for none, mild, and moderate/severe, respectively; p for trend=0.005). Among individuals stratified by the NFS, participants with advanced liver fibrosis had a significantly higher prevalence of elevated SF levels than participants without steatosis (56.3, 78.9, and 88.9% for none, simple steatosis, and advanced fibrosis, respectively; p<0.001 for advanced fibrosis vs. none, p for trend=0.002).

To assess whether NAFLD is independently associated with elevated SF levels in T2DM patients, multivariable logistic regression analysis was performed. Compared to

the absence of NAFLD, the presence of NAFLD was positively associated with a higher likelihood of elevated SF levels (OR, 1.56; CI, 1.18–2.02; p=0.022) after adjustment (Figure 1, Model 2). This association remained significant but was attenuated when the model was further adjusted for sex (OR 1.52; 95%CI 1.05–1.86; p=0.026) (Figure 1, Model 3). In the subgroup analysis, NAFLD did not have a heterogeneous effect on hyperferritinemia depending on the HbA1c level, duration of diabetes, hypertension, BMI, age, or sex (p>0.05 for all interaction terms with NAFLD). Notably, this association was significant in male patients (OR 2.58; 95%CI 1.24–5.48; p=0.021), whereas it was not significant in the female population (p=0.585, interaction term p=0.032).

Relationship between advanced liver fibrosis and elevated serum ferritin levels in type 2 diabetes mellitus patients

As shown in Figure 2, we investigated the relationship between the presence of advanced liver fibrosis with NAFLD and hyperferritinemia. In the stratification of NAFLD patients using the NFS score, advanced liver fibrosis was associated with hyperferritinemia (OR, 1.45; CI, 1.18–2.02; p=0.014) after adjustment (Figure 2, Model 3). No significant heterogeneity was found among the various subgroups defined by glycaemic control, duration of diabetes, hypertension, BMI, sex, and age (p>0.05 for all interaction terms with the NFS). However, this association disappeared in female patients (p=0.447), while it was maintained in male patients (OR 2.66; 95%CI 1.43–5.48; p=0.026, interaction term p=0.019).

DISCUSSION

First, we observed significantly higher SF levels in NAFLD patients than in non-NAFLD patients. This is consistent with previous research findings[13], suggesting the presence of disrupted iron metabolism in the development of NAFLD. Elevated SF levels may serve as predictive markers of NAFLD development, reflecting iron accumulation and abnormal iron distribution in the body. Furthermore, we found that NAFLD patients with advanced liver fibrosis were more likely to exhibit abnormal SF levels than patients with only steatosis, and the prevalence of elevated SF levels increased gradually with the severity of NAFLD. This further supports the relationship between elevated SF levels and NAFLD severity and liver fibrosis progression. Therefore, our study confirms the value of SF as a noninvasive biomarker for identifying NAFLD patients. Impaired or enhanced ferritin function can lead to the abnormal accumulation of iron ions, increasing the risk of ferroptosis. Elevated SF levels or disturbances in iron metabolism may increase the risk of cell ferroptosis[19-23].

Subsequently, we further conducted multivariate regression analysis in our study, which indicated that the association between NAFLD and elevated SF levels persisted after adjusting for other potential confounding factors. This further supports the independent relationship between NAFLD and elevated SF levels. This association may be influenced by sex, as the association between NAFLD and elevated SF levels was more significant in male patients, while no such association was observed in female patients in the subgroup analysis based on sex. Liang et al.[24] found that ferroptosis is modulated by the differential effects of sex hormones. The study identified MBOAT1 (an oestrogen

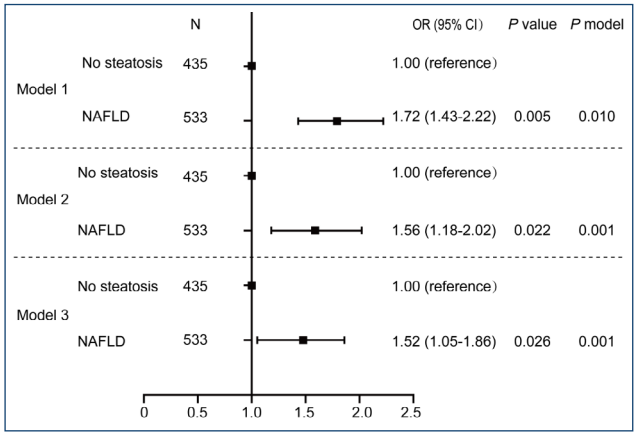

Figure 1. Adjusted odds ratio for hyperferritinemia by the presence of nonalcoholic fatty liver disease. Multivariable logistic regression in all subjects. Model 1, unadjusted; model 2, adjusted for age, BMI, hypertension, diabetes mellitus duration, fasting glucose, triglyceride, and cholesterol; model 3, further adjusted for sex. OR: odds ratio; CI: confidence interval; NAFLD: nonalcoholic fatty liver disease; BMI: body mass index.

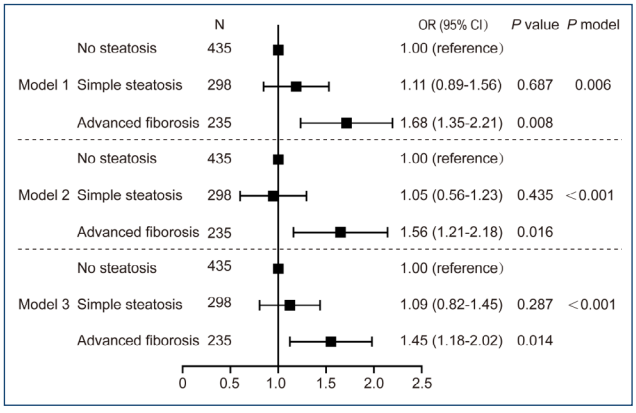

Figure 2. Adjusted odds ratio for hyperferritinemia by the presence of advanced liver fibrosis with nonalcoholic fatty liver disease. Multivariable logistic regression in all subjects. Model 1, unadjusted; model 2, adjusted for age, BMI, hypertension, diabetes mellitus duration, fasting glucose, triglyceride and cholesterol; model 3, further adjusted for sex. OR: odds ratio; CI: confidence interval; NAFLD: nonalcoholic fatty liver disease; BMI: body mass index.

receptor) and MBOAT2 (an androgen receptor) as novel regulators of ferroptosis by reshaping phospholipids. Compared to males, females exhibit significant resistance to ferroptosis in vivo; this resistance is significantly influenced by the female sex hormone environment. Based on in vivo single-cell resolution and unbiased computational inference, Shintaro et al.[25] found that the resilience of females to ferroptosis is both constitutive and adaptive. It is speculated that the regulation of ferroptosis sensitivity involves multiple interactions between cells and their environment. The promotion of BCL6 increased the susceptibility of male mice to NAFLD, suggesting that males limit their immunopathology[26]. The circadian rhythm can protect the liver by influencing hormone levels, and its disruption can lead to the liver undergoing "gender switching"[27].

Our study has several advantages. This study explored the relationship between the severity of liver steatosis and/or advanced liver fibrosis and high ferritin levels in T2DM patients, which can lead to the study of new ferroptosis pathogenic mechanisms and expand our understanding of the pathogenesis of NAFLD. Compared to those of previous studies, the large sample size of our study allowed us to further investigate the impact of liver fibrosis in subgroup analysis, and we found that males are more prone to having high ferritin levels, while females are less susceptible, possibly due to ferroptosis surveillance being differentially regulated by sex hormones. However, we also acknowledge some limitations in our study. First, the cross-sectional design precludes causal inferences between NAFLD and high ferritin levels. Second, due to its invasive nature, liver biopsy to confirm the presence of steatohepatitis was not feasible. Instead, we used the NFS, which has been well validated and widely used for screening for advanced liver fibrosis in NAFLD patients. Finally, this was a single-centre study from a Chinese hospital, and caution should be exercised when extrapolating the results to different clinical settings.

AUTHORS' CONTRIBUTIONS

TW: Conceptualization, Formal Analysis, Supervision, Writing – original draft. **LH:** Resources, Software, Supervision. **SW:** Resources, Software, Validation, Writing – review & editing. **DM:** Conceptualization, Data curation, Methodology, Software, Writing – original draft.

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ORIGINAL ARTICLE

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Influence of maternal and perinatal complications on therapeutic hypothermia in newborns with low Apgar scores

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SUMMARY

OBJECTIVE: The aim of this study was to evaluate the impact of therapeutic hypothermia on maternal and perinatal outcomes in newborns with Apgar score<7 at the 5th min.

METHODS: A retrospective cohort study was carried out with 55 newborns who had an Apgar score<7 at the 5th min (35 without and 20 with therapeutic hypothermia) from low-risk pregnancies between 33 and 41 weeks gestation. The Apgar score was calculated through an objective assessment by a neonatologist in the delivery room. Therapeutic hypothermia was indicated by a neonatologist in the delivery room, according to the protocol established by the Brazilian Society of Pediatrics. The maternal and perinatal outcomes of both groups (without and with therapeutic hypothermia) were compared.

RESULTS: A rate of Apgar score<7 at the 5th min was 1.02%. No statistical differences were observed between the two groups (without and with therapeutic hypothermia) regarding maternal/perinatal complications. The presence of maternal/perinatal complications did not increase the odds ratio of neonatal therapeutic hypothermia in newborns with Apgar score<7 at the 5th min.

CONCLUSION: The rate of Apgar score<7 at the 5th min was low, and it was not associated with any maternal/perinatal complications. There was no significant difference in maternal/perinatal complications between newborns who received therapeutic hypothermia and those who did not.

KEYWORDS: Newborn. Apgar score. Therapeutic hypothermia.

INTRODUCTION

The Apgar score was developed in 1952 by Dr. Virginia Apgar to evaluate the interference of obstetric conditions with the clinical condition of newborns shortly after delivery. It evaluates five parameters, namely, heart rate, breathing, muscle tone, skin color, and reflexes, in the newborn at the 1st and 5th min, and each of these parameters can be given from 0 to 2 points, where 0 means the parameter was absent and 2 shows the parameter in its best condition. . This score is currently used worldwide to assess the health status of newborns[1].

The 5th min Apgar score estimates the vitality of the newborn and the effectiveness of neonatal resuscitation, when applied[1]. The highest score is 10, representing the best condition of a newborn, and the lowest scores are generally associated with the findings suggestive of asphyxia in umbilical cord blood. While the Apgar score has some criticisms and considerations, it is very useful in evaluating the success of reanimation procedures[2].

There are many obstetric, maternal, and neonatal factors, including gestational age, use of anesthetics during delivery, congenital infections, cardiopulmonary alterations in the newborn, prenatal conditions, and birth weight, which can interfere with the Apgar score. An Apgar score that remains low after the 5th min of birth indicates severe asphyxia, so the clinical relevance of the score increases as the scoring decreases and the time of life increases, because low scores after the 5th min are associated with greater morbidity, neurological impairment, and neonatal mortality[3].

The Apgar score<7 (between 0 and 6) is a warning sign for pediatricians, and such newborns require special attention and care, according to the pathophysiology and the low Apgar grade[4]. Some factors related to neonatal asphyxia are unavoidable, but there are also preventable and reversible causes, as long as there is adequate obstetric care and relevant neonatal care protocols. Therefore, it is important to identify the risk

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factors for low Apgar scores so that the appropriate approach can be taken, thus reducing perinatal asphyxia[5].

Therapeutic hypothermia is a controversial treatment used to treat moderate or severe neonatal encephalopathy. While some studies showed a decrease in neonatal deaths, others have reported an increase[6,7]. A low Apgar score was a relevant factor in identifying newborns eligible for therapeutic hypothermia[8].

This study aims to evaluate the influence of therapeutic hypothermia on maternal and perinatal outcomes in newborns with Apgar score<7 at the 5th min.

METHODS

A retrospective cohort study was conducted by analyzing the medical records of pregnant women who had their delivery at the Amparo Maternal Hospital, which cares for low-risk obstetric pregnant women in the city of São Paulo between January 2021 and December 2021. This study was approved by the Research Ethics Committee of the Federal University of São Paulo (CAAE: 57974422.7.0000.5505). The consent form was not necessary because it was a retrospective study.

We included singleton pregnancies with newborns who had Apgar scores<7 at the 5th min and excluded cases with incomplete medical records or those that could not be accessed.

The Apgar score is calculated through an objective assessment by a neonatologist in the delivery room. Five parameters are assessed, namely, heart rate, breathing, muscle tone, skin color, and reflexes, with a score between 0 and 2. This assessment takes place between 1st and 5th min of life. If the score at the 5th min is<7, the assessment should be repeated every 5 min until the score is>7 or until the newborn reaches the 20th min of life[1]. The 5th min Apgar score is related to a good predictor of survival in infancy[9].

Therapeutic hypothermia is indicated by a neonatologist in the delivery room, according to the protocol established by the Brazilian Society of Pediatrics[10]. Evidence of perinatal asphyxia is required, together with signs of hypoxic–ischemic encephalopathy on physical examination. Evidence of perinatal asphyxia can be assessed by cord blood gases with a pH<7.0, by a history of any acute event at the time of delivery that could cause fetal distress, by an Apgar score ≤5 at the 5th min of life, or by the need for positive pressure ventilation for more than 20 min. The presence of hypoxic–ischemic encephalopathy is assessed using criteria defined by Shankaran et al.[11], before 6 h of life. These criteria consist of assessing the newborn's level of consciousness, spontaneous activity, posture, tone, primitive reflexes, and autonomic system. Alterations in at least three categories are required for the diagnosis of hypoxic–ischemic encephalopathy. Therapeutic hypothermia is contraindicated when the newborn is in situations of pre-death, complex malformations, genetic abnormalities, or when there is already a defined palliative care approach.

The study assessed several variables related to maternal and newborn clinical data, including maternal age, body mass index (BMI), number of pregnancies, number of previous deliveries, gestational age at admission, presence of prematurity, arterial hypertension during pregnancy, gestational diabetes mellitus, fetal growth restriction, positive culture for group B beta-hemolytic *Streptococcus* (GBS), premature rupture of ovular membranes (PPROM), characteristics of amniotic fluid (clear, fluid meconium, thick meconium, blood), labor (induced or spontaneous), type of delivery (vaginal, forceps, cesarean section), birth weight (<2,500 g, 2,500–4,000 g,>4,000 g), neonatal intensive care unit (NICU) admission, neonatal death, length of hospitalization in NCIU, length of invasive ventilation support, use of antibiotics, vasoactive drug, blood transfusions, intracranial hemorrhage, and whether the death was related with neonatal asphyxia. The cases included in the study will be separated into two groups: Group I—newborns without therapeutic hypothermia and Group II—newborns with therapeutic hypothermia.

The GPower 3.1 software (Heinrich-Heine-Universität, Düsseldorf, Germany) was used to calculate the sample size. To assess the association between the presence of neonatal hypothermia and maternal and newborn clinical data, a total sample of at least 52 patients was required. The sample size analysis was based on a w-effect of 0.50, a probability of error α of 0.05, and a power (probability of error 1-β) of 0.80, with five degrees of freedom (Df).

The data were analyzed using SPSS version 20.0 (Chicago, IL, USA) and Prisma GraphPad 7.0 (Boston, MA, USA). Quantitative variables were subjected to the D'Agostino-Pearson normality test and presented as means and standard deviations. Categorical variables were described as absolute and percentage frequencies and presented in tables and graphs. Differences between categorical variables and their proportions were analyzed using the chi-square test. The effect of the groups on the continuous variables was analyzed using the Mann-Whitney test (nonparametric distribution). The odds ratio (OR) for the development of neonatal hypothermia was calculated using binary logistic regression. The significance level adopted for all tests was p<0.05.

RESULTS

From January 2021 to December 2021, 55 cases with an Apgar score<7.0 at the 5th min were selected at the Amparo Maternal Hospital. The number of deliveries during this period of time was 5,348, corresponding to a rate of 1.02% of low Apgar score at the 5th min. The characteristics of the study population are shown in Table 1. The cases were separated into two groups:

Table 1. Characteristics of the study population.

	Group I (n=35)	Group II (n=20)	p-value
Maternal age (years)			
<18	0.0% (0/34)	5.3% (1/19)	0.358§
18–35	82.4% (28/34)	68.4% (13/19)	0.311§
>35	17.6% (6/34)	26.3% (5/19)	0.495§
BMI (kg/m²)	26.0 (22.0–34.0)	27.0 (24.0–36.0)	0.096ƒ
Number of pregnancies	1 (1–5)	1 (1–5)	0.984ƒ
Number of previous deliveries			
Nulliparous	54,3% (19/35)	50.0% (10/20)	0.759§
Multiparous	45.3% (16/35)	50.0% (10/20)	0.759§
Gestational age (weeks)	39.0 (33.0–41.0)	40.0 (38.0–41.0)	0.166ƒ
Prematurity	11.8% (4/34)	0.0% (0/19)	0.120§
Arterial hypertension during pregnancy	8.8% (3/34)	5.3% (1/19)	0.638§
Gestational diabetes mellitus	8.8% (3/34)	5.3% (1/19)	0.638§
Fetal growth restriction	0.0% (0/35)	0.0% (0/20)	
Positive culture for GBS	11.8% (4/34)	10.5% (2/19)	0.945§
PPROM	8.8% (3/34)	22.2% (4/18)	0.178§
Characteristics of amniotic fluid			
Clear	61.3% (19/31)	75.0% (12/16)	0.517§
Fluid meconium	9.7% (3/31)	0.0% (0/16)	0.541§
Thick meconium	25.8% (8/31)	25.0% (4/16)	>0.999§
Blood	3.2% (1/31)	0.0% (0/16)	>0.999§
Labor			
Spontaneous	76.5% (26/34)	61.1% (11/18)	0.336§
Induced	23.5% (8/34)	38.9% (7/19)	0.336§
Type of delivery			
Vaginal	44.1% (15/34)	52.6% (10/19)	0.579§
Forceps	0.0% (0/34)	0.0% (0/19)	
Cesarean section	55.9% (19/34)	47.4% (9/19)	0.579§
Birth weight			
<2,500 g	23.5% (8/34)	5.3% (1/19)	0.133§
2,500–4,000 g	70.6% (24/34)	84.2% (16/19)	0.334§
>4,000 g	5.9% (2/34)	10.5% (2/19)	0.611§
Apgar score at the 1st min	3.0 (0.0–8.0)	2.0 (0.0–5.0)	0.185ƒ
NICU admission	100.0% (35/35)	100% (20/20)	
NICU length (days)	3.0 (1.0–19.0)	7.0 (1.0–30.0)	0.032ƒ
Invasive ventilation support (h)	5.5 (0–456.0)	24.0 (0–480.0)	0.117ƒ
Use of antibiotics	30.3% (10/33)	42.1% (8/19)	0.389§
Vasoactive drug	28.1% (9/32)	57.9% (11/19)	0.035§
Blood transfusion	18.2% (6/33)	15.8% (3/19)	0.826§
Intracranial hemorrhage	6.3% (2/32)	26.3% (5/19)	0.044§
Neonatal death	5.7% (2/35)	5.0% (1/20)	0.911§

Group 1: without hypothermia; Group 2: with hypothermia; BMI: body mass index; GBS: Group B beta-hemolytic ***Streptococcus***; PPROM: premature rupture of ovular membranes; NICU: neonatal intensive care unit; Mann-Whitneyƒ: median (minimum–maximum); Chi-square§: percentage (n/N); p<0.05.

Group I—newborns without therapeutic hypothermia (n=35) and Group II—newborns with therapeutic hypothermia (n=20).

Newborns with therapeutic hypothermia had a longer stay in the NICU (7.0 vs 3.0 days, p=0.032, respectively), a higher prevalence of vasoactive drug (57.9% vs 28.1%, p=0.035) and intracranial hemorrhage (26.3% vs 6.3%, p=0.044), compared to newborns without therapeutic hypothermia. There was no significant differences between the groups in relation to BMI (p=0.096), Apgar score at the 1st min (p=0.185), maternal age<18 years (p=0.358), maternal age between 18 and 35 years (p=0.311), maternal age>35 years (p=0.495), number of pregnancies (p=0.984), nulliparity (p=0.759), multiparity (p=0.759), gestational age at admission (p=0.166), prematurity (p=0.120), arterial hypertension during pregnancy (p=0.638), gestational diabetes mellitus (p=0.638), positive culture for GBS (p=0.945), PPROM (p=0.178), clear amniotic fluid (p=0.517), fluid meconium (p=0.541), thick meconium (p>0.999), blood in the amniotic fluid (p>0.999), spontaneous onset of labor (p=0.336), induction of labor (p=0.336), vaginal delivery (p=0.579), cesarean section (p=0.579), birth weight<2,500 g (p=0.133), birth weight between 2,500 and 4,000 g (p=0.334), birth weight>4,000 g (p=0.611), NICU admission, invasive ventilation support (p=0.117), use of antibiotics (p=0.389), blood transfusion (0.826), and neonatal death (p=0.911). There was no case of neonatal death related to neonatal asphyxia (Table 1).

The vasoactive drug increased the risk of newborn therapeutic hypothermia (OR:3.51, CI 95% 1.07–11.6, p=0.039). The presence of prematurity (p=0.120), arterial hypertension during pregnancy (p=0.638), gestational diabetes mellitus (p=0.638), positive culture for GBS (p=0.945), PPROM (p=0.178), clear amniotic fluid (p=0.517), fluid meconium (p=0.541), thick meconium (p>0.999), blood in the amniotic fluid (p>0. 999), spontaneous onset of labor (p=0.336), induction of labor (p=0.336), vaginal delivery (p=0.579), cesarean section (p=0.579), birth weight<2,500 g (p=0.133), birth weight between 2,500 and 4,000 g (p=0.334), birth weight>4,000 g (p=0.611), NICU length stay (p=0.051), invasive ventilation support (p=0.183), use of antibiotics (p=0.389), blood transfusion (p=0.826), and intracranial hemorrhage (p=0.061) did not increase the OR of neonatal therapeutic hypothermia (Table 2).

DISCUSSION

A low Apgar score at the 5th min is associated with nonresponse to adequate resuscitation. The Apgar score at the 5th min is a good predictor of neonatal mortality[12] and severe morbidities such as neurological disabilities (seems to persist many years postnatally)[13] and pediatric infections[14]. Low Apgar scores at the 5th min have been associated with several maternal/obstetric complications, including non-vertex fetal presentation, prolonged labor, presence of meconium in amniotic fluid, induced labor, and low birth weight[15]. In Southwest China, hypertensive disorders in pregnancy were a more important factor for a low Apgar score (<7)[16]. In a study developed in Atlanta, Georgia, USA, maternal hypertensive disorders, PPROM, cesarean section, vaginal delivery after cesarean section, and male gender were associated with Apgar score<7 at the 5th min[17]. In our study, maternal arterial hypertension disorders, PPROM, thick meconium in amniotic fluid, induced labor, cesarean section, and birth weight<2,500 g were 7.5%, 25.5%, 13.4%, 28.3%, and 16.9%, respectively, in newborns with Apgar score<7 at the 5th min.

The rate of low Apgar scores at 5th min varies among continents, with higher rates in developing and underdeveloped countries. In an Ethiopian study, the rate of low Apgar score at the 5th min was 13.8%[15]. In a Nigerian study, the rate of low Apgar score at the 5th min was 4.0%[18]. In our study, the rate of Apgar score<7 at the 5th min was 1.02%, but we should point out that our service is a reference for low-risk pregnancies with few maternal/fetal risk factors for low Apgar scores. In a large low-risk population sample (31.5 million deliveries), Chen and Chauban[19] assessed the association between a low Apgar score at the 10th min and adverse perinatal outcomes. The authors observed that the rate of composite neonatal adverse outcomes was 403.5 per 1,000 live births with a low Apgar score at the 10th min (0–3). The rate of infant mortality was 201.0 per 1,000 live births among newborn infants with a low Apgar score at the 10th min. Another factor that may explain the low rate of Apgar scores at the 5th min in our study could be the small sample size.

Neonatal therapeutic hypothermia has been demonstrated to reduce death or disability in term and near-term infants with moderate–severe hypoxic–ischemic encephalopathy. The technique consists of whole-body cooling and selective head cooling. The cooling devices generally used are ice packs and phase change material for whole-body cooling and cooling caps for selective cooling[20]. Meta-analysis of 11 randomized controlled clinical trials of selective head cooling and whole-body cooling initiated within 6 h after delivery, involving 1,505 term and near-term infants (≥35 weeks gestation) with moderate to severe hypoxic–ischemic encephalopathy demonstrated the benefits of hypothermia[21].

In our study, we used the Apgar score<7 at the 5th min as a criterion for neonatal therapeutic hypothermia. Chen et al.[22] assessed 258 newborns (110 hypoxic–ischemic encephalopathy and 148 controls) and observed by multivariate logistic regression analysis that low birth weight (<2,500 g), amniotic fluid contamination, low Apgar score at the 1st min (≤3), and low Apgar score at the 5th min (≤7) increased the risk of hypoxic–ischemic

Table 2. Odds ratio of hypothermia in newborns with Apgar score <7 at the 5th min.

Variable	OR	95%CI	p-value§
Prematurity	0.174	0.009–3.41	0.120
Arterial hypertension during pregnancy	0.574	0.055–5.94	0.638
Gestational diabetes mellitus	0.574	0.055–5.94	0.638
Positive culture for GBS	0.95	0.159–5.01	0.945
PPROM	2.95	0.582–15.0	0.178
Characteristics of amniotic fluid			
Clear	1.89	0.533–6.26	0.517
Fluid meconium	0.00	0–2.20	0.541
Thick meconium	0.95	0.27–3.96	>0.999
Blood	0.00	0–17.44	>0.999
Beginning of labor			
Spontaneous	0.48	0.142–1.68	0.336
Induced	2.06	0.592–7.02	0.336
Type of delivery			
Vaginal	1.40	0.438–4.11	0.579
Cesarean section	0.71	0.243–2.28	0.579
Birth weight			
<2,500 g	0.18	0.015–1.16	0.133
2,500–4,000 g	2.22	0.568–8.33	0.334
>4,000 g	1.88	0.272–12.67	0.611
NICU length	1.09	1.00–1.19	0.051
Invasive ventilation support	1.00	0.99–1.08	0.183
Use of antibiotics	1.67	0.51–5.42	0.389
Vasoactive drug	3.51	1.07–11.6	0.039
Blood transfusion	0.84	0.18–3.85	0.826
Intracranial hemorrhage	5.36	0.92–31.08	0.061

Group 1: without hypothermia; Group 2: with hypothermia; CI: confidence interval; GBS: Group B beta-hemolytic ***Streptococcus***; PPROM: premature rupture of ovarian membranes; OR: odds ratio; binary logistic regression: percentage (n/N); p<0.05. Chi-square§.

encephalopathy. In our study, 5.3 and 25.0% of newborns who received therapeutic hypothermia had low birth weight and thick meconium in the amniotic fluid, respectively.

CONCLUSION

The rate of Apgar score<7 at the 5th min was low, and it was not associated with any maternal/perinatal complications. Furthermore, newborns with or without therapeutic hypothermia did not differ among maternal/perinatal complications.

AUTHORS' CONTRIBUTIONS

PTC: Data curation, Investigation. **MNA:** Data curation, Investigation. **ABP:** Formal Analysis. **EAJ:** Writing – original draft. **NM:** Methodology. **RM:** Writing – review & editing. **SYS**: Supervision.

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ORIGINAL ARTICLE

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The rs1862513 promoter variant of resistin gene influences susceptibility to nonalcoholic fatty liver disease

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SUMMARY

OBJECTIVES: Nonalcoholic fatty liver disease is the term used for a range of conditions in which fat builds up in the liver and exceeds 5% of hepatocytes without inordinate alcohol intake or other causes of lipid accumulation. Regarding the fact that insulin resistance and obesity play key roles in the pathogenesis of nonalcoholic fatty liver disease, as well as the connection between resistin and these metabolic diseases, the association between nonalcoholic fatty liver disease and a resistin gene (*RETN*) polymorphism was examined.

METHODS: In this genetic case–control association study, 150 biopsy-proven nonalcoholic fatty liver disease patients and 154 controls were enrolled and genotyped for the *RETN* rs1862513 (-420C>G) gene polymorphism using PCR–RFLP method.

RESULTS: The -420C>G genotype frequency distributions in both groups were consistent with Hardy-Weinberg equilibrium (HWE; p>0.05). The carriers of the *RETN* -420C>G "CC" genotype compared with the "GG" genotype occurred less frequently in the cases with nonalcoholic fatty liver disease than in the controls, and the difference remained significant even after adjustment for confounding factors (p=0.030; OR=0.47, 95%CI=0.36–0.93). Interestingly, the *RETN* -420C>G "C" allele was also associated with a decreased risk for nonalcoholic fatty liver disease too (p=0.042; OR=0.72, 95%CI=0.53–0.95).

CONCLUSION: We found for the first time an association between biopsy-proven nonalcoholic fatty liver disease and *RETN* -420C>G promoter polymorphism. The carriers of the *RETN* -420C>G "CC" genotype had a 53% decreased risk for nonalcoholic fatty liver disease. Our findings, however, need to be corroborated by further studies.

KEYWORDS: NAFLD. Polymorphism. Resistin. *RETN*.

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is the term used for a range of conditions in which fat builds up in the liver and exceeds 5% of hepatocytes without inordinate alcohol intake or other causes of lipid accumulation. NAFLD affects roughly 25% of adults worldwide and encompasses simple steatosis, nonalcoholic steatohepatitis (NASH), and cirrhosis[1]. Positive associations between NAFLD and circulating insulin levels, insulin resistance (IR), type 2 diabetes (T2D), and obesity have been found. Moreover, patients with NASH have a higher IR index than cases with simple steatosis, and the severity of the increased levels of liver enzymes is also higher in NAFLD patients with IR than those without IR. Finally, significant associations between variants in insulin signaling pathway genes including insulin receptor (*INSR*), insulin receptor substrate 2 (*IRS2*), insulin-like growth factor 1 (*IGF1*), and insulin-like growth factor binding protein 3 (*IGFBP3*) and the risk of NAFLD have been discovered[2-7].

Resistin, a 12-kDa cysteine-rich polypeptide hormone protein, is the product of the *RETN* gene and may be involved in the pathogenesis of NAFLD. It is predominantly secreted by macrophages and adipocytes and has a pivotal role in energy homeostasis. Resistin inhibits the ability of insulin to stimulate cellular glucose uptake and links obesity to insulin resistance[8]. IR, obesity, and NAFLD are all associated with alterations in circulating resistin levels. It has been demonstrated that resistin

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levels were positively associated with body mass index (BMI)[9], IR[10], and NAFLD[11]. Previous reports have also indicated significant associations between *RETN* gene polymorphisms and the expression of *RETN* gene[12], serum resistin levels[13], and obesity[14]. Thus, this study was designed to investigate the possible contribution of the *RETN* rs1862513 gene polymorphism to NAFLD. The inclusion criteria for selecting this single-nucleotide polymorphism (SNP) include (I) its common use in prior genetic studies; (II) functional importance; and (III) relatively high degree of heterozygosity.

METHODS

Study population

After informed consent, 150 patients with biopsy-proven NAFLD (age range, 32–86 years) and 154 controls (age range, 31–82 years) were enrolled. NAFLD patients were enrolled after fatty liver diagnosis, which in turn was defined by (a) ultrasonographic confirmation of fatty liver, (b) having high circulating levels of AST, ALT, and GGT, (c) excluding subjects with other causes of liver disease such as Wilson's disease, alpha-1 antitrypsin deficiency, viral hepatitis, and alcohol use of more than 70 g/week in women or more than 140 g/week in men, and (d) liver biopsy evidence of NAFLD using the Brunt's criteria by an experienced pathologist. The control group was recruited from the research staff of the Institute and medical students. Those who were free of elevated AST, ALT, GGT, viral hepatitis infection (examined by blood test), had no liver steatosis (examined by abdominal ultrasonography), and were not alcoholic or on regular medications were enrolled as controls. This study complied with the principles of the Declaration of Helsinki and was performed according to the Institute's Ethics Committee approval.

Genotyping

Genomic DNA purification from 5 mL EDTA-anticoagulated whole blood was performed using phenol–chloroform extraction and ethanol precipitation. Then, the DNA samples were stored at -20°C. To detect the genotypes of the *RETN* rs1862513 or -420C>G variant, we used PCR–RFLP method. In brief, genomic DNA was amplified using the primers: 5'-TCCTGGCTTGGCTAATAAGTC-3' and 5'-TACCAGTTCTATTGCTCATGGG-3' to discover the genotypes of the *RETN* gene. PCR conditions were as follows: (I) pre-degeneration at 95°C for 10 min, (II) 35 cycles for degeneration at 95°C for 45 s, annealing at 61°C for 40 s, and extension at 72°C for 40 s, and (III) final extension at 72°C for 10 min. The PCR products (500 bp) were then analyzed by RFLP: overnight digestion with the restriction enzyme of EarI (Fermentas, Leon-Rot, Germany) at 37°C in a water bath. Electrophoresis was performed on a 2.5% agarose gel stained with ethidium bromide and then the RFLP products (500 bp, 363 bp, and 137 bp) were visualized using ultraviolet light transillumination[15]. The "C" allele of the *RETN* -420C>G SNP had bands of 363 bp and 137 bp, whereas its "G" allele had a band of 500 bp, thus an individual with band(s) at 363 bp and 137 bp, at 500 bp only, or at 500 bp, 363 bp, and 137 bp was defined as "CC" homozygotic genotype, "GG" homozygotic genotype, and "CG" heterozygotic genotype, respectively. To verify the genotyping results, 20% of all the subjects were genotyped twice by different laboratory personnel; the reproducibility was 100%.

Statistical analyses

T-test was used to compare continuous variables. To compare categorical clinical variables and allele frequencies between the case and control groups, we used chi-square (χ^2) test. χ^2 test was also used to examine HWE. To assess the association between the genotype frequencies of the *RETN* -420C>G and the risk of NAFLD, logistic regression analysis was used. This analysis was also applied to adjust the confounding factors such as age and BMI. To evaluate the measure of the associations, the odds ratio (OR) and the corresponding 95% confidence interval (95%CI) were used. A p-value less than 0.05 was considered statistically significant (SPSS, version 25.0, Chicago, IL, USA).

RESULTS

Table 1 shows the clinical and biochemical data of controls and NAFLD patients. The patients were more likely to be male ($p<0.001$) and smokers ($p=0.02$). They also had higher age ($p<0.001$), BMI ($p<0.001$), systolic blood pressure ($p<0.001$), diastolic blood pressure ($p<0.001$), AST ($p<0.001$), ALT ($p<0.001$), and GGT ($p<0.001$) than the controls.

Both groups had a consistent genotype frequency distribution and presented HWE, hence we used a representative sample population. The carriers of the "CC" genotype of *RETN* -420C>G variant compared with the carriers of the "GG" genotype were associated with a decreased risk for NAFLD, and the difference remained significant after adjustment for confounding factors including age, BMI, sex, smoking status, SBP, and DBP ($p=0.030$; OR=0.47, 95%CI=0.36–0.93) (Table 2). Furthermore, the *RETN* -420C>G "C" allele in comparison to "G" allele was significantly underrepresented in the cases with NAFLD compared to controls ($p=0.042$; OR=0.72, 95%CI=0.53–0.95).

Table 1. Selected characteristics of the patients with nonalcoholic fatty liver disease and controls[a].

Variables	Patients (n=150)	Controls (n=154)	p-value
Age (years)	38.1 (9.3)	29.0 (7.4)	<0.001
Body mass index (kg/m^2)	29.4 (5.0)	23.2 (3.3)	<0.001
Sex			
Men	109 (72.7)	80 (51.9)	
Women	41 (27.3)	74 (48.1)	<0.001
Smoking history			
No	111 (74.0)	140 (90.9)	
Former	19 (12.7)	9 (5.8)	
Current	20 (13.3)	5 (3.3)	0.020
Systolic blood pressure (mmHg)	123.8 (15.3)	113.9 (13.4)	<0.001
Diastolic blood pressure (mmHg)	74.5 (9.8)	70.0 (8.1)	<0.001
Aspartate aminotransferase (IU/L)	39.4 (17.1)	19.8 (7.1)	<0.001
Alanine aminotransferase (IU/L)	71.1 (39.9)	19.5 (10.2)	<0.001
Gamma glutamyl transferase (IU/L)	57.8 (31.4)	19.1 (8.3)	<0.001
Steatosis			
Grade 0			
Grade 1	38 (25.3)		
Grade 2	81 (54.0)		
Grade 3	31 (20.7)		
Necroinflammation			
Grade 0	46 (30.7)		
Grade 1	57 (38.0)		
Grade 2	45 (30.0)		
Grade 3	2 (1.3)		
Fibrosis			
Stage 0	88 (58.7)		
Stage 1	55 (36.7)		
Stage 2	7 (4.6)		
Stage 3			
Stage 4			

[a]Variables presented as mean (SD) or number (%).

DISCUSSION

As a complex disease, NAFLD is caused by the interactions between many environmental and genetic factors, each of which has a somewhat small individual effect; hence, it is difficult to discover them. However, studying the candidate gene polymorphisms can be a useful approach to recognize the potential genes involved in NAFLD pathogenesis, although contradictory findings are not infrequent in genetic association studies. The discrepancies may be explicated by variations in the environmental factors, differences in the disease definition, racial differences in genetic makeup, and statistical analyses[16,17]. Regarding the fact that IR, obesity, and inflammation are of critical importance in the development and progression of NAFLD, and resistin plays an important role in these metabolic disorders, it is biologically reasonable to hypothesize that *RETN* gene may be involved in NAFLD pathogenesis. The release of free fatty acids from adipose tissue and their influx into liver can be accelerated by IR. To maintain glucose homeostasis in patients with NAFLD, insulin secretion seems to increase to make up for low insulin sensitivity in these patients[2-4,18].

Table 2. Distribution of resistin gene (*RETN*) rs1862513 polymorphism in the patients with nonalcoholic fatty liver and in the controls[a].

RETN (rs1862513)	Controls (n=154)	Patients (n=150)	Crude OR (95%CI) p-value	Adjusted OR (95%CI) p-value[b]
Genotype-wise comparison				
GG	73 (47.4)	86 (57.3)	1.0 (reference)	1.0 (reference)
GC	43 (27.9)	37 (24.7)	0.87 (0.71–1.58)0.750	0.92 (0.67–1.60)0.890
CC	38 (24.7)	27 (18.0)	0.43 (0.41–0.88)0.028	0.47 (0.36–0.93)0.030
GC and CC	81 (52.6)	64 (42.7)	0.72 (0.64–1.20)0.207	0.77 (0.6–1.21)0.219
CC versus others	38 (24.7)	27 (18.0)	0.61 (0.59–1.23)0.052	0.64 (0.5–1.27)0.056
Allele-wise comparison				
G	189 (61.4)	209 (69.7)	1.0 (reference)	–
C	119 (38.6)	91 (30.3)	0.72 (0.53–0.95)0.042	–

[a]Variables presented as numbers (%).
[b]Adjusted for age, body mass index, sex, smoking status, systolic blood pressure, and diastolic blood pressure in genotype-wise comparisons.

RETN gene consists of four exons and is situated on the short arm of chromosome 19. In this study, we demonstrated that there is a significant association between NAFLD and the -420C>G variant located in the promoter of *RETN* gene. The "CC" genotype of *RETN* -420C>G gene polymorphism in comparison to "GG" genotype was a protective factor for NAFLD. The "C" allele of the *RETN* -420C>G variant occurred less frequently in the NAFLD patients too. Alterations in promoter sequence may affect the expression of *RETN* gene and/or the function of RETN protein. The -420C>G promoter variant influences the expression of *RETN* gene. It has been shown that this polymorphism induces resistin mRNA synthesis through the generation of a Sp1/Sp3 binding site[12], which in turn increases the resistin transcription and finally leads to a higher circulating resistin level. Interestingly, previous studies confirm this hypothesis. The *RETN* -420C>G polymorphism is associated with the expression of *RETN* gene[12] and serum resistin levels[13,19]. There is a significant association between -420G allele and higher serum resistin levels[13,19] and obesity[14]. The *RETN* -420C>G "GG" genotype also increases the *RETN* promoter activity[12]. Resistin desensitizes fat, muscle, and liver cells to insulin and causes hepatic insulin resistance[20]. Consistently, resistin levels were positively associated with IR[10], obesity[9], NAFLD[11], and fibrosis severity[21]. Alternatively, resistin may cause NAFLD via inducing inflammation, which is a key contributor to NAFLD pathogenesis. It appears that the link between resistin and inflammatory markers is independent of BMI. Serum resistin levels were positively associated with C-reactive protein (CRP) as an inflammatory biomarker. Resistin is also implicated in inflammatory processes as a proinflammatory factor in liver fibrogenesis and upregulates the expression of proinflammatory cytokines such as TNF-α, IL6, IL12, and MCP-1 in monocytes, macrophages, and hepatic cells through the NF-κB pathway. Resistin also enhances the expression of TNF-α and IL-1β via MEK and ERK signaling pathways by inhibiting some microRNAs[8,22,23], Consequently, a growing body of evidence corroborates the hypothesis that resistin and its gene, *RETN*, may play a role in the development and progression of NAFLD, and the finding of the present study that the -420C>G polymorphism of *RETN* gene is associated with NAFLD is in line with the above evidence. Notwithstanding these findings, however, the exact molecular mechanism through which the -420C>G variant affects the function of *RETN* gene is largely undetermined and needs to be fully elucidated.

Potential limitations exist in our study. First, it was unreasonable to carry out sub-analyses owing to the modest sample size. Second, due to budget limitations we were unable to measure the circulating resistin level and genotype of more than one variant of the *RETN* gene. Despite the above limitations, the present study had a good design, and we used liver biopsy as the gold standard method to confirm NAFLD diagnosis. Moreover, it also had novel and interesting findings that were consistent with prior research.

CONCLUSION

Our findings indicated that *RETN* -420C>G "CC" genotype had a 53% decreased risk for NAFLD compared with its "GG" genotype counterpart. Interestingly, this observation is pertinent from a theoretical viewpoint, although further studies with larger samples of different populations are required to elucidate the participation of *RETN* gene polymorphisms in NAFLD susceptibility.

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AUTHORS' CONTRIBUTIONS

SN: Data curation, Writing – original draft. **MN:** Data curation, Writing – original draft. **RS:** Data curation, Writing – original draft. **TM:** Conceptualization, Formal Analysis, Supervision, Writing – original draft. **GR:** Data curation, Writing – original draft. **AA:** Data curation, Writing – original draft. **HN:** Data curation, Writing – original draft. **RD:** Data curation, Writing – original draft. **HF:** Data curation, Writing – original draft. **SPT:** Data curation, Formal Analysis, Writing – original draft.

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ORIGINAL ARTICLE

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A practical predictive model to predict 30-day mortality in neonatal sepsis

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SUMMARY

OBJECTIVE: Neonatal sepsis is a serious disease that needs timely and immediate medical attention. So far, there is no specific prognostic biomarkers or model for dependable predict outcomes in neonatal sepsis. The aim of this study was to establish a predictive model based on readily available laboratory data to assess 30-day mortality in neonatal sepsis.

METHODS: Neonates with sepsis were recruited between January 2019 and December 2022. The admission information was obtained from the medical record retrospectively. Univariate or multivariate analysis was utilized to identify independent risk factors. The receiver operating characteristic curve was drawn to check the performance of the predictive model.

RESULTS: A total of 195 patients were recruited. There was a big difference between the two groups in the levels of hemoglobin and prothrombin time. Multivariate analysis confirmed that hemoglobin>133 g/L (hazard ratio: 0.351, p=0.042) and prothrombin time >16.6 s (hazard ratio: 4.140, p=0.005) were independent risk markers of 30-day mortality. Based on these results, a predictive model with the highest area under the curve (0.756) was built.

CONCLUSION: We established a predictive model that can objectively and accurately predict individualized risk of 30-day mortality. The predictive model should help clinicians to improve individual treatment, make clinical decisions, and guide follow-up management strategies.

KEYWORDS: Hemoglobin. Model. Mortality. Neonatal sepsis. Prothrombin time.

INTRODUCTION

Neonatal sepsis is a serious and life-threatening disease that needs timely and immediate medical attention[1]. Despite advances in medical care, neonatal sepsis remains a significant cause of morbidity and mortality in neonates worldwide. The prevalence of neonatal sepsis and mortality rates varies across different regions and healthcare settings, with higher rates reported in low-resource areas[2]. Studies have reported that various biomarkers can affect the prognosis of neonatal sepsis, including gestational age, birth weight, presence of comorbidities, blood change, and the type of infecting organism[3-7]. Early identification and suitable treatment are essential for improving outcomes in patients with sepsis. To neonates, the clinical manifestations of sepsis can be nonspecific. Laboratory investigations, such as blood pressure monitoring, interleukin-18, and elevated neutrophil-to-monocyte ratio, are helpful for clinicians to evaluate the risk of adverse outcomes and guide treatment decisions[8-10]. So far, there is no specific prognostic biomarkers or model for dependable predict outcomes in neonatal sepsis. Our goal is to establish a predictive model based on readily available laboratory data to assess 30-day mortality in neonatal sepsis.

Patients

The investigation recruited neonates who were admitted to the hospital between January 2019 and December 2022. The admission information was obtained from the medical record retrospectively. The neonates were limited to those who were diagnosed with neonatal sepsis, with complete patient information, and aged within 28 days. Finally, 195 patients were recruited in the cohort. The diagnosis of neonatal sepsis was based on the International Consensus on Pediatric Sepsis definition[11]. The study was approved by the ethics committee at the local hospital and was conducted in accordance with the guidelines set out in the Declaration of Helsinki.

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Data

The following admission indexes were gathered in this investigation: age, gender, gestational age, weight, Apgar scores (5 min and 10 min), admission laboratory results, including routine blood test (neutrophils, lymphocyte, monocyte, platelet, hemoglobin), biochemical indicators [alanine aminotransferase (ALT), aspartate transaminase (AST), lactate dehydrogenase (LDH), direct bilirubin (DB), albumin (ALB), urea nitrogen (UREA), creatinine (CREA)], and coagulation function [prothrombin time (PT), international normalized ratio (INR), activated partial thromboplastin time (APTT) and thrombin time (TT)]. The primary outcome of neonatal sepsis was 30-day mortality, and the primary outcome was obtained from medical records or by telephone.

Statistical analysis

All statistical analyses were conducted with SPSS 21.0 (SPSS, Inc., IA, USA). Continuous variables, shown as mean±standard deviation, were compared with t-test and analysis of variance (ANOVA). Categorical variables (numbers and percentages) were compared using the chi-square test. Univariate or multivariate analysis was utilized to identify independent risk factors for 30-day mortality. Patients were divided into two groups according to the primary outcome. The receiver operating characteristic (ROC) curve was drawn to check the performance of the model in predicting the primary events and confirmed the optimal cutoff value of the predictive model. All tests were two-sided, and p-values <0.05 were considered statistically significant.

RESULTS

Baseline characteristics

The basic information of patients is presented in Table 1. Of these patients, the 30-day mortality rate was 17.5%. There were 123 males and 72 females, with a median age of 3 days (ranging from 1 day to 28 days). Compared with the nonsurvivor group, the significant elevated levels of platelet and ALB and the significant declined levels of ALT, AST, UREA, PT, INR, and TT were found in the survivor group (all p<0.05). Beyond that, obvious difference was also found in the numbers of culture positive, the levels of hemoglobin, LDH, DB, and CREA between the two groups, although statistical significance was not reached (all p<0.10) (Table 1).

Independent predictors of 30-day mortality

To further select independent factors of 30-day mortality, we conducted univariate and multivariate analyses on these variables with p<0.1 (Table 1). After adjusted these factors (culture positive, hemoglobin, platelet, ALT, AST, LDH, DB, UREA, CREA, PT, INR, TT, ALB), hemoglobin>133 g/L (hazard ratio (HR): 0.351, 95% confidence interval (CI): 0.128–0.961, p=0.042) and PT >16.6 s (HR: 4.140, 95%CI: 1.523–11.260, p=0.005) were independent risk markers of 30-day mortality (Table 2). Based on these results, a predictive model was built as follows:

Logit p=1.421×PT–1.046×hemoglobin–2.155.

Performance of the predictive model in the prediction of 30-day mortality

Sensitivity and specificity were determined to compare the performance of the predictive model and independent predictors. Figure 1A shows the ROC curve of the predictive model; the predictive model had the highest area under the curve (AUC) (0.756, 95%CI 0.666–0.847, P<0.001). The calibration curve of the predictive model had demonstrated good agreement (Figure 1B). Details of the performance are shown in Supplementary Table 1.

In addition, the patients were divided into two groups based on the predictive model. A comparison was made between the two groups; the results showed that the 30-day mortality rate was higher in the predictive model value>0.05588 groups than that in the predictive model value ≤ 0.05588 groups (24.3 vs. 3.40%).

DISCUSSION

In this study, we found a significant association among HB, PT, and the risk of 30-day mortality. Based on independent risk factors, we built a predictive model with good performance. In addition, we found that the 30-day mortality rate was higher in the predictive model value>0.05588 group than that in the predictive model value ≤ 0.05588 group.

Neonatal sepsis refers to a severe bloodstream infection that occurs in neonates. It is often accompanied by a characterized systemic inflammatory response syndrome[12]. Inflammation plays a vital role in the pathogenesis and progression of neonatal sepsis[13]. During neonatal sepsis, the presence of pathogens triggers an immune response, leading to the release of various pro-inflammatory molecules such as cytokines, chemokines, and acute-phase reactants[14,15]. However, the overproduction of pro-inflammatory molecules can result in widespread tissue damage, organ dysfunction, and complications associated with sepsis[16].

Hemoglobin is a major component of red blood cells, and its measurement provides information about anemia and oxygenation status[17]. In neonatal sepsis, the infection and inflammatory response can impact the hematopoietic system,

potentially leading to the development of anemia[18]. The release of inflammatory mediators and activation of inflammatory cells may suppress red blood cell production or promote their destruction, thereby reducing hemoglobin levels. Furthermore, sepsis can also cause systemic hemodynamic changes such as tissue hypoperfusion and circulatory disturbances, which can influence blood hemoglobin levels[19]. In line with prior studies, our study also confirmed that HB was an independent index. Therefore, monitoring hemoglobin levels in neonates with sepsis can provide valuable information about the severity of anemia, inflammatory response, and overall circulatory status. This aids clinicians in assessing the disease severity, guiding treatment strategies, and monitoring treatment effectiveness.

Prothrombin time, a test that measures the duration taken for the blood to clot, is an important indicator of coagulation function and can provide insights into the body's ability to form blood clots[20]. In neonatal sepsis, the inflammatory response and activation of coagulation pathways can lead to alterations in the coagulation system[21]. Sepsis-induced changes in the levels of coagulation factors, platelets, and endothelial cells can affect the clotting process and prolong PT[22,23]. Additionally, disseminated intravascular coagulation, a severe complication associated with sepsis, can further disrupt the coagulation cascade and contribute to abnormal PT results[24]. By monitoring PT in neonates with sepsis, clinicians can gain insights into the coagulation status and identify potential clotting abnormalities. This information is crucial for guiding appropriate treatment strategies, such as the administration of blood products or anticoagulants, and improving patient outcomes. Our study also confirmed this.

For clinical application, it is important to make the assessment of risk factors as convenient as possible. In our study, HB and PT are prevalent in clinical practice and convenient to acquire.

Table 1. Characteristics of patients.

General	Survivor (n=166)	Nonsurvivor (n=29)	p-value
Gender (male/female), n	106/60	s17/12	0.521
Median age (range), days	3 (1, 27)	5 (1, 28)	0.407
Gestational age at birth (weeks)	38.5 (29.0, 41.0)	38.0 (27, 41)	0.671
Weight (g)	3050 (1090, 3860)	750.19 (1088, 3510)	0.351
Apgar score (1 min)	10 (5, 10)	9 (4.5, 10)	0.570
Apgar score (5 min)	10 (8, 10)	10 (9, 10)	0.299
Culture positive, n (%)	50 (30.1)	6 (20.1)	0.070
CRP (mg/L)	7.85 (0.1, 96.4)	20.6 (0.1, 152.3)	0.187
Monocyte (×109/L)	0.92 (0.14, 2.96)	0.77 (0.13, 4.18)	0.655
Lymphocyte (×109/L)	3.66±1.80	4.23±3.62	0.309
Neutrophil (×109/L)	6.01 (1.91, 18.39)	6.87 (1.36, 17.98)	0.656
Hemoglobin (g/L)	149 (99.0, 190.6)	133 (91.5, 182.0)	0.066
Platelet (×109/L)	265.81±128.97	181.38±116.54	0.001
ALT (U/L)	10 (3.0, 44.0)	14 (3.0, 580.8)	0.001
AST (U/L)	37 (14.1, 191.4)	48 (15.8, 1791.2)	0.003
LDH (U/L)	405 (212, 1269)	515 (235, 3512)	0.050
DB (μmol/L)	8.4 (4.9, 23.5)	10.8 (4.4, 179.7)	0.072
UREA (mmol/L)	3.55 (1.38, 9.82)	5.25 (2.91, 18.42)	<0.001
CREA (μmol/L)	44 (18.8, 98.0)	55.5 (14.7, 145.3)	0.050
PT (s)	13.7 (10.8, 23.0)	17.4 (11.6, 31.3)	<0.001
INR	1.18 (0.93, 2.01)	1.51 (1.00, 2.89)	<0.001
APTT (s)	52.5 (33.9, 110.6)	57.6 (32.9, 112.3)	0.259
TT (s)	18.5 (15.2, 27.8)	19.4 (15.2, 54.2)	0.001
ALB (g/L)	33.4 (25.5, 40.1)	31.2 (21.4 to 36.6)	0.001

ALB: albumin; ALT: alanine aminotransferase; APTT: activated partial thromboplastin time; AST: aspartate aminotransferase; CRP: C-reactive protein; CREA: creatinine; INR: international normalized ratio; PT: prothrombin time; TT: thrombin time; UREA: urea nitrogen.

They were independent factors demonstrated by multivariate analysis and we built a simple, convincing, and readily available model with good performance. On subgroup analysis, the 30-day mortality rate was higher in the predictive model value>0.05588 groups than that in the predictive model value ≤ 0.05588 groups. Identifying high-risk patients may help clinicians improve the treatment efficacy and clinical outcome.

This study is limited by its retrospective nature and single-center data, which may cause selection bias. Second, only admission biomarkers were included in the present analyses, and it is

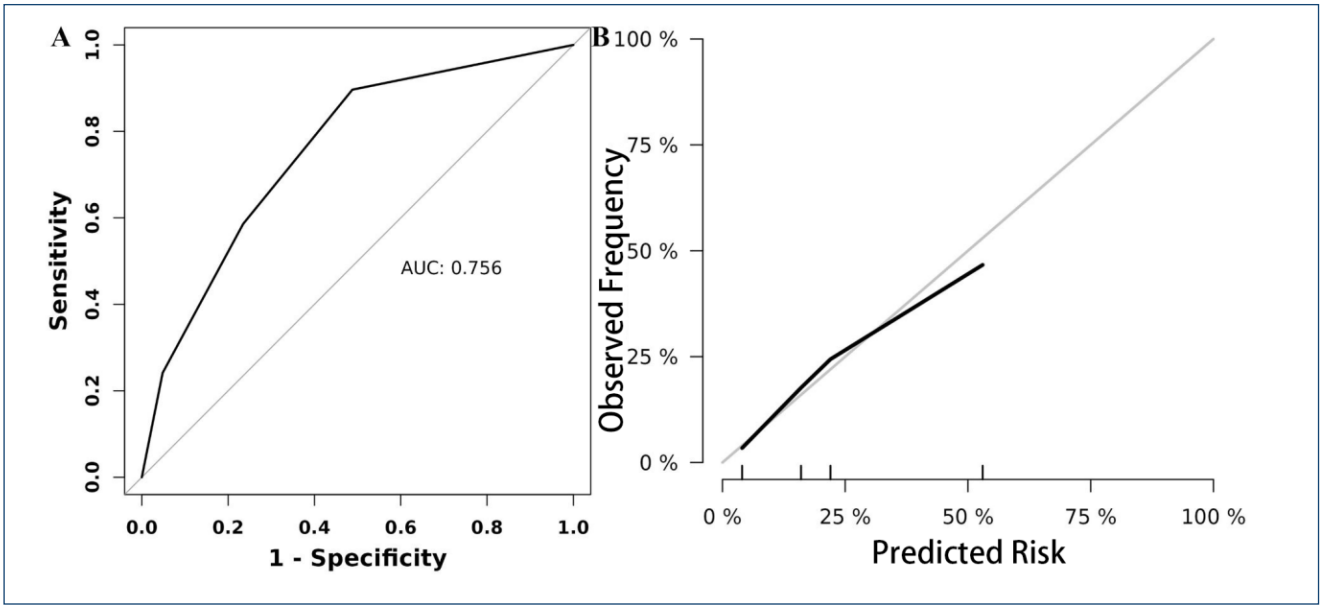

Figure 1. Performance of the predictive model. (A) Receiver operating characteristic curves of factors for predicting mortality. (B) Calibration curves.

Table 2. Analysis of in-hospital death.

Variables	Univariate analyses			Multivariate analyses			
	HR	95%CI	p-value	β	HR	95%CI	p-value
Culture positive	2.695	0.891–8.130	0.076				
Hemoglobin>133 g/L	0.350	0.157–0.782	0.011	-1.046	0.351	0.128–0.961	0.042
Platelet>244 ×109/L	0.210	0.085–0.519	<0.001				
ALT>32 U/L	5.775	2.163–15.415	0.001				
AST>41 U/L	2.104	0.850–4.769	0.133				
UREA>2.9 mmol/L	13.378	1.771–101.045	0.001				
PT>16.6s	4.613	2.029–10.490	<0.001	1.421	4.140	1.523–11.260	0.005
INR>1.45	4.183	1.846–9.474	0.001				
TT >18.35s	2.786	1.129–6.876	0.026				
ALB>33.9 g/L	0.204	0.068–0.612	0.002				
CREA>71 mmol/L	2.991	1.242–7.205	0.018				
LDH>387 U/L	0.905	0.860–0.952	0.134				
DB>13.4 μmol/L	1.955	0.433–8.828	0.538				
Logit p=1.421×PT−1.046×hemoglobin−2.155							

ALB: albumin; ALT: alanine aminotransferase; APTT: activated partial thromboplastin time; AST: aspartate aminotransferase; CI: confidence interval; CREA: creatinine; HR: hazard ratio; INR: international normalized ratio; PT: prothrombin time; TT: thrombin time; statistically significant p-values are denoted in bold (P<0.05).

possible that dynamic changes in biomarkers during the course of treatment might also influence outcomes in neonatal sepsis. Third, there were no test results for inflammatory factors, such as procalcitonin, and interleukin-6 (IL-6). Fourth, there are no external and internal cohorts. Thus, before clinical application, large, multicenter, prospective studies and validation cohort need to be conducted to determine the value of the predictive model.

CONCLUSION

Based on the clinical risk factors identified in this cohort, we established a model that can objectively and accurately predict individualized risk of 30-day mortality. The predictive model should help clinicians to improve individual treatment, make clinical decisions, and guide follow-up management strategies.

AVAILABILITY OF DATA AND MATERIALS

The datasets are available from the corresponding author on reasonable request.

STATEMENT OF ETHICS

The study was performed to conform with the Declaration of Helsinki and was approved by the Local Ethics Committee of the Hospital (2023-007).

AUTHORS' CONTRIBUTIONS

TQ: Writing – original draft. **XT:** Data curation, Formal Analysis.

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ORIGINAL ARTICLE

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A new effect of intravenous iron treatment in pregnancy: contraction in nonstress test and timing of labor

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SUMMARY

OBJECTIVE: The aim of this study was to elucidate the cause and results of contractions occurring in term pregnant women receiving intravenous iron therapy.

METHODS: During 2019–2020, 136 pregnant women beyond 35 weeks of gestation, who received intravenous iron treatment due to iron deficiency anemia, were included through retrospective screening. Iron deficiency anemia was defined as having hemoglobin levels < 10 g/dL and ferritin levels <15 ng/mL, and the pregnant women underwent nonstress test before and after treatment.

RESULTS: The average treatment week for the pregnant women was 36.82±0.74, and the presence of regular contractions in post-treatment follow-up nonstress tests was 72.1% (n=98). The average week of birth was 38.48±1.60. Pregnant women with contractions who had previous cesarean were found to have a mean delivery week of 36.82±0.67, which was statistically significant earlier than for nulliparous and multiparous women (p<0.001).

Conclusion: In pregnant women with iron deficiency anemia who were beyond 35 weeks, temporary regular contractions may be observed in the nonstress test following intravenous iron replacement. We think that this effect may lead to early term birth in pregnant women with a history of cesarean section. It needs to be confirmed by further prospective studies and animal studies.

KEYWORDS: Pregnancy. Iron. Calcium. Anemia. Iron-deficiency. Cesarean section. Fetal monitoring.

INTRODUCTION

Iron deficiency anemia during pregnancy is a global public health problem, affecting approximately 56% of pregnant women in developing countries[1]. Since iron deficiency anemia is associated with adverse maternal and neonatal outcomes during pregnancy, its treatment is crucial[1]. Among these adverse effects are first-trimester pregnancy losses, placental abruption, preterm birth, postpartum hemorrhage, hysterectomy, fetal malformation, growth restriction, stillbirth, and maternal death[2,3].

According to the World Health Organization guidelines, the criteria for anemia during pregnancy are defined as hemoglobin (Hb) levels <11 g/dL in the first and third trimesters, and <10.5 g/dL for the second trimester[4]. In cases where there is non-compliance with oral treatment or when anemia persists despite oral treatment, intravenous (IV) iron therapy is considered a good alternative option[5]. In addition, the importance of IV iron preparations in the rapid and effective treatment of prenatal anemia in the third trimester has been demonstrated. The recent UK guidelines especially recommend considering IV iron supplementation for anemia treatment after 34 weeks of pregnancy[6].

Iron (Fe) is an element that is absorbed intestinally through divalent metal transporter 1 (DMT1) receptors[7]. It has been demonstrated that high levels of calcium (Ca) competitively reduce the absorption of iron by competing with iron at the receptor level[8]. Additionally, some studies have shown that this effect is short-lived and does not significantly impact iron absorption[7,9]. However, it has not yet been clearly established whether calcium absorption is affected in cases of high iron concentration.

In our clinic, we applied pre- and post-treatment nonstress test (NST) to pregnant women beyond 35 weeks who require IV iron therapy to monitor fetal well-being. We observed that in the majority of our patients there were no uterine contractions before the treatment, but regular uterine contractions occurred afterwards. The aim of this study was to elucidate the cause of this situation and to understand whether these contractions occurring in term pregnant women receiving IV iron therapy lead to birth before the expected delivery date.

METHODS

As part of the research, a total of 159 pregnant women beyond 35 weeks, who received IV iron therapy due to iron deficiency anemia, participated in the study conducted at our hospital between 2019 and 2020. Local ethics committee approval was

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obtained from the same hospital (Ankara Bilkent City Hospital Ethics Committee No. 2, Approval Date: 2021, Approval No: 21/1029). This is a retrospective cohort study.

Pregnant women who had a singleton pregnancy between 35 and 37 weeks of gestation and received IV iron treatment due to existing anemia (despite oral iron therapy) according to WHO criteria were included in our study. Iron deficiency anemia was diagnosed when the hemoglobin was <10 g/dL and ferritin was <15 ng/mL[10]. Women who had any complications in pregnancy (preeclampsia, diabetes mellitus, IUGR, malpresentation, trauma, etc.), who had contractions detected on NST prior to IV iron treatment, and who had insufficient data were excluded from the study. IV ferric carboxymaltose treatment was given to the pregnant women. The reason for this was based on the effectiveness and safety of IV ferric carboxymaltose infusion in the treatment of iron deficiency anemia during pregnancy[11]. IV iron doses required to correct iron deficiency anemia when Hb was <10 g/dL according to body weight were 500 mg (<35 kg), 1,500 mg (>35 to<70 kg), and 2,000 mg (>70 kg). The maximum recommended iron infusion dose was 1,000 mg of iron/week (1,000 mg in the first session and the remaining amount in the second session after 1 week)[10]. Ferric carboxymaltose was administered as a single dose of 1,000 mg via IV drip infusion, to be completed in 15 min.

All pregnant women scheduled to receive IV iron therapy underwent pre-treatment NST. Due to the reported adverse effects of IV iron therapy[12], patients were subjected to post-treatment NST, and they were kept under observation for 2 h. The patients' files were reviewed, and NST records prior to and after IV iron therapy were collected. The contraction status during NST, gravida, parity, the hospitalization duration, previous mode of delivery, gestational week at which they received IV iron therapy, the gestational week of delivery, mode of delivery, and the need for neonatal intensive care were determined for these patients. Regular contractions in the NST were defined as the presence of at least four contractions lasting 40 s or longer within 20 min.

The sample size estimation was performed by using G*Power version 3.0.10 (Franz Faul, Universitat Kiel, Kiel, Germany). With a Cohen's effect size of 0.5, alpha error of 0.05, and power of 80%, the minimum number of participants required was calculated as 128. Statistical analyses were performed using SPSS version 22 (IBM Corp., Armonk, NY, USA). The normal distribution of data was examined using the Shapiro-Wilk test. Continuous and normal distributed variables were presented as mean±standard deviation, and within-group differences were evaluated using Student's t-test. Continuous and non-normal distributed variables were presented as median (minimum–maximum), and the differences between variables were analyzed using the Mann-Whitney U-test. Categorical variables were presented as numbers (percentages), and differences between categorical data were compared using the chi-square test or Fisher's exact test. p-Values below 0.05 were considered statistically significant.

RESULTS

Out of the 159 patients who were selected to receive IV treatment due to iron deficiency anemia, 23 were excluded from the study because they had contractions during the pre-treatment NSTs, 8 due to lack of data, and 2 due to complications, resulting in a total of 136 participants. The characteristics of the pregnant women included in the study are shown in Table 1. The average gestational week for the treatment was determined to be 36.82±0.74. In the post-treatment follow-up NST, the presence of regular contractions was observed in 72.1% (n=98) of the participants (Table 1).

The delivery weeks based on the presence of contractions in different conditions are shown in Table 2. Pregnant women who had contractions in the NST after IV iron therapy were found to have significantly earlier delivery weeks (p<0.001). In cases with contractions, the delivery week for women who had previous cesarean deliveries was determined as 36.82±0.67, which was statistically significant earlier than for nulliparous and multiparous pregnant women (p<0.001) (Table 3). This statistical significance is attributed to the cesarean group, and no statistically significant difference was found between nulliparous and multiparous pregnant women.

Table 1. Characteristics of the pregnant women included in the study.

Characteristics	n=136
Age	27.64±5.52
Hemoglobin (g/dL)	8.78±0.66
Ferritin (ng/mL)	4.34±2.78
Gravida	2 (1–7)
Parity	1 (0–6)
Nulliparity	38 (27.9)
Pre-existing cesarean delivery	56 (41.2)
Treatment week	36.82±0.74
Presence of post-treatment contraction	98 (72.1)
Delivery week	38.48±1.60
Post-treatment cesarean delivery	59 (43.4)

The data are presented as mean ± standard deviation, median (minimum-maximum), and number (%).

Table 2. The delivery weeks based on the presence of contractions in different conditions.

	Presence of contractions after treatment		p-value
	Contraction (+) n=98	Contraction (-) n=38	
Delivery week	38.03±1.56	39.64±1.00	**<0.001**
	Presence of contractions in nulliparous pregnant women		
	Contraction (+) n=20	Contraction (-) n=18	
Delivery week	39.51±1.25	39.82±0.94	0.395
	Presence of contractions in multiparous pregnant women		
	Contraction (+) n=32	Contraction (-) n=10	
Delivery week	38.83±1.32	40.32±0.80	**0.002**
	Pregnant women who had a previous cesarean delivery		
	Contraction (+) n=46	Contraction (-) n=10	
Delivery week	36.82±0.67	38.65±0.40	**<0.001**

Data are shown as mean±standard deviation. p-values <0.05 (denoted in bold) were considered statistically significant.

Table 3. Delivery week based on the previous mode of delivery in the presence of contractions.

	Previous mode of birth	n	Mean±SD	p-value
Contractions present (+)	Nulliparous	20	39.5±1.2	**<0.001**
	Multiparous	32	38.8±1.3	
	Cesarean	46	36.8±0.7	

The data are presented as mean ± standard deviation. Statistically significant p-value is denoted in bold.

A total of 38 pregnant women who did not have contractions in the NST after treatment were discharged on the same day. It was determined that 46 pregnant women, who had previously given birth by cesarean section and were found to have contractions in the NST after IV iron treatment, underwent cesarean section within 24 h due to the regular contractions that did not recede in the NST and the accompanying progressive cervical effacement and dilation. The hospitalization duration for nulliparous and multiparous (previously delivered vaginally) (n=52) pregnant women with contractions detected in the NST after IV iron therapy was determined to be 2.23±0.42 days. It was observed that the contractions in the NST regressed after an average of 36.26±2.82 h.

Out of the 21 newborns requiring intensive care, 18 were infants of pregnant women who had previously delivered by cesarean section and who underwent cesarean section at a mean of 36.8±0.7 weeks due to contractions during the NST post-IV treatment (p=0.013). Two of the newborns were the infants of nulliparous pregnant women (delivery week 39.5±1.2) and one of the newborns was the infant of multiparous pregnant women (delivery week 38.8±1.3) (p=0.270, p=0.762, respectively).

DISCUSSION

In our clinic, we identified that 72.1% of pregnant women with anemia who were 35 weeks and above had regular contractions in their NST after IV iron treatment. The average gestational age for birth in pregnant women with contractions after IV iron treatment was 39.51±1.25 for nulliparous women, 38.83±1.32 for multiparous women, and 36.82±0.67 for women with previous cesarean section. Pregnant women with previous cesarean section were found to deliver at early term.

In iron deficiency anemia, IV iron therapy is increasingly preferred due to its rapid improvement of hemoglobin levels, fewer gastrointestinal side effects, and improved safety profile[13]. Nevertheless, adverse effects such as infusion reactions and hypophosphatemia have been reported after IV iron administration[14]. However, there are no studies in the literature reporting contractions detected in the NSTs after IV iron therapy.

Calcium and iron are divalent elements that have a close relationship inside and outside the cells. It has been shown that high extracellular calcium concentration in the intestines inhibits iron absorption[15]. However, some studies have reported that this effect is temporary and that intracellular iron uptake improves shortly thereafter[9]. There are not many studies in the literature regarding whether high iron concentrations affect the intracellular uptake of calcium. In a study conducted by Núñez et al. on neuron cells, excessive iron levels were reported to increase uncontrolled calcium flux, promoting oxidative stress, and damaging mitochondrial function, as well as cell permeability[16]. The second study in the literature on this topic is by Paterek et al., who

conducted research on rat cardiac muscle cells. In their study, they administered high doses of ferric carboxymaltose to rats and found that L-type Ca^{2+} channels in cardiac muscle cells were permeable to Fe^{2+} cations, allowing iron ions passing through the channels to inhibit the Ca^{2+}-dependent inactivation of L-type Ca^{2+} currents, leading to increased permeability for both Ca^{2+} and Fe^{2+}. Therefore, it was observed that high iron concentrations temporarily increase calcium entry into the cell[17]. L-type calcium channels are important channels present in uterine smooth muscle cells and play a role in uterine contractions during labor[18]. Uterine L-type calcium channel activity is significantly regulated throughout pregnancy and becomes more sensitive during term (35–36 weeks)[19]. Therefore, in our study, in order to clearly demonstrate the effect of IV iron treatment on uterine muscle, which was our hypothesis, we included pregnancies of 35 weeks and above in which L-type Ca channels are more active.

In our study, we believed that the high iron concentrations resulting from IV iron treatment in pregnant women beyond 35 weeks increase calcium influx through more sensitive L-type Ca^{+2} channels in the matured uterus and initiate uterine contractions. At the same time, the regression of contractions 24–48 h after IV iron application in the NST follow-ups of both nulliparous and multiparous pregnant women and the average delivery occurring at around 39–40 weeks upon discharge support the notion that this situation is temporary. However, patients with previous cesarean section are immediately taken for a cesarean section by clinicians when regular contractions are observed in NST after IV iron treatment, due to the fear of uterine rupture risk, without waiting for the 39th week. Studies have shown that in pregnant women with previous cesarean section, there is no significant difference in maternal adverse outcomes for any gestational week at or above 36 weeks, but there is an impact on neonatal adverse outcomes[20,21]. Ma'ayeh et al. reported that birth at 37 weeks in women with repeated cesarean section was associated with reduced neonatal morbidities, respiratory distress syndrome, transient tachypnea of the newborns, and neonatal intensive care unit admissions compared to birth at 36 weeks[20]. Glavind et al. showed that even between 38 and 39 weeks of gestation, there were differences in the risk of neonatal morbidity, respiratory morbidity, and neonatal hospitalization, with lower risks at 38 weeks of gestation[21]. In our study, 85% of the newborns requiring intensive care were born to mothers who had previously given birth by cesarean section, and after IV treatment, these newborns were delivered by cesarean section at an average of 36.8±0.7 weeks due to contractions detected during NST. The presence of regular contractions on NST in women with previous cesarean section triggers concerns about the risk of uterine rupture. However, Stenson et al. reported that among 208 pregnant women with previous cesarean section, they induced labor using vaginal PGE2 (prostaglandin-E2), amniotomy, oxytocin, misoprostol, and balloon catheter, and only nine cases (4.3%) experienced uterine rupture[22].

An important limitation of our study is the absence of other adverse neonatal outcomes (neonatal morbidities, respiratory distress syndrome, transient tachypnea of the newborn) and cases of uterine rupture, excluding the need for neonatal intensive care in our analysis. Another limitation is that pregnant women under 35 weeks of gestation were not included and it could not been shown whether IV iron therapy leads to preterm delivery in them. The strength of our study is that this is the first study in the literature to question and discuss the effect of IV iron treatment on the uterus. However, animal experiments are needed to clearly demonstrate the effects of IV iron therapy on the uterus.

In conclusion, we have shown that temporary contractions may occur in NST after IV iron replacement in pregnant women with iron deficiency anemia at or above 35 weeks of gestation. We think that clinicians experience a lot of fear of uterine rupture in pregnant women who have had previous cesarean section. It is important to prevent early term labor because of concerns about adverse neonatal outcomes.

ETHICS APPROVAL

For studies with human subjects include the following. All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008. Local Ethics Committee approval was obtained from the same hospital (Approval No: 21/1029). Informed consent was obtained from all patients for being included in the study.

AUTHORS' CONTRIBUTIONS

MİH: Data curation, Formal Analysis, Writing – original draft. **İH:** Data curation, Formal Analysis. **MKK:** Data curation, Formal Analysis.

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ORIGINAL ARTICLE

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The impact of videolaparoscopic surgery in the treatment of endometriosis on depression levels

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SUMMARY

OBJECTIVE: The aim of the study was to evaluate the impact of laparoscopic surgical treatment of endometriosis on the levels of health-related depression in patients using a validated questionnaire.

METHODS: A prospective study was carried out between September 2020 and May 2022 in a private hospital (São Luís, Maranhão, Brazil), which analysed depression using the Beck Depression Inventory-II, on 103 patients undergoing surgical treatment for endometriosis, evaluated preoperatively and 3 and 6 months after the procedure. Patients with unsuccessful clinical treatment for endometriosis and pain level ≥7 on Visual Analog Scale and who agreed to participate in the study were included. Demographic data were acquired by consulting medical records.

RESULTS: The average age of the participants was 36±6.3 years; the majority of patients were brown (68.6%), married (66.6%), overweight (55.8%), had had hormonal treatments with progestogens (50.9%), low fertility (50.9%), severe endometriosis (39.3%), endometriosis surgery+myomectomy (29.4%) and one (1%) patient withdrew from the study. There was a statistically significant reduction in mean Beck Depression Inventory between the preoperative period and 6 months after surgery ($p<0.0001$).

CONCLUSION: Surgical treatment of endometriosis appears to have a positive impact on the symptoms of depression in the patients evaluated.

KEYWORDS: Endometriosis. Depressive symptoms. Surgical procedures. Operative.

INTRODUCTION

Endometriosis is an oestrogen-dependent chronic inflammatory condition characterised by the presence of the glands and stroma of endometrial-like tissue outside the uterine cavity, and the condition affects an average of 10% of women of reproductive age[1-3].

A characteristic of the disease is the delayed diagnosis, which varies according to country and study group and ranges from approximately 4 to 10 years from symptom onset[1,4]. The gold standard method of diagnosis of endometriosis is videolaparoscopy, but in recent years, imaging tests have been implemented and are widely used[3,5].

Endometriosis usually causes pain and infertility with reduced quality of life, sexual disorder, bipolar disorder, anxiety and depression, in addition to being related to chronic pelvic pain and the occurrence of alexithymia, somatisation, low self-esteem and pain catastrophising[6-8]. Individuals with chronic debilitating diseases have a higher prevalence of depressive symptoms, and this comorbidity can have harmful effects on the clinical condition of the patient[9].

Studies show that patients with endometriosis have more depressive states than patients without this pathology[5,7,10,11]. The impact of pain in the population with endometriosis is individualised and does not depend on the stage of the disease, suggesting that it is the intensity of the pain that leads to psychological distress and its consequences, not the endometriosis itself[10,11]. One study showed that psychological distress leads to pain catastrophising and predisposes patients to think repeatedly and amplify negative thoughts, which leads to an impact on mental health and pain intensity[11]. Catastrophising negatively influences depression and hinders responses to treatments, making early investigation and detection of this disorder essential for adequate treatment and cost reduction[11].

The treatment of endometriosis is clinical and/or surgical. Initially, clinical treatment is usually empirical with menstrual cycle blockade, and when this is not successful, surgical treatment may be indicated[1,2]. Surgical treatment also proceeds in cases of suspected ovarian cancer and intestinal or urinary tract obstructions[1,2]. The treatment involves a multidisciplinary team,

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including physical therapists, psychologists, nutritionists, and sometimes psychiatrists[1].

The search for solutions to improve depression in patients with endometriosis is essential to benefit these women. In this sense, this study aims to identify whether surgical treatment of endometriosis has an impact on improving the depressive state of these patients and evaluate its impact.

METHODS

This is a prospective cohort study, carried out on patients with an indication for surgical treatment for endometriosis that was performed by the gynaecological surgery team at Hospital São Domingos, São Luís, Maranhão, Brazil, from September 2020 to May 2022. Endometriosis was suspected based on clinical data and/or suggestive imaging tests. Demographic data were acquired by consulting medical records.

Symptomatic patients with clinical treatment for pain attributed to endometriosis, who had unsuccessful clinical hormonal treatment for at least 3 months and with pain level ≥7 according to the Visual Analog Scale (VAS) were included.

Patients with previous endometriosis surgeries, cancer diagnosis, major surgical complications, asymptomatic cases of endometriosis with surgical indication, pain level ≥7 on VAS ≥7, those who initially did not want to participate in the study and patients with chronic pelvic pain according to the American College of Obstetricians and Gynecologists' Committee on Practice Bulletins[12] were excluded.

One day before the scheduled date for surgery, the patients responded to the validated Portuguese version of the Beck Depression Inventory II (BDI)[13,14]. The questionnaire was readministered to the patients 3 and 6 months after surgery in a face-to-face format during follow-up appointments. These patients did not undergo any medical treatment for depression during the study (Figure 1).

The BDI assesses the presence of depressive symptoms such as sadness, guilt, past failure, and loss of pleasure, among others[13,14]. The instrument consists of 21 questions, and its results range from 0 to 63. In the validated Portuguese version, which was applied in this study, the intensity of depression is classified based on the score on the inventory as no/minimum depression (0–13), mild (14–19), moderate (20–28) and severe (29–63)[14]. It is important to emphasise that the BDI is not used to diagnose depression but to assess the level of depression.

The patients' data (age, ethnicity, marital status, if they were overweight, or had had hormone treatments and fertility) were assessed based on medical records. The degree of endometriosis was established intraoperatively according to the modified American Society for Reproductive Medicine (r-ASRM) classification into stages I to IV: minimal, mild, moderate and severe disease[15]. The type of surgery performed was recorded: excision of endometriosis foci, endometriosis+myomectomy, endometriosis+hysterectomy, endometriosis+rectosigmoidectomy, and endometriosis+myomectomy+rectosigmoidectomy and endometriosis+hysterectomy+rectosigmoidectomy. The surgical approach was defined according to the regions affected by the endometriosis foci, the degree of endometriosis involvement and comorbidity with other pelvic pathologies to be operated on.

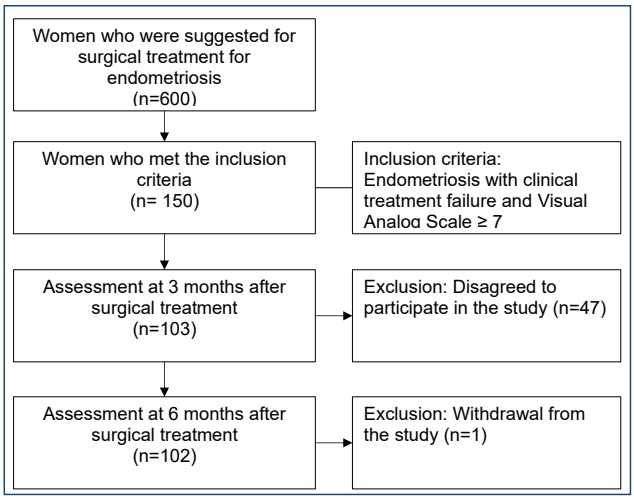

Figure 1. Flowchart of patients at the endometriosis outpatient clinic with indications for surgical treatment for endometriosis from September 2000 to May 2022.

After collection, the data were processed using the Microsoft Excel 2010 software, in which they were organised into tables and graphs. For statistical analysis, the Statistical Package for Social Sciences (SPSS) version 17.0 was used. Numerical and categorical variables were quantified using absolute and relative frequency measures. The Kolmogorov–Smirnov test was used to assess the normality of the questionnaires. Since the data distribution was not normal, nonparametric data expressed as the median (25th percentile–75th percentile) were used. To compare the different time points, the Friedman test was used, followed by Dunn's post-hoc test.

Data were tabulated in Microsoft Office Excel® (2016 version) (Redmond, WA, USA) and analysed in SPSS (version 21) (Chicago, IL, USA). Data are presented as the mean and standard deviation or median and range (minimum and maximum), and numerical and categorical variables are presented as the

absolute number (n) and relative (%) frequency. Normality was assessed using the Shapiro-Wilk test.

To compare the evaluations during follow-up (before, 3 and 6 months after surgery), the Friedman test was applied, with post-hoc analysis using Tukey's test. All statistical associations were set at a significance level of $p \leq 0.05$.

The Research Ethics Committee of Hospital São Domingos, São Luís do Maranhão (Brazil), approved and validated the performance of this study through the Brazil Platform. Each participant agreed to participate in the study and completed and signed an informed consent form.

RESULTS

In total, 600 gynaecological laparoscopic surgeries were performed. A total of 150 patients met the study criteria, of whom 47 did not agree to participate in the study and 103 agreed. One chose not to continue in the study upon return from the second evaluation without informing the reason (Figure 1). All participants had confirmation of endometriosis during the surgical procedure. The final sample consisted of 102 patients, with a mean age of 36±6.3 years. Regarding marital status, 34 (33.3%) were single, and 68 (66.6%) were married. Regarding colour, 18 (17.6%) patients were white, 70 (68.6%) were brown and 14 (13.7%) were black. Regarding body mass index (BMI), 57 (55.8%) were overweight. In clinical treatment prior to surgery, 52 (50.9%) underwent hormonal treatments with progestogens, 30 (29.4%) combined hormonal contraceptives and 21 (20.5%) both. Out of the patients, 40 (39.2%) were infertile and 52 (50.9%) were fertile or did not want to get pregnant. Regarding the degree of the disease, 9 (8.8%) had minimal endometriosis, 18 (17.6%) had mild endometriosis, 35 (34.3%) had moderate endometriosis and 40 (39.2%) had severe endometriosis (Table 1).

The patients underwent the following surgeries: 21 (20.6%) had exeresis of endometriosis foci, 30 (29.4%) had endometriosis+myomectomy, 16 (15.7%) had endometriosis+hysterectomy, 20 (19.6%) had endometriosis+rectosigmoidectomy, 1 (1%) had endometriosis+myomectomy+hysterectomy, 3 (2.9%) had endometriosis+myomectomy+rectosigmoidectomy, and 11 (10.9%) had other types of surgery (Table 1).

There was a reduction in BDI before surgery, from a median of 8 (25th–75th percentile: 3–13) to 2 (0–6) at 3 months after surgery and 0 (0–2) at 6 months after surgery (<0.0001). There was a significant reduction in the BDI mean between 3 and 6 months after surgery, as well as both when compared with the mean before surgery. However, as the mean BDI values in the three groups were within the normal range for the general population, these results should be analysed cautiously (Table 2).

Table 1. Socio-demographic and clinical characteristics of patients undergoing videolaparoscopy for the treatment of endometriosis.

Variables	n (%)
Age (years) Mean±SD	36±6.3
Colour/race	
White	18 (17.6)
Brown	70 (68.6)
Black	14 (13.7)
Marital status	
Single	34 (33.3)
Married	68 (66.7)
Overweight (BMI[3] 25.0 kg/m^2)	57 (55.8)
Hormone treatments	
Progestogens	52 (50.9)
Combined hormonal contraceptives	30 (29.4)
Both	21 (20.5)
Fertility	
Infertile	40 (39.2)
Fertile but did not want to get pregnant	52 (50.9)
Degree of endometriosis (rASRM criteria)	
Minimum	9 (8.8)
Lightweight	18 (17.6)
Moderate	35 (34.3)
Severe	40 (39.2)
Surgery	
Endometriosis	21 (20.6)
Endometriosis + myomectomy	30 (29.4)
Endometriosis + hysterectomy	16 (15.7)
Endometriosis + rectosigmoidectomy	20 (19.6)
Endometriosis + myomectomy + hysterectomy	1 (1.0)
Endometriosis + myomectomy + rectosigmoidectomy	3 (2.9)
Others	11 (10.9)

BMI: body mass index; rASRM: revised American Society for Reproductive Medicine; Both: changes the hormone composition due to unwanted effects; Endometriosis: peritoneal, ovarian and deep forms; Others: endometriosis with appendectomy, wall endometriosis, umbilical hernia and intestinal shaving.

A reduction was observed in the distribution of women during the study follow-up, where a more significant number of women with moderate or severe symptoms were observed in the first assessment (10.7%) and a lower number in the last assessment (1.0%) (p-value<0.001) (Table 3).

DISCUSSION

Studies that evaluated women with endometriosis undergoing surgical treatment found that the mean age ranges at the time of surgery were similar to those of our patients[1-16]. Regarding the ethnic profile of the patients in our study, previous studies found a higher prevalence of endometriosis in white patients[1-4].

The rate of being considered overweight in the female population in general is around 50%, which is compatible with the findings of this research, although studies indicate that being overweight is an indicator of protection against endometriosis[16,17].

The clinical treatment carried out prior to choosing the study group followed the guidance of the European Society of Human Reproduction and Embryology (ESHRE), where we initially opted for clinical treatment with progestins or oral combined contraceptives, both used continuously, and in cases of undesirable effects, we changed one for the other, forming a third group that used both[1]. The percentage of infertile patients with endometriosis varies in the literature at around 40%, similar to what was observed in the sample of this study[16].

A meta-analysis on endometriosis and depression showed that patients with chronic pain due to endometriosis have an increased prevalence of depression compared to women with asymptomatic endometriosis[17]. Nevertheless, other factors, such as the possibility of infertility, also influence the association between the two diseases[18].

There is a possible genetically based aetiological association between depression and endometriosis, as the two conditions share certain gene loci, suggesting a possible direct correlation between depression and endometriosis to some extent[19]. Meta-analyses of genomic association showed that nine reproductive disorders are genetically correlated with each other and are significantly related to perinatal depression, female depression and non-perinatal depression but are related to childbirth and depression in both men and women, with perinatal depression associated with endometriosis[20]. The difference in reproductive hormone levels has been suggested to be the cause of the prevalence of depression, which is more noticeable after puberty, as well as the perinatal period, is affected by hormonal fluctuation and is associated with an increased risk of depression[20]. Depression and anxiety in patients with endometriosis are associated with worse symptoms and a poor prognosis, regardless of pain levels[21].

Few studies have evaluated depression before and after surgical treatment of endometriosis[22,23]. One study analysed depression in women 2 weeks before and 3 months after undergoing laparoscopic surgery for endometriosis, with a significant reduction in depression[22]. In comparison, our study found lower mean BDI values at all evaluation stages.

Broeck et al. evaluated depression scores in patients surgically treated for endometriosis with and without rectosigmoidectomy and found a significant reduction in the prevalence of moderate or severe depression in both groups before treatment and 18 months after the procedure[23]. In the follow-up after surgery, lower mean values of BDI were achieved among women who had immediate reproductive desire before surgery and actually became pregnant compared to those who had this objective but were unable to become pregnant[23]. In this sample, the follow-up period was longer than that in our study. We should consider that the prevalence of women with severe depressive symptoms was lower before and after surgical treatment in our study. Another point to be considered is that patients with minimal and mild endometriosis were excluded from this study, whereas our study included all stages of endometriosis[23]. In both studies, there was a significant decrease in the BDI scores.

The limiting factors of this study were the size of the selected sample, selection bias, there was no blinding and an excellent team that made generalisation difficult, and the fact that the patients did not receive psychological or psychiatric follow-up during the 6-month study period. However, after the research was completed, all patients were referred for specialised treatment, despite there being a significant reduction in depression with surgical treatment for endometriosis, which mitigates the

Table 2. Comparison between Beck Depression Inventory assessment times for patients undergoing surgical treatment for endometriosis.

Beck Depression Inventory (BDI)	Median (min–max)
T1	8 (0–35)[a]
T2	2 (0–32)[a]
T3	0 (0–27)[a]
p-value £	<0.001

£: Friedman; [a]Equal letters indicate a statistically significant difference between the times evaluated.

Table 3. Comparison between the Beck Depression Inventory categories of patients undergoing videolaparoscopy for the treatment of endometriosis.

BDI categories	BDI T1		BDI T2		BDI T3		p-value
	n	%	n	%	n	%	
Minimal/mild	91	89.3	98	96.0	101	99.0	<0.001¥
Moderate/severe	11	10.7	4	4.0	1	1.0	

BDI: Beck Depression Inventory. ¥ McNemar.

postponement of specialised follow-up for depression. This may explain the low number of studies with this objective.

CONCLUSION

The results presented indicate that laparoscopic surgical treatment of endometriosis significantly reduces mild, moderate and severe depressive symptoms, with a possible positive impact on the quality of life of these patients. Long-term studies evaluating the outcome of the procedure with larger samples are needed.

AUTHORS' CONTRIBUTIONS

JNN: Formal Analysis, Writing – original draft. **VGM:** Writing – original draft. **ABCM:** Writing – original draft. **FNB:** Writing – original draft. **PCL:** Formal Analysis.

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ORIGINAL ARTICLE

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The relationship between premenstrual syndrome and personality traits in university students

Figen Alp Yılmaz[1*]

SUMMARY

OBJECTIVE: This study was conducted to determine the relationship between personality traits and premenstrual syndrome in university students

METHOD: This cross-sectional study was conducted with 616 female university students between February and June 2020.

RESULTS: The Premenstrual Syndrome Scale score was determined as a mean of 125.40±25.41. According to linear regression analysis, extrovert/introvert personality traits, emotional balance/neuroticism, and consistent/inconsistent personality traits were statistically significant predictive factors of premenstrual syndrome.

CONCLUSION: The results of this study demonstrated that two-thirds of the students had premenstrual syndrome and personality traits affected premenstrual syndrome. It is recommended that attention should be paid to personality traits when coping with premenstrual syndrome.

KEYWORDS: Personality traits. Premenstrual syndrome. Students.

INTRODUCTION

Premenstrual syndrome (PMS) is the umbrella term for a group of physical, emotional, and psychological symptoms of varying severity that occur in the late luteal phase of the menstrual cycle and end after the onset of menstruation[1]. Although the specific symptoms experienced vary greatly between individuals, commonly reported physical symptoms of PMS include breast tenderness, bloating, fatigue, headaches, and appetite changes. Emotional and psychological symptoms may include mood swings, irritability, anxiety, depression, and difficulty concentrating[2-4]. PMS symptoms can cause changes in body image and life activities, loss of workforce, economic losses, increased likelihood of accidents, decreased self-confidence, alcohol/drug use, increased tendency for crimes, deterioration in social relationships, and decreased academic performance[5,6].

Studies conducted in Turkey and other countries have shown that PMS is a common problem among women[3,7,8]. PMS is considered an important health problem due to its prevalence and the negative effects of the symptoms on daily living activities, academic/professional life, and the individual's productivity[9]. Although it is many years since PMS was first described, the mechanism behind its occurrence is not yet fully understood[4]. The fact that most PMS symptoms are psychological and behavioral suggests that they may be related to personality structure[10]. Individuals respond to events in line with their personality traits and determine ways to cope accordingly. There are studies in the literature showing that PMS is related to the personality structure of women, and it has been determined that the personality traits of "Neuroticism" and "Agreeableness" affect PMS and that neurotic personality is a predictor of PMS[11]. Determining the relationship between personality structures and PMS may be beneficial in coping with and treating premenstrual complaints. Therefore, the aims of this study were as follows: (1) to determine the frequency of PMS in university students, (2) to determine the level of PMS and personality traits in university students, (3) to examine the relationship between PMS and personality traits, and (4) to identify significant factors predicting PMS in university students.

METHODS

This descriptive, cross-sectional study was conducted between February and June 2020 at a university in the Central Anatolia region of Yozgat. The study sample consisted of 616 female students. The sample size was calculated with power analysis to provide an effect size of 0.05 and a 90% confidence interval determined with a 5% error level. The study group was formed of female students aged ≥18 years, who had a regular menstrual cycle and no known physical or psychological disorders. Students with any psychiatric illness were excluded from the study.

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Data collection

Data were collected with the Personal Information Form, Premenstrual Syndrome Scale (PMSS), and Cervantes Personality Scale (CPS).

Personal Information Form: This form contained questions to determine the socio-demographic characteristics of the university students.

Premenstrual Syndrome Scale

This 44-item scale with Likert-type responses was developed by Gençdoğan in 2006. Depressive affect, anxiety, fatigue, irritability, depressive thoughts, pain, appetite change, sleep change, and bloating are the sub-dimensions of the scale. The total score that can be obtained from the scale ranges from 44 to 220 points. A total PMSS score of ≥111 indicates the presence of PMS. In the original study, the Cronbach's alpha coefficient of the PMSS items was 0.75, while that of the subscales was between 0.75 and 0.91. In this study, the Cronbach's alpha coefficient was 0.88 for PMSS items and varied between 0.57 and 0.65 for subscales[12].

Cervantes Personality Scale

This scale was developed by Castelo-Branco et al. to evaluate the personality traits of women, and validity and reliability studies were conducted by Bal and Şahin. Each question in the scale is answered according to the individual's own experiences. The scale is a six-point Likert-type scale consisting of 20 items with three subscales (Extroversion/introversion, Emotional balance/neuroticism, Consistency, and inconsistency). As the mean scores from the sub-dimensions decrease, the personality traits of being extroverted, emotional balance, and consistency are seen more frequently. The Cronbach's alpha reliability coefficient of the CPS has been calculated as α=0.97 for the extroversion/introversion dimension, α=0.81 for the emotional balance/neuroticism dimension, and α=0.71 for the consistent/inconsistent dimension[12]. In this study, the reliability coefficient was found to be 0.82 for extroversion/introversion, 0.78 for emotional balance/neuroticism, and 0.69 for consistency/inconsistency.

Ethical considerations

This study was approved by Yozgat University Social Sciences Ethics Committee. Before starting the data collection process, written permission was obtained from the university where the research was conducted. Written informed consent for voluntary participation in the research was provided by all students.

Statistical analysis

After examining the normality distribution of the data, descriptive statistical methods such as frequency, percentage, mean, and standard deviation were used in the analyses. Differences between independent groups were analyzed using the independent-samples t-test and one-way ANOVA test. Pearson correlation analysis was used to evaluate the relationships between the scale average scores. Multiple linear regression analysis was performed to evaluate the relationship between the PMSS scores and the subscales of the CPS. The reliability of the scales was evaluated with Cronbach's alpha coefficient. The level of statistical significance was accepted as $p<0.05$ (Table 1).

RESULTS

The PMS scale total mean score was found to be 125.40±25.41, and the incidence of PMS was determined to be 72.4%.

The age of the students, father's education level, and menstruation duration were determined to be variables affecting PMS, and the difference between the groups was statistically significant ($p<0.05$). Students in the age group of 17–20 years, whose fathers were high-school graduates, and students whose menstruation lasted 9 days or more were found to have PMS. The average score of those students was higher than that of other groups (Table 2).

Of those experiencing PMSS, the mean score was determined as 25.55±4.79 in the extroversion/introversion dimension, 20.88±4.54 in the emotional balance/neuroticism dimension, and 17.02±4.93 in the consistent/inconsistent

Table 1. Distribution of the premenstrual syndrome scores of the students.

PMSS sub-dimensions	X ± SD	Minimum	Maximum
Depressive mood	20.91±5.39	7	35
Anxiety	16.56±4.95	6	30
Fatigue	17.81±4.61	6	30
Irritability	14.53±4.25	5	25
Depressive thoughts	20.09±5.31	7	35
Pain	8.78±2.77	3	15
Appetite changes	9.07±2.92	3	15
Sleep changes	8.94±2.66	3	15
Bloating	8.67±2.96	3	15
Total PMSS	125.40±25.41	56	215
PMSS		**N**	**%**
Present		446	72.4
Absent		170	27.6

PMSS: Premenstrual Syndrome Scale.

dimension. A statistically significant relationship was found between the emotional balance/neuroticism dimension and consistent/inconsistent personality and PMS scores of the students participating in the study (Table 3).

The predictive power of the linear regression model calculated using the backward elimination method (adjusted R^2) was determined to be 24.7%. Extrovert/introvert personality trait (β=-0.159), Emotional balance/Neuroticism (β=0.361), and Consistent/inconsistent personality trait (β=0.167) were found to be significant predictors of students experiencing PMS ($p<0.05$).

DISCUSSION

Premenstrual syndrome affects the lives of women of reproductive age, with prevalence varying from country to country. The results of this study showed that the average PMSS score was 125.40±25.41 and the frequency of PMS was 72.4%. In a systematic review and meta-analysis study conducted to determine the frequency of menstrual cycle disorders in university students in all geographical regions of the world, the prevalence of PMS was determined as 51.3%[13]. In other systematic review and meta-analysis studies, the prevalence of PMS has been reported to be 70.8% in Iran[14], 53% in Ethiopia[8], 43% in India[3], and 52.2% in Turkey[7]. The prevalence of PMS among university students in Turkey has been reported to vary between 33 and 91.8%[5,7,15-19]. It is thought that these differences in the prevalence of PMS may be due to unequal genetic, dietary, and lifestyle factors among young adult women and various socially accepted practices during the premenstrual and menstrual period[20,21].

The results of the current research showed that students exhibited the personality trait of being extroverted/introverted more than that has been reported in other studies[22,23]. The extrovert personality traits include being open to communication, energetic, warm-blooded, sociable, talkative, enthusiastic, and excited. It is known that these people are successful and happy in establishing ties with their environment and in business and family life. Individuals with introvert personality traits are calm, introverted, and non-social individuals[24]. Some personality traits may cause intense reactions to PMS-related complaints[10]. In this study, it was determined that the extrovert/introvert personality trait affected PMS, but there appears to be no consensus on this issue in the literature. Some studies have found that being extroverted/introverted did not affect PMS[22,23], while others have reported that PMS is less common in those with high levels of extroversion[25,26].

In this study, the emotional balance/neuroticism rates were found to be similar to the rates in other studies[22,23]. Neuroticism is directly related to depression and can cause both adaptation and health problems. Individuals with emotionally balanced personality traits are defined as people who are relaxed, have a high level of self-confidence, and are patient. Neurotic individuals are described as anxious, angry, introverted, and insecure. The results of this study regarding the predictive effect of personality traits on PMS showed that the emotional balance/

Table 2. Comparison of personality characteristics of students according to premenstrual syndrome status.

CPS	PMSS		Test/p
	Present	Absent	
Extroversion/introversion	25.55±4.79	26.31±5.15	0.077
Emotional balance/neuroticism	20.88±4.54	17.42±5.43	**0.019**
Consistent/inconsistent	17.02±4.93	12.65±5.35	**0.042**

Bold indicates $p<0.05$.

Table 3. Predictive factors of female students' perceptions of premenstrual syndrome.

Variables	B (95%CI)	SE	β	T	p
Constant	100.797	8.27		12.176	**0.000**
Age	0.385	1.806	0.008	0.213	0.831
Father's education level	-0.590	1.268	-0.016	-0.466	0.642
Menstrual frequency	-0.951	1.433	-0.023	-0.663	0.507
Extroversion/introversion	-0.824	0.184	-0.159	-4.485	**0.000**
Emotional balance/neuroticism	1.815	0.200	0.361	9.069	**0.000**
Consistent/inconsistent	0.784	0.185	0.167	4.233	**0.000**

R=0.504, Adj. R^2=0.247, F=34.560, p<0.001.
Adj. R^2: adjusted R square; B: partial regression coefficient; β: standard partial regression coefficient; 95%CI: 95% confidence interval.
Bold indicates $p<0.05$.

neuroticism sub-dimension predicts PMS at a significant level. A previous study conducted in Spain showed that the frequency of PMS was higher in people with neurotic personality traits[27]. A relationship between PMS and neurotic personality traits has also been reported in Nigerian university students, and PMS was seen to be significantly more common in women exhibiting neurotic personality traits[28]. From the personality traits sub-dimension, the trait of being consistent/inconsistent refers to a situation being persistent and not contradictory. In this study, consistent personality trait was determined as a predictive variable of PMS. Ölçer et al. evaluated university students and found a significant difference between the PMS and CPS subscale consistent/inconsistent, similar to the current research.

Strengths and limitations

It is thought that the results obtained from this study will guide healthcare professionals in protecting the mental health of university students experiencing PMS. However, this study also had some limitations. As the study was conducted in a single university, it cannot be generalized to all students experiencing PMS. Another limitation of the study was that because it was a cross-sectional study, a causal relationship could not be established. Therefore, there is a need for further studies with large samples to examine the causal relationship and variables between PMS and personality traits in university students.

CONCLUSION

The results of this study demonstrated that two-thirds of the students experienced PMS and that personality traits and PMS are interrelated dynamics.

AUTHORS' CONTRIBUTIONS

FAY: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

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ORIGINAL ARTICLE

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Etiology and perinatal outcomes between early and late-onset nonimmune hydrops fetalis

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SUMMARY

OBJECTIVE: We aimed to compare the etiology and perinatal outcomes of non-immune hydrops fetalis diagnosed early- and late-onset at our hospital.

METHODS: The records of the patients who applied to our department were reviewed, and we reached 42 non-immune hydrops fetalis cases retrospectively and examined the medical records. Hydrops diagnosis week, birth week, accompanying anomalies, and perinatal outcomes were compared as ≤12 weeks (early-onset) and >12 weeks (late-onset).

RESULTS: The prevalence of non-immune hydrops fetalis was 0.05%, and the median week of diagnosis for hydrops was 18 weeks. Consanguinity (16.7%) was found in seven pregnancies, and the other seven patients (16.7%) had a history of hydrops in previous pregnancies. Anomalies of the skeletal system, central nervous system, and gastrointestinal tract accounted for 66.7% of ≤12 weeks in non-immune hydrops fetalis cases. Cardiac abnormalities were more common (26.7%) in patients at > 12 weeks (p=0.078). A statistically significant difference was found between the distribution of week of birth and week of diagnosis (p=0.029). Notably, 66.7% of patients diagnosed before week 12 and 23.3% of patients diagnosed after week 12 delivered their babies before week 24. Spontaneous intrauterine death occurred before week 12 in 45.5% (n=5) of non-immune hydrops fetalis and after week 12 in 39.1% (n=9) of non-immune hydrops fetalis. Notably, 69.2% (n=9) of the patients who had prenatal invasive testing resulted in normal karyotype.

CONCLUSION: In this study, most of the fetuses diagnosed with early-onset non-immune hydrops fetalis were born in the first 24 weeks. Additionally, live birth rates and cardiac anomalies were observed to be higher in late-onset non-immune hydrops fetalis.

KEYWORDS: Cardiovascular abnormalities. Congenital abnormalities. Hydrops fetalis. Non-immune hydrops fetalis.

INTRODUCTION

Hydrops fetalis is defined as abnormal fluid accumulation in two fetal compartments, including fetal pleura, pericardium, intra-abdominal, and subcutaneous tissues[1]. Immune hydrops fetalis is characterized by fluid accumulation in the fetus due to erythrocyte destruction and anemia resulting from parental blood incompatibility. Hydrops cases that are not due to blood group incompatibility are referred to as nonimmune hydrops fetalis (NIHF). The prevalence of immune hydrops fetalis has decreased significantly due to the widespread use of anti-D immunoglobulin prophylaxis. Therefore, most cases of hydrops fetalis are now thought to be NIHF[2].

The incidence of NIHF varies from 1 in 1,500 to 1 in 4,000 births[3]. Although most NIHF cases are idiopathic, common causes include cardiovascular anomalies, infectious diseases, and aneuploidies[4]. Prognosis depends on etiology and hydrops subtype, and the perinatal mortality reported in NIHF cases ranges from 50 to 98%[5]. The diagnosis is made by observation of at least two of the following findings: ascites, hydrothorax, pleural effusion, pericardial effusion, and skin edema, defined as 7 mm or more of edema on the fetal scalp[6]. Decisions about the timing and course of delivery in patients diagnosed with NIHF are based on ultrasound findings and prenatal predictive fetal assessments[7]. This study was conducted to compare the

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gestational age at birth, pregnancy outcomes and associated fetal anomalies, consanguineous parentage status, and prenatal invasive test results in NIHF cases diagnosed before and after 12 weeks of gestation.

METHODS

This study was designed retrospectively by analyzing patients who applied to the Etlik Zubeyde Hanım Training and Research Hospital between 2017 and 2020 and gave birth. The ethics committee approved the protocol of this hospital study. We adhered to the principles of the Declaration of Helsinki in this study. A total of 90,000 outpatients presenting to our hospital between 2017 and 2020 were studied, and 92 pregnancies with hydrops fetalis in the prenatal period were identified. However, they were excluded from the study because 46 patients had immune hydrops fetalis. Four hydrops patients had multiple pregnancies. Therefore, 42 patients were retrospectively evaluated for etiology (Figure 1). Two clinicians (DŞ and SYE), both experts in fetal anomalies, performed all ultrasound examinations and confirmed the diagnosis of hydrops. For fetal morphology scanning, the Voluson E6 ultrasound system was used in our perinatology clinic (GE Healthcare, Zipf, Austria).

The required pregnancy/delivery data of the patients were obtained from the hospital computer records. Inclusion criteria were singleton pregnancies diagnosed with NIHF during the prenatal period and followed up by our department. NIHFs diagnosed before 12 weeks of gestation were defined as early pregnancy, and NIHFs that began after 12 weeks of gestation were defined as late pregnancy. Exclusion dcriteria for the study population were the presence of multiple pregnancies and immune hydrops fetalis. Gestational age was determined by the last menstrual period or first-trimester ultrasound results. Abortion/week of delivery, birthweight and pregnancy results (termination, intrauterine deaths, neonatal deaths, live births), fetal anomalies, status of consanguineous parents, and prenatal invasive tests as well as array analysis were evaluated. The diagnosis

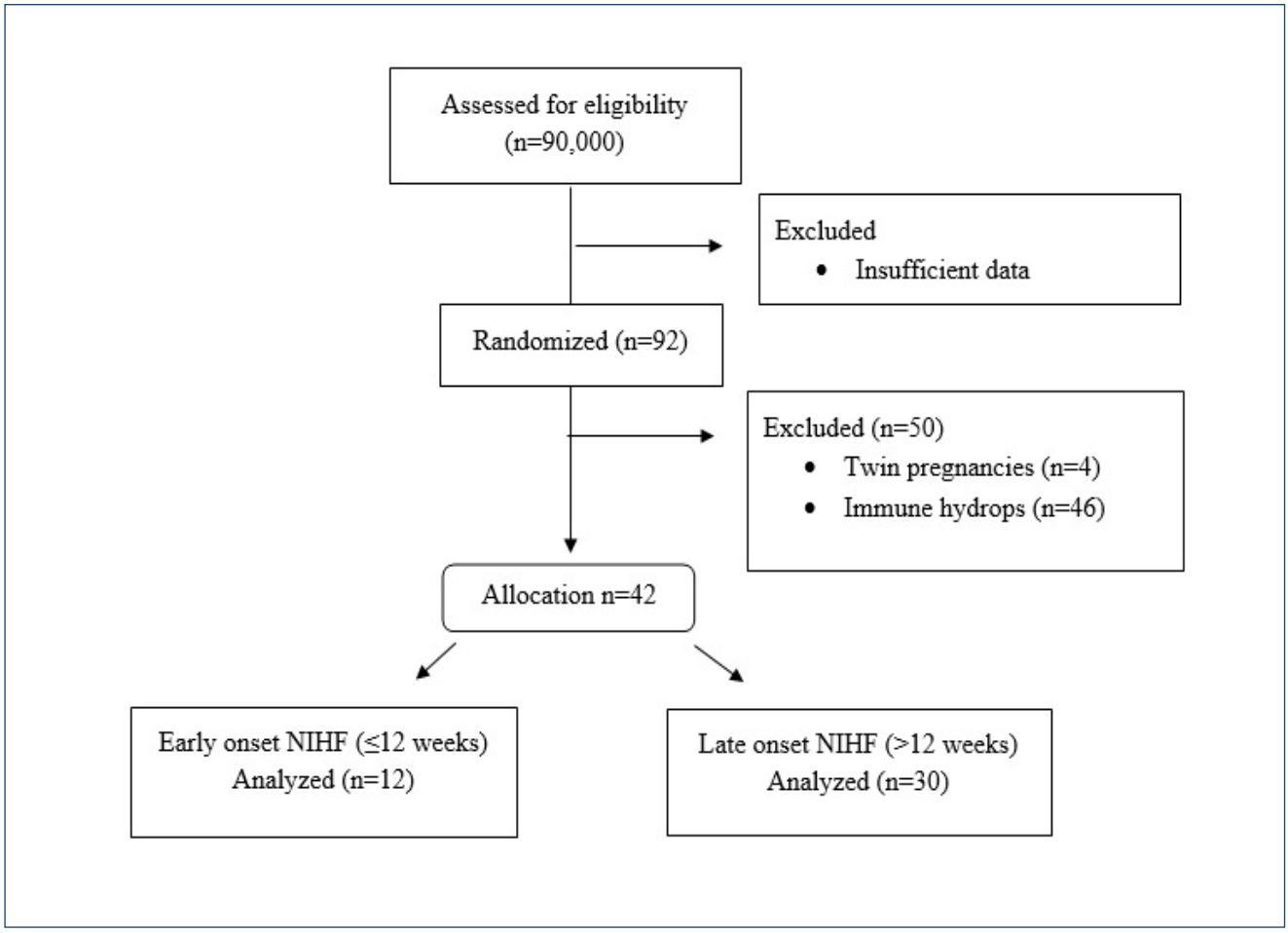

Figure 1. Flowchart of patient inclusion..

of fetal anemia was excluded by measuring the middle cerebral artery (MCA) peak systolic value and calculating it according to week. Since the cases did not have fetal anemia, a complete blood count was not performed in the neonatal period. In patients diagnosed with NIHF, the timing and course of birth were decided according to ultrasound findings, especially by analyzing fetal heart functions. Families whose babies were born alive were called to confirm defects.

STATISTICAL ANALYSES

The statistical package SPSS 23.0 IBM was used for statistical analyses. Descriptive statistics included mean, standard deviation, median, frequency, and percentages. Categorical variables were compared using the chi-square test and Fisher's exact test. The normal distribution of continuous variables was assessed using visual (histogram and Q-Q plots) and statistical methods (Kolmogorov-Smirnov tests). In cases where continuous variables were not normally distributed (nonparametric), two groups were compared using the Mann-Whitney U-test. When continuous variables were normally distributed, Student's t-test was performed. The accepted statistical significance level was $p<0.05$.

RESULTS

In this study, we found that the prevalence of NIHF in our hospital was 0.05%. In all hydrops groups, the mean age of pregnant women was 28.12±6.55 years, the median gravidity was 2 (range 1–6), and the median parity was 1 (content 0–5). The median week at which hydrops was diagnosed was 18 weeks. Consanguinity (16.7%) was found in seven pregnancies, and the other seven patients (16.7%) had a history of hydrops in previous pregnancies.

In patients diagnosed with NIHF in the first trimester, the diagnosis was confirmed in the second trimester by detailed ultrasonography and invasive test results. We found no statistically significant differences between the two groups with respect to the subgroup of structural abnormalities. Anomalies of the skeletal system, central nervous system, and gastrointestinal tract accounted for 66.7% of ≤12 weeks in NIHF cases. Cardiac abnormalities were more common (26.7%) in patients at>12 weeks (p=0.078). The distribution of these abnormalities by week is shown in Table 1. None of the parents consented to autopsy.

The analyses performed revealed no statistically significant difference between NIHF patients detected before 12 weeks of gestation and those detected after 12 weeks of gestation in

Table 1. Comparison of demographic data in the study by the week of nonimmune hydrops fetalis diagnosis.

	Diagnosis week		Total	Test statistics	p
	12 (n=12)	>12 (n=30)			
Age (years)	27.58±6.05	28.33±6.82	28.12±6.55	U=174	0.88
Birthweight (g)	2150.00±1299.7	1799.7±1005.2	1863.4±1038.5	U=28	0.538
NIHF history					
No	9 (75)	26 (86.7)	35 (83.3)	–	0.387F
Yes	3 (25)	4 (13.3)	7 (16.7)		
Consanguineous parentage					
No	9 (75)	26 (86.7)	35 (83.3)	–	0.387F
Yes	3 (25)	4 (13.3)	7 (16.7)		
Fetal anomalies					
No	2 (16.7)	9 (30)	11 (26.2)	=8.801	0.185
Cardiac	1 (8.3)	8 (26.7)	9 (21.4)		
Skeletal	3 (25)	1 (3.3)	4 (9.5)		
Central nervous system	3 (25)	3 (10)	6 (14.3)		
Gastrointestinal system	2 (16.7)	4 (13.3)	6 (14.3)		
Urinary system	0 (0)	3 (10)	3 (7.1)		
Pulmonary anomalies	1 (8.3)	2 (6.7)	3 (7.1)		

U: Mann-Whitney U-test statistic; F: Fisher's exact test; : chi-square test statistic; NIHF: nonimmune hydrops fetalis. Results were accepted as 95% confidence interval and p-value <0.05 significant.

terms of consanguineous parentage, hydrops history, and fetal anomalies (p=0.387 and =0.185, respectively). The clinical and demographic characteristics of the patients are shown in Table 1.

In our hospital, invasive prenatal screening and array analysis are recommended for all patients diagnosed with NIHF. Cases in which consanguinity was detected and all patients whose prenatal invasive test results were abnormal were referred to genetics. However, only 13 patients (30.9%) agreed to undergo these examinations. Amniocentesis (A/S) was performed in 23.8% (n=10) of these investigations, chorionic villus sampling in 4.8% (n=2), and cordocentesis in 2.4% (n=1). Invasive testing revealed a normal karyotype in nine patients, Trisomy 21 in one patient, Turner syndrome in two patients, and Trisomy 18 in one patient (Table 2). No abnormal results were detected in array analysis.

A statistically significant difference was found between the distribution of the week of birth and the week of diagnosis (p=0.029). Notably, 66.7% of women diagnosed before week 12 and 23.3% of women diagnosed after week 12 delivered before week 24. Meaningful pregnancy data were available in 34 of the 42 patients included in this study. Spontaneous intrauterine death occurred before week 12 in 45.5% (n=5) of NIHFs and after week 12 in 39.1% (n=9) of NIHFs. Overall, 17.6% of pregnancies (n=6) were terminated, 32.4% of babies (n=11) were born alive, and neonatal death occurred in 8.8% of newborns (n=3). When analyzed by weeks of diagnosis, no significant association was found between patients in terms of pregnancy results (p=0.477, Table 2). The prevalence of neonatal mortality in all cases was 25% [(number of infant deaths between 0 and 27 days of life/number of live births) * 1,000] [10]. The delivery method of the live births included in the study is known. Also, 56% of these patients (n=14) delivered their babies vaginally and 44.0% (n=11) by cesarean section (Table 2). Cesarean section was performed in three patients (6.5%) for fetal distress, in five patients (10.8%) for previous uterine surgery, in two patients (4.4%) for abnormal fetal presentation, and in one patient (2.2%) for chorioamnionitis. Intrauterine syphilis infection was detected in one case and cytomegalovirus (CMV) infection in one case. These two cases, detected with

Table 2. Comparison of perinatal outcomes according to diagnosis week of nonimmune hydrops fetalis.

	Diagnosis week		Total	Test statistics	p
	≤12 (n=12)	>12 (n=30)			
Prenatal invasive test type No	7 (58.3)	22 (73.3)	29 (69)		
A/S	0 (0)	7 (23.3)	10 (23.8)	=5.689	0.128
CVS	5 (41.7)	0 (0)	2 (4.8)		
Cordocentesis	0 (0)	1 (3.3)	1 (2.4)		
Prenatal invasive test results					
Normal	3 (60)	6 (75)	9 (69.2)		
Trisomy 21	0 (0)	1 (12.5)	1 (7.7)	=2.438	0.487
Turner	1 (20)	1 (12.5)	2 (15.4)		
Trisomy 18	1 (20)	0 (0)	1 (7.7)		
Pregnancy results					
Termination	3 (27.3)	3 (13)	6 (17.6)		
İntrauterine deaths	5 (45.5)	9 (39.1)	14 (41.2)	=2.491	0.477
Neonatal deaths	0 (0)	3 (13)	3 (8.8)		
Live births	3 (27.3)	8 (34.8)	11 (32.4)		
Delivery type of alive fetuses					
Vaginal delivery	3 (60)	11 (55)	14 (56)	–	1.000F
Cesarean section	2 (40)	9 (45)	11 (44)		
Birth week					
<24	8 (66.7)	7 (23.3)	15 (35.7)		
25–36	1 (8.3)	8 (26.7)	9 (21.4)	=7.101	0.029
>37	3 (25)	15 (50)	18 (42.9)		

F: Fisher's exact test; : chi-square test statistic; NIHF: nonimmune hydrops fetalis; A/S: amniocentesis; CVS: chorionic villus sampling. Results were accepted as 95% confidence interval and p-value <0.05 significant.

maternal blood samples, were also confirmed in the neonatal period. These two patients did not accept prenatal invasive tests. Fetal anemia and parvovirus B19 were not detected in our study population. Maternal complications were not observed during pregnancy and the postpartum period.

DISCUSSION

This study provides a comparison of the etiology and perinatal outcomes of fetuses diagnosed with NIHF. This is the first study on the etiology and perinatal outcomes of early and late-onset NIHF. The most important finding of this study was that the fetuses diagnosed with early-onset NIHF were also born early, that is, in the first 24 weeks. In addition, live birth rates were higher in fetuses diagnosed with late-stage NIHF.

Early-onset NIHF is mentioned in a limited number of articles in the literature. According to Jauniaux, early hydrops cases were defined between approximately 11 and 14 weeks of age, with no unique week specified[8]. Smeland et al., in their study of a patient with recurrent pregnancy loss due to NIHF at different weeks, defined NIHF as early onset but did not specify a specific week for this definition[9]. Ranganath et al. defined the case of hydrops fetalis beginning at 14 weeks as early-onset hydrops[10]. When analyzing hydrops cases by subdivision after week 12 in our study, we found that the hydrops cases that started in the early weeks, which is an important perinatal outcome, were born earlier than the hydrops cases that started in the later weeks. This allowed us to divide the NIHF cases into early onset and late onset.

In a meta-analysis of 6,361 patients, cardiovascular disease (21.7%) and chromosomal abnormalities (13.4%) were reported as the most common abnormalities in NIHF patients[2]. According to a recent study, the etiology was unknown in 46% (30/65), suspected in 9.2% (6/65), and confirmed in 44.6% (29/65). Of the confirmed cases, 11 resulted from aneuploidy, 7 from fetal structural abnormalities, 2 each from fetal arrhythmias, Noonan syndrome, and generalized lymphocytic dysplasia, and 1 from arthrogryposis, parvovirus, neonatal alloimmune thrombocytopenia, fetal goiter, and Kasabach-Merritt syndrome[11]. In our study, similar to the literature, these were the most common anomaly groups. Although the most common anomaly groups in our study were identical to those in the literature, there was no statistically significant difference between these groups in terms of early- and late-onset NIHF (Table 2). We think that the low rate of prenatal invasive testing in a high-risk population such as NIHF is due to religious reasons. Pediatricians recommended genetic testing to these patients in the neonatal period, but the patients did not undergo it.

According to a retrospective study, the mean week of diagnosis of patients referred with a diagnosis of NIHF was 29.1±4.4, whereas the mean week of delivery of live fetuses was 34.3±2.7[12]. However, all patients included in this study were those diagnosed with hydrops in the third trimester. According to another study, 31.7% of patients diagnosed with NIHF were born before 32 weeks of gestation, 9.4% were born before 28 weeks, and 77.7% of NIHF pregnancies were associated with preterm birth[13]. However, in these two studies, the week in which patients were diagnosed with NIHF was not reported. Notably, 66.7% of NIHF cases with early onset were born before 24 weeks, and 50% of NIHF cases with late onset were born after 37 weeks (Table 2). Our study differs from the literature in this regard.

According to one study, 33.3% of patients diagnosed with NIHF in the previous pregnancy also developed NIHF in the subsequent pregnancy, and 36% of them were diagnosed with lysosomal storage disease[14]. In our study, 16.7% of the patients confirmed that NIHF had complicated their previous pregnancies. Although not statistically significant, the fact that patients with a history of hydrops had more late-stage NIHF than early-stage NIHF could be due to late hospitalization because of problems with previous pregnancies. Future screening of these recurrent NIHF cases for lysosomal storage disorders may be useful.

The consanguinity rate in our country was reported to be 8.4%[15]. Because a high risk of developing autosomal recessive genetic diseases is associated with consanguineous ancestry, screening for NIHF cases is essential. In our study, seven patients (15.2%) had a consanguineous ancestry. We believe that consanguineous ancestry is necessary for the pathophysiology of NIHF and should be questioned.

The prognosis of NIHF depends on the underlying etiology, gestational and birth week, and neonatal status. Even in the absence of chromosomal abnormality, survival rates of less than 50% have been reported in the literature[16,17]. In our study, the neonatal mortality rate was low compared to the literature[18]. However, intrauterine mortality was still higher, supporting the "all or nothing" rule in first-trimester obstetric practice[19].

Our study has some limitations. First, this is a case–control study with a limited number of patients rather than a prospective study. Most patients did not give consent for invasive prenatal testing,

so etiological reasons could not be fully elucidated. It is difficult to confirm the diagnosis of hydrops in fetuses from terminated pregnancies. Because none of the parents consented to autopsy and postpartum genetic testing, we had to limit our diagnosis and confirmation of abnormalities to ultrasonography. In a study of tauopathies in hydrops patients, new-generation rasopathy genes were found in 56% of 26 patients with hydrops fetalis. It has been reported that testing these genes is beneficial in such patients[20]. However, in our country, these genes cannot yet be studied in the perinatal period.

CONCLUSION

As a result, fetuses diagnosed with NIHF at an early stage may have a more severe course. Although there was no significant difference in etiology between trimesters, cardiac abnormalities were observed to be more common in late-onset NIHF patients. Further studies and new genetic tests are needed to improve treatment and prognosis, better identify ultrasound findings, and perform predictive fetal assessments to justify the indication.

ETHICS COMMITTEE APPROVAL

The study protocol was approved by the hospital's Medical Research Ethics Department. The authors have confirmed that they have complied with the World Medical Association Declaration of Helsinki regarding the ethical conduct of research involving human subjects (the institutional review board approval number is 14.02.2020/03/16).

INFORMED CONSENT

Written informed consent for the use of the data was obtained from all persons who participated in this study.

AUTHORS' CONTRIBUTIONS

SYE: Writing – original draft, Writing – review & editing. **MCİ:** Writing – review & editing. **AÇ:** Data curation. ÇA**:** Data curation. **GÖ:** Writing – review & editing. **NVT:** Conceptualization. ÖYÇ**:** Formal Analysis. **DŞ:** Writing – original draft, Writing – review & editing.

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ORIGINAL ARTICLE

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Can cognitive behavioral therapy improve vasomotor symptoms and recurrent depression in postmenopausal women?

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SUMMARY

OBJECTIVE: The aim of this study was to evaluate the effectiveness of cognitive behavioral therapy in the treatment of vasomotor, sexual dysfunction, and recurrent depression in postmenopausal women.

METHODS: This prospective, open study evaluated 112 postmenopausal women with vasomotor symptoms. Sexual dysfunction has cultural, social, biological, and emotional issues and divided into two groups: G1, without depression (n=65) and G2, with recurrent depression (n=47). The subjects underwent 12 sessions of in-person cognitive behavioral therapy and 12 sessions of home-based activity over a period of 6 months. They were evaluated at 3 months following the completion of therapy. Depression, memory, and attention-related functions, as well as climactic symptoms, were assessed using a questionnaire.

RESULTS: In the depression questionnaire, the G1 group had a lower initial score than the G2 group ($p<0.01$). Following 6 months of therapy, both groups had similar improved scores. In the depression questionnaire, the women in group G1 had higher baseline values. In the assessment of vasomotor symptoms, the values in both groups were similar and showed an improvement in vasomotor symptoms after 24 weeks of treatment, but these effects disappeared after the follow-up of 48 weeks in the G2 group. Both groups improved the sexual dysfunction after 24 weeks.

CONCLUSION: Cognitive behavioral therapy may be effective in reducing vasomotor symptoms and ameliorate the sexual dysfunction and recurrent depression in postmenopausal women after 24 weeks of treatment.

KEYWORDS: Cognitive behavioral therapy. Menopause. Depression. Recurrent. Cognition.

INTRODUCTION

Cognitive behavioral therapy (CBT) in postmenopausal women has been evaluated as a treatment for recurrent depression and sexuality[1,2]. In fact, evidence indicates that CBT has the potential to effectively promote mental and sexual health in clinical trial participants[3,4]. The attention and amnestic function tests are also the most common methods used to evaluate the success of CBT[5].

Recurrent depression is a disorder characterized by the repeated occurrence of this symptom in the absence of any antecedent independent episodes of mood exaltation and increased energy. This affection also has variable length from a few weeks to a few months, and at least two episodes might have lasted for at least 2 weeks, separated by months[6,7]. This may worsen in the postmenopausal women with vasomotor symptoms[6,7]. In addition, the highest incidence of depression and decline in cognitive functions occurs during this period[5,6]. Difficulties arise in the performance of professional and domicile tasks, which may further negatively reduce emotional stability[6].

Cognitive behavioral therapy may be an alternative therapy to change behavior and emotional state[7] as well as has a positive influence on psychic status and vasomotor symptoms[8]. CBT may also affect sexual dysfunction, such as hypoactive sexual desire disorder, sexual arousal dysfunction, orgasmic dysfunction, psychogenic dyspareunia, and sexual dysfunction penetration that correspond to HAOO.2, HA01.03, HA02.02, HA20, and HA40.1 CID 11, respectively[9]. The aim of this study was to evaluate the effectiveness of CBT in the treatment of vasomotor, sexual dysfunction, and recurrent depression in postmenopausal women[10,11].

METHODS

We performed a prospective, case–control, open study to assess the changes in vasomotor symptoms and depression in

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postmenopausal women following CBT. The study was approved by the Institutional Review Board of the Faculty of Medicine of the University of Sao Paulo Research project approved by the Ethics Committee for analysis of CAPPesq Projects and Research at HC-FMUSP under protocol opinion 235.365 (number 235,375), and all subjects provided informed written consent.

Women were evaluated by psychiatrists of the Women's Mental Health Program (ProMulher) Institute of Psychiatry – HCFMUSP without other psychiatric disorders[12,13]. They were evaluated at ProMulher ambulatory second-floor south wing, a private room, for local recruitment psychology, data collection, and CBT sessions, from 1 p.m. to 7 p.m. on Monday and 4 p.m. to 7 p.m. on Thursday. Each session comprised six patients, lasting for 1 h and carried out by a research psychologist. Depressed women in the group with clinical demographic data were verified and recruited from the Climacteric Ambulatory Division of the Discipline of Gynecology IC-HCFMUSP and Women's Mental Health Program (ProMulher) IPq – HCFMUSP.

Inclusion criteria were postmenopausal women between 50 and 60 years of age, not using hormone replacement therapy, being postmenopausal between 2 and 6 years, and having more than 4 years of schooling. Exclusion criteria included primary ovarian insufficiency, hysterectomy with bilateral annexectomy, use of psychotropic drugs in the last 6 months prior to evaluation, impairment in cognitive processes, other psychiatric disorders, low intelligence quotient (IQ) (<79), severe and moderate depression with psychotropic treatment, and uncompensated endocrinopathies.

Groups

A total of 269 women were screened, of which 82 (55.0%) did not meet the inclusion criteria, 45 (30.2%) were evaluated (fear of cognitive degeneration), 13 (8.72%) were excluded (less than 75% attendance), and 9 (6.0%) left without justification. Women completed the program (n=120), but when called for re-evaluation, 8 (6.6%) changed their residence (city and state). A total of 112 women in the postmenopausal study were divided into two groups: G1 without depression (n=65) and G2 with recurrent depression (n=47).

Procedure

Participants gave written informed consent. The interviews were conducted face-to-face and recorded for transcription. The mean length of all evaluation through the questionnaires was 90 min±10 min.

Interview guide

At the initial visit, women's clinical history was ascertained to establish baseline clinical data. The evaluation of depression/anxiety and climacteric symptoms was performed using the Beck Depression Inventory (BDI)[14] and Greene Climacteric Scale[15], respectively. A diagnostic questionnaire to characterize recurrent depression was applied. The patients underwent neuropsychological analysis to evaluate attention and memory before completing the specific neuropsychological questionnaires.

A questionnaire structured with demographic factors (age, marital status, education, professional occupation) was used to assess clinical pretreatment status. It also included menstrual, gynecological, obstetric, puerperal, psychiatric, and sexual history, as well as information about specific complaints during their premenstrual period and following menopause.

The BDI[14] questionnaire has 21 groups of affirmations with four items and evaluates the presence and intensity of depressive symptoms. It also describes how the individual has felt in the previous week, including the day of the evaluation, using a cutoff of 18 to indicate moderate depression.

The validated GCS[15] comprises psychological symptoms of anxiety and depression, physical (somatic) and vasomotor symptoms, and sexual dysfunction in four degrees of intensity: symptoms absent, mild—not bothersome without interfering with everyday life, and intense—bothersome and interfere with daily life. It also contains 6 questions for anxious symptoms, 5 for depressive symptoms, 7 for somatic symptoms, 2 for vasomotor symptoms, and 1 for sexual symptoms, with a total of 21 questions (1. heart beating quickly or strongly; 2. feeling tense or nervous; 3. difficulty in sleeping; 4. excitable; 5. attacks of anxiety, and panic; 6. difficulty in concentrating; 7. feeling tired or lacking in energy; 8. loss of interest in most things; 9. feeling unhappy or depressed; 10. crying spells; 11. irritability; 12. feeling dizzy or faint; 13. pressure or tightness in head; 14. parts of body feel numb; 15. headaches; 16. muscle and joint pains; 17. loss of feeling in hands or feet; 18. breathing difficulties; 19. hot flushes; 20. sweating at night; and 21. loss of interest in sex). The following scores are based on the symptoms: 0 (absence); 1 (mild); 2 (moderate), and 3 (intense). The total maximum score is 63 points for all questions. The five domains of GCS are anxiety (#1–#5 questions), depression (#6–#11 questions), somatic symptoms (#12–#18 questions), vasomotor symptoms (#19 and #20), and sexual function (#21).

The questionnaire on Women's Mental Health (QSM)[16] depicts the quality of life, assessing physical and mental symptoms, specifically in women of middle age, period of life between maturity and old age. It is grouped into nine domains that evaluate somatic symptoms, depressive mood, concentration/memory problems, anxiety/fear, sexual satisfaction, vasomotor symptoms, sleep disorders, menstrual changes, and attractiveness.

The 20-item Psychiatric Morbidity Scale (SRQ 20)[17] evaluates the presence of nonpsychotic psychiatric disorders in the population in several countries of different cultures using a cutoff of 8 for women.

The Wechsler Abbreviated Scale Intelligence[18] was used to estimate IQ. The matrices contain 35 groups of figures with an incomplete grid model to be examined and completed with one correct choice out of five possibilities. Vocabulary contains 42 items representing verbal knowledge. The score was transformed into a weighted note, with its summation providing the total score and the estimated IQ.

Neuropsychological

The Stroop Color Word Test (SCWT)[19] evaluates selective attention, inhibitory control, and executive functions, and is composed of three cards with different levels of difficulty. The colors are distributed in six series in a random manner. Evaluation is by execution time, punctuated by mean and standard deviation, including errors.

The Trail Making Test (TMT)[20] evaluates the speed of attention, sequencing, mental flexibility, visual tracking, and motor function. Part A involves the collection of randomly distributed numbers 1–25. Part B involves an alternate sequence of numbers and letters, numbers 1–13 and letters A–M, distributed in a random order. The evaluation is by execution time, punctuated by means and standard deviation, including errors.

The Numbers and Letters (sub-scale)—Wechsler Adult Intelligence Scale-III[21] subtest evaluates working memory. It is composed of a list of numbers and letters disordered with seven items and three sequences each. After reading each item, you must sort them alphabetically first and then numerically without making mistakes.

The Digits—Direct and Reverse—Digit Span in Wechsler Memory Scale-Revised[22] test is divided into two parts. In the first part, the direct order is related to attention, with seven series of numbers, with two attempts each. In the second part, the inverse order is linked to working memory, and executive functions consist of six series of numbers, with two attempts each. The gross score is obtained and consequently the percentile[23].

The Digital Windows—Visuals—Visual Subtests—Wide Range Assessment of Memory and Learning. Second Edition—WRAML II[24] evaluates immediate memory and visuospatial learning. A plate is used with nine patient-cast circles, randomly numbered from 1 to 9 for the applicator. Following the same sequence carried out by the applicator, corrections and errors are noted on the answer sheet.

The List of Words—Verbal Subtest—Wide Range Assessment of Memory and Learning—WRAML II[24] evaluates short-term verbal memory and systematic verbal learning ability. There is a list of 16 words to be repeated. The task repeats itself four times and retrieves them later, evaluating the retention in its memory.

Cognitive behavioral therapy procedure

The CBT procedure was done the next day of questionnaire application with a group of six women following the protocol described in other study[1]. The CBT for each group was performed each 15 days during 24 weeks. Specific CBT exercises help empty stored negative feelings and emotions[4,11]. Through test of amnestic functions and attention, it allows us to indicate a better treatment strategy. The focus of CBT is to change one's behavior and reinforce the emotion[1]. The length of each CBT was 1 year. Two facilitators participated in each CBT. The CBT was held in the specific room only for this procedure in the Women's Mental Health Program (ProMulher) IPq – HCFMUSP.

Breathing exercises were performed before each CBT session and at home. Each woman was evaluated for her breathing pattern. This exercise brought calm, balance, better concentration, reducing anxiety[25-27]. Relaxation exercises bring a better quality of sleep and a decrease in daily stress[1,11].

Evaluation

The evaluations of effectiveness were performed at (a) baseline (before CBT), (b) after 24 weeks of CBT (at the end of procedure), and (c) 24 weeks after the end of procedure (follow-up—48 months after baseline).

Statistical analysis

The sample size and power estimation were calculated based on the mean and standard deviation differences between the WHQ scores in the pre- and post-treatment group in women with depression and menopausal symptoms (published) in the literature[28,29]. With a power of 90% and a significant p-value of 5%, the minimum number of patients calculated for this study was 89. With the inclusion of 120 initial and final 112 patients, the power of this study was 97 and 95%, respectively.

Student's t-test or the Mann-Whitney test was used, according to the data distribution. In the proportion of variables, Fisher's exact test or chi-square test was used. Repeated-measures ANOVA or Kruskal-Wallis was used for comparison among baseline, 24 months, and follow-up in the same group. The tests were performed with a significance level of 5%. Spearman´s correlation test was applied for evaluating the influences of amnestic and attention tests on the Green domains, BDI, and SRQ-20 scale.

RESULTS

Table 1 summarizes the clinical demographic data of the two groups of participants. There were also no significant differences between the two groups in the clinical demographic data of patients.

Table 2 summarizes the evaluation of amnestic and attention tests in both groups. In the SCWT-"D", SCWT-Erros-D", SCWT-W, SCWT-Erros-W, SCWT-CV, and SCWT-Erros-CW, there was a significant increase in values at 24 weeks in G2 comparing baseline and after 24 weeks ($p<0.05$). The G1 presented improved in SCWT-W and SCWT-CW ($p<0.05$). There is no difference in the comparison of baseline and 24 weeks of CBT when comparing both groups. After the follow-up evaluation (48 weeks), the values of G2 were higher than the ones of G1 ($p<0.05$).

The attention test results are summarized in Table 2. The values of TMT-B, number and letters, indirect digits, digital windows, list of words, list of words recovery of G1 increased after 24 weeks of CBT and maintained after follow-up of 48 weeks (24 weeks without CBT). The G2 group presented an improved TMT-A and other parameters, except those of TMT-Erros A, TMT-Erros B, and list of words. There were no differences in comparison between both groups in relation to baseline, 24 weeks of treatment, and 48 weeks of follow-up (24 weeks without CBT).

Table 1. Clinical demographic data of patients.

	G1: no depression (n=65)	G2: depression (n=47)	p
Age (M /SD)	55.5 (2.7)	54.8 (3.1)	0.72
Education (n)			
Middle school	05 (7.7%)	03 (6.4%)	
High school	17 (26.2%)	21 (44.7%)	
University degree	43 (66.2%)	23 (48.9%)	0.88
Marital status (n)			
Single	13 (20%)	10 (21.3%)	
Married	26 (40%)	27 (57.4%)	
Divorced	22 (33.8%)	06 (12.8%)	
Widowed	04 (6.2%)	04 (8.5%)	0.55
Professional status (n)			
Work	33 (50.8%)	33 (70.1%)	
Pensioned off	14 (21.5%)	10 (21.3%)	
Housewife	18 (27.7%)	04 (8.5%)	0.66
Body mass index (kg/m^2)			
< 25	10 (15.4%)	07 (14.9%)	
≥25	55 (84.6%)	40 (85.1%)	0.94
Physical exercise (n)			
Yes	14 (21.5%)	09 (19.1%)	
No	51 (78.5%)	38 (80.9%)	0.75

n: number of patients; G: group; M: mean; SD: standard deviation; p: p-value.

The BDI, SRQ-20, and Domains of Scale Greene are summarized in Table 3. The BDI baseline values of G2 (recurrent depression) were higher than those of G1 (no depression). The baseline values were 12.14 (3.93) and 25.21 (4.62) for G1 and G2, respectively ($p<0.001$). The scores of BDI decreased in both groups after 24 weeks of treatment and follow-up. The comparison between the groups did not find any differences after 24 weeks of treatment or during the follow-up period. Similar results were observed using the SRQ-20 scale. In relation to domains of Scale Greene, the anxiety, depression, sexual dysfunction, and physical symptoms at baseline in G2 were significantly superior to those of G1 ($p<0.05$), except the vasomotor symptom domain. Both groups presented significant improvements in all domains after 24 weeks of treatment, except the physical domain, where only the G2 group presented a significant decrease in this parameter ($p<0.01$). The anxiety and depression domain values of G2 were lower than those of G1 ($p<0.01$). The values of depression and vasomotor symptom domain of G1 during follow-up were significantly lower than baseline. The values of all domains of G2 during the follow-up were significantly different compared with baseline, expect the vasomotor symptom domain. No differences in all domains were found between the groups during the follow-up.

Correlation test

We assessed whether the SCWT-D and SCWT-W scales would be influenced by other conditions such as the Greene Climacteric Scale and SRQ-20. At baseline, the SCWT-W scale (Spearman's rho=0.3939, $p<0.01$), the total Greene Climacteric Scale (Spearman's rho=0.2776, $p<0.01$), and the SRQ-20 scale (Spearman's rho=0.3200, $p<0.01$) were all positively correlated with physical symptoms. At 24 weeks, the SCWT-D variable was correlated with vasomotor symptoms (Spearman's rho=0.2579, $p<0.01$). The SRQ-20 scale correlated with vasomotor symptoms (Spearman's rho=0.194, $p<0.01$), while the SCWT-W variable was only marginally related to vasomotor symptoms (Spearman's rho=0.1887, $p<0.01$). After 48 weeks, the SCWT-D variable score was related to vasomotor symptoms (Spearman's rho=0.2571, $p<0.01$). The SCWT-W variable was similarly correlated to vasomotor symptoms.

DISCUSSION

Recurrent depression is a major cause of professional incapacitation and disruption of family ties, leading to global affective

Table 2. Evaluation of amnestic and attention tests in both groups.

Evaluation/ time weeks	G1: no depression (n=65)			G2: recurrent depression (n=47)			Comparison among periods in G1			Comparison among periods in G2			Comparison between groups in different periods		
	Basal	24	48	Basal	24	48	Basal X 24	24X48	Basal X 48	Basal X 24	24X48	Basal X 48	Basal	24	48
SCWT-"D"[1]	17.48 (3.86)	18.72 (3.72)	32.40 (7.89)	17.40 (3.61)	20.70 (5.11)	35.80 (9.41)	NS	<0.001	<0.001	NS	<0.001	<0.001	NS	NS	<0.05
SCWT-Erros-"D"[1]	0.27 (0.64)	0.24 (0.55)	1.64 (1.84)	0.17 (0.43)	0.27 (0.57)	2.14 (2.43)	NS	<0.001	<0.001	NS	<0.001	<0.001	NS	NS	NS
SCWT –"W"[1]	14.47 (2.85)	17.72 (3.82)	29.91 (7.80)	17.40 (3.61)	20.70 (5.11)	35.80 (9.41)	<0.05	<0.001	<0.001	NS	<0.001	<0.001	NS	NS	<0.001
SCWT-Erros- "W"[1]	0.07 (0.26)	0.01 (0.12)	0.10 (1.60)	0.02 (0.14)	0.06 (0. 24)	0.85 (1.23)	NS	<0.001	<0.001	NS	<0.001	<0.001	NS	NS	NS
SCWT– "CW"[1]	13.48 (2.67)	16.73 (3.10)	29.02 (7.7)	13.69 (2.14)	17.98 (2.58)	30.27 (6.79)	<0.01	<0.001	<0.001	<0.005	<0.001	<0.001	NS	NS	NS
SCWT-Erros- "CW"[1]	0.01 (0.12)	0.10 (0.31)	0.53 (1.22)	0.0	0.08 (0.28)	0.48 (1.1)	NS	<0.01	<0.01	NS	NS	<0.05	NS	NS	NS
TMT –"A"[1]	1.10 (14.28)	24.46 (8.00)	27.54 (7.12)	31.87 (19.88)	25.05 (15.67)	28.67 (8.28)	NS	NS	NS	<0.05	<0.01	NS	NS	NS	NS
TMT- Erros-"A"[1]	0.23 (0.63)	0.10 (0.35)	0.13 (0.42)	0.02 (0.14)	0.04 (0.20)	0.14 (0.41)	NS	NS	NS	NS	NS	NS	NS	NS	NS
TMT –"B"[1]	76.84 (23.09)	53.39 (14.02)	63.58 (16.39)	84.14 (36.81)	54.17 (17.24)	61.93 (14.65)	<0.001	NS	<0.01	<0.001	NS	<0.001	NS	NS	NS
TMT- Erros-"B"[1]	1.16 (1.45)	0.86 (1.04)	0.46 (0.88)	0.68 (0.95)	0.53 (0.77)	0.40 (0.82)	NS	NS	<0.05	NS	NS	NS	NS	NS	NS
Number and letters[1]	8.24 (2.53)	11.02 (2.25)	14.61 (2.00)	7.34 (2.28)	11.17 (2.11)	13.91 (2.26)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	NS	NS	NS
Span digts – direct[2]	10 [4–12]	11 [5–12]	11 [7–12]	10 [4–12]	11 [7–12]	12 [6–12]	NS	NS	<0.001	<0.001	NS	<0.001	NS	NS	NS
Indirect digits[2]	5 [2–12]	7 [2–12]	8 [3–12]	5 [2–11]	8 [2–12]	9 [3–12]	<0.01	NS	<0.001	<0.001	NS	<0.001	NS	NS	NS
Digital windows[1]	6.23 (2.47)	9.10 (2.97)	7.86 (2.42)	6.14 (2.53)	9.10 (2.01)	7.68 (2.26)	<0.001	NS	<0.01	<0.001	NS	0.05	NS	NS	NS
List of words[2]	8 [3–14]	10 [7–16]	11 [6–17]	8 [3–14]	9 [6–15]	12 [9–19]	<0.001	NS	<0.001	NS	<0.001	<0.001	NS	NS	NS
List of words recovery[2]	8 [0–15]	11 [4–16]	14 [5–16]	7 [0–15]	10 [5–16]	14 [7–16]	<0.001	<0.05	<0.001	<0.05	<0.001	<0.001	NS	NS	NS

SCWT: Stroop Color Test; TMT: Trail Making Test. [1]Mean (standard deviation) and unpaired Student's t-test was used. Repeated-measures ANOVA and post-hoc Bonferroni tests were applied for comparison in the same group among periods. [2]Median [interquartile range] and Mann-Whitey test were used. Repeated-measures Kruskal-Wallis and post-hoc Dunn tests were applied for compassion in the same group.

and professional problems[10]. In addition, when there are other associated stressors, such as postmenopausal vasomotor waves, the recurrence and intensity of this condition can increase greatly[8]. Our present findings show that CBT can reduce the symptoms associated with recurrent depression as well as the occurrence of menopausal symptoms such as hot flashes and sexual dysfunction. The amnestic parameters have a positive correlation with the improvement in recurrent depression.

Hot flashes interfere with sleep, quality of life, and recurrence and severity of depressive symptoms[27], which encompass not only mood changes but also a deficiency in concentration and difficulty in carrying out daily activities[26]. In our study with CBT, the most patients' frequent dysfunctional automatic thoughts were (a) assuming responsibility for some external situation when in fact others were actually responsible, (b) interpreting their experiences as being totally good or totally bad, (c) concluding that she knows what others are thinking of her and focuses on only one aspect of the situation that she is facing while other aspects are ignored, (d) rejecting positive information about themselves or a situation, and only seeing

Table 3. Results of Beck Depression Inventory, SRQ-20, and domains of the Greene Climacteric Scale.

G1: no depression (n=65)				G2: recurrent depression (n=47)			Comparison among periods in G1			Comparison among periods in G2			Comparison between groups in different periods		
Questionnaires or domains	Basal	24 weeks	48 weeks	Basal	24 weeks	48 weeks	Basal X 24 weeks	24 weeks X 48 weeks	Basal x 48 weeks	Basal X 24 weeks	24 weeks X 48 weeks	Basal x 48 weeks	Basal	24 weeks	48 weeks
BDI[1]	12.14 (3.93)	5.26 (2.78)	8.10 (4.37)	25.21 (4.62)	6.10 (2.83)	8.04 (3.94)	<0.001	<0.05	<0.001	<0.001	NS	<0.001	<0.001	NS	NS
SRQ-20[1]	7.78 (4.40)	4.49 (3.84)	3.89 (2.84)	12.55 (3.84)	6.10 (4.39)	4.00 (2.96)	<0.001	NS	<0.001	<0.001	NS	<0.001	<0.001	NS	NS
Domains of Greene Climacteric Scale															
Anxiety[1]	7.12 (3.47)	3.70 (2.44)	4.92 (3.27)	9.55 (2.94)	5.61 (3.19)	5.63 (2.90)	<0.01	NS	NS	<0.01	NS	<0.01	<0.01	<0.05	NS
Depression[1]	8.52 (4.78)	2.47 (1.89)	3.38 (3.08)	12.94 (3.71)	5.63 (4.58)	3.83 (2.83)	<0.01	NS	<0.01	<0.01	NS	<0.01	<0.01	<0.01	NS
Sexual dysfunction[1]	1.84 ± 1.12	2.30 ± 0.98	1.60 ± 1.04	2.36 ± 1.03	1.16 ± 0.83	1.59 ± 0.99	<0.01	<0.01	NS	<0.01	NS	<0.01	<0.05	NS	NS
Vasomotor symptoms[1]	2.80 ± 2.15	1.10 ± 1.58	0.67 ± 1.07	1.66 ± 2.13	1.06 ± 1.35	0.72 ± 1.05	<0,01	NS	<0,01	<0.01	NS	NS	NS	NS	NS
Physical symptoms[1]	5.76 ± 4.24	4.04 ± 3.17	4.44 ± 3.56	8.93 ± 5.01	3.23 ± 2.75	4.12 ± 2.93	NS	NS	NS	<0.01	NS	<0.01	<0.01	NS	NS
Total[1]	25.85 ± 11.43	13.55 ± 7.71	14.95 ± 8.46	36.60 ± 11.21	18.00 ± 9.51	15.96 ± 7.24	<0.01	NS	<0.01	<0.01	NS	<0.01	<0.01	NS	NS

BDI: Beck's Depression Inventory; SRQ: Self-Reporting Questionnaire; [1]Mean (standard deviation) and unpaired Student's t-test were used. Repeated-measures ANOVA and post-hoc Bonferroni tests were applied for comparison in the same group among periods.

the negative, (e) maximizing negative experiences while positive ones are minimized, (f) placing a global and rigid label on someone or a situation instead of evaluating it as a whole, (g) interpreting events in terms of how they should be instead of focusing on how they are, and (h) believing that what has happened may recur and will be terrible, intolerable, or unbearable[27]. All these items have negative consequences after CBT.

Our study had some limitations: (a) no control group with recurrent depression without CBT; (b) subjects also had an unsatisfactory response to convectional drug therapy and, therefore, may be a select group; (c) we remain unaware of the duration of gains achieved by CBT; (d) long period of follow-up to check whether there is the residual benefit of CBT; and (e) vasomotor and sexual dysfunction were evaluated using the Greene Climacteric Scale. Therefore, further study using specific questionnaire on sexuality and digital vasomotor symptom registration is necessary to evaluate the real effects of CBT.

The strengths of our study are the evaluation of amnestic and attention parameters on the results of CBT and the follow-up of patients: the benefits on anxiety, depression, and physical symptoms of recurrent depression may remain after CBT therapy.

CONCLUSION

Cognitive behavioral therapy may be effective in reducing vasomotor symptoms and ameliorating the sexual dysfunction and recurrent depression in postmenopausal women after 24 weeks of treatment.

AUTHOR'S CONTRIBUTIONS

LADT: Conceptualization, Data curation, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing. **JMS:** Writing – original draft, Writing – review & editing. **JR:** Conceptualization, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing – original draft, Writing – review & editing. **JAOT:** Formal Analysis, Writing – original draft, Writing – review & editing. **IML:** Writing – original draft, Writing – review & editing. **ECB:** Writing – original draft, Writing – review & editing. **NRM:** Conceptualization, Funding acquisition, Investigation, Methodology, Supervision, Validation, Writing – original draft, Writing – review & editing.

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ORIGINAL ARTICLE

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Determinants of health-promoting behaviors in pregnant women

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SUMMARY

OBJECTIVE: The aim of the study was to examine the relationship between social support, marital dissatisfaction, psychological factors, and health-promoting behaviors in pregnant women.

METHODS: This cross-sectional study was conducted on 1,265 pregnant women who visited the outpatient clinic of a maternity hospital between May and August 2023. The Health Promotion Lifestyle-II Questionnaire was used to measure the healthy lifestyle behaviors of pregnant women. The mental health status of pregnant women was measured using the Depression Anxiety and Stress Scale-21. The Marital Disaffection Scale was used to assess the level of disaffection toward a spouse. Perceived social support was measured by the Multidimensional Perceived Social Support Scale.

RESULTS: Pregnant women had a mean age of 26.46±5.09 years. Multivariate linear regression analysis revealed that there was a positive association between perceived social support and health-promoting behaviors. It was also found that marital disaffection was negatively associated with health-promoting behaviors ($p<0.001$).

CONCLUSION: The present study suggests that stress, anxiety, depression, and marital disaffection are negatively associated with health-promoting lifestyle behaviors, while social support is positively associated with the adoption of health practices in pregnant women. Understanding the complex interplay between psychosocial factors and healthy behaviors is crucial to improving healthy behaviors in pregnant women.

KEYWORDS: Health promotion. Pregnancy. Social support. Lifestyle.

INTRODUCTION

Unhealthy behaviors and lifestyles are responsible for a significant number of deaths globally[1]. During pregnancy, women undergo significant mental and physical changes that can impact their lifestyle and behaviors. Therefore, promoting healthy behaviors during this transformative period is crucial to ensure the well-being of both the mother and the developing fetus[2-4].

Health-promoting behaviors encompass a range of practices that contribute to favorable health status and pregnancy outcomes. These practices include nutrition, physical activity, health responsibility, stress management, interpersonal relationships, and self-actualization[5]. For instance, there is an increased risk of adverse birth outcomes for pregnant women who are overweight or obese, or who experience greater weight gain during pregnancy. Smoking during pregnancy is related to higher rates of low birth weight and fetal heart rate abnormalities. High levels of stress during pregnancy have also been shown to significantly impact pregnancy outcomes[6].

Health promotion is a process that enables individuals to have better control over their behaviors. Social, environmental, cultural, and political factors and individual characteristics play a critical role in health promotion. Social support is a major contributor to health promotion, both through behavioral and psychological means. Furthermore, social support may positively impact mental and physical health by reducing stress levels[7].

Healthcare providers need to be aware of the intricate connection between social support, marital disaffection, psychological factors, and healthy habits in pregnant women in order to promote maternal and child health[8,9]. Effective interventions should focus on removing barriers and strengthening facilitators, with an emphasis on moderating social factors and personal expectations. However, there is a lack of evidence-based interventions in this area due to the varying prevalence rates of psychological factors in different settings. A deeper understanding of the complex interplay between social factors and healthy behaviors is needed. Therefore, the present study aims to explore the association between social support, marital disaffection, psychological factors, and health-promoting behaviors in pregnant women.

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METHODS

This cross-sectional study was conducted on pregnant women who attended the outpatient clinic of a maternity hospital between May and August 2023. The research was conducted under the ethical standards set by the Helsinki Declaration. The Scientific Research Ethics Committee granted approval for the study (approval number: 21/30). All pregnant women gave written informed consent after being fully informed about the study.

Data were collected by a trained midwife under the supervision of the principal investigator. The principal investigator followed the data collection procedure plan. The data were collected using a structured questionnaire administered by a face-to-face interviewer. The data collector received a 3-day training on data collection, sample selection, study tool administration, and data handling procedures. The data collector was familiar with the study's objectives, methods, and ethical aspects.

The inclusion criteria for this study were singleton pregnant women between the ages of 18 and 45 years, who had a gestational age greater than 28 weeks, gave birth to a healthy infant, and were literate. Exclusion criteria were high-risk pregnancy or experienced any stressful life events within the past 6 months. In addition, women with major depression or psychiatric illness, those who delivered babies with anomalies, and those who refused to participate were excluded.

The sociodemographic data form created by the researchers included questions about age, income, family type, body mass index (BMI), education level, and occupation of pregnant women and spouse.

The Health Promotion Lifestyle-II Questionnaire (HPLP-II) was developed by Walker et al.[5] and later adapted into Turkish by Esin[10]. It consists of 52 items measured on a four-point Likert scale from 1 to 4 (never, 1; sometimes, 2; often, 3; and always, 4). The assessment's total score ranges between 52 and 208 points, with higher scores indicating better health-promoting behaviors. The scale evaluates six dimensions of individual behavior that contribute to a healthy lifestyle: self-efficacy, responsibility for health, interpersonal relationships, stress management, exercise and physical activity, and nutrition. The HPLP-II showed high internal consistency with a Cronbach's alpha value of 0.91.

Depression Anxiety and Stress Scales-21 (DASS-21) was created by Lovibond and Lovibond[11] and then adapted to Turkish by Yılmaz et al.[12] to assess participants' mental health status. The DASS-21 has been empirically validated with various populations from diverse cultures, demonstrating high internal consistency[13-15]. This 21-item scale consists of three dimensions: anxiety, depression, and stress. Each item is scored on a 4-point Likert-type scale. A higher total score is indicative of more severe symptoms of depression, anxiety, and stress. Cronbach's alpha internal consistency ranges between 0.755 and 0.822.

Marital Disaffection Scale (MDS) was developed by Kayser[16] and adapted to Turkish by Çelik[17], and was utilized to assess the level of disaffection towards a spouse. It is a 21-item, 4-point Likert-type scale with a single-factor structure. Each item is scored from 1 to 4 (Not at All True, 1; Not Very True, 2; Somewhat True, 3; and Very True, 4). The total score ranges from 21 to 84, with higher scores indicating higher levels of marital disaffection. The scale has high internal consistency, as reflected by the Cronbach's alpha coefficient of 0.89.

Multidimensional Scale of Perceived Social Support (MSPSS) is a psychometric tool designed by Zimet et al.[18] and adapted to Turkish by Eker et al.[19] to measure individuals' perceived social support from three sources: "family, friends, and a significant other." This scale comprises 12 items, rated on a 5-point Likert-type scale from strongly disagree to strongly agree. The internal consistency coefficients reported for the scale are within the range of 0.80 to 0.85, with a Cronbach's alpha coefficient of 0.92.

Statistical analysis

Statistics were performed with the IBM SPSS 25 software. Qualitative variables were presented as frequencies and percentages, while quantitative variables were presented as means and standard deviations. Independent-sample t-tests were employed for comparisons between variables with two categories and quantitative variables, whereas one-way ANOVA was employed for variables with more than two categories. Pairwise comparisons between categories were done using the Tukey test if a significant difference was detected. To examine the relationship between two quantitative variables, we employed the Pearson correlation. Additionally, we conducted multivariate linear regression to determine the variables influencing the HPLP-II. The significance level was set at $p<0.05$.

RESULTS

This study included 1,265 pregnant women who met the eligibility criteria. The pregnant women had a mean age of 26.46+5.09 years. Among the participants, 37.3% had completed secondary school, 84.9% were housewives, and 36.0% had husbands who graduated from secondary school. A great majority of the women (97.5%) had a nuclear family, and 66.6% of the women had a monthly income that was equal to their expenses.

Pregnant women with primary school education had lower HPLP-II scores than those pregnant women with higher levels

of education (p<0.001), and those whose spouses graduated from primary school had lower scores compared to those who graduated from university (p=0.017). Pregnant women with income equal to expenses showed higher HPLP-II scores than those with expenses higher than their income (p<0.001). Pregnant women in nuclear households exhibited higher HPLP-II scores than those in extended households (p=0.031) (Table 1).

The study found no significant correlations between HPLP-II scores and age or pre-pregnancy BMI. However, there was a weak positive correlation between HPLP-II scores and the MSPSS total score and a weak negative correlation between the MDS total score. In addition, a negative correlation was observed between the HPLP-II score and depression, anxiety, and stress scores (all p<0.001) (Table 2).

It was found that increases in educational status (β=0.191, p=0.008) and MSPSS scale scores (β=0.196, p<0.001) led to increases in HPLP-II scale scores, while an increase in MDS scores led to a decrease in HPLP-II scale scores (β=-0.195, p<0.001). Age (β=0.191, p=0.570), pre-pregnancy BMI (β=0.191, p=0.336), income (β=0.191, p=0.856), family type (β=0.191, p=0.090), husband's educational status (β=0.191, p=0.079), depression score (β=0.191, p=0.269), anxiety score (β=0.191, p=0.161), and stress score (β=0.191, p=0.181) had no significant relationship with HPLP scores (Table 3).

Table 2. Relationship between Health Promotion Lifestyle-II Questionnaire and marital disaffection, multidimensional perceived social support, and Depression Anxiety and Stress Scale-21.

		Total HPLP-II
Age	r	-0.057*
	p	0.044
Pre-pregnancy BMI	r	-0.064*
	p	0.023
MSPSS-total	r	0.309**
	p	<0.001
MDS-total	r	-0.314
	p	<0.001
DASS depression	r	-0.221**
	p	<0.001
DASS anxiety	r	-0.194**
	p	<0.001
DASS stress	r	-0.228**
	p	<0.001

MDS: Marital Disaffection Scale; DASS: Depression Anxiety Stress Scale; MSPSS: Multidimensional Scale of Perceived Social Support; BMI: body mass index; HPLP-II: Health Promotion Lifestyle-II Questionnaire. *p<0.05. **p<0.001.

Table 1. The mean Health Promotion Lifestyle-II Questionnaire scores and sociodemographic characteristics.

Variables	HPLP-II Mean±SD	t/F	p-value	Post-hoc comparisons
Educational status				
Primary school	130.34±26.64	7.332	<0.001*	1<2,3,4
Secondary school	139.75±27.19			
High school	138.75±27.12			
University	144.1±32.23			
Income				
Income is less than expense	133.34±29.27	-5.417	<0.001	2>1
Income is equal to expense	142.46±27.68			
Educational status of spouse				
Primary school	134±26.53	3.417	0.017*	1<4
Middle school	139.1±27.66			
High school	138.43±26.88			
University	143.32±32.2			
Family type				
Nuclear family	139.69±28.58	2.157	0.031*	1>2
Extended family	128.52±24.9			

HPLP-II: Health Promotion Lifestyle-II Questionnaire. *p<0.05.

Table 3. Predictors of the pregnant women's Health Promotion Lifestyle-II Questionnaire scores using multivariate linear regression analysis (n=1,265).

	B	Std. Error	St. B	t	p	95%CI
(Constant)	132.289	11.32		11.687	<0.001*	-0.331, -0.182
Age	-0.074	0.131	-0.015	-0.568	0.570	-0.656, -0.224
Pre-pregnancy BMI	-0.216	0.224	-0.026	-0.963	0.336	1.507, -10.014
Educational status	5.761	2.168	0.191	2.657	0.008	-3.016, -3.633
Income	0.309	1.694	0.005	0.182	0.856	-17.585, -1.286
Family type	-8.149	4.809	-0.044	-1.694	0.090	-8.205, -0.45
Educational status of spouse	-3.877	2.206	-0.126	-1.758	0.079	0.39, -0.694
MSPSS-total	0.542	0.077	0.196	7.005	<0.001*	-0.754, -0.423
MDS-total	-0.589	0.085	-0.195	-6.964	<0.001*	-1.109, -0.31
DASS depression	-0.4	0.362	-0.071	-1.106	0.269	-0.192, -1.154
DASS anxiety	0.481	0.343	0.075	1.403	0.161	-1.242, -0.235
DASS stress	-0.503	0.376	-0.085	-1.338	0.181	-0.331, -0.182

MDS: Marital Disaffection Scale; DASS: Depression Anxiety Stress Scale; MSPSS: Multidimensional Scale of Perceived Social Support; BMI: body mass index; HPLP-II: Health Promotion Lifestyle. *p<0.001.

DISCUSSION

The present study revealed an inverse correlation between stress, anxiety, depression, and marital disaffection with health-promoting lifestyle behaviors and a positive correlation between social support and the adoption of healthy behaviors.

The relationship between social support and health promotion practices in women is complicated and not well understood. The present study found that social support increases health-promoting behaviors. Similarly, Fathnezhad-Kazemi et al.[20] and Jung and Chun[21] found that women with higher social support demonstrated better performance in adopting health-promoting lifestyles. However, some studies have failed to demonstrate such an association. It has been reported that support provided by others can sometimes have a negative or ineffective impact, although some studies have reported a positive relationship. Differences in study design and research communities could account for the varying results, highlighting the need for prospective studies that assess pregnant women at each trimester.

Research on the relationship between mental health and health-promoting behaviors has yielded mixed results. While some studies have found a negative relationship, others have found no such relationship. For example, several studies found that there was a significant inverse relationship between pregnancy anxiety and the overall health-promoting behavior score[22,23]. Kemp and Maker[24], on the contrary, did not find such a relationship between anxiety levels and overall HPLP-II scores. It is possible that the discrepancies in findings between these studies are due to cultural, environmental, and economic differences.

This study demonstrates a negative correlation between depression symptoms and health-promoting behaviors among pregnant women. This aligns with a recent research that highlighted that depression may act as a direct risk factor in compromising healthy practices by reducing self-care during pregnancy[25]. In addition, our research has established a clear relationship between marital disaffection and poor health behaviors. This finding is in agreement with a previous study that also highlighted the negative impact of marital dissatisfaction on healthy pregnancy lifestyles[6].

Limitations

Despite the strengths of our study, such as the large sample size, some limitations need to be considered. One of the limitations of this study is the inability to establish cause and effect due to its cross-sectional design. In contrast to previous studies that examined pregnant women regardless of the trimester, our study only focused on only one-time interval during the pregnancy. Therefore, future studies would benefit more from cohort studies in various trimesters on this issue.

CONCLUSION

The findings of the current study suggest that stress, anxiety, depression, and marital disaffection are negatively associated with health-promoting lifestyle behaviors, while social support is positively associated with the adoption of health practices in pregnant women. It is therefore crucial to identify psychological risk factors during pregnancy and provide appropriate

interventions to enhance the lifestyle of pregnant women. The psychological factors identified in the present study can help healthcare providers develop prevention strategies to promote healthy behaviors in pregnant women.

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AVAILABILITY OF DATA AND MATERIALS

The dataset used and analyzed in the study is available from the corresponding author upon reasonable request.

ETHICAL STATEMENT

Ethical approval for this study was provided by the Hamidiye Scientific Research Ethics Committee (approval number: 21/30). The database management is in accordance with privacy legislation, and the presented study is in accordance with the ethical principle of the Declaration of Helsinki.

AUTHORS' CONTRIBUTIONS

GB: Conceptualization, Writing – original draft, Writing – review & editing. **EK:** Writing – original draft, Writing – review & editing. **LK:** Data curation, Writing – review & editing. **KNB:** Formal Analysis, Writing – original draft, Writing – review & editing.

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ORIGINAL ARTICLE

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Thyroid surgery in children: a single-center experience of 20 years

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SUMMARY

OBJECTIVE: Thyroidectomy is a relatively uncommon procedure in pediatric patients. We aimed to review our 20-year experience of thyroid surgery.

METHODS: A total of 39 patients who underwent thyroid surgery from 2003 to 2023 were retrospectively evaluated. All patients were followed preoperatively and postoperatively by our institutional multidisciplinary board. Patients were divided into two groups based on their pathologies: benign and malignant.

RESULTS: In total, 39 patients (27 girls and 12 boys) underwent 47 thyroid surgeries (total thyroidectomy in 19 patients and subtotal thyroidectomy in 20 patients, with 8 of them having completion thyroidectomy). Notably, 20 (51%) patients had benign and 19 (49%) patients had malignant pathologies. Median age at operation was 157 (9–223) months in the benign group and 182 (1–213) months in the malignant group. In the benign group, 12 (60%) patients had colloidal goiter and 8 (40%) patients had other conditions. In the malignant group, 12 (63%) patients had papillary thyroid carcinoma, 3 (16%) patients had follicular thyroid carcinoma, 2 (11%) had medullary thyroid carcinoma, and 2 patients had other thyroid malignancies. Overall permanent complication rate was 2 out of 39 (5%), which was similar for both groups (1 hypocalcemia in each group). The median follow-up was 38 months (1–179 months) with no local recurrence or distant metastasis.

CONCLUSION: Pediatric thyroidectomies are performed on a heterogeneous group of pediatric patients due to a diverse group of pathologies. A multidisciplinary approach is required for proper initial management and surgical strategy with decreased complication rate and event-free survival of these patients in experienced tertiary centers.

KEYWORDS: Hypocalcemia. Pediatrics. Thyroid gland. Thyroid nodule. Thyroidectomy.

ABBREVIATIONS: PTC: papillary thyroid carcinoma; FTC: follicular thyroid carcinoma; MTC: medullary thyroid carcinoma; MEN2: multiple endocrine neoplasm; FNA: fine needle aspiration; DTC: differentiated thyroid carcinomas.

INTRODUCTION

Thyroidectomies are performed in approximately 50,000 cases annually, but only 500 of these procedures are performed in children[1]. Even though thyroidectomy is one of the most commonly performed surgeries, it is still relatively uncommon in children[1-4]. Pediatric thyroidectomy is indicated for various conditions such as goiter that causes compressive symptoms, Graves' disease, thyroid nodules, thyroid cancer, and prophylaxis in familial endocrine syndromes such as multiple endocrine neoplasm 2[2,5,6]. Thyroid nodules have an incidence of 1–2% in the pediatric population, which is less common than in adults[6-8]. However, the malignancy rate is 22–26% and is more likely in the pediatric population, with papillary thyroid carcinoma (PTC) (80–90%) accounting for the majority, followed by follicular thyroid carcinoma (FTC) (10%) and medullary thyroid carcinoma (MTC) (3–5%) and rarely less differentiated malignancies of the thyroid[6-9]. This results in a more aggressive approach in children than in adults[6,8]. Even though thyroid cancer in children is rare and accounts for less than 1% of all childhood cancers, overall malignancy rates are increasing throughout the years[5,6,10-13]. The reported risk factors for malignancy include autoimmune disorders such as Hashimoto's thyroiditis and Graves' disease, iodine deficiency, and radiation exposure[9].

Physical examination, radiological imaging, and pathological evaluations can be utilized for the differential diagnosis of thyroid nodules[9]. Thyroid nodules are usually present as an asymptomatic neck mass, cervical lymphadenopathy, or compression symptoms may accompany[9]. On ultrasonographic imaging, irregular shape and margins, microcalcifications, marked hypoechogenicity, intranodular hypervascularization, enlargement of the nodule over time, invasion of extra thyroid tissues, anteroposterior diameter larger than the transverse diameter, and the presence of cervical lymphadenopathies suggest

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malignancy[9,14]. Ultrasound-guided fine needle aspiration (FNA) biopsy can be performed on hypofunctioning thyroid nodules, and histopathological evaluation according to the Bethesda classification can be evaluated to predict the risk of malignancy[9].

In the literature, complications related to thyroidectomies have a higher incidence in children than adults, mainly due to less experience in the pediatric population[2,4]. These complications include hematoma, hypoparathyroidism, hypocalcemia, vocal cord paralysis, nerve injury and vascular, and tracheal or esophageal injury[2-4].

The aim of this study is to review 20 years of pediatric thyroidectomy experience with a multidisciplinary approach in a single institution and to compare complication rates between malignant and benign thyroid disease.

METHODS

Study design and data collection

A total of 39 pediatric patients (age below 18 years) who underwent thyroid surgery in a single tertiary center from 2003 to 2023 were evaluated retrospectively by an institutional multidisciplinary board. All procedures performed in this study were in accordance with the ethical standards of the institutional and national research committee and with the Helsinki Declaration and its later amendments or comparable ethical standards. This study was approved by our institutional ethics committee (KAEK-454). Written consent was obtained from the patient's parents or legal guardians prior to surgery.

Data were collected from the Akdeniz University School of Medicine, Department of Pediatric Surgery and Pediatric Endocrinology. Gender, age at operation, concordant diseases, number of operations performed and surgical approaches, pathological results, and postoperative complications were noted. Vascular sealing devices and nerve monitoring were used selectively. All patients were followed preoperatively and postoperatively by our institutional multidisciplinary board. Patients were divided into two groups according to benign and malignant pathologies. The follow-up duration of each patient was noted.

Statistical analysis

Descriptive statistics were calculated for all variables. The association between benign and malignant disease with specific outcomes, including hypocalcemia and vocal cord paralysis, was analyzed using Fisher's exact probability test using SPSS for Windows (SPSS 23.0; IBM, Armonk, NY, USAC). The level of statistical significance was set to $p<0.05$.

RESULTS

A total of 39 patients underwent 47 thyroid surgeries, of whom 27 were girls and 12 were boys. Of note, 19 children had a total thyroidectomy, and 20 children had a subtotal thyroidectomy. Eight children who underwent subtotal thyroidectomy primarily had a completion thyroidectomy after pathological results. In addition, 20 (51%) patients had benign and 19 (49%) patients had malignant pathologies (6 of them underwent modified neck dissection) (Table 1). The median age at operation was 157 (9–223) months for benign pathologies and 182 (1–213) months for malignant pathologies. In the benign group, 12 (60%) patients had colloidal goiter and 8 (40%) had other conditions. In the malignant group, 12 (63%) patients had PTC, 3 (16%) had FTC, 2 (11%) patients had MTC, and 2 patients had other thyroid malignancies. Five children had prophylactic total thyroidectomy due to a RET oncogene mutation.

Preoperatively, FNA was performed with ultrasound guidance on 25 patients (64.1%) who presented with thyroid nodules. Notably, 8 of these children (32%) had nondiagnostic unsatisfactory results, while 9 (36%) had benign and 8 (32%) had malignant results. Two children with nondiagnostic FNA results were diagnosed with malignant conditions after thyroidectomy.

Hypocalcemia was significantly more frequent in the malignant group ($p<0.05$). The overall permanent complication rate was 2 out of 39 (5%), which was similar for both groups (one hypocalcemia in each group). Three patients who underwent total or completion thyroidectomy along with modified neck lymph node dissection had intensive care unit

Table 1. Number of benign and malignant thyroid disease.

Diagnosis	Number of patients (n=39)
Benign (goiter)	20
Colloidal hyperplasia/goiter	12
Dishormogenetic goiter	3
Benign (other conditions)	5
Hashimoto's thyroiditis	3
Normal thyroid (thyroid lobectomy for parathyroid hyperplasia)	1
Thymus (intrathyroidal)	1
Malignant	19
Papillary thyroid carcinoma	12
Follicular thyroid carcinoma	3
Medullary thyroid carcinoma	2
Hurtle cell carcinoma	1
Immature teratoma	1

admission due to vocal cord paralysis (p=0.106). One of these patients had a tracheostomy while in the intensive care unit. All the vocal cord paralysis was transient and the tracheostomy was decannulated before discharge. The recurrent laryngeal nerve injury was not seen in either group. The overall rate of complications was higher for operations for malignancy (p<0.01) (Table 2). Radioactive iodine therapy was applied to 13 patients. The median follow-up period was 38 months (1 month– 179 months). There was no local recurrence or distant metastasis.

DISCUSSION

Thyroidectomy is a relatively uncommon procedure in the pediatric population and is indicated for hyperthyroidism, thyroid nodules, malignancy, goiter with compressive symptoms, and prophylaxis in children with familial endocrine syndromes that cause a predisposition to thyroid malignancies[1-4]. This study aims to compare complication rates between benign and malignant thyroid diseases in pediatric patients with a multidisciplinary approach in a single institution.

Benign thyroid diseases in children are mainly Graves' disease, chronic autoimmune thyroiditis (Hashimoto's thyroiditis), colloid goiter, and thyroid adenomas[15]. Benign thyroid pathologies can be managed with anti-thyroid medication. However, if these treatments fail, surgical management or radioactive iodine treatment is an alternate viable option[6,16]. The literature shows that radioactive iodine treatment in children can increase secondary malignancy rates, which causes concern, especially in children younger than 5 years of age[16]. In this study, 20 patients (51%) underwent thyroidectomy for benign thyroid pathologies. One of the patients who had a thyroidectomy for a benign pathology had permanent hypocalcemia. Hypocalcemia reportedly presents up to 20% in children following thyroidectomies, 8% of which can become permanent[3]. Young patient age, hyperthyroidism, and lymph node dissection are known risk factors for hypocalcemia[3].

Table 2. Number of complications in benign and malignant thyroid disease groups.

Complications	Benign (n=20)	Malign (n=19)
Hypocalcemia (n=8)		
Transient	0	6
Permanent	1	1
Vocal cord paralysis (n=3)		
Transient	0	3
Permanent	0	0

Thyroid cancer in children is rare and accounts for less than 1% of all childhood cancers[6]. Thyroid nodules are also less common in children and are not necessarily malignant but have a higher rate of malignancy compared to adults[6,8]. Due to this increased malignancy risk of nodules in children, FNA is recommended to be performed under ultrasonography guidance[7]. Studies show that about 35% of FNA results were indeterminate[7]. In our study, 32% of the FNA results were indeterminate and 25% of them were malignant after operation.

Radiation, iodine deficiency, and genetic predisposition syndromes are known risk factors for thyroid carcinoma. Children especially have thyroid tissue that is more vulnerable to radiation, and Hashimoto's thyroiditis increases the risk of malignancy of a thyroid nodule[6]. Furthermore, overall malignancy rates are increasing throughout the years both for adults and children[5,6,10-13]. Pediatric thyroid malignancies are mostly well-differentiated thyroid carcinomas (DTC), accounting for 0.5–3% of all childhood cancers[6,13,17]. PTC is the most common type of DTC in children, accounting for about 60–80% of all pediatric DTCs; FTC and MTC are less common[6,13]. In children, PTC is more aggressive, and local and regional involvement at the time of diagnosis is around 60%, which is more common at diagnosis than in adults[5,6,18]. In the literature, the most common pathology in pediatric thyroidectomy cases is PTC, followed by FTC and Graves' disease[1]. In this study, 12 patients (31%) underwent thyroidectomy due to PTC. Five of these patients who had undergone thyroid lobectomy due to benign or inconclusive FNA results underwent completion thyroidectomy. Chen et al. argued that the rate of lymph node metastasis is about 60–80%[19]. All 12 patients underwent neck lymph node sampling at the time of operation, and 6 of these patients were positive for carcinoma and underwent modified neck lymph node dissection. Modified neck lymph node dissection and total thyroidectomy are known risk factors for hypoparathyroidism[1]. In our series, persistent hypoparathyroidism developed in two patients.

Follicular thyroid carcinoma and MTC account for less than 10% of all pediatric thyroid malignancies[6,20,21]. In this study, three cases were diagnosed as FTC accounted for 16%. MTC in children is usually familial and related to RET oncogene mutations and syndromes such as MEN 2A and 2B[6]. Prophylactic thyroidectomy at different ages is recommended based on the risk stratification of these patients[6]. In our series, five patients underwent prophylactic thyroidectomy based on the RET mutation, and two of these patients had MTC on pathological evaluation. On the contrary, one patient who underwent total thyroidectomy for a degenerative thyroid nodule was diagnosed with Hurtle cell carcinoma.

Thyroid teratomas are rare and generally benign pathologies[3]. However, immature teratomas have been reported rarely in literature[3]. Generally, this malignancy rate increases with age[3]. In our series, one patient underwent total thyroidectomy due to a neonatal immature teratoma without any complications postoperatively.

Postoperative complications of thyroidectomy are most commonly hypocalcemia due to trauma or devascularization of parathyroid glands[7]. According to the literature, 35.5% transient and 4.2% permanent hypocalcemia occur following thyroidectomy in children[7]. Permanent hypocalcemia is associated with central or lateral neck dissection[22]. In our series, transient and permanent hypocalcemia complications occurred in 15 and 5.1% of patients, respectively. In our series, hypocalcemia, both transient and permanent, was related to lymph node dissection. Both the patients who developed permanent hypocalcemia had undergone lateral lymph node dissection. Another possible complication of thyroidectomy is laryngeal nerve injury, which can be unilateral or bilateral. Unilateral injury results in hoarseness, choking, and aspiration of food or water, while bilateral injury can be a life-threatening condition blocking the airway[7]. The literature shows that recurrent laryngeal nerve injury rates are higher in children than in adults[16]. In our series, three patients with PTC, who underwent total or completion thyroidectomy along with modified neck lymph node dissection, developed bilateral vocal cord paralysis, which was resolved in follow-up. In recent years, the use of nerve stimulation to protect the laryngeal nerve has become a standard approach. We presume that the rates of laryngeal nerve injury will decrease in the following years.

In the tertiary center where this study was conducted, all patients were evaluated by the pediatric endocrinology, pediatric surgery, and radiology departments prior to surgery to form a treatment plan. The medical treatment of the patients was planned by the pediatric endocrinology department. Ultrasonographic evaluation and FNA were performed by the radiology department. All patients who required thyroid scintigraphy were referred to the nuclear medicine department. All of the operations were performed by the pediatric surgery department after a multidisciplinary meeting of all the departments involved in the evaluation of the patient.

This study has several limitations. First of all, this is a retrospective study, which may cause data-related bias. Although the single-center design of this study allows for a more standard protocol of surgery procedure and follow-up, it also accounts for another limitation of the small sample size.

CONCLUSION

Pediatric thyroidectomies are performed on a heterogeneous group of pediatric patients due to a diverse group of pathologies. Higher recurrent laryngeal nerve injury and hypocalcemia rates in thyroidectomies have been associated with low-volume centers, especially in the pediatric population. This study supports that a multidisciplinary approach is crucial for the proper initial management and surgical strategy and can decrease complication rates and event-free survival of these patients in experienced tertiary centers.

ETHICAL APPROVAL

Written informed consent was given by the patients parents/legal guardians. This study was approved on 07/06/2023 by the İnstitutional Ethical Committee (KAEK- 454). The study was carried out in accordance with Decleration of Helsinki.

AUTHORS' CONTRIBUTIONS

KB: Conceptualization, Data curation, Formal Analysis, Writing – original draft. **SU:** Conceptualization, Data curation, Formal Analysis, Writing – original draft. **JA:** Conceptualization, Data curation, Writing – original draft. **AK:** Data curation, Formal Analysis. **MP:** Data curation, Formal Analysis. **AB:** Data curation, Formal Analysis. **GK:** Conceptualization, Data curation, Formal Analysis.

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ORIGINAL ARTICLE

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The predictive value of hemogram parameters for early preterm delivery in pregnant women undergoing cervical cerclage

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SUMMARY

OBJECTIVE: This study aims to investigate the predictive value of hemogram parameters in early preterm delivery (32 gestational weeks and below) among pregnant women who have undergone cervical cerclage, based on cervical changes determined before the cerclage procedure.

METHODS: Between 2010 and 2020, a total of 161 patients underwent cervical cerclage. The participants were divided into three groups. Group 1 (n=92) consisted of pregnant women who underwent prophylactic cerclage. Group 2 (n=31) included those with cervical shortening (<5 mm) and/or dilation (≤3 cm). Group 3 (n=38) comprised pregnant women with cervical dilation >3 cm. Each group was further divided based on delivery weeks, with a cutoff at 32 weeks. Demographic parameters and laboratory parameters were assessed.

RESULTS: In Group 1, all hemogram parameters showed no significant differences between deliveries below and above 32 weeks. In Group 2, the neutrophil-to-lymphocyte ratio value before cerclage was higher in the early preterm delivery group (p=0.002), with a cutoff value of 4.75 in receiver operating characteristic analysis. In Group 3, the white blood cell value before cerclage was higher in the early preterm delivery group (p=0.005), with a cutoff value of $13.05\times10^3/\mu L$ in receiver operating characteristic analysis.

CONCLUSION: The use of hemogram parameters to predict early preterm delivery in pregnant women undergoing prophylactic cerclage is not appropriate. However, neutrophil-to-lymphocyte ratio value can predict early preterm delivery when cervical dilation is 3 cm or less and/or cervical shortening is 5 mm or less. When cervical dilation exceeds 3 cm, the white blood cell value is more appropriate for predicting early preterm delivery.

KEYWORDS: Preterm birth. Cervical cerclage. Neutrophil lymphocyte ratio. Inflammatory markers. Perinatology.

INTRODUCTION

Cervical insufficiency is a rare condition, impacting around 1% of pregnancies and frequently leading to premature delivery[1]. Cervical cerclage procedures are employed to extend pregnancy; however, accurately predicting the success of this surgical intervention can be challenging. The examination of inflammatory markers in maternal blood and/or amniotic fluid is currently underway to assess their potential in predicting the progression of pregnancy. Indeed, there are publications indicating that intra-amniotic infections can contribute to premature delivery[2,3]. Certainly, some previous studies have proposed the significance of assessing infection markers in amniotic fluid through amniocentesis before the placement of emergency cervical cerclage[4,5]. Inflammatory markers such as C-reactive protein, white blood cell (WBC) count, and neutrophil-to-lymphocyte ratio (NLR) have been the subject of extensive research for their role in predicting pregnancy outcomes in women undergoing cerclage procedures[6].

White blood cell count has long been employed as an inflammation parameter. NLR has garnered attention in recent years as an inflammatory marker with potential predictive value in various medical conditions. It is a straightforward parameter for assessing the inflammatory status and has been utilized as a prognostic marker in cardiovascular diseases[7], determining the prognosis in some cancer types[8], as well as in cases of infection and inflammation[9]. The NLR ratio is defined as 1–3 normal, 6–9 mild stress, and above 9 moderate and severe stress[10], and the clinical significance for NLR between 3 and 6 is uncertain.

The objective of this study is to assess the effectiveness of hemogram parameters in predicting early preterm delivery in pregnant women undergoing cervical cerclage.

METHODS

Participants and study design

This retrospective clinical study took place at a tertiary center from September 2020 to December 2020, receiving approval from the local ethics committee (document number 71556).

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The study included patients undergoing cerclage surgery between 2010 and 2020, adhering to specific criteria.

Inclusion criteria comprised having a singleton pregnancy, undergoing cerclage using the McDonald procedure, delivering at the same hospital, and delivering due to the onset of labor. Exclusion criteria included multiple pregnancies, known uterine anomalies, delivery within 48 h after the cerclage procedure, fetal anomalies, other systemic infections, conditions leading to elevated inflammatory parameters in the mother, and signs of chorioamnionitis.

A total of 201 pregnant women were screened, with 161 meeting the criteria and enrolling in the study. Participants were divided into three groups based on cervical length and/or the degree of cervical dilation.

Group 1: Comprising 92 pregnant women receiving prophylactic cerclage based on obstetric history, without evidence of cervical shortening or dilation.

Group 2: Consisting of 31 pregnant women who underwent cerclage due to cervical shortening (≤5 mm) and/or dilation (≤3 cm).

Group 3: Including 38 pregnant women who underwent cerclage due to cervical dilation exceeding 3 cm.

The groups were further analyzed by dividing them into two subgroups based on weeks of delivery, with a cutoff value of 224 days (32 weeks). Hemogram results were compared between individuals delivering before or after the 30-s gestational week.

Surgical and medical procedures

Tocolytic treatment was administered to every patient scheduled for the cervical cerclage procedure, starting just before the procedure and continuing for 48 h post-procedure. Tocolytic therapy included either indomethacin (100 mg rectally followed by 25 mg orally four times a day) or nifedipine (initiated at 30 mg/h followed by 10 mg orally every 4 h). The cerclage procedure used the McDonald technique, employing Mersilene suture material. Following cerclage application, each pregnant woman received progesterone gel at a dose of 90 mg administered vaginally once daily until delivery.

In cases where amniotic sludge was detected along with cervical changes, broad-spectrum antibiotic treatment was administered to selected patients in our clinic in mid-2021. Pregnant women included in the study did not receive broad-spectrum multi-antibiotic therapy.

Demographic parameters and evaluated variables

Demographic parameters included maternal age, gravidity, and parity. Evaluated clinical parameters included gestational age at cerclage placement, gestational age at delivery, days of hospitalization post-cerclage application, and duration of pregnancy prolongation from cerclage application (latency period). Evaluated laboratory parameters included WBC, neutrophil, lymphocyte, NLR, and hemoglobin values just before cerclage application, 6 h after cerclage application, just before delivery, and 6 h after delivery.

Statistical analysis

Data were analyzed using IBM SPSS version 23. Prior to statistical analyses, checks were conducted to ensure no data entry errors and to assess parameter adherence to expected ranges. Normality assumptions of continuous variables were examined using the Kolmogorov-Smirnov test, and the homogeneity of variances was assessed using Levene's test. Descriptive statistics, including mean and standard deviation, were provided for continuous variables. In cases of non-normal distribution, the Mann-Whitney U test was employed. Receiver operating characteristic (ROC) analysis was applied to significant findings. A significance level of $p<0.05$ was considered for all analyses.

RESULTS

Demographic and evaluated parameters are presented in Tables 1, 2, and 3. Across all groups, except for the cerclage application-latency period, maternal age, gravid, parity, hospitalization (days), and gestational days at cerclage application were statistically similar between those delivering earlier and later than 32 weeks. The cerclage application-latency period was significantly shorter in deliveries below 32 weeks of gestation ($p\leq0.001$).

In Group 1, there were 12 (13%) pregnant women ≤32 gestational weeks, 9 (9.8%) pregnant women between >32 and ≤36 gestational weeks, and 71 (77.2%) pregnant women with gestational weeks >36 who have given birth. In Group 2, there were 11 (35.5%) pregnant women ≤32 gestational weeks, 5 (16.1%) pregnant women between >32 and ≤36 gestational weeks, and 15 (48.4%) pregnant women with gestational weeks >36 who have given birth. In Group 3, there were 26 (68.4%) pregnant women ≤32 gestational weeks, 6 (15.8%) pregnant women between >32 and ≤36 gestational weeks, and 6 (15.8%) pregnant women with gestational weeks >36 who have given birth.

In Group 1 (Table 1), early preterm delivery showed lower hemoglobin at the sixth hour after delivery ($p=0.013$). Other parameters, including WBC, neutrophil, lymphocyte, NLR, and Hbg values, were similar between groups at various time points.

Group 2 (Table 2) demonstrated higher NLR and lower hemoglobin concentrations before cerclage in deliveries below 32 weeks ($p=0.002$; $p=0.018$). Elevated NLR and WBC values persisted at the sixth hour after cerclage ($p=0.048$; $p=0.042$),

Table 1. Comparison of hemogram and demographic parameters according to the gestational weeks of delivery in group 1.

Group 1	Gestational weeks of delivery		p
	≤224 days (n=12)	>224 days (n=80)	
Mean±SD			
Maternal age (years)	37.42±4.21	35.26±6.34	0.243
Gravid	4.25±2.66	2.67±1.23	0.065
Parity	0.88±0.84	0.98±0.71	0.679
Hospitalization (days)	1.75±0.62	1.80±0.97	0.768
Gestational days at cerclage applied	99.25±9.21	97.43±7.97	0.572
Cerclage application-latency period (days)	86.17±40.05	166.44±13.76	<0.001*
Before cerclage application			
WBC×10^3/µL	9.87±3.22	9.34±2.13	0.459
Neu×10^3/µL	7.80±2.91	6.88±1.90	0.144
Lym×10^3/µL	1.82±0.64	1.94±0.52	0.323
NLR	4.68±2.51	3.79±1.97	0.164
Hbg g/dL	12.04±1.01	12.43±1.03	0.217
Six hours after cerclage application			
WBC×10^3/µL	11.77±3.47	9.95±2.66	0.112
Neu×10^3/µL	9.58±3.69	7.87±2.60	0.202
Lym×10^3/µL	1.77±0.63	1.64±0.53	0.645
NLR	6.34±4.22	5.35±2.77	0.755
Hbg g/dL	11.22±1.16	11.42±1.16	0.501
Before delivery			
WBC×10^3/µL	12.93±5.40	10.26±2.71	0.100
Neu×10^3/µL	10.39±5.61	7.68±2.34	0.173
Lym×10^3/µL	1.83±0.72	1.95±0.50	0.352
NLR	6.66±4.67	4.07±1.29	0.274
Hbg g/dL	11.60±1.27	12.26±1.47	0.111
Six hours after delivery			
WBC×10^3/µL	16.02±3.98	14.82±4.31	0.415
Neu×10^3/µL	14.15±3.90	4.13±12.5	0.207
Lym×10^3/µL	1.39±0.61	1.46±0.49	0.563
NLR	12.18±7.07	10.08±6.64	0.320
Hbg g/dL	10.16±0.82	11.33±1.72	0.013*

WBC: white blood cell; Neu: neutrophil; Lym: lymphocyte; NLR: neutrophil/lymphocyte ratio; Hbg: hemoglobin. *p<0.05.

and both NLR and neutrophil values before delivery were higher in early preterm delivery (p=0.007; p=0.029).

Group 3 (Table 3) indicated higher WBC and neutrophil levels before and after cerclage in early preterm delivery, while NLR levels remained similar. However, the NLR value before delivery was higher in individuals with early preterm delivery (p=0.034). Postpartum hemoglobin values did not significantly differ between groups.

Receiver operating characteristic analyses for Group 2 revealed an NLR cutoff of 4.75 before cerclage placement for predicting early preterm delivery (area=0.836, std. error=0.084, p=0.002, confidence interval; 95%). ROC analyses for Group 3, a WBC count equal to or exceeding 13.05×10^3/µL before cerclage, predicted early preterm delivery (area=0.762, std. error=0.076, p=0.010, confidence interval; 95%).

Table 2. Comparison of hemogram and demographic parameters according to the gestational weeks of delivery in group 2.

Group 2	Gestational weeks of delivery		p
	≤224 days (n=11)	>224 days (n=20)	
Mean ± SD			
Maternal age (years)	33.64±8.08	30.55±6.13	0.338
Gravid	1.88±0.84	2.50±1.59	0.569
Parity	0.75±0.88	0.81±0.91	0.928
Hospitalization (days)	3.91±3.18	2.15±0.93	0.072
Gestational days at cerclage applied	137.27±34.57	137.85±30.15	0.974
Cerclage application-latency period (days)	32.45±21.21	125.85±35.65	<0.001*
Before cerclage application			
WBC×10^3/µL	13.19±5.82	10.58±2.52	0.157
Neu×10^3/µL	10.41±6.34	8.06±2.16	0.104
Lym×10^3/µL	2.19±1.99	1.96±0.45	0.197
NLR	7.99±5.40	4.24±1.43	0.002*
Hbg g/dL	11.23±0.79	12.27±1.28	0.018*
Six hours after cerclage application			
WBC×10^3/µL	14.83±5.77	10.77±2.15	0.042*
Neu×10^3/µL	12.44±6.05	8.32±2.28	0.048*
Lym×10^3/µL	1.52±0.58	1.84±0.52	0.188
NLR	10.12±8.17	4.98±2.45	0.048*
Hbg g/dL	10.43±1.17	11.04±1.47	0.410
Before delivery			
WBC×10^3/µL	13.37±3.34	10.51±2.05	0.072
Neu×10^3/µL	10.83±3.19	7.56±1.66	0.029*
Lym×10^3/µL	1.65±0.61	2.07±0.56	0.072
NLR	7.37±3.22	3.78±1.09	0.007*
Hbg g/dL	10.22±1.48	12.38±0.89	0.007*
Six hours after delivery			
WBC×10^3/µL	13.77±3.96	15.61±4.71	0.482
Neu×10^3/µL	11.23±3.81	12.05±5.70	0.820
Lym×10^3/µL	1.10±0.37	2.55±3.20	0.024*
NLR	11.35±6.36	8.88±3.45	0.553
Hbg g/dL	9.57±1.50	10.63±1.44	0.120

WBC: white blood cell; Neu: neutrophil; Lym: lymphocyte; NLR: neutrophil/lymphocyte ratio; Hbg: hemoglobin. *p<0.05.

DISCUSSION

The key findings from our study suggest that hemogram parameters, when assessed based on obstetric history, are not predictive of early preterm delivery in pregnant women who have undergone prophylactic cerclage. Instead, these parameters have demonstrated predictive value for early preterm delivery in cases where emergency cerclage is performed due to cervical shortening and/or dilation.

Specifically, in situations where cervical length is <5 mm and/or cervical dilation is £3 cm, the NLR value emerges as a useful predictor for early preterm delivery. Conversely, in pregnant women undergoing emergency cerclage with cervical dilation exceeding 3 cm, the WBC value proves valuable in predicting early preterm delivery. These distinctions highlight the importance of tailoring predictive

Table 3. Comparison of hemogram and demographic parameters according to the gestational weeks of delivery in group 3.

Group 3	Gestational weeks of delivery		P
	≤224 days (n=26)	>224 days (n=12)	
Mean±SD			
Maternal age (years)	33.58±7.93	36.17±6.18	0.343
Gravid	2.11±1.45	2.83±2.32	0.555
Parity	0.53±0.84	1.17±2.40	0.999
Hospitalization (days)	7.50±11.7	4.0±2.34	0.792
Gestational days at cerclage applied	142.81±29.54	146.75±34.85	0.865
Cerclage application-latency period (days)	25.69±21.75	114.25±55.53	<0.001*
Before cerclage application			
WBC×10³/µL	12.19±2.73	9.84±2.52	0.005*
Neu×10³/µL	9.59±2.32	7.31±2.02	0.008*
Lym×10³/µL	1.85±0.63	1.81±0.59	0.963
NLR	5.78±2.51	4.45±1.97	0.129
Hbg g/dL	12.07±1.53	12.19±1.07	0.988
Six hours after cerclage application			
WBC×10³/µL	13.02±3.83	9.58±2.40	0.028*
Neu×10³/µL	10.58±3.95	7.69±2.60	0.039*
Lym×10³/µL	1.79±0.61	1.49±0.70	0.250
NLR	7.52±6.82	6.85±4.98	0.999
Hbg g/dL	10.89±1.22	10.93±0.84	0.917
Before delivery			
WBC×10³/µL	14.85±4.04	10.15±3.22	0.005*
Neu×10³/µL	12.30±4.05	7.77±2.98	0.004*
Lym×10³/µL	1.78±0.88	1.95±0.66	0.512
NLR	9.01±6.01	4.29±1.68	0.034*
Hbg g/dL	11.86±1.21	11.82±1.68	0.753
Six hours after delivery			
WBC×10³/µL	16.53±5.44	16.45±6.82	0.677
Neu×10³/µL	14.22±5.25	14.27±6.47	0.677
Lym×10³/µL	1.47±0.44	1.53±0.91	0.853
NLR	10.02±3.77	21.85±34.7	0.129
Hbg g/dL	10.94±1.60	10.80±1.56	0.547

WBC: white blood cell; Neu: neutrophil; Lym: lymphocyte; NLR: neutrophil/lymphocyte ratio; Hbg: hemoglobin. *p<0.05.

measures based on the specific circumstances surrounding cerclage procedures.

The study highlights the significance of elevated WBC values, a high NLR, and increased levels of interleukin-6 (IL-6) and interleukin-8 (IL-8) in amniotic fluid as indicators with predictive value for preterm delivery[6]. Additionally, cervical dilation, vaginal prolapse of the amniotic membrane, and cervical funneling are identified as key indicators for predicting preterm delivery subsequent to cerclage procedures[11,12].

Our findings suggest that the predictive significance of various inflammatory markers for preterm delivery varies based on the degree of cervical dilation. Specifically, maternal blood WBC and NLR values determined before emergency cerclage can effectively predict early preterm delivery.

However, as the degree of cervical dilation detected before cerclage increases, the WBC value becomes more meaningful in predicting preterm delivery.

Furthermore, in a scoring system utilized for prognostic assessment in emergency cervical cerclage procedures, higher scores are assigned with an increase in the amount of cervical dilation and a WBC value exceeding $13.60\times10^3/\mu L$. This underscores the importance of considering both cervical dilation and WBC values in assessing the prognosis of emergency cerclage interventions[13]. In pregnant women who underwent cerclage due to cervical dilation exceeding 3 cm, the increase in WBC values before the cerclage procedure holds greater significance in predicting preterm delivery. The ROC analysis conducted for this patient group determined the WBC cutoff value for predicting early preterm delivery as $13.05\times10^3/\mu L$.

In pregnant women who underwent cerclage due to cervical shortening (<5 mm) or cervical dilation level ≤3 cm, the increase in NLR values before the cerclage procedure holds greater significance in predicting preterm delivery. The ROC analysis conducted for this patient group determined the NLR cutoff value for predicting early preterm delivery as 4.75. Notably, a similar planned study demonstrated a cutoff value of 4.8 for this parameter[14].

Second, the findings suggest that in Group 2 and Group 3, where emergency cerclage was applied and resulted in preterm delivery, the pre-delivery NLR value is statistically higher. In the group that underwent prophylactic cerclage, however, the pre-delivery NLR value did not exhibit statistically significant differences. It is conceivable that in cases of prophylactic cerclage, the underlying cause of preterm delivery may be non-intrauterine infection-related.

Another significant result is that in pregnant women who underwent emergency cerclage, the detection of a high NLR value during pregnancy follow-ups could serve as a predictor of preterm delivery. Additionally, the study indicates that the treatment of intrauterine infections leads to an improved obstetric prognosis when predicting preterm delivery. Furthermore, the use of antibiotic therapy in pregnancies, especially those with the presence of amniotic sludge, has been shown to improve the timing of childbirth[15]. The study concludes that the presence of amniotic sludge in patients with cervical shortening may function as an indicator for predicting preterm delivery[16]. In the monitoring of pregnant women undergoing emergency cerclage, the observation of a high NLR value can be utilized as an indicator for predicting preterm delivery. However, in the case of prophylactic cerclage applied based on obstetric history, neither pre-cerclage nor pre-delivery hematological parameters showed statistically significant data that could be used in predicting preterm delivery.

It has been demonstrated that in pregnant women who underwent prophylactic cerclage, second-trimester cervical length measurements and elevated plasma cytokine levels in maternal blood could potentially serve as data for predicting preterm delivery, contributing to forecasting the prognosis of pregnancy[17,18]. However, in our study, we have concluded that none of the hematological parameters we evaluated can be used for prediction.

In conclusion, our study suggests that the use of hemogram parameters for predicting early preterm delivery in pregnant women undergoing history-based prophylactic cerclage is not appropriate. However, for pregnant women who undergo cerclage due to cervical shortening or dilation, the NLR value can effectively predict early preterm delivery when cervical dilation is 3 cm or less and/or cervical shortening is 5 mm or less. Conversely, when cervical dilation exceeds 3 cm, it is more appropriate to use the WBC value to predict early preterm delivery.

ETHICS COMMITTEE APPROVAL

The study was performed in accordance with the ethical standards for human research established by the Declaration of Helsinki and Good Clinical Practice guidelines and was approved by the local Ethics Committee of Süleyman Demirel University School of Medicine.

INFORMED CONSENT

Informed consent was obtained from all participants, the purpose of the study was explained to them, and their participation was voluntary. Participants were also informed about their right to withdraw at any time without any negative consequences.

AUTHORS' CONTRIBUTIONS

ÜKT: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **EE:** Data curation, Formal Analysis, Methodology, Software, Visualization. **CD:** Conceptualization, Data curation, Formal analysis, Investigation, Resources, Software. **MOÖ:** Conceptualization, Data curation, Formal Analysis, Methodology, Software, Supervision. **MS:** Data curation, Formal Analysis, Methodology, Project administration, Supervision, Validation, Visualization, Writing – original draft.

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ORIGINAL ARTICLE

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Hepatitis B reactivation risk and physician awareness in rheumatological patients receiving anti-tumor necrosis factor-α treatment

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SUMMARY

OBJECTIVE: We aimed to evaluate the risk of hepatitis B virus reactivation in rheumatic patients using anti-tumor necrosis factor-alpha drugs and the awareness of physicians about hepatitis B virus reactivation.

METHODS: Demographic characteristics, pre- and post-treatment hepatitis markers, and laboratory parameters of patients receiving anti-tumor necrosis factor-alpha therapy in our rheumatology clinic were retrospectively examined.

RESULTS: A total of 448 patients, 240 (53.6%) female and 208 (46.4%) male, were evaluated. Their mean age was 48.02±14.64 years. While HBsAg was examined in 443 (98.9%) patients before treatment, 7 (1.6%) patients were found to be HBsAg positive. While anti-HBc IgG was examined in 405 (90.4%) patients, it was positive in 69 (17%) patients. HBs Ag (total 446–99.6%) test was performed in three patients who were not tested for HBsAg before the treatment, and anti-HBc total (431–96.2% total) test was performed in 26 patients who were not tested for anti-HBc total. All HBsAg positive patients and 17 (24.6%) of those with previous hepatitis B received antiviral treatment. While the median follow-up period of the patients was 24 (6–60) months, no patient developed hepatitis B virus reactivation.

CONCLUSION: The screening rates and awareness of physicians providing anti-tumor necrosis factor-alpha therapy for hepatitis B virus infection were found to be higher compared to similar studies. Hepatitis B virus reactivation did not develop in any patient. Since the risk of hepatitis B virus reactivation is low, especially in patients with previous hepatitis B, it would be more appropriate to follow up the patients without giving antiviral prophylaxis.

KEYWORDS: Rheumatic diseases. Tumor necrosis factor alpha inhibitor. Hepatitis B virus.

INTRODUCTION

Anti-tumor necrosis factor-alpha (TNF-α) and disease-modifying antirheumatic drugs (DMARDs) are used in the treatment of various rheumatic diseases, especially rheumatoid arthritis (RA)[1]. 'Traditional DMARDs used in rheumatic treatment include methotrexate (MTX), hydroxychloroquine, sulfasalazine and leflunomide. DMARDs with potential hepatotoxic and immunosuppressive effects, such as MTX, can cause activation of the hepatitis B virus (HBV). In addition to these drugs, combining steroids with anti-TNF-α and other biologicals poses a risk for HBV activation[2]. TNF-α plays an important role in host defense. Patients treated with anti-TNF-α agents have increased susceptibility to infections. TNF-α is a cytokine that can suppress HBV replication and has an important role in the elimination of HBV by stimulating HBV-specific cytotoxic T-cell responses[3]. Prophylactic antiviral therapy has proven to be of significant benefit in preventing HBV reactivation in HBsAg positive patients treated with anti-TNF-α agents or DMARDs[4]. Therefore, it is recommended to initiate antiviral prophylaxis or treatment for chronic HBV infection in rheumatic patients receiving anti-TNF-α therapy or DMARDs[2].

In this study, we aimed to evaluate the HBVr risk and physician awareness of HBVr in patients using anti-TNF-α due to inflammatory rheumatological disease.

METHODS

Study population

The data of patients who received anti-TNF-α treatment at the rheumatology outpatient clinic of Recep Tayyip Erdoğan University Training and Research Hospital between June 2018 and June 2023 were retrospectively examined.

Data collection

Using our hospital's electronic record system, the patients' diagnoses, demographic characteristics, anti-TNF-α starting

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dates and treatment durations, HBV serology before and after anti-TNF-α treatment, and clinical and laboratory results of the patients were evaluated.

Exclusion criteria

Patients who received anti-TNF-α treatment for less than 2 months, patients under 18 years of age, cancer patients, patients with insufficient viral marker data, patients without clinical follow-up and laboratory results, and those who were found to be positive for hepatitis C virus RNA were excluded from the study.

Definitions

HBsAg and/or anti-HBc tests were performed within 6 months before the start of treatment with anti-TNF-α drugs, HBV screening, and patients with HBsAg positivity detected for more than 6 months were defined as chronic hepatitis B patients. HBsAg negative/anti-HBc positive patients are defined as having recovered from HBV infection[5,6]. While monitoring patients, HBV reactivation was defined as a 10-fold increase in viral load from baseline negative to HBV DNA positivity and/or a change from baseline negative to HBsAg positivity[5-7]. Hepatitis was defined as an increase in the serum alanine aminotransferase (ALT) level of at least three times the upper limit of normal (45 U/L for serum ALT)[6]. HBV-related hepatitis was defined as clinical and biochemical evidence of hepatitis with an increase in HBV DNA[5].

Serological and virological evaluation for hepatitis B virus infection

Hepatitis B virus serological markers, including HBsAg, anti-HBs, and anti-HBc levels, were evaluated by electrochemiluminescence immunoassay method using the Roche Cobas e6001 device (Roche Diagnostics, Mannheim, Germany). Serum HBV DNA levels were measured by Rotor-Gene Q (QIAGEN, Hilden, Germany) real-time polymerase chain reaction method (lower detection limit was 12 IU/mL). Routine biochemical parameters were tested using the Roche Hitachi Cobas 8000 Modular Analyzer system (Roch Diagnostics, Germany).

Statistical analysis

The SPSS Windows version 22 program was used in statistical tests. Continuous variables were evaluated for normal distribution with histogram, Q–Q graph, and Shapiro-Wilk or Kolmogorov-Smirnov test depending on the number of variables. Among continuous variables, those with normal distribution were presented as mean±standard deviation throughout the entire study, and independent variables t-test was used to compare the two groups. Other continuous variables were presented with median (minimum–maximum) values, and the non-parametric Mann-Whitney U test was used to compare the groups. Categorical variables were presented as frequency and percentage, and Pearson chi-square test or Fisher's exact probability test was used to compare the groups. Tests with a p-value of 0.05 or less within the 95% confidence interval were considered statistically significant.

RESULTS

A total of 448 patients were included in the study, of whom 240 (53.6%) patients were female and 208 (46.4%) were male. The average age of the patients was 48.02±14.64 years, and the average age of women was significantly higher than that of men (p<0.001) (Table 1).

Patients received anti-TNF-α treatment due to 226 (50.4%) ankylosing spondylitis, 160 (35.7%) RA, 54 (12.1%) psoriatic arthritis, and 8 (1.8%) Behçet's disease. Notably, 150 (33.5%) patients received etanercept, 137 (30.6%) received golimumab, 112 (25%) received adalimumab, 33 (7.4%) received infliximab, and 16 (3.6%) received certolizumab treatment. The most common comorbid conditions found in the patients were hypertension in 118 (26.3%), diabetes in 37 (8.3%), coronary artery disease in 26 (5.8%), hyperlipidemia in 12 (2.7%), and chronic obstructive pulmonary disease in 8 (1.8%).

HBsAg was examined in 443 (98.9%) patients before treatment. While anti-HBc IgG was examined in 405 (90.4%) patients, it was not examined in 43 (9.6%) patients. Anti-HBc IgG test was positive in 69 (17%) patients. HBsAg (total 446–99.6%) test was performed in three patients who were not tested for HBsAg before the treatment, and an anti-HBc total (431–96.2% total) test was performed in 26 patients who were not tested for anti-HBc total. While the total number of HBsAg positive patients was 7 (1.6%), the number of HBsAg negative/anti-HBc total positive patients was 69 (16%).

A total of 24 patients received antiviral treatment. In addition, 11 patients received entecavir, 12 received tenofovir disoproxil fumarate (TDF), and 1 received lamivudine treatment.

Table 1. Demographic characteristics of the patients.

Variable		P
Male/female, n (%)	208 (46.4)/240 (53.6)	
Age, mean±SD	48.02±14.64	**<0.001[a]**
Male	43.78±14.56	
Female	51.70±13.71	

[a]T-test, SD: standard deviation. Statistically significant value is denoted in bold.

Antiviral treatment was started prophylactically in all HBsAg positive patients, 1 with entecavir and 6 with TDF. Antiviral treatment was started prophylactically in 17 (24.6%) patients, including entecavir in 10 patients, TDF in 6 patients, and lamivudine in 1 patient with HBsAg negative and anti-HBc total positive.

HBsAg negative/anti-HBc total positive patients

The average age of 69 patients with HBsAg negative/anti-HBc total positive was 54.33±13.07 years and 45 of them (65.2%) were women. The average age of women was higher than men (p=0.016). The median follow-up period was 24 (6–60) months. Of the patients receiving anti-TNF-α therapy, RA was noted in 30 (43.5%), ankylosing spondylitis in 29 (42%), psoriatic arthritis in 7 (10.1%), and Behçet's disease in 3 (4.3%). The treatment received by the patients was 29 (42) golimumab, 22 (31.9%) etanercept, 13 (18.8%) adalimumab, 4 (5.8%) infliximab, and 1 (1.4%) certolizumab (Table 2). While HBV DNA test was performed in 22 (31.9%) patients before treatment, all of them were found to have negative HBV DNA levels.

DISCUSSION

Hepatitis B infection is one of the most common infections worldwide. Chronic hepatitis B infection is an important cause of morbidity and mortality in society, leading to the development of hepatocellular cancer and cirrhosis. HBVr occurs with the reactivation of the patients' immune response against HBV. In cases of immunosuppression from any cause, immune-mediated control of HBV replication is impaired and reactivation may subsequently occur. Anti-TNF-α inhibitors, steroids, and other immunosuppressive drugs used in rheumatological diseases may cause the functions of T and B lymphocytes to deteriorate, thus suppressing the immune response to HBV[8,9].

Table 2. Demographic characteristics of HBsAg negative/anti-HBc total positive patients.

Variable		P
Male/female, n (%)	24 (45.8)/45 (65.2)	
Age, mean±SD	54.33±13.07	**<0.016[a]**
Male	49.21±14.79	
Female	57.07±11.31	
Diagnosis, n (%)		
Rheumatoid arthritis	30 (43.5)	
Ankylosing spondylitis	29 (42)	
Psoriatic arthritis	7 (10.1)	
Behcet's disease	3 (4.3)	
Treatment, n (%)		
Golimumab	29 (42)	
Etanercept	22 (31.9)	
Adalimumab	13 (18.8)	
Infliximab	4 (5.8)	
Sertozulimab	1 (1.4)	

[a]T-test, SD: standard deviation. Statistically significant value is denoted in bold.

In a study conducted by Lan et al., it was reported that 40% of chronic hepatitis B patients developed HBVr due to the use of anti-TNF-α, and 5% of them had a mortality risk[10]. In another study, the HBVr rate was found to be between 27 and 39% in HBsAg positive patients receiving anti-TNF-α. In the study conducted by Pérez Alvarez et al., HBVr was reported in 35 (39%) out of 87 HBsAg positive patients receiving anti-TNF-α and in 9 (5%) out of 168 HBsAg negative/anti-HBc positive patients. One of the HBsAg negative/anti-HBc positive patients died due to fulminant liver failure[11,12]. In a study, it was reported that in patients receiving anti-TNF-α therapy with an indication of autoimmune disease, 9 out of 23 patients (39%) who were HBsAg positive at the beginning of treatment developed HBVr, but none of the 178 HBsAg negative/anti-HBc positive patients developed HBVr[7]. In our study, no patient developed HBVr, regardless of hepatitis serology and antiviral prophylaxis.

The Asian Pacific Association for the Study of the Liver and the American Association for the Study of Liver Diseases guidelines recommend prophylactic antiviral treatment during treatment and for up to 12 months after discontinuation of treatment in all HBsAg positive patients receiving immunosuppressive therapy, regardless of HBV DNA level. The European Association for the Study of Liver Diseases Study of the Liver recommends preemptive treatment during treatment and for up to 12 months after discontinuation of treatment in all HBsAg positive patients who are candidates for immunosuppression or chemotherapy, regardless of HBV DNA level. Regarding HBsAg negative/anti-HBc positive patients, all guidelines recommend that if the HBV DNA level is detectable, the patients should be treated as if they were HBsAg positive[13-15]. In our study, prophylactic antiviral treatment was started in all 7 HBsAg positive patients and in 17 (24.6%) HBsAg negative/anti-HBc total positive patients, even though 10 of them had negative HBV DNA levels and 7 of them had not had HBV DNA levels checked.

Most reported cases of HBVr in HBsAg negative/anti-HBc positive patients occurred in patients receiving concomitant use of anti-TNF-α therapy and other immunosuppressive drugs[16,17]. In our study, although 32 (46.3%) of the negative/anti-HBc

positive patients were receiving steroid treatment before treatment, no patient developed reactivation.

Guidelines recommend that patients who will be given anti-TNF-α therapy should be screened for hepatitis B. HBsAg, anti-HBc, and anti-HBs should be checked in these patients. If HBsAg and/or anti-HBc are positive, HBV DNA should be examined. HBV seronegative patients should be vaccinated. Those diagnosed with chronic hepatitis B should be evaluated for antiviral treatment for hepatitis[17-19]. In our study, before starting anti-TNF-α treatment in rheumatological patients, screening was performed in a very high proportion of patients compared to the literature, although not all patients were screened according to guideline recommendations. In addition, patients in whom HBsAg/anti-HBc total was not considered during the anti-TNF-α treatment were examined for screening during follow-up. In our study, 98.9% patients were suggested for HBsAg test and 90.4% patients were suggested for anti-HBc total test.

Limitations

As a single-centered and retrospective study, our failure to access all data is the limitation of our study. Failure to follow up the majority of patients in terms of HBV DNA level limits the study.

CONCLUSION

Anti-TNF-α treatment of physicians in terms of HBV infection rates and awareness of the ratio were found to be higher than similar studies. HBVr has not developed in any patient who has been passed by hepatitis B and did not receive antiviral treatment. Since the risk of HBVr is low in such patients, it will be appropriate to follow up patients with gastroenterological or infectious diseases without giving antiviral professional physicians and to increase the awareness of physicians who provide immunosuppressive treatment for HBVr.

ETHICS COMMITTEE APPROVAL

The study was conducted in accordance with the principles of the Declaration of Helsinki and was approved by the Recep Tayyip Erdoğan University Local Ethics Committee (No. 2023/209).

AUTHORS' CONTRIBUTIONS

OC: Conceptualization, Writing – original draft. **BK:** Investigation, Writing – original draft. **SD:** Formal Analysis, Project administration. **KI:** Methodology, Supervision.

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ORIGINAL ARTICLE

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Evaluation of the efficacy of labor induction with vaginal misoprostol in a low-risk pregnant women population

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SUMMARY

OBJECTIVE: The aim of this study was to evaluate the success rate and predictors of labor induction using vaginal misoprostol in a low-risk pregnant women population.

METHODS: A prospective cohort study was carried out with 196 pregnant women. Groups 2 and 4 of the Robson Classification admitted for induction of labor with vaginal misoprostol (25 μg tablets every 6 h, up to 4 tablets, for a maximum of 24 h). The success of labor induction was considered the achievement of vaginal delivery. Binary logistic regression was used to determine the best predictors of successful induction of labor with vaginal misoprostol.

RESULTS: Of all the pregnant women analyzed, 140 (71.4%) were successful and 56 (28.6%) were unsuccessful. Pregnant women who achieved successful induction had a higher number of pregnancies (1.69 vs. 1.36, p=0.023), a higher number of deliveries (0.57 vs. 0.19, p<0.001), a higher Bishop score (2.0 vs. 1.38, p=0.002), and lower misoprostol 25 μg tablets (2.18 vs. 2.57, p=0.031). No previous deliveries [$x^2(1)=3.14$, odds ratio (OR): 0.24, 95% confidence interval (CI): 0.10–0.57, R^2 Nagelkerke: 0.91, p=0.001] and the presence of one previous delivery [$x^2(1)=6.0$, OR: 3.40, 95% CI: 1.13–10.16, R^2 Nagelkerke: 0.043, p=0.029] were significant predictors of successful induction of labor with vaginal misoprostol.

CONCLUSION: A high rate of labor induction success using vaginal misoprostol in a low-risk population was observed, mainly in multiparous and with gestational age>41 weeks. No previous delivery decreased the success of labor induction, while one previous delivery increased the success of labor induction.

KEYWORDS: Labor induction. Misoprostol. Maternity hospital. Bishop.

INTRODUCTION

Labor induction is defined as the artificial induction of labor in a pregnant woman whose gestational age is within the limits of fetal viability and who has no signs of active labor[1]. Its main indication is to ensure maternal and fetal well-being[2], and it is mainly used in pregnancies of more than 41 weeks gestation[3].

Rates of induced labor have been increasing over the years, with North American literature reporting an increase of more than 100% since 1990[4]. In developed countries, the proportion reaches about 25% of all births. In low- and middle-income countries, induction rates are typically lower, but in some places they can still approach those of high-income countries[5].

Success in inducing labor depends on several factors and is more likely in multiparous and younger women, and those with a lower body mass index (BMI)[6]. Some biochemical markers can also predict this success, such as fetal fibronectin and IGFBP-1[1]. However, the condition of the cervix before the onset of labor remains the most important predictor[6].

There are pharmacological and mechanical alternatives to cervical ripening. Pharmacological options include prostaglandin analogs such as E1 (misoprostol) or E2 (dinoprostone). Misoprostol has the advantage of being a cheap, accessible drug that can be stored at room temperature. However, prostaglandin analogs are contraindicated in patients with uterine scarring due to the associated risk of tachysystole and uterine rupture. In these cases, cervical ripening can be performed with a transcervical balloon, in which a Foley tube is inserted through the internal opening of the cervix and the balloon is inflated with 30–50 mL of volume[7].

The International Federation of Gynaecology and Obstetrics (FIGO) recommendation for induction of labor with misoprostol is 25 μg vaginally every 6 h or orally every 2 h[8]. However, there

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are few studies that directly evaluate the recommended dose and duration of use of this drug. Repeated administration may prolong the latent phase of labor, which is associated with higher rates of cesarean section, chorioamnionitis, endometritis, and uterine atony[9].

The purpose of this study was to evaluate the success rate and predictors of labor induction using vaginal misoprostol in a low-risk pregnant women population.

METHODS

A prospective cohort study was conducted in the Amparo Maternal Hospital, a low-risk maternity hospital in the city of São Paulo, Brazil, between February and April 2022. This study was approved by the Ethics Committee of the Federal University of São Paulo (CAAE: 54185521000005505).

The inclusion criteria were pregnant women in Groups 2 and 4 of the Robson Classification admitted for induction of labor with vaginal misoprostol. The exclusion criteria were fetal malformations, uterine myomatosis, fetal death, and women not fluent in Portuguese. The participants were divided into two groups: (1) success—pregnant women who had a vaginal delivery and (2) unsuccess—pregnant women who had a caesarean section. Robson Classification Group 2 includes nulliparous, singleton pregnancy ≥37 weeks, and induced or cesarean section before labor, and Group 4 includes multiparous (excluding previous cesarean section), singleton pregnancy ≥37 weeks, and induced or cesarean section before labor[10].

The care of the pregnant women followed the Amparo Maternal protocol for induction of labor, based on the current recommendations of the FIGO and the protocol of the Municipality of São Paulo. It consists of the vaginal introduction of misoprostol 25 µg tablets every 6 h, up to 4 tablets, for a maximum duration of 24 h.

During the study period, a book was provided to record pregnant women admitted for induction of labor and the respective Bishop score rates in each of the two inpatient rooms at the hospital. This record allowed us to identify the patients who were eligible to participate in the study. The researchers visited the maternity ward every day and, after the patients agreed and signed the informed consent form, they collected data on their care during labor induction and delivery.

The data were analyzed using SPSS version 20.0 (SPSS Inc., Chicago, IL, USA) and Prisma GraphPad 7.0 (GraphPad Software, San Diego, CA, USA). Quantitative variables were subjected to the D'Agostino and Pearson normality test. Parametrically distributed variables were presented as mean and standard deviation. Non-parametrically distributed variables were presented as median, 25th percentile, and 75th percentile. Categorical variables were described as absolute and percentage frequencies and presented in tables and graphs. Differences between categorical variables and their proportions were analyzed using the chi-square test. The effect of the groups on the continuous variables was analyzed using the Student's t-test (parametric distribution) or the Mann-Whitney test (non-parametric distribution). Binary logistic regression was used to determine the best predictors of successful induction of labor with vaginal misoprostol. The significance level adopted for all tests was $p<0.05$.

RESULTS

In our study, we evaluated 196 cases of pregnant women who underwent labor induction with vaginal misoprostol. Of all the pregnant women analyzed, 140 were successful and 56 were unsuccessful. Among the patients who successfully induced labor, 44 also used oxytocin in the induction/conduction of labor, while in the group of unsuccessful patients, 20 used oxytocin. The clinical maternal/neonatal characteristics and the process of induction of labor of the entire population included in the study are identified in Table 1.

Pregnant women who achieved successful induction had a significantly higher mean number of pregnancies (1.69 vs. 1.36 pregnancies, $p=0.023$) and deliveries (0.57 vs. 0.19 deliveries, $p<0.001$) than pregnant women who were unsuccessful in inducing labor with vaginal misoprostol. There was a significant association between induction success and type of delivery ($p<0.001$). Pregnant women with successful induction of labor had a higher mean Bishop score (2.0 vs. 1.38, $p=0.002$) and lower misoprostol 25 µg tablets used (2.18 vs. 2.57, $p=0.031$) than those with unsuccessful induction of labor. The time between the use of the first misoprostol tablet and delivery was shorter in pregnant women with successful induction compared to those with unsuccessful induction of labor (18.0 vs. 25.0 h, $p<0.001$) (Table 2).

A binary logistic regression model was created to assess whether the number of previous deliveries, BMI ≥30 kg/m^2, Bishop's score ≤5, and Bishop's score ≥6 were predictors of successful induction of labor with vaginal misoprostol. It was found that no previous deliveries [$x^2(1)=3.14$, odds ratio (OR): 0.24, 95% confidence interval (CI): 0.10–0.57, R^2 Nagelkerke: 0.91, $p=0.001$] and the presence of one previous delivery [$x^2(1)=6.0$, OR: 3.40, 95%CI: 1.13–10.16, R^2 Nagelkerke: 0.043, $p=0.029$] were significant predictors of successful

Table 1. Clinical maternal/neonatal characteristics of the study population.

Variable	Included cases (196)
Maternal age (years)	24.0 (21.0–29.0)
Body mass index (kg/m²)	29.0 (26.0–31.8)
Number of pregnancies	1.59 (0.93)
Number of deliveries	0.46 (0.80)
Nulliparous	69.9% (137/196)
At least one previous delivery	30.1% (59/196)
Gestational age (weeks)	40.3 (39.0–41.0)
Comorbidities	
Diabetes mellitus	9.7% (19/196)
Arterial hypertension	7.1% (14/196)
Oligohydramnios	0.5% (1/196)
Hypothyroidism	2.6% (5/196)
Urinary tract infection	0.5% (1/196)
Other	5.1% (10/196)
None	74.5% (146/196)
Type of delivery	
Vaginal	68.3% (134/196)
Forceps	2.6% (5/196)
Cesarean section	29.1% (57/196)
APGAR score at 5th min	9.0 (9.0–10.0)
Birth weight (g)	
<2,500	5.1% (10/195)
2,500–2,999	23.6% (46/195)
3,000–3,499	46.7% (91/195)
3,500–4,000	21.0% (41/195)
>4,000	3.1% (6/195)
Postpartum hemorrhage	1.03% (2/194)
Neonatal intensive care unit admission	8.2% (16/194)
Indication of labor induction	
Diabetes mellitus	5.6% (11/196)
Arterial hypertension	6.7% (13/196)
Premature rupture of ovular membranes	34.2% (67/196)
Gestational age ≥41 weeks	46.4% (91/196)
Other	7.1% (14/196)
Bishop score at admission	1.82 (1.4)
Total number of misoprostol 25 µg tablets	2.3 (1.15)
Oxytocin use	35.0% (64/183)
Success of labor induction	71.4% (140/196)
Time until delivery (h)	20.0 (12.0–28.0)

Mean (standard deviation); median (25th–75th percentile); percentage (absolute number/total number of cases analyzed).

induction of labor with vaginal misoprostol. No previous delivery decreased the odds of successful induction of labor by 0.24 times, while one previous delivery increased the odds of successful induction of labor by 3.40 times. The presence of two previous deliveries (p=0.058), three previous deliveries (p=0.670), BMI ≥30 kg/m² (p=0.797), Bishop's score ≤5 (p=0.515), and Bishop's score ≥6 (p=0.515) were not significant predictors of successful induction of labor with vaginal misoprostol (Table 3).

A weak significant negative correlation (r=0.29, p<0.0001) was observed between the Bishop score and the number of vaginal misoprostol tablets used. A weak but significant negative correlation (r=0.28, p<0.0001) was also observed between the Bishop score and time to delivery.

DISCUSSION

Vaginal misoprostol was the most effective option for cervical preparation compared with oxytocin, dinoprostone (prostaglandin E2), and placebo, without increasing cesarean section rates or tachysystole with changes in fetal heart rate[2]. Despite a higher incidence of tachysystole when induction was performed with misoprostol, this did not imply differences in cesarean section rates or neonatal outcomes[11]. In a meta-analysis published by Wang et al.[12], including 8 studies with 1,669 pregnant women, the use of vaginal misoprostol showed less oxytocin augmentation when compared with dinoprostone. The other obstetric/neonatal outcomes, such as tachysystole, uterine hyperstimulation, vaginal delivery within 24 h, cesarean section, neonatal intensive care unit admission, and Apgar score at 5th min<7, were similar between the groups.

In our study, we included pregnant women with Robson Classification Groups 2 and 4 for labor induction with vaginal misoprostol. Most of the pregnant women included in our study belonged to Robson Group 2a (nulliparous), which is to be expected considering that the most common indication for induction of labor was a gestational age of>41 weeks. There was also a significant relationship between the Robson group and type of delivery, with multiparous women more likely to have a vaginal delivery. Vargas et al.[13] performed a retrospective cohort study to assess the impact of induction of labor on cesarean section rates using the Robson Classification. We included 1,166 pregnant women, and the cesarean section rate was 20.9%. The highest cesarean section rate was observed in Robson Classification Groups 5 (65.2%) and 8 (32.3%). Robson Classification Group 2 was the highest contributor to the overall cesarean section rate, since it represented 56.7% of the pregnant women.

Table 2. Clinical characteristics of pregnant women who induced labor with vaginal misoprostol.

	Success of labor induction (140)	Unsuccess of labor induction (56)	p
Maternal age (years)	24.0 (21.0–28.3)	25.0 (21.0–29.3)	0.523†
Body mass index (kg/m²)	29.0 (26.6–31.6)	29.0 (27.0–32.3)	0.551†
Number of pregnancies	1.69 (0.99)	1.36 (0.72)	0.023∂
Number of deliveries	0.57 (0.85)	0.19 (0.58)	<0.001∂
Nulliparous	62.9% (88/140)	87.5% (49/56)	<0.001†
At least one previous delivery	37.1% (52/140)	12.5% (7/56)	<0.001
Gestational age (weeks)	40.1 (39.0–41.0)	40.8 (39.7–41.2)	0.034†
Comorbidities			
Diabetes mellitus	9.3% (13/140)	10.7% (6/56)	0.747ƒ
Arterial hypertension	6.4% (9/140)	8.9% (5/56)	
Oligohydramnios	0.7% (1/140)	0.0% (0/56)	
Hypothyroidism	3.6% (5/140)	0.0% (0/56)	
Urinary tract infection	0.7% (1/140)	0.0% (0/56)	
Other	4.3% (6/140)	7.1% (4/56)	
None	75.0% (105/140)	73.2% (41/56)	
Type of delivery			
Vaginal	95.7% (134/140)	0.0% (0/56)	<0.001ƒ
Forceps	3.6% (5/140)	0.0% (0/56)	
Cesarean section	0.7% (1/140)	100% (56/56)	
APGAR score at 5th min	9.0 (9.0–10)	9.0 (9.0–10)	0.944∂
APGAR score at 5th min <7	0.71% (1/140)	0.0% (0/55)	>0.999
Birth weight (g)			
<2,500	6.4% (9/140)	1.8% (1/55)	0.313ƒ
2,500–2,999	20.7% (29/140)	30.9% (17/55)	
3,000–3,499	49.3% (69/140)	40.0% (22/55)	
3,500–4,000	20.7% (29/140)	21.8% (12/55)	
>4,000	2.1% (3/140)	5.5% (3/55)	
Postpartum hemorrhage	1.4% (2/139)	0.0% (0/55)	0.371ƒ
Neonatal intensive care unit admission	7.9% (11/140)	9.3% (5/54)	0.594ƒ
Indication of labor induction			
Diabetes mellitus	5.7% (8/140)	5.4% (3/56)	0.327ƒ
Arterial hypertension	5.7% (8/140)	8.9% (5/56)	
Premature rupture of ovular membranes	37.1% (52/140)	26.8% (15/56)	
Gestational age ≥41 weeks	42.9% (60/140)	55.4% (31/56)	
Other	8.6% (12/140)	3.6% (2/56)	
Bishop score at admission	2.0 (1.39)	1.38 (1.34)	0.002∂
Total number of misoprostol 25 μg tablets	2.18 (1.10)	2.57 (1.23)	0.031∂
Oxytocin use	31.9% (44/138)	44.4% (20/45)	0.303ƒ
Time until delivery (h)	18.0 (12.0–26.0)	25.0 (16.0–33.5)	<0.001†

∂: Student's t mean (standard deviation); †: Mann-Whitney median (25th percentile–75th percentile); ƒ: Chi-square percentage (absolute number/total number of cases analyzed). p<0.05.

Table 3. Odds ratio for successful induction of labor using vaginal misoprostol considering the number of deliveries, body mass index, and Bishop score.

	OR	95% CI	p
No previous delivery	0.24	0.10–0.57	0.001
One previous delivery	3.40	1.13–10.16	0.029
Two previous deliveries	4.20	0.95–18.83	0.058
Three or more previous deliveries	1.61	0.17–14.80	0.670
BMI ≥30 kg/m^2	0.88	0.35–2.19	0.797
Bishop score ≤5	2.52	0.15–41.1	0.515
Bishop score ≥6	0.40	0.02–6.43	0.515

CI: confidence interval; OR: odds ratio; BMI: body mass index; Binary logistic regression. p<0.05.

In our study, we evaluated 196 cases of pregnant women who underwent labor induction, of which 140 (71.4%) were successful and 56 (28.6%) were unsuccessful. Among the patients who successfully induced labor, 44 also used oxytocin in the induction/conduction of labor, while in the group of unsuccessful patients, 20 used oxytocin. In a retrospective cohort study, Berkley et al.[14] evaluated the efficacy of labor induction with vaginal misoprostol (25 µg every 3–6 h) in nulliparous pregnant women with severe preeclampsia (145) and an unfavorable Bishop score. The rate of successful vaginal delivery was 65.5% (95). Vaginal delivery was associated with a shorter postpartum stay and less neonatal respiratory distress. In our study, using a majority low-risk population (75% without any risk factor), we obtained a higher rate of vaginal delivery (71.4%, 140/196). Yosef and Getachew[15] performed a retrospective cross-sectional study with 294 mothers (undefined risk) who delivered in their service. The prevalence of labor induction was 20.4% (75% with oxytocin and 25% with vaginal misoprostol), and the most prevalent cause of induction was preeclampsia (41.6%). Of the 60 induced mothers, 23.3% had failed induction. In our study using a majority low-risk population, the main indication was gestational age>41 weeks (46.4%), and the preeclampsia indication occurred in only 6.6%; however, the rates of unsuccessful labor induction were higher in our study (28.6 vs. 23.3%).

In our study, no previous deliveries and the presence of one previous delivery were significant predictors of successful induction of labor with vaginal misoprostol. No previous delivery decreased the odds of successful induction of labor by 0.24 times, while one previous delivery increased the odds of successful induction of labor by 3.40 times. Caliskan et al.[16] assessed the possible predictors of unsuccessful labor induction with vaginal misoprostol (50 µg each 6 h) in 1,030 pregnant women with single fetuses,>34 weeks of gestation, and Bishop score<5. Increasing gestational age in the Bishop score decreased the risk of unsuccessful labor induction. Corrêa et al.[17] determined the predictive factors for the success of labor induction with vaginal misoprostol (1 tablet of 25 µg vaginally every 4 h for the first 5 doses and 2 tablets of 25 µg vaginally 50 µg every 6 h for the 6th, 7th, and 8th doses) in 873 high-risk pregnant women. The successful labor induction rate was 72% with vaginal delivery. They observed that maternal age<24 years, previous vaginal deliveries, lower gestational age, and greater cervical dilation were predictors of successful labor induction. We believe that none or one previous delivery were predictors of successful induction of labor because the majority of our sample consisted of nulliparous pregnant women.

In our study, a weak but significant negative correlation was observed between the Bishop score and the number of vaginal misoprostol tablets used. Drakopoulos et al.[18] evaluated the number of oral misoprostol tablets needed to achieve a Bishop score of ≥6 in a retrospective study of 400 pregnant women. The incremental probability of achieving a significant change in Bishop score after 7 tablets was low (+2.0%). This study is consistent with our findings of a weak correlation between the Bishop score and the number of vaginal misoprostol tablets.

The strengths of this study were its prospective design, the fact that it was carried out in a referral maternity hospital for low-risk pregnancies, and the fact that it followed international recommendations for inducing labor with vaginal misoprostol. Possible limitations would be the relatively small sample size.

CONCLUSION

We observed a high rate of labor induction success using vaginal misoprostol in a low-risk population, mainly in multiparous and with gestational age>41 weeks. No previous delivery decreased the success of labor induction, while one previous delivery increased the success of labor induction with vaginal misoprostol.

AUTHORS' CONTRIBUTIONS

LSVB: Data curation, Investigation. **MPRS:** Data curation, Investigation. **EAJ:** Writing – original draft. **ABP:** Formal Analysis. **LRRS:** Methodology. **DBSP:** Writing – review & editing. **SYS:** Supervision.

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ORIGINAL ARTICLE

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Investigation of the effect of Myricetin on Cisplatin-induced liver hepatotoxicity

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SUMMARY

OBJECTIVE: Cisplatin, a widely used anticancer agent, induces hepatotoxicity alongside organ damage. Understanding Cisplatin's toxicity mechanism and developing preventive measures are crucial. Our study explores Myricetin, a flavonoid, for its protective effects against Cisplatin-induced hepatotoxicity.

METHODS: In our study, a total of 32 Wistar albino male rats were utilized, which were categorized into four distinct groups: Control, Myricetin, Cisplatin, and Myricetin+Cisplatin. For the histological assessment of hepatic tissues, hematoxylin–eosin and periodic acid Schiff staining were employed, alongside immunohistochemical measurements of TNF-α, interleukin-17, and interleukin-6 immunoreactivity. Additionally, aspartate transaminase and alanine transaminase values were examined by biochemical analysis.

RESULTS: In the histological evaluation of the tissues, a normal healthy cell structure and a strong periodic acid Schiff (+) reaction were observed in the hepatocyte cells in the tissues of the Control and Myricetin groups, while intense eosinophilia, minimal vacuolization, congestion, and sinusoidal expansions were observed in the hematoxylin–eosin stainings, and a decrease in the positive reaction in the periodic acid Schiff staining was observed in the Cisplatin group. Consistent with these histological findings, an increase in TNF-α, interleukin-17, and interleukin-6 expressions ($p<0.0001$) and a concomitant increase in aspartate transaminase and alanine transaminase values were observed in the Cisplatin group. In the group protected by Myricetin, a significant improvement was observed in all these histological and biochemical values.

CONCLUSION: Cisplatin induces notable histopathological alterations in the liver. In this context, Myricetin exhibits the potential to alleviate Cisplatin-induced damage by modulating histological parameters and biochemical processes.

KEYWORDS: Antioxidant. Cisplatin. Myricetin. Hepatotoxicity. Rat.

INTRODUCTION

Cisplatin (Cis) is a derivative of platinum salts and is a chemotherapy drug used to inhibit the growth of cancer cells[1]. Cis, a teratogenic, mutagenic, and carcinogenic effective agent, is used in various cancer treatments, such as ovarian, cervix, and head and neck cancer[2]. In addition to its anti-tumoral effects, Cis causes many undesirable effects, such as hepatotoxicity[1-3].

Myricetin (Myr) is a member of the flavonoid group called flavonols. It is obtained from various fruits, vegetables, tea, berries, and similar plants. Myr has been found to have anti-proliferative and anti-angiogenic effects in many types of cancer[4]. Myr is reported to be effective in many diseases, including different types of tumors, inflammatory diseases, atherosclerosis, thrombosis, cerebral ischemia, diabetes, Alzheimer's disease, and pathological microbial infections[5]. Myr has demonstrated therapeutic potential in reducing alcohol-induced liver damage, indicating its effectiveness in alleviating hepatic injury. It may serve as a specific protective agent against liver damage[6]. Studies conducted with Myr suggest that it reduces liver DNA damage induced by chemical substances and reduces increased serum levels of aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), and total bilirubin[7].

In response to liver damage, specific intracellular processes are initiated to maintain liver integrity. TNF is the main mediator of these processes and activates different cellular responses such as proliferation, survival, and death[8]. TNF-α cooperates with interleukin-17 (IL-17) to synergistically induce a massive production of interleukin-6 (IL-6) and interleukin-8 (IL-8) by endothelial cells, skin and synovial fibroblasts, and hepatocytes[9].

Our study aimed to investigate the potential treatment effects of Myr, a natural ingredient, to alleviate liver damage caused by Cis and offer a protective approach. Additionally, in this study, we aimed to elucidate the effects of these cytokines on liver damage and Myr treatment by evaluating the immunoreactivity of pro-inflammatory cytokines TNF-α, IL-17, and IL-6.

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This important study may contribute to the understanding of a potential new approach to reduce liver toxicity associated with cancer treatment and may help develop better treatment strategies in the future.

METHODS

Animals

In this study, conducted at the Department of Histology and Embryology, Erciyes University in Kayseri, Turkey, a total of 32 male Wistar albino rats, aged 9 weeks and weighing between 220 and 240 g, were utilized. These rats were procured from the Erciyes University Experimental and Clinical Research Center. The rats were housed in standard cages, maintained at a temperature of 21°C, and subjected to a 12-h light/dark cycle, with their nutritional and hydration requirements met. Prior to the study, the rats were individually weighed, and animals of similar weight were grouped. Ethical considerations and established guidelines for the care and well-being of all animals were strictly adhered to throughout the study.

Experimental design

The rats were randomly assigned to four groups of eight. Control group: rats that had access only to water and food throughout the experiment; Cis group: a single dose (7.5 mg/kg) of Cis was administered intraperitoneally on the seventh day[10]; Myr group: Myr (10 mg/kg) was administered intraperitoneally for 7 days[11]; Myr+Cis group: Myr (10 mg/kg) was administered intraperitoneally for 7 days, and at the end of the seventh day, a single intraperitoneal dose of Cis (7.5 mg/kg) was given. After the experimental procedure, the rats were anesthetized and then sacrificed.

Chemicals

Cisplatin (Koçak Farma, Istanbul, Turkey) was used intraperitoneally as an inducer of liver damage. Myr (Sigma-Aldrich, St. Gallen, Switzerland) was used as a protective and therapeutic substance in the experiment.

Histological examination

At the end of the experiment, rats were anesthetized using anesthetic agents [ketamine (75 mg/kg)+xylazine (10 mg/kg)]. Liver tissues were fixed in a 4% formaldehyde solution. Then, the routine light microscopic procedure was applied. For this procedure, dehydration was first applied to the tissues. Then, it was made transparent by holding it in xylene, and fixed blocks were made with paraffin. Sections were taken from paraffin blocks and stained with hematoxylin–eosin and periodic acid-Schiff (PAS). Sections were examined under a light microscope[12].

To determine the changes occurring as a result of damage to the liver tissue, the immunohistochemical staining method was applied to show the expressions of TNF-α, IL-17, and IL-6[12].

Biochemical analysis

Alanine aminotransferase and AST values of blood serum samples taken at the end of the experiment were analyzed by the service in the Erciyes University Central Biochemistry Laboratory.

RESULTS

Histopathological findings

The histological structure of normal healthy cells was observed in the liver sections of the control and Myr groups. It is seen that some of the hepatocytes in the Cis-treated group have more intense eosinophilic staining. It is seen that there is irregularity in the arrangement of the cell cords and widening and distortions in the sinusoidal spaces in some sections. However, areas of congestion and mononuclear cell infiltration were detected in the tissues belonging to the damage group. In addition, in the liver tissues of the Myr+Cis-applied group, there was a decrease in eosinophilic staining compared to the damage group, the hepatocyte arrangement around the central vein was more regular, and the widening in the sinusoidal spaces decreased (Figure 1).

Periodic acid Schiff staining was performed to evaluate the glycogen content of liver tissues. In the sections of the control and Myr groups, it was observed that hepatocyte cells gave a strong PAS-positive reaction. However, in the Cis-applied group, there was a decrease in PAS positivity compared to the control group. In addition, an increase in PAS positivity density was observed in the Myr+Cis-applied group (Figure 1).

Immunohistochemical findings

In the study, immunohistochemical staining was performed to determine the TNF-α, IL-17, and IL-6 immunoreactivity of the experimental groups. When TNF-α protein expression was examined, a significant increase was observed in the Cis group applied alone compared to the other groups, while this increase was observed to be minimally reduced in Cis applied together with Myr. Similarly, a significant increase in IL-17 and IL-6 expression was observed in the Cis group administered alone, while a statistically significant improvement was observed in the Cis group administered together with Myr (Figure 2 and Table 1).

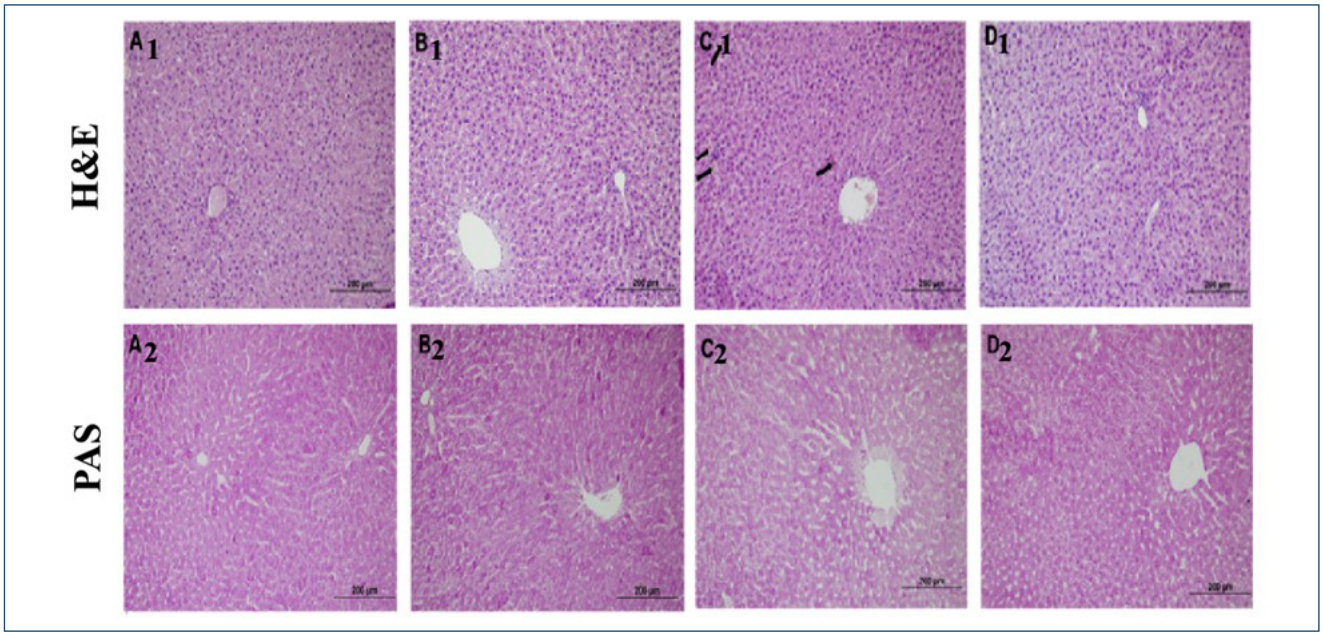
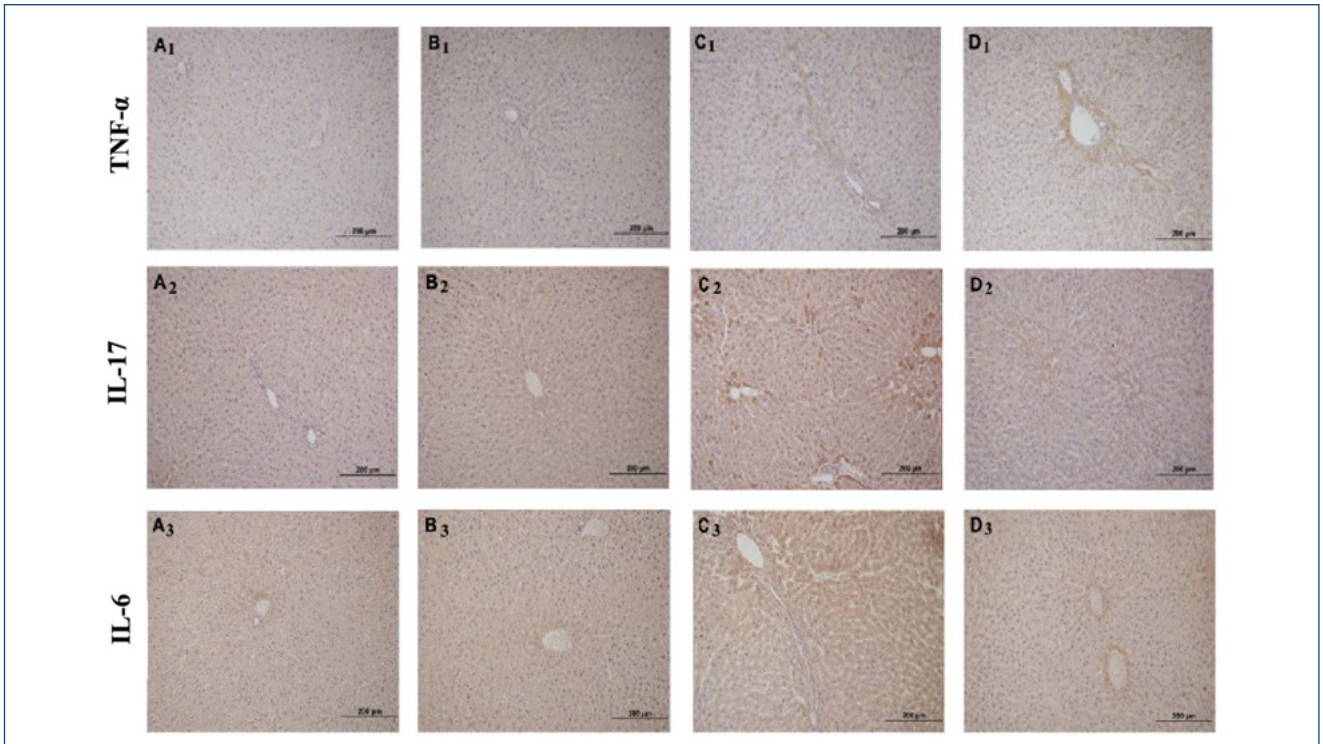


Figure 1. Hematoxylin–eosin (H&E) staining of liver tissue of experimental groups. A_1: Control group, B_1: Myricetin group, C_1: Cisplatin group, D_1: Cisplatin+Myricetin group. Black arrows: It shows areas with high eosinophilic staining in hepatocyte cells. Liver tissue periodic acid Schiff (PAS) staining of the experimental groups. A_2: Control group, B_2: Myricetin group, C_2: Cisplatin group, D_2: Cisplatin+Myricetin group. 20× objective, scale bar 200 μm.



'Most reported cases of HBVr in HBsAg negative/anti-HBc positive patients occurred in patients receiving concomitant use of anti-TNF-α therapy and other immunosuppressive drugs.

Figure 2. TNF-α immunohistochemistry staining image A_1: Control group, B_1: Myricetin group, C_1: Cisplatin group, D_1: Cisplatin+Myricetin group. IL-17 immunohistochemistry staining image. A_2: Control group, B_2: Myricetin group, C_2: Cisplatin group, D_2: Cisplatin+Myricetin group. IL-6 immunohistochemistry staining image. A_3: Control group, B_3: Myricetin group, C_3: Cisplatin group, D_3: Cisplatin+Myricetin group. 20× objective, scale bar 200 μm.

Table 1. Liver tissue TNF-α, interleukin-17, and interleukin-6 immunoreactivity measurement results and serum aspartate transaminase and alanine transaminase results of the experimental groups.

Groups	Control	Myr	Cis	Myr+Cis	p
TNF-α	74.7±2.4[a]	79.4±2.5[b]	80.8±5.5[cd]	79.9±3.5[bd]	<0.0001
IL-17	88.2±4.9[a]	88.7±5.8[a]	95.1±6.1[b]	91.4±3.9[c]	<0.0001
IL-6	81.8±2.2[a]	86.3± 4.7[b]	90.8±9.7[c]	85.1±2.8[bd]	<0.0001
Groups	**Control**	**Myr**	**Cis**	**Myr+Cis**	**p**
AST	80.8±10.3[a]	68.6±16.1[ab]	87.1±10.6[ac]	84.6±6.3[a]	0.0503
ALT	45.0±4.0[a]	40.3±4.8[a]	47.3±5.5[ab]	39.1±2.9[ac]	0.0143

IL-17: interleukin-17; IL-6: interleukin-6; AST: aspartate transaminase; ALT: alanine transaminase; Myr: Myricetin; Cis: Cisplatin. Data are expressed as mean±standard deviation. There is no significant difference between groups containing the same letter (a–d). $p<0.05$ was considered significant.

Biochemical findings

While minimal changes were observed between the groups in AST values, in the comparison of the Cis group applied alone and the Cis group applied together with Myr in ALT values, it was seen that Myr corrected the increase in the damage group statistically significantly (Table 1).

Statistical analyses

In the study, statistical analysis of the results obtained from biochemical and immunoreactivity data was performed using GraphPad (Prism 8.00 for Mac, GraphPad Software, La Jolla, California, USA). The D'Agostino Pearson omnibus test was used to check the normal distribution of the data. Data were expressed as mean±SD and analyzed by one-way ANOVA test and Tukey's post-hoc test for parametric tests. $p<0.05$ was considered significant in the analysis.

DISCUSSION

It is known that Cis causes damage to many tissues, and one of these negative effects is liver hepatotoxicity[13,14]. Cis causes morphological changes in the arrangement of hepatocyte cords[15]. For example, in the liver, irregularity in the hepatic cords, portal triad fusion and central vein obstruction[16], degenerative hepatocytes[14], pyknosis of hepatocyte nuclei around the vena centralis in some and hypertrophy, inflammation, hypertrophy in some hepatocytes, vascular occlusion, sinusoidal dilatation[17], and congestions are a few of them[18].

Similar to the results of these studies, according to our histological data, in the liver sections of rats administered Cis alone, compared to the control group, there were changes in the classical lobule structure, intense eosinophilia in hepatocytes, thickening of the vena centralis wall, minimal vacuolar changes in the cytoplasm, sparse mononuclear cell infiltration, and enlargements of the sinusoids. It was determined that the strong PAS-positive reaction seen in the control group decreased in the damage group. This decrease in PAS positivity may be due to damaged mitochondria and decreased glucose levels. Histological data of the Cis group applied together with Myr show that cellular deteriorations were improved and there was an increase in the PAS-positive reaction in hepatocytes compared to the damage group. After Cis administration, significant glycogen loss is observed in hepatocytes. Myr prevents this glycogen loss. Glycogen positivity in hepatocytes is confirmed by amylase incubation, which abrogates the PAS reaction in these compartments[15].

Single-dose Cis administration increases AST, ALT, and ALP activities[19] and causes a significant increase in serum TNF-α levels compared to the control group[16,20]. Our data in our study increased the serum AST and ALT values of the group administered a single dose of Cis, similar to the literature. However, a significant improvement was observed in liver enzyme values, especially ALT values, in the group protected by Myr against Cis-induced damage.

Similarly, in Cis-induced damage studies, severe TNF-α expressions in the Cis group[3] and an increase in oxidant parameters, a decrease in antioxidant parameters, and a severe increase in TNF-α and Caspase-3 expressions in immunohistochemical evaluations were noted[21]. Our findings in our study are similarly manifested by the upregulation of TNF-α, IL-17, and IL-6 in the damage group. In hepatotoxicity, Myr prevents hepatotoxicity by modulating the production of free radicals and inflammatory markers. Additionally, Myr treatment reduced hepatotoxicity and ethanol-induced inflammatory markers such as IL-6[6]. Apart from this, hemorrhagic necrosis of liver tissues in hepatotoxicity and inflammatory cell infiltration in the portal area were significantly reduced by Myr pretreatment, resulting in less bleeding and cell infiltration, indicating that Myr has a protective effect on liver

tissues[22]. In our current study, although the histological disorders and biochemical changes occurring in the injury group showed a partial improvement in TNF-α expression in the immunohistochemical values of the Myr group applied for protective purposes along with Cis, a significant improvement was observed in IL-17 and IL-6 protein expressions. Biochemical values similarly support these findings.

CONCLUSION

The decrease in histological damage markers and biochemical activities of Myr against Cis-induced hepatotoxicity unequivocally demonstrates its protective effect on cellular structure, highlighting the need to enhance the dose and duration of Myr application to optimize its effectiveness, which constitutes a crucial avenue for further research.

ACKNOWLEDGMENTS

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ETHICS COMMITTEE APPROVAL

All procedures were carried out with the approval of the Ethical Committee (date 2021, decision no: 21/187 and date 2023 23/052) of Erciyes University Experimental Animals.

AUTHORS' CONTRIBUTIONS

SA: Data curation, Formal Analysis, Writing – original draft. **NK:** Data curation, Formal Analysis, Writing – original draft. **DK:** Data curation, Formal Analysis. **BY:** Data curation, Formal Analysis.

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ORIGINAL ARTICLE

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The effect of compression stockings on the complaints well-being and sleep quality of pregnant women with restless legs syndrome: a randomized controlled study

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SUMMARY

OBJECTIVE: The aim of this study was to determine the effect of compression stockings on complaints, well-being, and sleep quality in pregnant women with restless legs syndrome.

METHODS: This randomized placebo-controlled study was conducted on 63 pregnant women (placebo group [PG]=31; experimental group [EG]=32) at the Perinatology Outpatient Clinic of a Health Research and Application Centre in Turkey. Pregnant women in the experimental group wore compression stockings when they got up in the morning for 3 weeks and took them off at bedtime. Placebo group women wore a placebo stocking. Data were collected using the restless legs syndrome Severity Rating Scale, the Pittsburgh Sleep Quality Index, the World Health Organization-5 Well-Being Index, and the Application Satisfaction Form on the 22nd day of the first interview. Statistical significance was accepted as $p<0.05$.

RESULTS: Post-test mean scores of both the experimental group and placebo group in the restless legs syndrome Severity Rating Scale (post-test:;8.87±5.27, 12.19±5.60; pre-test:;21.28±5.63, 21.0±5.61; $p<0.05$), the Pittsburgh Sleep Quality Index (post-test:;5.34±3.28, 6.12±3.12; pre-test:;10.15±4.23, 9.61±4.59; $p<0.05$), and Well-Being Index (post-test:;18.06±4.59, 19.00±4.47; pre-test:;12.71±5.85, 15.09±5.62; $p<0.05$) showed recovery according to the pre-tests. However, the post-test restless legs syndrome Severity Rating Scale of the experimental group was lower than that of the placebo group ($p<0.05$). The effect of their application started in 3.93±1.74 days on average in the experimental group, while it started in 5.09±1.55 days in the placebo group ($p<0.05$).

CONCLUSION: Both applications reduced the severity of restless legs syndrome in pregnant women and increased sleep quality and well-being. However, compression stockings were more effective in reducing restless legs syndrome severity. Nurses can use compression and placebo stockings in the care of pregnant women with restless legs syndrome.

Clinical Trial Registration Number: NCT05795868.

KEYWORDS: Compression stockings. Obstetric nursing. Restless legs syndrome. Sleep quality. Quality of life.

INTRODUCTION

Restless legs syndrome (RLS) is a chronic sensory-motor disorder that causes an irresistible urge to move and discomfort in the legs. Symptoms begin, especially during long-term inactivity, such as sleeping and resting[1]. The syndrome is seen twice as often in women[2]. In addition, RLS is more common in pregnant women compared to other women, and it is seen in 15.4–29.2% of pregnant women in Turkey[3,4].

It has been stated that genetic factors, the brain dopamine system, and pregnancy-specific factors such as multiparity, hemoglobin, iron and folate deficiency, estrogen level, and nerve tension may be influential in the formation of the syndrome during pregnancy[1,4-6].

Although RLS is a condition that can be seen in every trimester, its incidence and severity peak in the third trimester[7]. It states that as symptoms worsen, there may be significant distress in sleep, well-being, cognitive health, activities of daily living, and social areas of essential functioning[5-9]. It was also found that there was an increased rate of pre-eclampsia, difficult labor, cesarean section, and depression in women with RLS. Early treatment is therefore essential for a healthier pregnancy and fetus[5,8-10].

Nonpharmacological treatments are primarily recommended for RLS. One recommended treatment is to use a pneumatic compression device, proven to work in hemodialysis patients[5]. RLS symptoms in pregnant women can be relieved by using compression stockings, which can have a similar effect at the

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same pressure. However, no studies have been found in the literature to report the effectiveness of compression stockings on RLS symptoms. In this context, the aim was to determine the effect of compression stockings on the complaints, well-being, and sleep quality of pregnant women with RLS.

METHODS

Study design

We conducted a randomized, placebo-controlled study (Clinical Trials: NCT05795868).

Settings and samples

This study was conducted in the perinatology outpatient clinic of a university hospital in Turkey. The sample size was determined to be 29 people in each group (differences=1.51; power=0.95; standard deviation=2.5; n2/n1=1)[11]. However, 35 pregnant women were included in each group to allow for data that might be lost. Notably, 70 women who met the inclusion criteria were allocated to groups according to pre-prepared randomization lists (fsl 1). Inclusion criteria for the study: the women should be RLS according to the RLS Diagnostic Criteria Questionnaire Form and doctor's examination, symptom severity >10, literate, ages of 18–40 years, with a single pregnancy at 27–37 weeks of gestation, take iron, vitamin D, magnesium, and calcium supplements, and have hemoglobin ≥11 g/dL. The women with communication barriers, high-risk pregnancy, pre-pregnancy RLS, chronic disease, body mass index (BMI) >30, sleep apnea, dermatological problem in the feet and legs, varicose veins, who used antipsychotic, antidepressant, or heparin, antihistamine, antiemetic, calcium channel blocker, dextromethorphan, and decongestant-type drugs were excluded from the study (Figure 1).

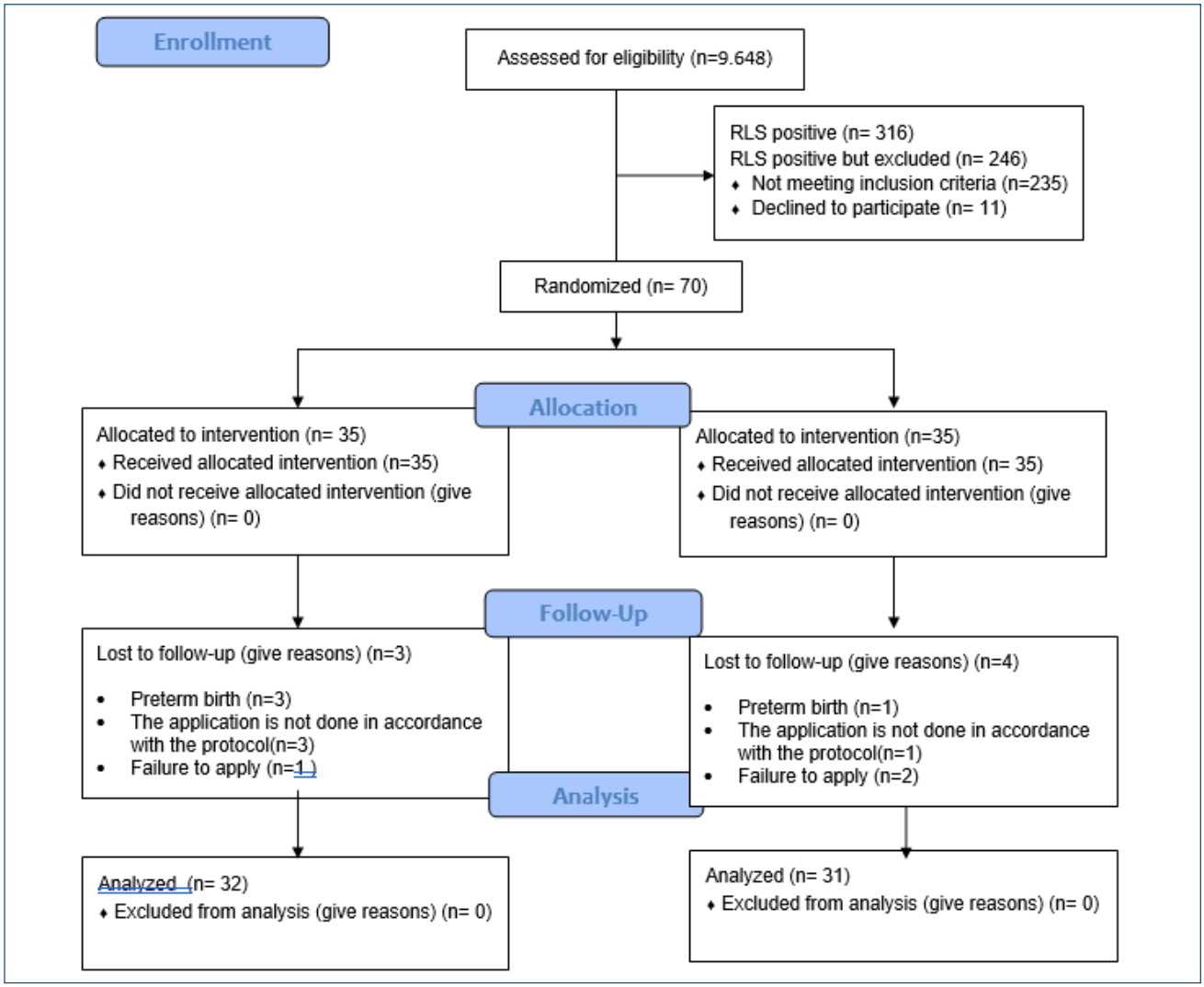

Figure 1. Consort flowchart.

Measures

Personal Information Form: The form consists of questions including socio-demographic and obstetric characteristics[2-7].

The RLS Diagnostic Criteria Questionnaire Form: The International Restless Legs Syndrome Study Group (IRLSSG) created the diagnosis form[12]. RLS is diagnosed by answering "yes" to all five questions[3,9].

Restless legs syndrome Severity Rating Scale: The scale was developed by IRLSSG and consists of 10 questions[13]. The score range varies between 0 and 40. A score of 1–10 indicates mild, a score of 11–20 indicates moderate, a score of 21–30 indicates severe, and a score of 31–40 indicates very severe. The validity and reliability study of the scale was conducted in Turkey[14]. In this study, Cronbach's alpha coefficient was 0.79–0.90.

Pittsburgh Sleep Quality Index (PSQI): PSQI was developed in 1989 by Buyyse et al.[15] It evaluates sleep quality. The PSQI consists of 24 questions. The total PSQI score ranges from 0 to 21. Scores greater than 5 indicate poor sleep quality. The validity and reliability study was conducted by Ağargün et al in Turkey[16]. In this study, Cronbach's alpha coefficient was found to be 0.76–0.79.

World Health Organization-5 Well-Being Index (WHO-5): The WHO-5 questionnaire comprises five items related to the participant's feelings. Each item is evaluated between 0 and 5. The total score ranges from 0 to 25. A total score below 13 indicates a poor quality of life[17]. The validity and reliability study of the scale was conducted in Turkey[18]. In this study, Cronbach's alpha coefficient was found to be 0.87–0.88.

Application Satisfaction Form: The researcher created the form to determine the participants' satisfaction levels toward the application. Satisfaction levels consist of two parts: positive and negative feedback about the application is expressed numerically on a decimal scale and is open-ended.

Procedures

The data collection instruments were administered to pregnant women in a face-to-face interview with the researcher. Data were collected in outpatient clinics and it took approximately 20–25 min to complete the questionnaires. The Personal Information Form, IRLSSG, PSQI, and WHO-5 forms were administered to all of the pregnant women as a pre-test. For 3 weeks, the women in the groups wore the socks they were given before. The women were administered the IRLSSG, PSQI, WHO-5, and a follow-up form at the end of 3 weeks.

Experimental group intervention

The experimental group (EG) used CCL2 (below-knee medium pressure) stockings (23–32 mm/Hg) with a graduated pressure system and size variation. The size of compression stockings suitable for pregnant women was determined, and stockings were provided. Women in groups were taught how to wear the stockings and were given written instructions. For 3 weeks, the women in the groups wore the socks they were given before getting up in the morning at home and took them off when they slept.

Placebo group intervention

The placebo group (PG) used 100-denier knee-high stockings with no therapeutic effect. Women in groups were taught how to wear the stockings and were given written instructions. For 3 weeks, the women in the groups wore the socks they were given before getting up in the morning at home and took them off when they slept.

Statistical analyses

The data were analyzed using SPSS 24.0, and values $p<0.05$ were accepted. The normality of the data of the numerical variables was evaluated with the QQ plot, the kurtosis, and skewness measures. Homogeneity between groups was analyzed by t-test. Due to the normal distribution of the data, the independent sample t-test was used in the independent groups, and the dependent-sample t-test was used in the dependent groups. Descriptive analysis was used in cases that expressed feedback about the application.

Ethical aspect of the study

Approval (2020/627) to conduct the study was obtained from the Clinical Research Ethics Committee, and the Helsinki Declaration ethical principles were followed at all stages. Informed voluntary written consent was obtained from those included in the study.

RESULTS

The groups were similar in terms of socio-demographic and obstetric characteristics ($p>0.05$; Table 1).

The IRLSSG pre-test mean scores of the EG and PG were similar (21.28±5.63 and 21.0±5.61, respectively; $p>0.05$). The post-test mean score of the EG (8.87±5.27) was lower than the PG (12.19±5.60) ($p<0.05$). However, the IRLSSG post-test mean score of both groups was statistically significantly lower than the pre-test mean scores of the groups ($p<0.001$; Table 2).

The PSQI pre-test scores of the groups were similar ($p>0.05$). Although the mean post-test PSQI score was smaller in the EG (5.34±3.28) than in the PG (6.12±3.12), this difference was not statistically significant ($p>0.05$; Table 2).

Table 1. Comparison of socio-demographic and obstetric characteristics by groups.

Features	EG (n=32)	PG (n=31)	t p
	$\bar{x} \pm$ SD	$\bar{x} \pm$ SD	
Age (years)	27.75±4.99	26.48±5.54	0.953 0.344
BMI	26.93±2.65	26.65±3.03	0.393 0.696
Gestational weeks	30.78±3.82	30.12±3.67	0.690 0.493
Gravida	2.21±1.28	2.32±1.27	−0.321 0.749
Weight gained during pregnancy	9.03±3.64	7.77±3.33	0.833 0.159

t=independent sample test. EG: experimental group; PG: placebo group; SD: standard deviation; BMI: body mass index.

Table 2. Comparison of the pre-test and post-test mean scores of the scales according to the groups.

Scales	EG (n=32)	PG (n=31)	Test p
	$\bar{x} \pm$ SD	$x \pm$ SD	
IRLSSG			
Pre-test	21.28±5.63	21.0±5.61	0.198[t] 0.843
Post-test	8.87±5.27	12.19±5.60	−2.421[t] 0.018
Test p	11.625[y] <0.001	7.285[y] <0.001	
Difference	12.40±6.03	8.80±6.73	0.381 0.029
PSQI total score			
Pre-test	10.15±4.23	9.61±4.59	0.488[t] 0.627
Post-test	5.34±3.28	6.12±3.12	−0.971[t] 0.336
Test p	6.654[y] <0.001	4.057[y] <0.001	
Difference	4.81±4.09	3.48±4.78	0.668 0.240
WHO-5 total score			
Pre-test	12.71±5.85	15.09±5.62	−1.643[t] 0.106
Post-test	18.06±4.59	19.00±4.47	−0.876[t] 0.105
Test p	7.169[y] <0.001	3.987[y] <0.001	
Difference	5.34±4.21	3.96±5.54	0.192 0.271
Benefit from the application			
Yes	31 (96.9)	29 (93.5)	0.378[x2] 0.613
No	1 (3.1)	2 (6.5)	
Day of benefit *(x ± SD)	3.93±1.74	5.09±1.55	−2.784 0.007
Satisfaction level (x ± SD)	8.18±1.61	8.16±1.61	0.064[t] 0.949

*Responses were received from pregnant women who benefited from the application. [t]Independent sample t-test. [y]Paired sample t-test. [x2]Chi-square. EG: experimental group; PG: placebo group; IRLSSG: International Restless Legs Syndrome Study Group; PSQI: Pittsburgh Sleep Quality Index; WHO-5: World Health Organization-5 Well-Being Index; SD: standard deviation.

The groups were similar in terms of WHO-5 pre-test scores and post-test scores (p>0.05). The mean post-test WHO-5 score was similar in the EG (18.06±4.59) and PG (19.00±4.47) groups (p>0.05; Table 2). Women in the EG reported that they experienced relief, on average, 3.93±1.74 days after application, while women in the PG reported that they experienced relief, 5.09±1.55 days after application (p<0.05; Table 2).

The most common positive and negative codes in the EG were pain relief/reduction (n=13) and discomfort (n=14). For PG, the most commonly reported positive and negative codes were pain relief/reduction (n=15) and sweaty legs (n=4) (Table 3).

DISCUSSION

In the study, the severity of RLS decreased to a mild level in the EG after application, while it decreased to a moderate level in the PG. Although improvements in the severity of RLS were observed in both groups, this effect was greater in the EG. There is evidence that the compression device and enhanced external counterpulsation (EECP), which have a similar effect on the venous system, may be effective in groups other than pregnancy[11,19]. A study of 10 people found that using a pneumatic compression device for 1 h in the evening could relieve RLS symptoms and improve their quality of life[20]. In another randomized, placebo-controlled trial, people with RLS were given 1 h of therapeutic or placebo compression per day. The study found a beneficial effect of placebo compression. At the same time, therapeutic compression was found to improve the severity of the disease compared to placebo[11]. In their pilot study, Rajaram et al. [19] found that enhanced EECP for 35 days significantly improved RLS symptoms in six patients with RLS. These investigators then conducted

Table 3. Distribution of positive and negative statements of pregnant women in the experimental group and placebo group (n=32)*.

Codes	Participants																								
EG group positive statements	**P-2**	**P-3**	**P-4**	**P-6**	**P-9**	**P-10**	**P-11**	**P-17**	**P-18**	**P-19**	**P-21**	**P-23**	**P-24**	**P-27**	**P-29**	**P-32**	**P-34**	**P-41**	**P-43**	**P-44**	**P-47**	**P-49**	**P-60**	**P-65**	**P-67**
Reduction in leg pain, relief				x	x	x	x	x		X	x						x		x	x	x		x	x	
No pain in the legs		x	x						x			x	x		x			x				X			
Comfortable sleep		x	x		x	x		x		X	x	x	x	x			x	x							
Reduction in leg edema/low back pain																x									
Psychological relief																				x					
EG group negative statements																									
Sock slip	x				x		x																		
Sock tightening		x	x	x				x	x	x	x		x			x	x	x							
Difficulty wearing			x	x	x		x	x	x	x	x	x		x		x	x	x						x	
Discomfort such as sweating and itching in the legs			x											x										x	
Continuing leg pain							x																		x
PG group positive statements	**P-14**	**P-15**	**P-16**	**P-20**	**P-22**	**P-25**	**P-26**	**P-29**	**P-30**	**P-31**	**P-33**	**P-35**	**P-42**	**P-45**	**P-46**	**P-50**	**P-56**	**P-61**	**P-64**	**P-70**					
Reduction in leg pain, relief	x	x	x	x	x				x	x	x	x	x	x	x	x	x		x						
No pain in the legs						x	x	x																	
Comfortable sleep		x		x		x	x	x			x	x		x											
Getting one's life in order											x		x												
PG group negative statements																									
Sock tightening															x					x					
Difficulty wearing					x				x	x															
Sweating in the legs					x						x		x		x										
Continuing leg pain	x													x				x							

*One participant is listed under more than one code. P: participant.

a randomized, double-blind, and sham-controlled study of EECP in RLS patients. The study was completed with six people and found that although both groups experienced an improvement in RLS severity scores, this could not be statistically evaluated[21]. Based on the results of this study, it is suggested that pneumatic compression can be used to reduce the severity of RLS symptoms during pregnancy and that the placebo effect should be investigated[5]. In this context, the findings obtained in the study are similar to those found in the literature[19,20]. The fact that compression stockings reduce the severity of RLS supports the theory that RLS may be associated with venous insufficiency[19,20,22,23]. Pneumatic compression devices, compression stockings, and EECP are thought to reduce the sensory symptoms of RLS by affecting the peripheral or central nervous system by regulating vascular flow. The significant effect of the placebo socks is similar to that found in the literature and shows that psychological factors can affect the severity of RLS[11,20].

The negative impact on sleep disturbance and quality of life in patients with RLS generally varies in direct proportion to the severity of the disease[6,8,9]. This study, like others, found that pregnant women in the groups experienced severe symptoms and had impaired sleep and quality of life. However, after the applications, the severity of RLS decreased in both groups, and there were similar improvements in sleep quality and quality of life. In a randomized, placebo-controlled study, positive effects of therapeutic or placebo compression applied to people with RLS were observed in both groups. However, in contrast to our study, therapeutic compression was found to improve daytime sleepiness, fatigue measures, and quality of life compared to placebo[17]. Another study evaluated polysomnographic measurements in six patients with RLS after EECP (four people) or placebo (two people) treatment and concluded that although there was a reduction in RLS severity, there was no significant effect on sleep[21].

The reduction of RLS severity and symptoms during pregnancy is very important. However, characteristics such as time to start, ease of use, satisfaction, and positive or negative aspects of the effectiveness of the application should also be identified. This study reported that almost all pregnant women in both the intervention and placebo groups benefited from the application, with the effect of the application starting on average from day 4 in the intervention group and from day 5 in the placebo group. Satisfaction with the treatment was similar in both groups. Reduction/relief of leg pain was reported as a positive feature of the applications in both groups. However, there were more negative reports about the use of compression stockings. However, the reported negative effects were more related to discomfort due to the tightness of the stockings or the enlargement of the abdomen than to health problems.

Strengths and limitations of the study

This research is one of the limited studies conducted to reduce the severity of symptoms in pregnant women with RLS. It is also the first study in the literature to investigate the effectiveness of compression stockings in reducing the severity of RLS. The results suggest that applying compression and placebo stockings may effectively reduce RLS symptoms and improve sleep and quality of life. In addition, how long the applications took effect and the women's opinions about the applications were also questioned. Another strength of this study is that compression and placebo stockings are cost-effective and easy to obtain. In addition, there is no need for hospitalization since technical knowledge and personnel are not needed for its use. Since people will not have to spend additional time or effort using socks, they can continue their daily activities. The limitation of the study is that the results were based on self-report rather than objective measurement.

CONCLUSION

The study found that wearing compression stockings and placebo stockings reduced the severity of RLS in pregnant women and improved their sleep and quality of life. The effect of the stockings was felt within 3–5 days, and the women were satisfied with the stockings and did not report any serious complications. In this context, health professionals, especially nurses, should develop institutional policies to reduce the severity of symptoms in pregnant women with RLS and develop solutions, including the use of compression stockings.

AUTHORS' CONTRIBUTIONS

ÖK: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resource, Software, Supervision, Validation, Visualization, Writing – original draft, writing – review & editing. **MB:** Conceptualization, Formal Analysis, Investigation, Methodology, Project administration, Software, Supervision, Validation, Writing – review & editing. **MTÖ:** Conceptualization, Data curation, Investigation, Methodology, Project administration, Resource, Software, Supervision, Validation, Writing – review & editing.

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ORIGINAL ARTICLE

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A preliminary study on the association between prognostic nutritional index and neutrophil-to-lymphocyte ratio with nutritional status and inflammation in febrile children's susceptibility to seizures

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SUMMARY

OBJECTIVE: The aim of this study was to evaluate the association between nutritional status, inflammation, and susceptibility to seizures in febrile children.

METHODS: This observational single-center study was carried out from January 2020 to December 2023 with 324 children aged 6 months and 6 years; 106 were diagnosed with febrile seizure, 108 were febrile children, and 110 were healthy controls. The prognostic nutritional index and neutrophil-to-lymphocyte ratio were calculated, and the cutoff threshold was established through receiver operating characteristics. The study utilized correlation and univariate–multivariate logistic regression analysis. The comparison between simple and complex febrile seizure was conducted to analyze differences.

RESULTS: The optimal cutoff values were identified as 61.25 for prognostic nutritional index and 1.04 for neutrophil-to-lymphocyte ratio. Our findings showed a significant negative association between febrile seizure and platelet count, high C-reactive protein, and high ferritin levels. Additionally, the febrile seizure group showed a significant positive correlation with high neutrophil-to-lymphocyte ratio values (≥1.04) and body temperature (≥38). Our findings revealed that high neutrophil-to-lymphocyte ratio, high C-reactive protein, and age less than 18 months were independently associated with seizure susceptibility in febrile children.

CONCLUSION: High neutrophil-to-lymphocyte ratio values and low prognostic nutritional index scores may serve as novel surrogate independent factors for seizure susceptibility in febrile children. Febrile children who are less than 18 months old are more prone to experience seizures than older febrile children. Moreover, there was a correlation between febrile seizures and elevated C-reactive protein levels and neutrophil-to-lymphocyte ratio values.

KEYWORDS: Prognostic nutritional index. Neutrophil, lymphocyte. Febrile seizure. Children.

INTRODUCTION

Febrile seizures (FSs) are the result of an immature brain reacting to a high body temperature. Simple FSs are considered harmless, and there is no evidence indicating an increased risk of death, neurodevelopmental problems, or epilepsy compared to the general population[1]. However, recent studies have connected complex FSs to sudden unexpected death in epilepsy, brain injury, and febrile status epilepticus[2].

Malnutrition makes a child more susceptible to infections that result in fever, which causes seizures in susceptible children. In opposition to the body of research concerning the correlation between malnutrition and other diseases, there is a scarcity of studies investigating the nutritional status of children with FS. Heightened levels of vitamin D and B12 in the serum of febrile children (FC) can serve as a prophylactic marker against FS[3]. Various studies have corroborated that iron deficiency is also a risk factor for FS[4,5].

Inflammation is a critical factor in the progression of spontaneous seizures[6]. Elevated neutrophil-to-lymphocyte ratio (NLR) has been noted in inflammatory conditions such as COVID-19 infection[7], gastrointestinal diseases[8], and thyroiditis[9]. Previous research has focused on the relationship between inexpensive and readily available blood biomarkers, such as the NLR, red blood cell distribution width (RDW), and platelet (PLT) count, in FSs[10].

Prognostic nutritional index (PNI) was independently linked to the presence and severity of neonatal sepsis in a negative manner[11]. Additionally, PNI has been linked with inflammatory conditions such as type 2 diabetes mellitus and its complications[12]. There is also evidence suggesting a connection between PNI and patient outcomes[13]. Despite the recognized

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prognostic significance of PNI in the pediatric population, it is surprising that no investigation has scrutinized the correlation between FS and measures of PNI. Furthermore, the relationship between NLR and PNI scores and susceptibility to FS remains unexplored in existing literature.

We aimed to investigate the association of NLR, PNI, and the related factors with seizure susceptibility in FC.

METHODS

This retrospective observational study, conducted in a tertiary hospital from January 2020 to December 2023, adhered to ethical principles outlined in the Declaration of Helsinki and was approved by the institutional ethics committee (November 22, 2023; number: 2023/174), and written informed consent was obtained from the participant.

Children aged 6 months to 6 years who manifest FSs are defined as displaying convulsions coinciding with a body temperature of 38°C or above, without any evidence of central nervous system infections or other causative factors for seizures. Furthermore, the participants were categorized as simple and complex FS in accordance with the American Academy of Pediatrics guidelines[14]. Simple FS lasts no more than 15 min, is generalized, and occurs only once within 24 h. Complex FS lasts longer than 15 min, has a focal origin, or occurs multiple times within 24 h. The FC group comprised children of the same age and gender who experienced fever due to acute infections but did not have seizures. Fever causes of FS and FC were classified as upper respiratory tract infection (URTI), lower respiratory tract infection (LRTI), acute gastroenteritis (AGE), urinary tract infection (UTI), and cellulitis. The final group is made up of healthy children (HC) who are brought to the hospital for routine health checks without presenting any complaints. These children are age- and sex-matched with the other groups and do not have seizures or fever.

Children with afebrile seizures, born before 37 gestational weeks, neurodevelopmental problems, metabolic abnormalities, central nervous system infections, diagnosed with other hematological problems, on nutritional supplements, and chronic systemic disease were excluded.

A total of 324 children's medical records were evaluated using a form: age, sex, body mass index (weight/height^2), tests [for hemoglobin, RDW, white blood cells, lymphocytes, neutrophils, platelet PLT counts, C-reactive protein (CRP), vitamin B12, D, ferritin, and albumin]. The PNI is calculated as 10×serum albumin (g/L) +5×lymphocyte (10^3 cells/μL), and NLR is calculated by dividing the percentage values of neutrophils and lymphocytes.

Statistical analyses were performed using SPSS for Windows 25.0. The test of normality was investigated using the Kolmogorov-Smirnov test. The Kruskal-Wallis tests were conducted to compare these parameters among the participants (FS, FC, and HC). Mann-Whitney U test was used for pairwise difference significance, with Bonferroni correction for multiple comparisons. The cutoff value was accessed by receiver operating characteristic (ROC) analysis. Correlations were determined by the Spearman test. Factors from univariate analyses were used for multivariate analysis via logistic regression. Differences were considered significant at $p<0.05$, with a coefficient interval of 95%.

RESULTS

A total of 324 participants, 106 (32.7%) were FS, 108 (33.3%) were FC, and 110 (34%) were HC. Female gender was similar among FS (47, 44.3%), FC (54, 50%), and HC (56, 50.9%) (p=0.582). The body mass index exhibited no significant difference among FS, FC, and HC (p=0.475). The fever etiology did not differ between the FS and FC in terms of URTI, LRTI, UTI, AGE, and cellulitis (47.2 vs. 42.6%, 20.8 vs. 21.3%, 5.7 vs. 13%, 15.1 vs. 15.7%, and 11.3 vs. 7.4%, respectively; p=0.742). The difference in the age distribution among groups was similar; FS (3±1.3), FC (3.4±1.6), and HC (3±1.5) (p=0.086). In terms of age, there were no significant variations among the three groups, with FS (3±1.3), FC (3.4±1.6), and HC (3±1.5) (p=0.086).

When the nutritional status was assessed, it was found that there were no significant differences in terms of vitamin D and B12, hemoglobin, and RDW (Table 1). The analysis suggested a significant difference among groups in terms of ferritin, CRP, PNI, NLR, and PLT. Pairwise comparison revealed that the PLT of the FS was significantly higher than in the FC and HC groups (p=0.003 and 0.004 vs. 0.867). The HC's PNI and NLR were assessed through pairwise comparison and significantly higher than those of the FS and FC [p=0.002, 0.015 vs. 0.620, and p<0.001, 0.006 vs. 0.032, respectively].

The ROC curve analysis calculated the cutoff values of PNI and NLR to be 61.25 (AUC=0.570, p=0.041) and 1.04 (AUC=0.633, p<0.001), respectively.

Age, gender, body temperature, duration of fever prior to seizure, recurrence of FS, and positive family history of FS did not significantly differ between simple and complex FS (Table 2). The complex FS group showed a significantly longer duration of FS (p<0.001). The simple FS showed significantly higher PNI, whereas the complex FS had significantly higher CRP (p=0.001 and 0.001, respectively). No significant differences were found in NLR, RDW, or PLT between the simple and complex FS. Among complex FS, a larger proportion of children

Table 1. Comparison of biochemical and complete blood count parameters of groups in the study.

Variables	Febrile seizure n=106	Febrile children	Healthy children	p-value
Mean ± SD		n=108	n=110	
Vitamin D (ng/mL)	24±10.4	25.8±12.1	27.2±11.7	0.138[a]
Vitamin B12 (pg/mL)	346.5±172.7	365.4±193.7	377.6±196.1	0.569[a]
Ferritin (µg/L)	33.7±25.7	31.2±22	26.5±27.6	**0.006[a]**
C-reactive protein (mg/L)	32.2±33.1	18.7±25.4	–	**<0.001[b]**
Body temperature (°C)	39.3±1	39±1.1	–	**0.029[b]**
Hemoglobulin (g/dL)	11.7±1	11.6±1.5	11.8±1.2	0.576[a]
Prognostic nutritional index	61.05±9.9	62.2±10	65.3±10.8	**0.006[a]**
Neutrophil-to-lymphocyte ratio	2±2.1	1.5±2.2	1.4±2.8	**<0.001[a]**
White blood cell	10.1±4.4	9.8±3.7	9.6±3.2	0.888[a]
Platelet count (10^9/L)	323.1±116.2	357.5±100	358.6±107.2	**0.004[a]**
Red cell distribution width (%)	14.7±1.9	14.9±1.7	14.5±1.4	0.290[a]

[a]Kruskal-Wallis test and [b]Student's t-test. Statistically significant p-values are denoted in bold.

Table 2. Biochemical and clinical parameters in children with simple versus complex febrile seizures.

Variables	Simple febrile seizure n=67	Complex febrile seizure n=39	p-value
Age (years)*	3±1.3	3±1.5	0.672
Gender** female	26 (38.8)	21 (53.8)	0.135
Prognostic nutritional index*	63.1± 9.4	57.6± 9.8	**0.001**
Neutrophil-to-lymphocyte ratio*	1.8±2	2.3±2.3	0.142
Ferritin (µg/L)*	33.5±27.3	34.2±22.8	0.564
Platelet count (10^9/L)*	338.2±126.9	297±90.8	0.206
Red cell distribution width (%)*	14.9±1.9	14.4±1.7	0.21
C-reactive protein (mg/L)*	12.6±16.9	29.3±33.1	**0.001**
Vitamin D (ng/mL)*	24.8±10	22.6±11	0.175
Fever duration before seizure (min)*	51.2±70.8	54.3±79.2	0.471
Body temperature (°C)*	39.3±1.03	39.2±1	0.722
Duration of febrile seizure*	3.03±1.6	15.1±11.2	**<0.001**
Times of febrile seizure recurrences*	2.2±1.8	2.2±1.6	0.673
Positive family history of febrile seizure**	35 (52.2)	21 (53.8)	0.874
Children with low PNI**	29 (43.3)	29 (74.4)	**0.002**
Children with high NLR**	38 (56.7)	29 (74.4)	0.071

*Mean±SD, **Number (%), Mann-Whitney U test was used. Statistically significant p-values are denoted in bold.

were observed to have low nutritional status (PNI≤61.25) and high inflammatory response (NLR≥1.04) (p=0.002 and 0.071, respectively). Among the 106 cases of FS, 49.1% were characterized by a single occurrence, while the remaining 50.9% (n=54) were marked by recurrent episodes.

The study examined FS and FC by correlation analysis to determine the relationship between clinical characteristics, lab tests, low PNI scores, and NLR. There was a significant negative correlation in FS between high NLR and age >18 months (r=0.216, p<0.001), PLT (r=0.116, p=0.037), and ferritin (r=0.212, p<0.001). High NLR is positively correlated with low PNI (r=0.425, p<0.001). Low PNI was significantly negatively correlated with age >18 months (r=0.207, p<0.001), PLT (r=0.254, p<0.001), and ferritin (r=0.124, p=0.026) in

FS. The FS was significantly negatively correlated with PLT (r=0.185, p=0.001), elevated CRP (r=0.329, p<0.001), and ferritin (r=0.149, p=0.007). On the other hand, FS was significantly positively correlated with high NLR (r=0.245, p<0.001) and elevated body temperature (r=0.135, p=0.049).

The study utilized both univariate and multivariate logistic regression methodologies to evaluate the factors contributing to the susceptibility of FC to seizures (Table 3). Age was categorized into two specific groups: children below and above 18 months of age, in accordance with a previous study. The multivariate analysis revealed that age below 18 months (p=0.039), high CRP (p<0.001), and high NLR (p=0.004) were independent factors related to seizure susceptibility in FC.

DISCUSSION

To the best of our knowledge, this is the first study to explore the relationship between PNI and NLR in FC and evaluate their susceptibility to FSs. We found a significant difference in HC's PNI and NLR compared to FS and FC. The FS group demonstrated significantly higher body temperature, serum ferritin, and CRP, along with a marked decrease in PLTs. FS patients had significantly lower PNI (≤61.25) compared to HC and FC. In addition, the relationship between PNI and FS subtypes has been investigated in this article for the first time. Our study demonstrates that patients with complex FS exhibit significantly lower PNI, higher NLR, and higher CRP compared to those with simple FS. Our research highlights the significance of addressing both low nutritional status and high inflammation in FC, as they may play a key role in the mechanism of FS.

The nutritional well-being and prognostic potential of FS children have been understudied. Kumari et al. reported iron deficiency as one of the risk factors for first-episode simple FS[4]. Our findings showed that FS has significantly higher serum ferritin than other groups, and those with complex FS have even higher levels than those with simple FS. Since ferritin is an acute-phase reactant, it rises in nonspecific inflammatory conditions such as FS and FC groups. We tried to explain this discrepancy with previous studies by examining ferritin levels of HC. Vitamin D has a beneficial effect on the brain in epilepsy, both

Table 3. Univariate and multivariate logistic regression analysis to determine risk factors for susceptibility to seizure in febrile children.

Variables	Univariate analysis			Multivariate analysis		
	OR	95%CI	p-value	OR	95%CI	p-value
Age	1.1	0.69–1.8	0.670	2.03	1.04–3.95	**0.039**
>18 months						
≤18 months						
Gender	1.3	0.8–2.04	0.302	1.3	0.7–2.3	0.453
Female						
Male						
Ferritin value≥11 µg/L	2.6	1.3–5.5	0.009	0.7	0.3–1.8	0.434
No						
Yes						
PNI score≤61.25	0.12	0.9–2.3	0.117	1.5	0.7–3.1	0.240
Yes						
No						
NLR value≥1.04	2.9	1.8–4.7	<0.001	2.8	1.4–5.6	**0.004**
No						
Yes						
Platelet count (×10^9/L)	1	0.9–1	0.007	1	0.9–1	0.058
Body temperature≥38°C	2.1	1–45	0.052	2.3	1–5.4	0.059
No						
Yes						
C-reactive protein≥5 mg/L	0.16	0.07–0.35	<0.001	7.3	3–17.4	**<0.001**
Yes						
No						

SD: standard deviation; OR: odds ratio; CI: confidence interval. Statistically significant p-values are denoted in bold.

by transmitting signals between nerve cells and by protecting the central nervous system. Bhat et al. showed that vitamin D deficiency is associated significantly with simple FS, and their recurrence is negatively correlated with low 25OH vitamin D[15]. Çığrı et al. proposed that high vitamin D and B12 levels prevent the development of FS in children[3]. Deficiency in vitamin B12 could potentially lower the threshold for seizures. Surprisingly, no significant differences were found in vitamin D or B12 levels between groups in the present study. These intriguing results could be related to the dietary practices of native children. Regularly consuming animal-based foods from our land may have boosted their vitamin D and B12 levels. To fully understand the potential benefits of vitamin B12 in raising the seizure threshold and vitamin D in reducing inflammation, further studies with larger sample sizes on FSs are warranted.

A reliable way to assess nutritional and immunological well-being is through the PNI. Elevated PNI levels are strongly related to positive health outcomes, as they reflect better functioning in these essential areas. In children, the PNI was used to predict coronary artery involvement and intravenous immunoglobulin resistance in Kawasaki disease[16-18], the outcome of human parainfluenza virus-induced pneumonia[19], the prognosis of chronic kidney disease[20], trace the response to treatment in pediatric leukemia[21], and as a predictor for severity of neonatal sepsis[11]. This study confirms that the PNI differed significantly between groups. Compared to other children, those with lower PNI scores were linked to FSs. We posit that the relationship between poor dietary habits and potential impaired management of inflammation is the contributing factor in the manifestation of FS.

The NLR is often relied on as a valuable tool to predict the prognosis of infectious diseases through monitoring systemic inflammation in children[22]. High levels of NLR are strongly linked to negative outcomes, so it is advisable to consider NLR as an unfavorable prognostic factor. Aside from assessing infectious diseases in children, the NLR is also utilized to predict the prognosis of autism[23], surgical site infection[24], asthma diagnosis and severity[25,26], and attention-deficit/hyperactivity disorder[27]. This study shows that NLR is strongly associated with seizure susceptibility in FC. There was a clear correlation between high NLR and FSs. This finding raises intriguing questions regarding the nature of the relationship between seizure burden and inflammation. Unfortunately, these findings are rather difficult to interpret due to the retrospective design of the study.

Immature neurons tend to synchronize and generate seizures. We found that age <18 months increased the risk of seizures twofold in FC. Another surprising variable that was found to be significantly associated with complex and simple FS was low PNI. This finding might indicate that the existence of nutritional disparities among individuals may be the underlying cause for the development of FS subtypes. The present results are significant in at least two major respects. First, it is crucial to recognize the wide-reaching advantages of a properly nourished infant, extending beyond just the expected benefits. This responsibility should be upheld by all social policies. Second, proper nutrition can help alleviate the impairments caused by inflammation in an immature brain network.

There are a number of limitations to our study, such as the lack of data on feeding techniques and daily protein intake. The study's relatively small sample size highlights the need for future studies to have larger sample sizes, a prospective design, and a longer duration of follow-up. This was a retrospective single-center study, and the research data cannot be generalized. The PNI and NLR were only calculated at admission, so continuous monitoring of the PNI and NLR might provide more significant insights into the seizure susceptibility of FC.

CONCLUSION

Given the child's infection-related nonspecific inflammation, it would be prudent to take precautionary measures against FSs by providing appropriate nutrition. Our findings indicate that FC older than 18 months are more likely to experience seizures. FC with elevated CRP levels are more susceptible to seizures than those with normal CRP levels. Additionally, compared to children with normal NLR levels, FC with elevated NLR levels are more prone to experiencing seizures.

ETHICAL APPROVAL

All the procedures followed by the authors were in accordance with the ethical standards of the responsible committee on human experimentation (institutional or regional) and with the Helsinki Declaration of 1975, as revised in 1983. The privacy rights of our patients were always observed. This study was approved by the Institutional Ethical Committee of Balikesir University (November 22, 2023; number: 2023/174). The groups' caregivers agreed to participate in the study by signing the relative informed consent form.

AUTHORS' CONTRIBUTIONS

İDÇ: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Methodology, Project administration, Resources, Software, Supervision, Validation, Writing – review & editing. **OÇ:** Conceptualization, Formal Analysis, Funding acquisition, Investigation, Methodology, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

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ORIGINAL ARTICLE

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The effect of mindfulness-based childbirth education intervention on fear of childbirth: systematic review and meta-analysis

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SUMMARY

OBJECTIVE: The aim of this study was to describe the effect size of mindfulness-based childbirth education on the fear of childbirth.

METHODS: In this study, the meta-analysis method, one of the methods of synthesising quantitative research, was used. EBSCO, PubMed, Google Scholar, WOS, and CINAHL databases were used to determine the studies to be included in the meta-analysis. The keywords such as "mindfulness", "fear of childbirth", "mindfulness-based childbirth", "mindfulness education" and "childbirth" were searched in the international literature. Four experimental studies published between 2013 and 2022 that aimed to determine the effect of mindfulness-based childbirth education on the fear of childbirth, had a full text available and met the inclusion criteria, were included in the study.

RESULTS: On the analysis of the data, mindfulness-based childbirth education was found to be effective in reducing the fear of childbirth (standard mean difference [SMD]=0.117, 95%CI: -1.049: -0.419, p<0.001, I^2=36.98%). The results of this meta-analysis indicated that mindfulness-based education provided to pregnant women was found to be effective in reducing the fear of childbirth.

CONCLUSION: Mindfulness-based childbirth education is considered to be used as an effective non-pharmacological midwifery and nursing intervention in reducing the fear of childbirth in pregnant women. This review was preregistered on PROSPERO (Ref No: CRD42022316472).

KEYWORDS: Meta-analysis. Mindfulness. Childbirth. Fear.

INTRODUCTION

The fear of childbirth is defined as the fear felt before, during, and after childbirth. Evaluating childbirth as negative cognitively and approaching childbirth with anxiety and fear are also used to express fear of childbirth[1]. In a study on fear of childbirth and the related factors in pregnancy involving 203 pregnant women, it was found that pregnant women showed high levels of fear of childbirth[2]. This fear prevents women from getting pregnant and giving birth[1]. The fear of childbirth has negative effects on the pregnancy process and childbirth[3]. The hormonal changes that take place in the body due to fear of childbirth suppress contractions. This prolongs the childbirth process and requires surgical interventions for the realisation of labour[1].

Factors that may be associated with fear of childbirth have been identified in the literature. Concerns about the health condition of the infant, attitudes and behaviours of healthcare professionals and the mother's health condition lead to an elevated fear of childbirth[4,5]. The primary concerns experienced during pregnancy are related to the health of the infant[4]. The healthcare professionals should comfort the pregnant woman with appropriate techniques and avoid negative behaviours in order to prevent the pregnant woman from having a negative experience and to have a healthier childbirth process. It is considered a necessity of the care service provided by midwives and nurses to provide properly the necessary counselling to the mother and partner during the childbirth process. These methods can reduce the risks related to the childbirth process and ensure a more successful and comfortable childbirth. Trainings about the childbirth process have been found to contribute to the reduction of negative thoughts and stress levels observed in pregnant women due to labour[4,5]. Recent studies have indicated that mindfulness-based training is used as a supplement to routine care in pregnant women. Based on this information, the aim of this study was to determine the effect of mindfulness-based childbirth education on fear of childbirth by meta-analysis method.

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METHODS

Research model

This research is a meta-analysis study. A meta-analysis refers to an analysis done to obtain an overall result by combining the results of different studies[6]. The study protocol was registered in the database of the International Prospective Register of Systematic Reviews (PROSPERO), allowing meta-analysis studies to be recorded (ID: CRD42022316472).

Search strategy

Before the data were collected, research questions were set in accordance with the PICOS (Participants, Intervention, Comparison, Outcomes, and Study Design) method, and a literature review was conducted based on these questions. Since the national literature lacks any studies in this field, the papers in the international literature constituted the database of the study. EBSCO, PubMed, Google Scholar, Web Of Science and CINAHL (Cumulative Index to Nursing and Allied Health Literature) online databases were searched for international articles. The keywords "mindfulness", "fear of childbirth", "mindfulness-based childbirth", "mindfulness education" and "childbirth" were used during the search.

Inclusion and exclusion criteria

On the literature review, 18 papers related to the study were reached and the sample of the study consisted of four studies that met the inclusion criteria (Table 1). The Preferred Reporting Items for Systematic Reviews and Meta Analyses (PRISMA) model was used as a guide for reporting the study data[7]. The following criteria were used to determine which studies would be included in the meta-analysis.

1. The analysis included the studies which were published between 2013 and 2022 and aimed to determine the effect of mindfulness-based childbirth education on the fear of childbirth.
2. Randomised controlled experimental or quasi-experimental studies.
3. The experimental group consisted of pregnant women and the Wijma Birth Expectation/Experience Questionnaire (W-DEQ-A) was used in the evaluation.
4. The studies in which the effectiveness of mindfulness-based childbirth education on the experimental group was reported were included.
5. The meta-analysis included the studies that met the inclusion criteria from the studies with full text available.

Study selection and data extraction

The coding, a data extraction process, is to remove the data eligible for the study from the complex data in the studies[8]. The data were coded in a coding form prepared in Excel format before statistical analysis. The coding form included the authors and year, study design, number of people in the experimental and control groups, mean age, intervention period, and gestational week.

Risk of bias assessment

The quality of the selected articles was evaluated by two researchers (SD and EE) with the Quality Assessment Tool (EPHPP) checklist. The evaluation of the risk of bias in all selected articles was done by two authors (SD and EE) independently using modified Cochrane tools for assessing the risk of bias, following the criteria outlined in the Cochrane Handbook for Systematic Reviews of Interventions. The other author (RAD) checked the results. The risk of bias was classified into seven domains. The bias risk for each area was classified as "low risk," "high risk" or "uncertain risk," according to the decision criteria in the "Risk of bias" assessment tool.

Table 1. Characteristics of the included studies.

Authors	Study design	Participants			Mean age	Intervention period	Gestational week
		Sample (n)	Intervention group	Control group			
Veringa-Skiba et al.[15]	RCT	141	75	66	Intervention: 33.11±3.92 Control: 32.72±3.86	30 min (until the moment of childbirth)	Between 16th and 26th week
Duncan et al.[13]	RCT	29	15	14	Unspecified	18 h (2 days only)	29th week
Kuo et al.[14]	RCT	106	53	53	Intervention: 34 ± 4.0 Experiment: 33.7 ± 4.8	1 h (8 week)	Between 12th and 24th week
Byrne et al.[12]	Quasi-experimental	12	12	–	Mean: 30.1±3.7	2.5 h (throughout 8 weeks)	Between 18th and 28th week

RCT: randomised controlled trial.

Data analysis

A comprehensive Meta-Analysis programme (CMA) (Version 3.0) was used for statistical analyses of the data, effect sizes and heterogeneity analyses. While calculating the effect size, the size was determined by Hedges G[9], a statistic that focuses on the standardisation of the outcomes achieved and the number of samples in the study. A random-effects model was used to take into account differences between subjects, intervention methods, durations and assessment tools in the included studies. Heterogeneity analyses were made by examining Tau, I_2, H_2 and Q values. The heterogeneity of effect sizes was assessed using Q and I_2 statistics. The I_2 values indicate low (25–50%), medium (51–75%) or high (>75%) heterogeneity[10,11]. As a result of the assessment made in the study, a Q value of 4.761 (p=0.19) and an I^2 value of 36.989% were obtained. These values indicated that there was a heterogeneous structure. Due to the heterogeneous structure, the fixed effect model was analysed, and an effect size of -0.734 (95%CI: -1.049: -0.419) was found to be statistically significant at the medium level (p=0.00). In order to render the effect size of -0.734 obtained according to the fixed-effect model insignificant (taking 0.001), Orwin's fail-safe N value was obtained as 716. This means that the study should include approximately 179 articles with statistically insignificant results for each study included in the meta-analysis in order to render the effect size insignificant. In Kendall's tau analysis, a test value of 0.34 was obtained, which indicated that there was no publication bias (p=0.367). Based on Egger's regression analysis method, the β^0 value was obtained as -1.203, the t-value as 0.555 and the p-value as 0.317. This result indicated that there was no publication bias.

RESULTS

When the total of the four studies included in the study was considered, the mean age of the pregnant women ranged between 30 and 33 years. The participants consisted of both multiparous and nulliparous pregnant women who were in the 12th–29th gestational week, received mindfulness-based childbirth education during pregnancy and for whom the effectiveness of the education was assessed with the Wijma Childbirth Expectancy/Experience Questionnaire (W-DEQ-A). Table 1 shows the characteristics of the participants, the intervention details, the outcome measures of the studies and additional information about the intervention in experimental and control conditions (Table 1).

In the study, Q statistics and I^2 values were analysed for the heterogeneity test. As a result of the analysis, the Q value was 4.761 (p=0.19) and the I^2 value was 37.99%. These values indicated that there was a heterogeneous structure. Due to the heterogeneous structure, the fixed-effect model was analysed, and an effect size of -0.734 (95%CI: -1.049: -0.419) was found to be statistically significant at the medium level (p=0.00). In order to render the effect size of -0.734 obtained according to the fixed-effect model insignificant (taking 0.001), Orwin's fail-safe N value was obtained as 716. Kendall's tau analysis resulted in a test value of 0.34, which indicated that there was no publication bias (p=0.367). Based on Egger's regression analysis method, the β^0 value was obtained as -1.203, the t-value as 0.555 and the p-value as 0.317 (Figure 1 and Table 2).

All four articles showed that awareness-based childbirth education provided to pregnant women alleviated the fear of childbirth[12-15]. The studies compared routine antenatal care with mindfulness-based childbirth education. The results of two studies showed that mindfulness-based childbirth education was more effective in alleviating the fear of childbirth compared to routine care (Byrne et al., p<0.01; Kuo et al., p<0.001), while the other two studies found that W-DEQ scores were lower than the scores obtained before the education and the difference between them was not statistically significant (Duncan et al., p=0.48; Veringa et al., p=0.045).

DISCUSSION

Fear of childbirth is an experience with negative consequences for maternal and newborn health[16]. Fear of childbirth, experienced at mild, moderate and severe levels, can lead to complications during childbirth, difficulties in the mother–infant relationship and depression and anxiety disorders in pregnant women[1]. Birth, known as a miraculous experience in the life cycle of women, is perceived as a threat for some women and fear of childbirth appears. Here, it is highly important to transform the woman's perception of childbirth from "fear" to a positive perception. One of the methods reported to be effective in reducing fear of childbirth in recent years is mindfulness-based approaches. The mindfulness-based practices, executed from the prenatal period, focus on breathing practices, attachment with the infant and feeling emotions and the use of these practices during childbirth[16]. In this present comprehensive meta-analysis of the effectiveness of mindfulness-based childbirth education in reducing the fear of childbirth in pregnant women, it was found that mindfulness-based education provided to pregnant women was effective in reducing fear of childbirth.

Risk factors for fear of childbirth should be determined with a detailed history taken during pregnancy follow-up,

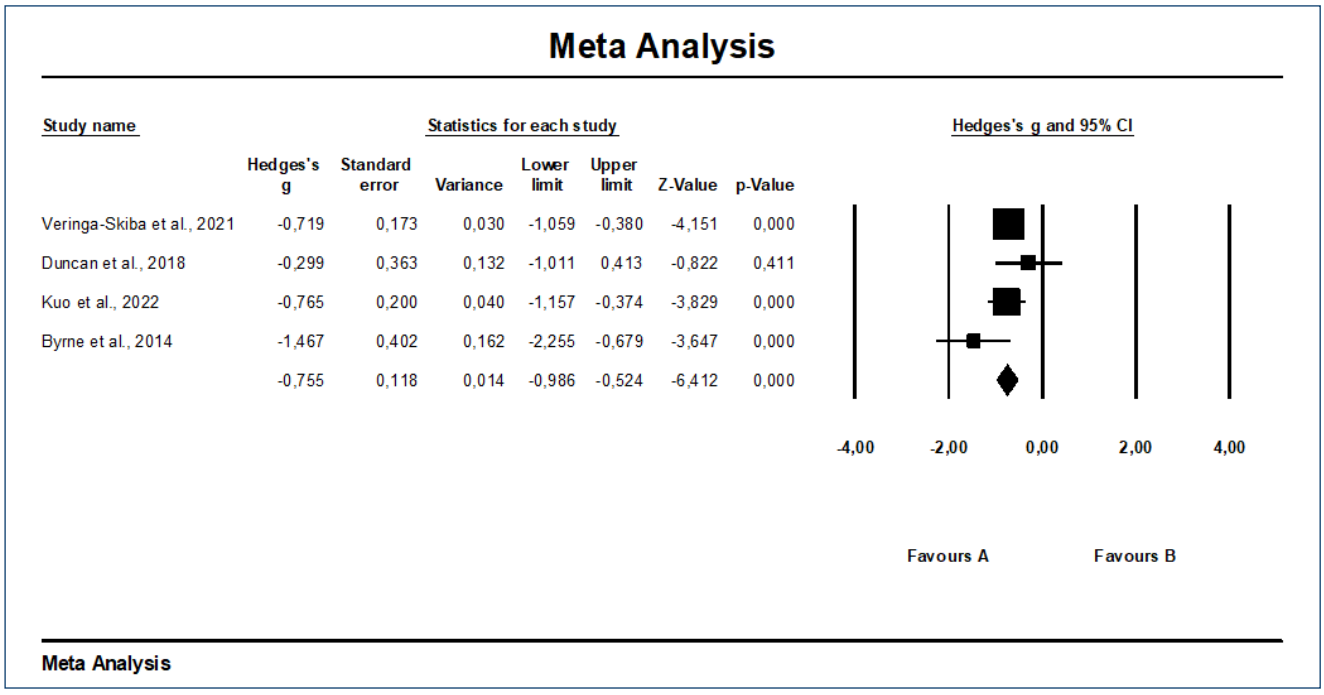

Figure 1. Meta-analysis outcomes.

Table 2. Homogeneity results of the meta-analysis and random-effects model (k=4) results of the meta-analysis.

Tau	Tau²	I²	H²	R²	Df	Q	p
0.038	0.194 (SE=0.085)	37.99%	-0.714		3	4.761	0.000
Random-effects model (k=4) results of the meta-analysis	Estimate	se	Z	p	CI lower bound	CI upper bound	
Intercept	-0.714	0.117	-6.086	0.000	-0.944	-0.484	

and fear of childbirth in pregnant women should be assessed. Once the level of fear of childbirth has been determined, interventions such as education, counselling and childbirth support can be provided to reduce the fear of childbirth and to inform about childbirth[1]. In a study, it was reported that women's fear of childbirth reduced with the training provided by healthcare professionals[2]. A systematic review study assessing mindfulness and perinatal mental health showed that mindfulness-based programmes of 8 weeks applied to pregnant women lowered perceived stress, anxiety and depressive symptoms and the level of postpartum depression in pregnant women. It was concluded that mindfulness-based programmes elevated the levels of mindfulness and self-compassion of pregnant women[17].

The use of a mindfulness-based model in training for childbirth preparation has a positive effect on reducing fear of childbirth and on maternal and neonatal health[16]. A randomised controlled study conducted with 63 pregnant women showed that participants in the intervention group underwent mindfulness-based cognitive behavioural therapy, while women in the control group had only routine antenatal care. In the study, it was found that the mean anxiety and depression scores of the intervention group were significantly lower than the scores of the control group[18]. A randomised controlled study conducted with 96 pregnant women showed that the intervention group attended a mindfulness-based childbirth and parenting programme, while the control group attended routine childbirth preparation education classes. The 8-week mindfulness-based programme effectively lowered the perceived stress level and depression in pregnant women and raised self-efficacy and mindfulness in childbirth[19]. In another study conducted on 60 pregnant women having their first pregnancy with 24–36 gestational weeks, the pregnant women in the intervention group were subjected to a mindfulness-based stress alleviation programme along with routine care. Immediately after the intervention and 1 month

later, it was observed that there were significant reductions in anxiety symptoms of pregnant women[20]. Studies in the literature show that mindfulness-based childbirth education is effective in improving the mental health condition of pregnant women and reducing the fear of childbirth.

CONCLUSION

Mindfulness-based education provided to pregnant women was found to be effective in reducing the fear of childbirth. It is considered that the integration of mindfulness-based education into routine pregnancy follow-ups may have positive effects on the psychological well-being of pregnant women. It is considered that reducing the fear of childbirth would increase vaginal childbirth rates and lower caesarean section rates, as well as provide a comfortable childbirth experience for pregnant women. Large-scale studies that have been meticulously designed are required to further confirm the results of this meta-analysis.

DATA AVAILABILITY STATEMENT

Example template data collection spreadsheets are available from authors on reasonable request.

AUTHORS' CONTRIBUTIONS

SD: Conceptualization, Data curation, Investigation, Methodology, Writing – original draft. **EE**: Data curation, Investigation, Methodology, Writing – original draft. **RAD**: Formal Analysis, Supervision, Writing – review & editing.

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ORIGINAL ARTICLE

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Study of tumor budding and its association with clinicopathological parameters in breast carcinoma

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SUMMARY

OBJECTIVE: Tumor budding is a phenomenon in which the tumor cells detach from the main mass and are present at the invasive front. The present study was conducted to study tumor budding in invasive breast carcinoma and to correlate it with clinicopathological parameters and molecular subtypes.

METHODS: The study was conducted over a period of 1 year, and tumor budding was studied as a single or group of cells at the invasive front of breast carcinoma counted in a high-power field (40×). The grading was statistically correlated with tumor size, grade, lymph node status, lymphovascular invasion, pathological TNM staging, molecular subtype, and survival of patients.

RESULTS: A total of 50 cases of invasive breast carcinoma were included, out of which 66% (n=33) showed high-grade tumor budding, which was statistically significantly higher in grade 2 invasive ductal carcinoma ($p<0.05$). High tumor budding was associated with lymphovascular invasion, lymph node metastasis, and a high Ki-67 proliferative index. All cases showing low-grade budding were alive until 6 months of diagnosis, but there was no statistically significant association between stage and budding.

CONCLUSION: Tumor buds are significantly higher in grade 2 invasive ductal carcinoma with lymphovascular invasion, lymph node metastasis, and a high Ki-67 proliferative index. Immunohistochemistry may prove helpful in distinguishing tumor buds from their mimickers. Further studies with extended follow-up are recommended to predict tumor budding as a prognostic marker in breast carcinoma, which may play an important role in cancer therapy.

KEYWORDS: Breast neoplasms. Tumor budding. Tumor. Buds.

INTRODUCTION

Breast cancer is the most common cancer in the world, with an age-standardized incidence rate of 47.8 and a mortality rate of 13.6 per 100,000 population[1]. It has been estimated that slightly more cases of breast cancer are present in less developed areas of the world than in more developed areas. Tumor budding is a phenomenon in which the tumor cells become detached from the main tumor mass and are present at the invasive front[2]. It has been considered to play an important role as a prognostic factor[3]. Tumor budding has been studied in different carcinomas, and the International Tumor Budding Consensus Conference (ITBCC) has highlighted a scoring system for the reporting of tumor budding in colorectal cancer[4]. The present study was therefore conducted to study tumor budding in invasive breast carcinoma and to correlate it with clinicopathological parameters and molecular subtypes.

METHODS

The study was conducted in the Department of Pathology over a period of 1 year and included all the newly diagnosed cases of invasive breast carcinoma. The core biopsies were excluded from the study. Relevant clinical details were noted for every case, and hematoxylin and eosin-stained sections were studied for histomorphological features, grading, and TNM staging according to the WHO classification of breast tumors[5]. Tumor budding was studied in every case as per the recommendations of the ITBCC, 2017[6]. Either a single or a group of five cells at the invasive front of breast carcinoma were counted in a high-power field (40×) as tumor buds (Figure 1). These tumor buds were counted in 10 high-power fields and documented as low- or high-grade depending on the number of buds. High-grade tumor budding was considered when tumor buds were >20/10 HPF and low when tumor buds were ≤20/10 HPF. The immunohistochemical examination was done for every case for ER, PR, HER2 neu, and Ki-67 to

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determine the molecular subtype of breast carcinoma. Pan-CK immunohistochemical staining was also done for confirmation of tumor buds (Figure 2). The tumor buds grading was then statistically correlated with clinical features and histopathological parameters, including tumor size, grade, lymph node status, lymphovascular invasion, pathological TNM staging, molecular subtype, and survival of breast carcinoma patients.

Statistical analysis of the observations was performed using the SPSS software (Statistical Package for Social Sciences) version 23 and Microsoft Excel. Categorical data was expressed as frequencies, and continuous data as mean±standard deviation or median. The association of categorical variables was analyzed using Pearson's chi-square test. A p-value of <0.05 was considered significant. The study was approved by the institutional research and ethics committee via letter no. SRHU/HIMS/RC/2022/108 dated April 2, 2022.

RESULTS

The study included 50 cases of invasive breast carcinoma, with all the cases being female. The mean age was 48.66±12.25 years, the median was 47 years, and the age range was 25–79 years.

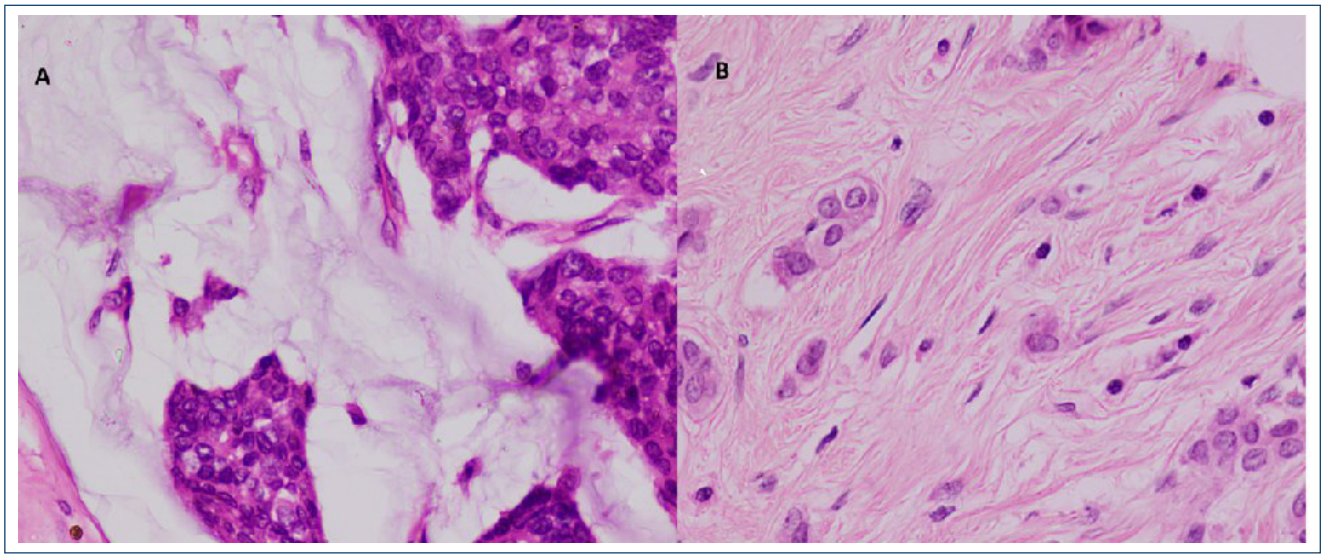

Figure 1. (A) Section shows invasive ductal carcinoma having low-grade tumor budding and (B) section shows invasive ductal carcinoma having high-grade tumor budding (H&E, 40×).

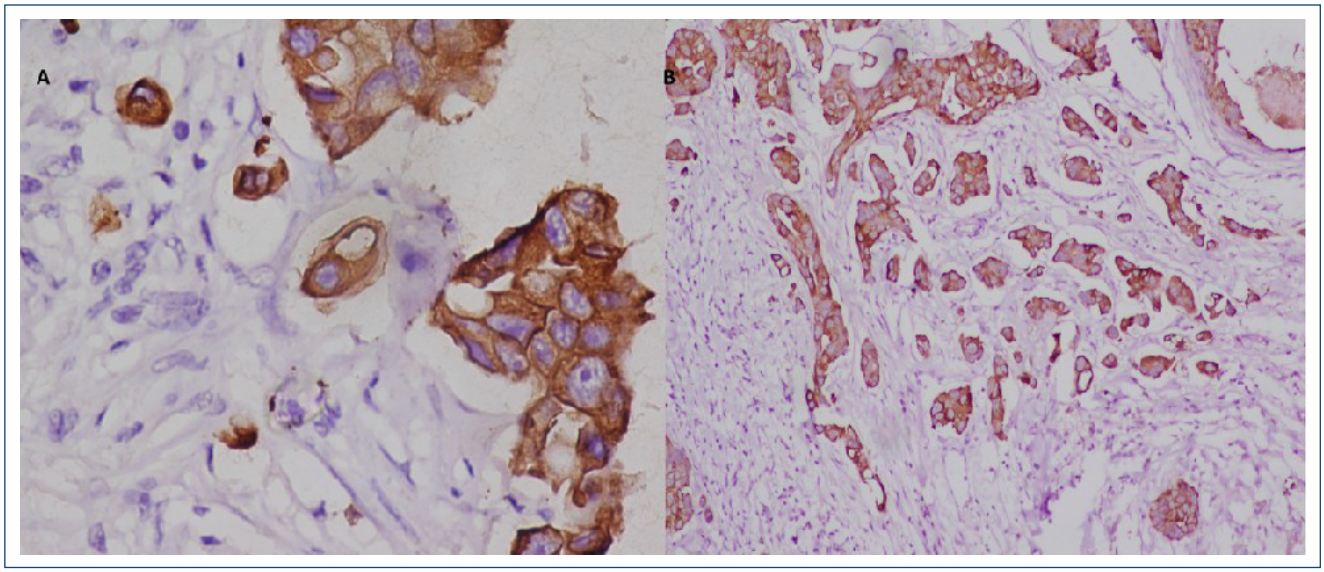

Figure 2. (A) A pan-CK-stained section demonstrates low-grade tumor buds in invasive ductal carcinoma and (B) a pan-CK-stained section demonstrates high-grade tumor buds in invasive ductal carcinoma (immunohistochemical pan-CK stain, 40×).

The right breast was involved in 56% of cases (n=28), while 2% (n=1) of cases showed bilateral breast involvement, with upper quadrant involvement in 62% of cases (n=31). Most commonly, the cases (n=43, 86%) presented with a lump in the breast for a duration of more than 6 months and bloody nipple discharge in 2% of cases. On mammography, 58% of cases (n=29) were in the BIRADS 4c (Breast Imaging Reporting and Data System) category, and on FNAC, 98% of cases were diagnosed as ductal carcinoma. On gross examination of the mastectomy specimen, a tumor size of 2–5 cm was observed in 60% of cases (n=30), with ulcero-infiltrative growth in 98% of cases. Histologically, 47 cases were of invasive ductal carcinoma, 2 were of invasive lobular carcinoma, and 1 was of mucinous carcinoma. Table 1 shows the distribution of histopathological findings in the carcinoma cases. It shows that grade 2 (RB score of 6–7) was present in 72% (n=36) of cases. The maximum number of cases, 22% (n=11), were pT2N0Mx, followed by pT2N1aMx at 16% (n=8), and 96% (n=48) of cases were alive after 6 months of diagnosis. The immunohistochemical staining for ER, PR, HER2 neu, and Ki-67 revealed that 48% (n=24) were luminal B breast carcinoma, while 24% were triple-negative breast carcinoma. It was observed that 66% of cases (n=33) showed high-grade tumor budding, which was statistically significantly higher in invasive ductal carcinoma, grade 2 ($p<0.05$). It was also observed that high tumor budding was associated with grade 2 breast carcinoma and lymphovascular invasion, and 77.14% (n=27) of cases showing high-grade tumor budding had lymph node involvement by the carcinoma. It was also observed that 91.4% of cases with a high Ki-67 proliferative index showed high-grade tumor budding. Table 2 shows the association between tumor budding and the molecular classification of breast carcinoma. It shows that triple negative and luminal B type of breast cancer had low-grade tumor budding in 35.3% (n=6) of cases, and luminal B type had high-grade tumor budding in 54.5% (n=18) of cases. Although it was observed that 100% of cases showing low-grade tumor budding were alive until 6 months of diagnosis, there was no statistically significant association between stage and tumor budding.

Table 1. Distribution of breast carcinoma cases according to the histopathological findings.

Histopathological findings			Number of cases (n)	Percentage (%)
RB score	Grade I (score 3–5)		4	8
	Grade II (score 6–7)		36	72
	Grade III (score 8–9)		10	20
Intratumoral DCIS (>25%)			8	16
Intratumoral DCIS (<25%)			42	84
Extratumoral DCIS (>10%)			4	8
Necrosis		Not seen	0	0
		Focal	18	36
		Extensive	32	64
Calcification			3	6
Lymphovascular invasion			39	78
Perineural invasion			4	8
Uninvolved breast		Fibrocystic breast disease	44	88
		Chronic mastitis	6	12

TNM: tumor node metastasis; FNAC: fine-needle aspiration cytology; DCIS: ductal carcinoma in situ.

Table 2. Association between tumor budding and molecular classification of breast carcinoma.

Molecular classification	Tumor budding			p-value
	Low grade	High grade	Total	
Luminal A	0 (0.0%)	3 (9.1%)	3 (6.0%)	0.219
Luminal B	6 (35.3%)	18 (54.5%)	24 (48.0%)	
HER2 neu positive	5 (29.4%)	6 (18.2%)	11 (22.0%)	
Triple negative	6 (35.3%)	6 (18.2%)	12 (24.0%)	
Total	17 (100.0%)	33 (100.0%)	50 (100.0%)	

DISCUSSION

Tumor budding, which is the phenomenon of the separation of a cluster of tumor cells from the main tumor mass, is considered the initial stage of metastasis[7]. It has been studied in various carcinomas, including lung carcinoma and head and neck carcinoma, and is considered to have prognostic significance[8,9]. Although the present study observed tumor budding in different histomorphological types of breast carcinoma, including ductal, lobular, and mucinous carcinoma, most of the previous studies have studied it in only invasive ductal carcinoma[10,11]. The observation of tumor buds may be done with 40× or 20× objective lens, but it is considered that at low power, it may become difficult to differentiate tumor buds from other cells[2,10,12]. The present study observed tumor buds at 40× and also confirmed them by doing immunohistochemical staining with cytokeratin. Liang et al. also confirmed tumor buds by doing immunohistochemical staining, which excluded any mimickers[13]. The authors therefore suggest that immunohistochemical cytokeratin stain may be helpful if there is any confusion regarding the presence of tumor buds, but in routine practice, observation at 40× may be sufficient.

An important finding observed in the present study was that high tumor budding was associated with grade 2 breast carcinoma, lymphovascular invasion, lymph node metastasis, and a high Ki-67 proliferative index. Previous studies have also observed similar findings, but the correlation with a high proliferative index is limited[14,15]. This suggests that tumor budding may emerge as an important prognostic factor in breast carcinoma. However, Mozarowski et al. observed in their study that there is no statistically significant difference in the frequency of complete or partial responses between the group having tumor budding and another without it[16]. In contrast, Silva et al. concluded that tumor budding in early breast cancer is a novel factor in the determination of adjuvant therapy decisions by identifying patients at a high risk of relapse and benefiting from treatment intensification[17].

It was observed in the present study that all the patients with low tumor budding survived for at least 6 months. Although this may suggest that tumor budding may be associated with survival, the follow-up period is too short for a definite opinion about it. Okcu et al. recently concluded that tumor budding is a reliable predictor of death and metastasis in invasive ductal breast cancer[18]. It has also been observed previously that a sentinel lymph node biopsy showing extracapsular extension was associated with additional positive axillary lymph nodes[19].

It has been reported that tumor budding is associated with epithelial-mesenchymal transition and interacts with the tumor microenvironment for metastasis[20]. Recently, partial epithelial-mesenchymal transition, which is a hybrid state in which both epithelial and mesenchymal characteristics are studied in relation to tumor budding, may be helpful in adjuvant therapy planning[20-22]. Previously, it has been observed that breast-conserving surgery is adequate for overall survival than mastectomy, even in large lesions, and is associated with a higher pathological complete response[23]. The combination of estrogen with melatonin has also been studied for breast cancer survivors, especially in females with intense vasomotor symptoms, and further studies are recommended for optimal hormonal replacement[24].

An important limitation of the present study was that only a limited number of cases were studied, with a survival period of only 6 months, which may not be enough to sufficiently comment on tumor budding as a prognostic marker in breast carcinoma.

Thus, to conclude, tumor buds are significantly higher in grade 2 invasive ductal carcinoma and are associated with lymphovascular invasion, lymph node metastasis, and a high Ki-67 proliferative index. It has to be histomorphologically studied at 40× to differentiate from other mimicking cells. Although immunohistochemistry using the epithelial marker pan-CK may prove helpful if there is any difficulty in differentiation of malignant cells from inflammatory cells, mostly routine HE-stained sections are sufficient. Further studies with extended follow-up are recommended to predict tumor budding as a prognostic marker in breast carcinoma and thus may play an important role in cancer therapy.

AUTHORS' CONTRIBUTIONS

SK: Conceptualization, Data curation, Writing – original draft. **SC:** Conceptualization, Writing – original draft. **AA:** Formal Analysis, Writing – review & editing.

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ORIGINAL ARTICLE

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Comparative performance of artificial ıntelligence models in physical medicine and rehabilitation board-level questions

Ahmet Kıvanç Menekşeoğlu[1*], Enes Efe İş[2]

SUMMARY

OBJECTİVES: The aim of this study was to compare the performance of artificial intelligence models ChatGPT-3.5, ChatGPT-4, and Google Bard in answering Physical Medicine and Rehabilitation board-style questions, assessing their capabilities in medical education and potential clinical applications.
METHODS: A comparative cross-sectional study was conducted using the PMR100, an example question set for the American Board of Physical Medicine and Rehabilitation Part I exam, focusing on artificial intelligence models' ability to answer and categorize questions by difficulty. The study evaluated the artificial intelligence models and analyzed them for accuracy, reliability, and alignment with difficulty levels determined by physiatrists.
RESULTS: ChatGPT-4 led with a 74% success rate, followed by Bard at 66%, and ChatGPT-3.5 at 63.8%. Bard showed remarkable answer consistency, altering responses in only 1% of cases. The difficulty assessment by ChatGPT models closely matched that of physiatrists. The study highlighted nuanced differences in artificial intelligence models' performance across various Physical Medicine and Rehabilitation subfields.
CONCLUSION: The study illustrates the potential of artificial intelligence in medical education and clinical settings, with ChatGPT-4 showing a slight edge in performance. It emphasizes the importance of artificial intelligence as a supportive tool for physiatrists, despite the need for careful oversight of artificial intelligence-generated responses to ensure patient safety.
KEYWORDS: Artificial intelligence. Physical Medicine and Rehabilitation. Academic performance.

INTRODUCTION

In the rapidly advancing domain of artificial intelligence (AI), various models such as ChatGPT-3.5, ChatGPT-4, and Google Bard have demonstrated notable proficiency in numerous academic studies, particularly within the context of medical examinations[1-3]. The integration of AI into clinical practices requires that these technologies not only comply with but also augment the procedural framework of medical professionals, with an emphasis on enhancing efficiency, accuracy, and reliability[4]. Consequently, evaluating these AI models' proficiency in interpreting and responding to specialized, board-style examination questions becomes a pivotal step in assessing their potential clinical utility. This research contributes to the scientific discourse by offering a detailed comparative analysis of these AI systems, specifically examining their relevance and efficacy in the specialized field of Physical Medicine and Rehabilitation (PMR), thereby laying the groundwork for future integration of AI in healthcare settings.

Physical Medicine and Rehabilitation is a discipline characterized by its holistic approach to patient care, necessitating an extensive understanding of a multifaceted treatment spectrum. The benchmark for our investigation is the PMR100, issued by the American Board of Physical Medicine and Rehabilitation (ABPMR). This compilation is reflective of the content scope and complexity inherent to the Part I Certification Examination in PMR[5].

The primary objective of our study was to critically assess and compare the capabilities of ChatGPT-3.5, ChatGPT-4, and Google Bard in interpreting and responding to the intricate and specialized questions encompassed within the PMR100. Additionally, we aimed to examine the performance of these AI systems within various subfields of PMR, offering a comprehensive assessment of their proficiency and applicability across the spectrum of this discipline.

METHODS

This was a comparative, cross-sectional study designed to evaluate and compare the performance of AI language models, specifically Bard and different versions of ChatGPT (3.5 and 4), in the context of PMR. The study aimed to assess the ability of these AI models to answer board-style questions and

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categorize them based on difficulty (easy, medium, and difficult). In this study, the AI models Bard (Google AI, Mountain View, CA, USA), ChatGPT-3.5 (OpenAI, L.L.C., CA, USA), and ChatGPT-4 (OpenAI, L.L.C., CA, USA) were used between January 20 and 25, 2024. Part I practice questions (PMR100) published by the ABPMR were used as a sample for the board exam. In the set of 100 questions, each question has one correct answer out of four options, and the answer key was provided by ABPMR. Out of a total of 100 questions, six were excluded from the study, and the data related to 94 questions were evaluated. Six questions were not evaluated because they contained videos or photographs.

Each AI model was presented with the questions, accompanied by a short introduction: "The following is a national board-level exam question for physiatrists. Read the question and indicate the level of difficulty as easy, medium, or difficult, then choose the correct option." After the first answer, each AI model was asked, "Are you sure?" to assess its confidence in the answer. Both answers and the level of difficulty were recorded. The answers were compared with the correct answer key provided by ABPMR.

The questions were also jointly graded by two European Board-certified physiatrists into three difficulty categories: easy, moderate, and difficult. Bloom's Taxonomy for Learning and Assessment Framework was employed to categorize the questions based on the necessary cognitive engagement[6,7].

The performance of each AI model was evaluated based on the following criteria: accuracy of answers, reliability of answers, compatibility of difficulty categorization, correct answer rate by difficulty category, and correct answer rate by subtypes of questions. Analyses were performed using the chi-square test or Fisher's exact test. All statistical analyses were performed using the SPSS software package (version 25; IBM Corp., Armonk, NY, USA). The statistical significance of all tests was set at $p \leq 0.05$.

RESULTS

The answers given by three different AI models were evaluated, and it was observed that ChatGPT-4 answered 74% of the questions correctly, Bard 66%, and ChatGPT 63.8%. It was found that the success rates decreased after asking, "Are you sure?" (66, 64.9, and 48.9%, respectively). There was no statistically significant difference between the three different AI models in the first response to the questions (p=0.254), but Bard (p=0.027) and ChatGPT-4 (p=0.018) were more successful than ChatGPT-3.5 in the second response to the questions. In the evaluation of the difficulty level of the questions determined by three different AI models, it was observed that Bard (p<0.001) categorized the questions more at medium difficulty compared to the other models (Table 1).

It was evaluated in terms of consistency of answer, and it was observed that ChatGPT-3.5 changed its answer in 66.7% of the questions. This rate was 32.2% in ChatGPT-4 and 1% in Bard. It was found that Bard changed answers to statistically significantly fewer questions than other AI models (p<0.001). The distribution of questions where ChatGPT-3.5 and 4 changed the answers was analyzed, and it was found that ChatGPT-3.5 changed answers from wrong to right in 20 questions, from wrong to wrong in 10 questions, and from right to wrong in 34 questions. In ChatGPT-4, these numbers were 10, 3, and 18, respectively.

In determining the difficulty distribution of the questions, it was found that Bard categorized the questions mostly as medium difficulty (p<0.001). Another important finding is that there was no significant difference in the difficulty distribution of the questions between the distribution made by physiatrists and ChatGPT-3.5 and ChatGPT-4.

The questions were categorized by the physiatrists into three categories: easy, medium, and difficult and the correct answer

Table 1. Analysis of the answers of three different artificial intelligence models.

		ChatGPT-3.5 n (%)	Bard n (%)	ChatGPT-4 n (%)	p	p#
1st answer	Incorrect	34 (36.2%)	32 (34.0%)	24 (25.5%)	0.254	
	Correct	60 (63.8%)	62 (66%)	70 (74.5%)		
2nd answer	Incorrect	48 (51.1%)	33 (35.1%)	32 (34%)	**0.028**	**0.027[1]**
	Correct	46 (48.9%)	61 (64.9%)	62 (66%)		**0.018[2]**
Diffuculty of questions	Easy	47 (50%)	1 (1.1%)	33 (35.1%)	**<0.001**	**<0.001[1]**
	Medium	45 (47.9%)	91 (96.8%)	57 (60.6%)		**<0.001[3]**
	Hard	2 (2.1%)	2 (2.1%)	4 (4.3%)		

#:Post-hoc analysis, 1: between ChatGPT-3.5 and Bard, 2: between ChatGPT-3.5 and ChatGPT-4, 3: between ChatGPT-4 and Bard.

rates of the AI models were evaluated. In the intra-group evaluation, ChatGPT-3.5 answered 82.7% of the easy questions correctly and had a significantly higher accuracy rate than the medium-hard questions (p<0.001). For ChatGPT-4, this value was 82.7%, and a statistically significant difference was found (p=0.020). No statistically significant difference was found between question difficulty and correct answer rate in the intergroup analysis (Table 2). The questions were also divided into two different difficulty levels: low order and high order, according to Bloom's taxonomy method, and three different AI models were evaluated in terms of the correct answer rates of these questions. No significant difference was found within or between the groups.

The questions were categorized as specified by ABPMR, and the correct answers of different models were evaluated. It was found that ChatGPT-3.5 achieved 80.6% success in musculoskeletal system questions, while ChatGPT-4 achieved 85.2%, and Bard and ChatGPT-3.5 achieved 77.8% success in patient assessment and diagnosis. In addition, no statistically significant difference was found between different AI models in the question subheadings (Table 3).

DISCUSSION

This study compares the performance of ChatGPT-3.5, ChatGPT-4, and Google Bard in the field of PMR, uncovering subtle differences in their abilities. All models performed similarly, but ChatGPT-4 led with a 74% success rate. Further testing showed ChatGPT-4 and Bard outperformed ChatGPT-3.5, especially in consistent answer quality, with Bard changing answers the least. The difficulty of questions as perceived by the ChatGPT closely matched expert opinions. Using Bloom's Taxonomy for question classification, all models showed similar performance across different cognitive demands. The study did reveal each model's strengths in patient assessment and diagnosis, with slight differences in specific areas.

In contrast to prior studies suggesting a clear superiority of ChatGPT-4 over its counterparts, our results present a more nuanced picture in the context of PMR-focused queries[3,8,9]. ChatGPT-4 indeed led the group with a 74% success rate, followed closely by Bard at 66%, and ChatGPT-3.5 at 63.8%, thereby not establishing a substantial margin of superiority for ChatGPT-4 as anticipated. When interpreting the results, it is notable that in a hypothetical examination with

Table 2. Assessment of initial artificial intelligence responses by difficulty level as determined by the authors.

	ChatGPT-3.5			Bard			ChatGPT-4			p
	Incorrect n (%)	Correct n (%)	p	Incorrect n (%)	Correct n (%)	p	Incorrect n (%)	Correct n (%)	p	
Easy	9 (17.3%)	43 (82.7%)		13 (25%)	39 (75%)		9 (17.3%)	43 (82.7%)		0.525
Medium	18 (52.9%)	16 (47.1%)	**<0.001**	14 (41.2%)	20 (58.8%)	0.062	10 (29.4%)	24 (70.6%)	**0.020**	0.143
Hard	7 (87.5%)	1 (12.5%)		5 (62.5%)	3 (37.5%)		5 (62.5%)	3 (37.5%)		0.446

Table 3. Comparison of artificial intelligence model performance by question categories as defined by ABPMR.

	ChatGPT-3.5		Bard		ChatGPT-4		p
	Incorrect n (%)	Correct n (%)	Incorrect n (%)	Correct n (%)	Incorrect n (%)	Correct n (%)	
Neurological disorders	11 (42.3%)	15 (57.7%)	10 (38.5%)	16 (61.5%)	6 (23.1%)	20 (76.9%)	0.304
Musculoskeletal medicine	6 (19.4%)	25 (80.6%)	11 (35.5%)	20 (64.5%)	8 (25.8%)	23 (74.2%)	0.354
Amputation	3 (60%)	2 (40%)	1 (20%)	4 (80%)	2 (40%)	3 (60%)	
Medical rehabilitation	3 (42.9%)	4 (57.1%)	2 (28.6%)	5 (71.4%)	1 (14.3%)	6 (85.7%)	
Rehabilitation problems	7 (46.7%)	8 (53.3%)	5 (33.3%)	10 (66.7%)	3 (20%)	12 (80%)	0.301
Basic sciences	4 (40%)	6 (60%)	3 (30%)	7 (70%)	4 (40%)	6 (60%)	
Patient evaluation and diagnosis	6 (22.2%)	**21 (77.8%)**	6 (22.2%)	**21 (77.8%)**	4 (14.8%)	**23 (85.2%)**	0.732
Electrodiagnosis	8 (72.7%)	3 (27.3%)	5 (45.5%)	6 (54.5%)	6 (54.5%)	5 (45.5%)	
Patient management	10 (32.3%)	21 (67.7%)	12 (38.7%)	19 (61.3%)	7 (22.6%)	24 (77.4%)	0.386
Equipment and assistive technology	2 (25%)	6 (75%)	3 (37.5%)	5 (62.5%)	3 (37.5%)	5 (62.5%)	
Applied sciences	8 (47.1%)	9 (52.9%)	6 (35.3%)	11 (64.7%)	4 (23.5%)	13 (76.5%)	0.357

a passing threshold of 70%, ChatGPT-4 would have passed, potentially setting it apart from other AI models. However, this distinction, albeit statistically subtle, could be significant in practical terms. Yet, this interpretation is constrained by two pivotal factors. First, the ABPMR employs a unique scoring methodology, using scaled scores rather than raw percentages, which complicates direct comparisons. A study by Cuthbert and Simpson employed the United Kingdom and Ireland In-Training Examination (UKITE) as a stand-in for the Section 1 examination of the Fellowship of the Royal College of Surgeons (FCRS). The performance of ChatGPT was notably lower, at 35.8%, falling 30% short of the FCRS pass mark and 8.2% below the average human score. The authors attributed this shortfall to ChatGPT's limited capability for higher-order judgment and multilogical reasoning, essential for selecting the optimal answer in clinical scenarios. Their study highlighted a stark contrast between a 53% success rate in basic science versus a 0% in trauma, a disparity not observed in our research, even after categorizing questions and applying Bloom's taxonomy[10]. Isleem et al. focused on ChatGPT's performance on Orthopedic In-Training and Self-Assessment Examination questions from the American Academy of Orthopaedic Surgeons (AAOS)[11]. Out of 301 questions, ChatGPT correctly answered 183 (60.8%), hinting at varying performance levels across similar medical exams and possibly underscoring a lack of consistency in the model's medical proficiency.

Artificial intelligence is increasing its use in the field of medicine, as it is all over the world, and it affects healthcare in different ways. Today, AI is increasing its effectiveness in patient assessment, and the personalization of treatment plans, especially in areas such as radiology, pathology, and dermatology, thus creating an unprecedented change in patient care and medical practices[12-14]. The advantages of the use of AI systems in the field of health include the ability to predict potential health problems by analyzing individual health data, the recognition of diseases in the preclinical stage or early stage and the possibility of effective treatment, and the monitoring and care of the patient outside the hospital environment[15].

In parallel with the increase in AI-mediated products used in the diagnosis, treatment, and follow-up of patients, regulatory rules are also being set. The concept of a medical device as software also encompasses AI-mediated products[16]. Therefore, to ensure patient safety and have certain standards, it must comply with the regulations of the medical device regulation. In addition, there are also ethical issues regarding the use of AI in the field of health. In this field, the guidelines published by different organizations, such as WHO and the European Union, also indicate increasing concerns and aim to create solutions[17]. Accordingly, there are still rules that need to be determined on vital points such as the openness of the algorithms used by AI technologies in decision-making, informing patients and obtaining informed consent, ensuring data confidentiality, and compliance with human rights and legal regulations[18].

In parallel with technological developments, the term telerehabilitation is gaining importance in the field of PMR. In this period, when the elderly population and chronic diseases are increasing, the use of technological applications is gaining importance for the sustainability of health systems and public health. Studies have shown that the use of virtual reality systems in rehabilitation improves patients' quality of life, exercise compliance, and motivation[19,20]. In the near future, it will be possible to create patient assessment and therapy programs by combining virtual reality (VR) systems with AI systems. In this way, it will be possible to remotely assess the functional status of patients, create a personalized rehabilitation program, and remotely monitor their functional status.

While there are concerns about AI-mediated language models, there is growing evidence that they can be used in medical education. It is predicted that it will increase its weight in medical education due to its features such as enabling faster evaluation of students' written exam results and reducing the burden of instructors, thereby creating personalized learning suggestions and materials for students[21].

The strengths of this study include the use of three different AI models and the first AI study on board-level questions in the field of PMR. However, this study has some limitations. The study lacks real-life data except for the authors' categorization of difficulty. Furthermore, this study used study questions from 2015 as the question set. It is suggested that future studies should be based on the use of real board questions and comparisons with real exam statistics.

CONCLUSION

Overall, ChatGPT-4 achieved a 74% success rate in responding to PMR board-style questions, followed by Bard with 66% and ChatGPT-3.5 with 63.8%. The success rate of all three AI models was considered satisfactory. This shows that AI technologies, even in their current form, can solve complex clinical problems within the scope of PMR. Although it is predicted that AI systems will be used more by medical professionals in the future, it is recommended that the content suggested by AI should be carefully reviewed by medical professionals to reduce the risk of harm to patients.

DATA AVAILABILITY

The data associated with the paper are not publicly available but are available from the corresponding author on reasonable request.

ETHICS APPROVAL

As this study involved no human or animal subjects and solely relied on AI-generated data, no ethical approval was required. However, the study was conducted in adherence to general ethical principles of research integrity and data confidentiality.

AUTHORS' CONTRIBUTIONS

AKM: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **EEİ:** Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

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ORIGINAL ARTICLE

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The effect of serum biochemical parameters on clinical prognosis in children presenting with diabetic ketoacidosis

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SUMMARY

OBJECTIVE: The aim of this study was to determine whether diabetes mellitus has a high risk of diabetic ketoacidosis-related complications. Biochemical parameters affect the resolution time of diabetic ketoacidosis.

METHODS: The present study is based on a retrospective evaluation of the records of patients who presented to the Pediatrics Clinic of Adiyaman University Hospital between January 1, 2017, and October 1, 2022, with a diagnosis ofdiabetic ketoacidosis. The demographic characteristics, serum biochemical parameters, blood gas results, and time to transition to subcutaneous insulin therapy were all recorded.

RESULTS: This study included 49 (49%) female and 51 (51%) male patients aged 1–17 years (mean age: 9.05±4.33 years). The average time to clinical improvement of the sample, that is, transition to subcutaneous insulin therapy, was 21.04±7.8 h. An evaluation of the presence of acute kidney injury based on serum urea and creatinine levels and eGFR values revealed no significant effect on the rate of clinical recovery (respective p-values: p=0.076, p=0.494, and p=0.884). A univariate analysis identified blood glucose (p=0.025), blood gas pH (p<0.001), and blood bicarbonate (p=0.004) values as prognostic factors, while a multivariate analysis revealed pH values had an independent and significant effect on the resolution time of diabetic ketoacidosis.

CONCLUSION: Serum glucose, pH, and bicarbonate levels are the most important determinants of clinical prognosis in patients with diabetic ketoacidosis. These findings can serve as a guide for clinicians in the follow-up and treatment of such patients.

KEYWORDS: Child. Biochemical parameters. Diabetic ketoacidosis. Resolution time.

INTRODUCTION

Type 1 diabetes mellitus (DM) is a chronic metabolic disease that leads to polyuria, polydipsia, and weight loss and is managed with insulin, exercise, and dietary planning[1]. Diabetic ketoacidosis (DKA) is a mortal complication of type 1 DM characterized by acidosis, ketosis, and hyperglycemia and frequently accompanied by dehydration and electrolyte disorders. Previous studies have reported that approximately 30–60% of patients present with DKA at the time of initial diagnosis, while DKA is identified in 7–10% of patients during follow-up[2-4]. Prolonged or complicated DKA can lead to organ dysfunction, prolonged hospitalization, and increased treatment costs[5]. Severe acidemia in DKA leads to decreased cardiac contractility, cardiac arrhythmias, and hemodynamic instability, and is the leading factor affecting clinical prognosis due to the damage sustained by vital organs (brain and kidneys). Acidosis also inhibits the binding of insulin to its receptor and the reduction in ketone formation[5-7]. DKA develops suddenly, progresses rapidly, and is accompanied by life-threatening complications, with mortality rates of 5% reported[8]. The regulation of fluids, electrolytes, and acid-base balance disorders is the primary treatment approach to the management of DKA, among which fluid and electrolyte therapies are organized based on International Society for Pediatric and Adolescent Diabetes (ISPAD) guidelines[9]. The management, follow-up, and treatment of DKA are important for the long-term prognosis of patients, given the potential adverse effect on vital organs. The present study investigates the effect of biochemical parameters on DKA resolution time, that is, clinical improvement, in pediatric patients and evaluates the approaches used in its follow-up and treatment. The study further seeks to identify the patient population associated with worse prognosis. Determining the parameters affecting clinical prognosis will contribute to the search for new target drugs for the treatment of DKA.

METHODS

This study included patients under the age of 18 years who applied to the Pediatrics Clinic of Adiyaman University Hospital between January 1, 2017, and October 1, 2022, with a new

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diagnosis of type 1 DKA, whose records were evaluated retrospectively. DKA diagnoses were based on ISPAD guidelines, specifically on the presence of hyperglycemia (blood glucose>200 mg/dL), metabolic acidosis (venous blood gas pH<7.3 and/or plasma bicarbonate<18 mmol/L), and the presence of ketonemia and ketonuria[1]. Demographic characteristics, complete blood count, urine density, serum biochemical parameters, blood gas results, time to transition to subcutaneous insulin therapy, and results of renal function tests were all recorded. Patients with chronic metabolic, nephrological, or neurological diseases and those on drugs that may cause fluid-electrolyte or acid-base balance disorders were excluded from the study. The ISPAD guidelines were used as the basis for fluid therapy, and volume expansion was performed with one or more boluses of 0.9% saline infused over 20–30 min until peripheral perfusion was restored. Maintenance and deficit therapy were administered with 0.45% saline over 24–48 h.

Calculations

Anion gap, corrected sodium, and blood osmolarity were estimated using the following formulas (1):

Anion gap=Na–(Cl+HCO_3): mean value:12±2 mmol/L.

Corrected sodium=measured Na+1.6 ([plasma glucose–5.6]/5.6) mmol/L or measured Na+1.6 ([plasma glucose–100]/100) mg/dL.

Effective osmolality (mOsm/kg)=2×(plasma Na)+plasma glucose mmol/L; normal range is 275–295 mOsm/kg.

Diabetic ketoacidosis resolution time/transition to subcutaneous insulin therapy

DKA resolution is defined as pH ≥ 7.30, serum bicarbonate >18 mmol/L, and resolution of ketosis or ketonemia[1]. Time to transition to subcutaneous insulin therapy within 24 h of hospitalization was indicated.

Statistical analysis

IBM SPSS Statistics for Windows (Version 24.0; Armonk, NY: IBM Corp.) was used for the statistical evaluation of the data obtained in the study. Frequency values were used to define the gender distribution of the patients examined in the study, as were mean and standard deviation values for all other parameters. Pearson's correlation coefficient was used for the assessment of the relationship between the parameters and the recovery time of the patients. The optimum cutoff values for glucose, blood gas pH, blood gas bicarbonate, blood gas CO_2, and serum osmolarity were determined based on the receiver operating characteristic (ROC) curve and the area under the curve (AUC), and median values were used for all other laboratory parameters, with cutoff values used for the categorization of the two groups as "low" or "high." Univariate and multivariate logistic regression analyses were used to determine predictors of patients who recovered within the first 24 h of starting treatment. Variables with significant differences between the treatment-responsive and non-responder groups were included in the logistic regression analysis. All continuous variables were categorized according to clinically identified thresholds. The odds ratio (OR) was reported with corresponding 95% confidence intervals (95%CI). The ROC curve and the area under the ROC curve (ROC-AUC) were calculated for the comparison of independent prognostic factors. The results were accepted as statistically significant within confidence limits of 99% ($p<0.01$) and 95% ($p<0.05$).

Ethics

The study was granted appropriate Institute Review Board approval and was conducted with the approval of the Ethics Commission of the Adiyaman University Hospital in Adiyaman (No. 2022/8-2), in accordance with the Declaration of Helsinki.

RESULTS

This study included 49 (49%) female and 51 (51%) male patients aged 1–17 years (mean age: 9.05±4.33 years). The mean body mass index of patients was 16.93±3.03 kg/m^2. Consistent with a diagnosis of DKA, elevated blood glucose, metabolic acidosis, elevated white blood cell counts, prerenal renal failure, and increased urine densities were observed in the sample. The mean time to clinical improvement, that is, the transition to subcutaneous insulin therapy, was 21.04±7.8 h. The laboratory values of the patients are presented in Table 1. None of the patients with prerenal acute kidney injury required renal replacement therapy (hemodialysis/peritoneal dialysis).

Correlation between diabetic ketoacidosis resolution rates and laboratory parameters

A correlation analysis was performed to examine the relationship between DKA resolution rates and laboratory parameters, and the resulting resolution rates were found to be weakly correlated with blood glucose (r:0.201, p:0.045<0.05) levels and anion gap values (r:0.264, p:0.008<0.01); moderately correlated with blood gas pH (r:-0.588, p:0.001<0.01) and HCO_3 (r:-0.552, p:0.001<0.01) levels; weakly and directly correlated with white blood cell counts (r:0.262, p:0.008<0.01)

Table 1. Laboratory test results of the patients.

Laboratory parameters	Min	Max	Mean	SD
Glucose (mg/dL)	238	800	529.75	149.53
BUN (mg/dL)	12	61	27	9.51
Creatinine (mg/dL)	0.20	1.72	0.88	0.26
eGFR (mL/min/1.73 m^2)	32.65	337.48	73.61	33.44
Na (mmol/L)	120	143	132.37	4.56
Corrected Na (mmol/L)	128.78	150.20	139.24	4.09
K (mmol/L)	3.10	6.10	4.34	0.61
Cl (mmol/L)	88	120	103.05	5.95
Mg (mg/dL)	1.65	2.10	1.84	0.14
pH (mmol/L)	6.78	7.31	7.13	0.12
HCO_3 (mEq/L)	2.40	18	9.57	3.61
CO_2 (mEq/L)	2.70	45	22.62	7.11
Anion gap (mmol/L)	6	39.50	24.08	5.75
Urine density	1010	1059	1032.69	8.98
WBC ($\times 10^9$/L)	36	39190	12437.26	6320.84
Serum osmolarity (mOsm/kg)	275.83	333.66	03.81	10.98
HbA1c (%)	7.40	18.10	12.67	2.34
Insulin (µU/mL)	0.18	60.70	4.85	9.25
C-Peptide (ng/mL)	0.10	1.65	0.31	0.28
Time to resolution (h)	6	50	21.04	7.86

SD: standard deviation; WBC: white blood cells.

and serum osmolarity (r:0.219, p:0.029<0.05); and weakly but inversely correlated with CO_2 (r:-0.371, p:0.001<0.01). As the glucose, anion gap, white blood cell count, and serum osmolarity values increased, so did the time to clinical recovery, whereas as pH and HCO_3 values increased, the time to clinical recovery decreased.

Regression analysis results

An analysis of the time to transition to subcutaneous insulin therapy revealed DKA resolution rates both within and after 24 h of hospitalization, with 32 (32%) patients recorded as transitioning to subcutaneous insulin therapy within the first 24 h.

Parameters cutoff values

In the ROC-AUC analysis, the optimal cutoff and AUC values within the respective confidence intervals for glucose, blood gas pH, blood gas bicarbonate, blood gas pCO_2, anion gap, and serum osmolarity shown in Table 2 were recorded. Median values were used for all other parameters for which cutoff values could not be determined from the ROC curve analysis (Figure 1).

Regression analysis

A logistic regression analysis was carried out to determine the prognostic factors of patients who recovered within the first 24 h. A univariate analysis revealed glucose (p=0.025), blood gas pH (p<0.001), and blood gas bicarbonate (p=0.004) to be prognostic factors, while in a multivariate model created using the factors found to be prognostic for treatment response time in the univariate analysis, only blood gas pH remained an independent prognostic factor (OR=0.23, 95%CI 0.07–0.77, p=0.017) (Table 2).

DISCUSSION

Diabetic ketoacidosis is a life-threatening and acute complication of type 1 DM[1], so determining the parameters affecting clinical prognosis and time to resolution is crucial. In the present study, based on the time to resolution of DKA, that is, the time to subcutaneous insulin therapy, an increase in serum glucose (p=0.045), serum osmolarity (p=0.029), and anion gap (p=0.008) were all found to delay clinical resolution, and a delay in clinical resolution was

Table 2. Univariate and multivariate analysis for factors affecting response to treatment within the first 24 h of hospitalization.

Variables	Category	Univariate analysis		Multivariate analysis	
		OR (95% CI)	p	OR (95% CI)	p
Age	<9.1/≥9.1	0.61 (0.26–1.43)	0.252		
Sex	Female/male	0.54 (0.23–1.27)	0.157		
Weight (kg)	<28/≥28	0.65 (0.28–1.51)	0.313		
Height (cm)	<133.3/≥133.3	0.65 (0.28–1.51)	0.321		
Laboratory parameters					
Glucose (mg/dL)	<527.5/≥527.5	2.69 (1.13–6.41)	**0.025**	2.48 (0.97–6.31)	0.057
BUN (mg/dL)	<26/≥26	2.08 (0.89–4.87)	0.093		
Creatinine (mg/dL)	<0.84/≥0.84	0.88 (0.42–1.81)	0.718		
eGFR (mL/min/1.73 m^2)	<66.9/≥66.9	0.57 (0.25–1.34)	0.200		
Corrected Na (mmol/L)	<138.8/≥138.8	1.99 (0.84–7.70)	0.117		
K (mmol/L)	<4.3/≥4.3	0.80 (0.40–1.60)	0.522		
Cl (mmol/L)	<104/≥104	1.05 (0.45–2.44)	0.917		
pH (mmol/L)	<7.13/≥7.13	1.18 (0.07–0.44)	**<0.001**	0.23 (0.07–0.77)	**0.017**
HCO_3 (mEq/L)	<8.8/≥8.8	0.27 (0.11–0.65)	**0.004**	0.69 (0.21–2.25)	0.535
CO_2 (mEq/L)	<22.9/≥22.9	0.61 (0.26–1.43)	0.259		
Anion gap (mmol/L)	<24.4/≥24.4	1.74 (0.74–4.09)	0.200		
Urine density	<1033/≥1033	0.73 (0.31–1.70)	0.460		
WBC (×10^9/L)	<11305/≥11305	1.45 (0.62–3.37)	0.392		
Serum osmolarity (mOsm/kg)	<303.28/≥303.2	2.09 (0.89–4.91)	0.091		
HbA1c (%)	<12.7/≥12.7	1.44 (0.62–3.34)	0.401		
Insulin (μU/mL)	<2.4/≥2.4	1.53 (0.64–3.65)	0.342		
C-Peptide (ng/mL)	<0.22/≥0.22	0.68 (0.29–1.61)	0.380		

Significant p-values are indicated in bold. OR: odds ratio; CI, confidence interval; WBC: white blood cells.

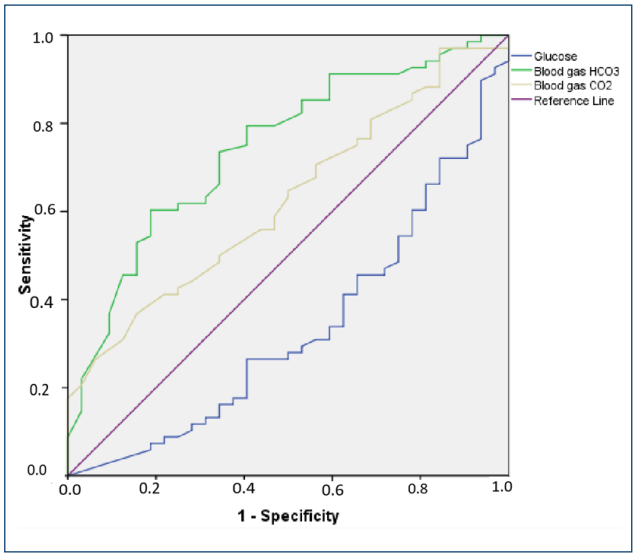

Figure 1. Receiver operating characteristic curve analysis for the predictors of the treatment response within the first 24 h after hospitalization.

also identified in cases in which the white blood cell count increased (p=0.008). Ying Wei et al. revealed increased serum osmotic pressure to be an independent risk factor for severe DKA and identified an association between increased blood glucose and osmotic pressure and severe DKA[10]. These findings are similar to those reported in the study by Shaltout et al.[11] In the present study, elevated blood glucose and osmolarity were found to have a negative effect on clinical prognosis and resolution time.

Guzman et al. identified a significant negative correlation between pH, HCO_3, and pCO_2 levels at resolution time and reported the pH value at the time of the initial diagnosis to have a significant and independent effect on the time to resolution in patients[12]. Similarly, in the present study, low pH, bicarbonate, and pCO_2 values were found to delay clinical recovery based on blood gas parameters (p=0.001). After dividing the patients into two groups based on the occurrence of clinical improvement within the first 24 h

and after, glucose (p=0.025), blood gas pH (p<0.001), and blood gas bicarbonate (p=0.004) levels were found to be prognostic factors.

In a multivariate model created using the factors found to be prognostic for treatment response time in the univariate analysis in the present study, only blood gas pH remained as an independent prognostic factor (OR=0.23, 95%CI 0.07–0.77, p=0.017), which can be attributed to the worsening of the clinical prognosis by severe acidemia through damage inflicted on vital organs (brain and kidneys).

Previous studies have reported that acute kidney injury negatively affects prognosis[8,10,13]. Huang et al. reported that metabolic acidosis improved later in patients with severe acute kidney injury (AKI)[14], which suggests that patients with severe AKI who develop intrinsic AKI due to impaired tubular function and renal inflammation have a poor prognosis[15]. In the present study, when the presence of AKI was evaluated with serum urea and creatinine levels and eGFR values, it was found to have no significant impact on the clinical recovery rate (p=0.076, p=0.494, and p=0.884, respectively). AKI seen in the course of DKA can be attributed to decreased intravascular volume, usually leading to a mild course and rapid resolution. The polyuria and vomiting often associated with hyperglycemia lead to a decrease in intravascular volume. AKI developing after hypovolemia is a prerenal condition that can be attributed to decreased renal perfusion and is reversible with appropriate fluid therapy. In the present study, fluids were given based on the degree of dehydration of the patient (4,000 mL/m^2/day), and this led to a rapid recovery of dehydration and AKI. This rapid recovery may explain the lack of any significant difference in the clinical improvement of patients with AKI when compared to those without AKI. Similar to the present study, Mishra et al. found no correlation between the severity of DKA and that of AKI[16], and again, similar to our findings, there have been previous studies reporting that the presence of AKI did not affect the duration of hospitalization or the resolution of metabolic acidosis[16]. In the present study, only the first episode of DKA in patients with newly diagnosed DM was evaluated, and the resolution time was recorded as 21.04±7.8 h, compared to the resolution time of 16.25 h reported by Ying Wei et al[10]. The longer resolution time in the present study than that reported by Ying Wei et al. can be attributed to the fact that only the first episode of DKA was evaluated. Guzman et al. showed that resolution times are prolonged with first-episode DKA[12].

Studies have shown that an increase in the anion gap worsens the clinical prognosis and is an indicator of extracellular fluid loss and dehydration. Isaac Lazar et al. reported that the DKA resolution time becomes longer as the normalization time of the anion gap is prolonged[17]. In line with the literature, the present study found that clinical recovery took longer time as the anion gap increased (p=0.008).

The early diagnosis and treatment of DKA and the recognition of factors associated with a poor prognosis during follow-up are essential for the reversal of DKA.

CONCLUSION

To the best of our knowledge, there has been no study in the literature to date comparing the resolution time of DKA within and after the first 24 h of hospital admission and evaluating only the attack at the time of the first diagnosis of DKA in a pediatric age group. The results of the present study reveal that serum glucose, pH value, and serum bicarbonate levels all affect resolution time, and in addition, pH value has an independent and significant effect on clinical prognosis. These prognostic markers are easily accessible and can aid clinicians during patient follow-up and treatment.

STUDY LIMITATION

The retrospective, single-center design of the present study can be considered a limitation. Multi-center, prospective studies are needed to contribute further to the literature.

CONSENT TO PARTICIPATE

Written informed consent was garnered from the parents of all those included in the study.

DATA AVAILABILITY

The authors confirm that all data supporting the findings of this study are included within the article.

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AUTHORS' CONTRIBUTIONS

GI: Formal Analysis, Writing – original draft. **CA:** Formal Analysis, Writing – original draft.

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The Adolescent Insomnia Questionnaire: the Turkish validity and reliability study

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SUMMARY

OBJECTIVE: This study aimed to evaluate the Turkish validity and reliability of the Adolescent Insomnia Questionnaire.

METHODS: The study was carried out with 265 adolescents. Data were collected with the Adolescent Insomnia Questionnaire and the Cleveland Adolescent Sleepiness Questionnaire. Exploratory factor analysis and confirmatory factor analysis were used to analyze the construct validity of Adolescent Insomnia Questionnaire. The scale reliability was tested using test-retest, Cronbach's α test, Pearson correlation analysis, and inter-item correlation analysis.

RESULTS: The Cronbach's α coefficients were found to be above 0.80 for all sub-dimensions and the total scale. Correlations between Adolescent Insomnia Questionnaire and Cleveland Adolescent Sleepiness Questionnaire scores were positively highly significant. The test-retest correlation analysis of Adolescent Insomnia Questionnaire was 0.675. The results of confirmatory factor analysis were χ^2/df=2.861, comparative fit index=0.966, incremental fit index=0.966, Tucker-Lewis index=0.956, normed fit index=0.949, root-mean-square error of approximation=0.084. The suitability of the data for exploratory factor analysis was evaluated with Bartlett's test of sphericity ($p<0.05$), and the sample adequacy was evaluated with the Kaiser-Meyer-Olkin test (0.77).

CONCLUSION: The Adolescent Insomnia Questionnaire Turkish version is a valid and reliable tool for measuring insomnia in adolescents aged 11–18 years. Adolescent Insomnia Questionnaire is a brief, practical, self-reported, age-appropriate, easily applicable, valid, and reliable tool in Turkish. This is the first Turkish validity and reliability study of Adolescent Insomnia Questionnaire.

KEYWORDS: AIQ. Reliability. Validity.

INTRODUCTION

Sleep, which covers one-third life of a human and is central to maintaining the health status, has an important place in the health maintenance of adolescents. Sleep problems experienced by adolescents cause life-threatening accidents and significant disorders in psycho-social functions[1]. In adolescents, insomnia is the most common problem among sleep disorders[2-4]. Notably, 7–40% of the general adolescent population appears to experience clinically classified insomnia. This rate varies according to characteristics such as population, age group, and gender[3].

There are various tools for the assessment of sleep problems in adolescents[5]. These tools do not directly measure insomnia in adolescents. There is a critical need for insomnia tools that are validated for use with adolescents. However, there is no insomnia questionnaire for adolescents other than the Adolescent Insomnia Questionnaire (AIQ)[6].

The AIQ was developed to measure the level of insomnia, and it consists of three sub-dimensions in adolescents[6]. Testing the AIQ by applying it to adolescents in a different country, language, and culture will give important results in terms of evaluating the screening sensitivity of the questionnaire. This is the first Turkish validity and reliability study of AIQ.

METHODS

Study design, sample size, and characteristics

This cross-sectional study was conducted between March and April 2022 in randomly selected three schools in the center of Amasya city in the Black Sea region. Data were collected from adolescents aged 11–18 years. The study was approved by the Amasya University Social Sciences Ethics Committee (04 January 2022, numbered: 50769). The research was conducted in accordance with the Declaration of Helsinki. Informed consent forms were signed by parents and children in the study. The sample size was required to be 10–20 times the number of scale items[7], so the data were collected from

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265 adolescents. A retest application of the AIQ Turkish version was conducted on 65 adolescents 15 days later from the first test. Data were evaluated with the IBM SPSS 20 statistical package program, and the confirmatory factor analysis (CFA) was determined by AMOS.

Data collection tools

Demographic variables were collected from adolescents with demographic information forms. The AIQ is a 5-point Likert-type scale (0=Never to 4=Almost Always), which consists of 13 items and 3 sub-dimensions (factors 1, 2, and 3, respectively). Factor 1 is sleep dissatisfaction and impairments and has items 3, 5, 10, 11, 12, and 13. Factor 2 is sleep onset and has items 1, 4, 7, and 9. Factor 3 is sleep maintenance and has items 2, 6, and 8. Items 3, 4, 8, and 9 are reverse-scored in the AIQ. AIQ scores range from 0 to 52, and the higher the score, the higher the insomnia. The cutoff point of the AIQ was accepted as 15 points. To analyze the validity of the AIQ, the Cleveland Adolescent Sleepiness Questionnaire (CASQ) was used[5].

Validity and reliability stages of the questionnaire

Language validity

The AIQ translation was completed as forward and backward translations[8]. The original English-to-Turkish version was made by three researchers who were native speakers of both Turkish and English and had not seen the AIQ before. The Turkish-to-English version was translated by three researchers who were natives to both Turkish and English and had not seen the questionnaire before. The translation was completed by evaluating the suitability for Turkish.

Content validity

The content validity study was evaluated with the Davis technique in this study[9]. Experts from seven different fields reported that the AIQ Turkish version is suitable. The content validity ratio (CVR) and content validity index (CVI) were calculated by taking the opinions of these experts. CVR and CVI are determined as suitable (CVR: 0.87–0.99 for each item and CVI:0.95).

Construct validity

In this study, factor analysis is one of the commonly used methods to construct validity[10]. Kaiser-Meyer-Olkin (KMO) and Bartlett's test of sphericity (BTS), exploratory factor analysis (EFA)[11], and CFA were carried out sequentially.

Reliability

Inter-item internal consistency was evaluated with Cronbach's α coefficient, Pearson correlation (PC) analysis, and inter-item correlation analysis.

RESULTS

Of the adolescents participating in the study, 52.1% were male, 20.0% were in the ninth grade, and 76.9% had a nuclear family structure, and it was determined that the income of 49.8% of them was equal to their expenses. The mean age of the adolescents was 14.49±2.28 years. The mean scores of the AIQ and CASQ were 22.02±4.23 and 38.34±8.31 in adolescents, respectively (Table 1).

The internal consistency of all items of the AIQ Turkish version was good (Cronbach's α=0.82). Factor 1 was good in

Table 1. Descriptive characteristics of adolescents.

Descriptive characteristics	n	%
Gender		
Female	127	47.9
Male	138	52.1
Class		
5th grade	23	8.7
6th grade	40	15.1
7th grade	20	7.5
8th grade	17	6.4
9th grade	53	20.0
10th grade	31	11.7
11th grade	42	15.8
12th grade	39	14.7
Family type		
Nuclear	196	76.9
Extended	43	16.9
Fragmented	16	6.3
Income level (monthly)		
Income less than expenses	35	13.8
Income equals expenses	126	49.8
Income more than expenses	92	36.4
	Mean ± SD	Med (min–max)
Age (years)	14.49±2.28	11–18
AIQ score	22.02±4.23	11–33
CASQ score	38.34±8.31	22–62

SD: standard deviation; AIQ: Adolescent Insomnia Questionnaire; CASQ: Cleveland Adolescent Sleepiness Questionnaire.

terms of internal consistency (Cronbach's α=0.83). Factors 2 and 3 were shown as excellent consistency (Cronbach's α=0.90 and Cronbach's α=0.93, respectively). The intraclass correlation coefficient (ICC) for the total AIQ score was found to be 0.822 and p<0.001 (Table 2).

It was analyzed between the AIQ and CASQ total scores using PC coefficient analysis, and the criterion-related validity (CRV) was examined by calculating the correlation coefficients. It was determined that there was a high level of positive (r=0.634, p<0.001) significant correlation between the AIQ and the CASQ total scores. In the correlation analysis, there was a significant correlation between AIQ factors 1 and 3 and CASQ total score (r=0.580 and r=0.200, p<0.010, respectively) (Table 2).

The mean score of AIQ is not statistically different between the test and retest results. According to the PC analysis results, a high level of positive correlation was found between the total AIQ test and retest scores (r=0.675, p<0.001) (Table 2). These results indicate the reliability of the AIQ Turkish version.

According to the EFA analysis, it was determined that there was a three-factor structure in accordance with the original questionnaire model. The explanation rate of the model was determined as 71% in the EFA. The factor loadings were found like the original AIQ sample. The rotated factor loadings ranged from 0.53 to 0.96 (Table 3). The results of KMO (0.77) and BTS (p<0.05) indicate that the sample was sufficient for the EFA.

To test the fit of the data to the model, CFA was conducted according to various fit indices such as chi-square/degree of freedom (χ^2/df), root-mean-square error of approximation (RMSEA) with its 90% confidence interval (CI), comparative fit index (CFI), incremental fit index (IFI), normed fit index (NFI), and Tucker-Lewis index (TLI). It shows that the model is at an acceptable level when CFI, IFI, and TLI are greater than 0.90 (0.96, 0.95) and the RMSEA value of 0.08. The χ^2/df ratio can be used as a measure of fit. It was determined that the χ^2/df value is below the desired value of 3 (χ^2/df=2.861; CFI=0.966; IFI=0.966; TLI=0.956; NFI=0.949; RMSEA=0.084). The CFA

Table 3. Factor loadings in the three-factor model of Adolescent Insomnia Questionnaire (13 items).

Questionnaire factors		Rotated factor loadings
Item 3*	Factor 1 Sleep dissatisfaction and impairments	0.858
Item 5		0.836
Item 10		0.534
Item 11		0.676
Item 12		0.831
Item 13		0.650
Item 1	Factor 2 Sleep onset	0.575
Item 4*		0.964
Item 7		0.966
Item 9*		0.961
Item 2	Factor 3 Sleep maintenance	0.895
Item 6		0.947
Item 8*		0.956

*Item is reverse-scored.

Table 2. The validity and reliability results of Adolescent Insomnia Questionnaire.

Internal consistency analysis of AIQ								
Internal consistency				Cronbach's α		ICC		p
AIQ total score				0.82		0.822		<0.000
Sleep dissatisfaction and impairment subscale				0.83				
Sleep onset subscale				0.9				
Sleep maintenance subscale				0.93				
AIQ and CASQ correlation								
AIQ		CASQ		Pearson correlation coefficient				p
Mean	SD	Mean	SD	R				
22.026	4.238	38.343	8.319	0.634				<0.001
Test-retest reliability analysis of AIQ								
AIQ	Test		Retest		Paired t-test results			
	Mean	SD	Mean	SD	Difference Test-retest	Difference	t	p
	22.098	3.948	23.114	3.768	-1.016	3.117	-2.547	<0.01

ICC: intraclass correlation coefficient; SD: standard deviation.

results of this study are accepted as excellent values. χ^2/df, NFI, CFI, IFI, and TLI values show a perfect fit, while the RMSEA value shows an acceptable fit. The three sub-dimension structure of AIQ is confirmed according to CFA.

DISCUSSION

In terms of both duration and quality, sleep is important for adolescent health. Unhealthy sleep during adolescence includes quantitative (short sleep duration, irregular sleep schedule) and qualitative aspects (night awakenings and difficulties falling asleep)[12]. These aspects lead to the development of insomnia. Although insomnia is the most common problem among sleep disorders in adolescents[3,4], there is no scale/instrument other than the AIQ that directly measures insomnia in adolescents.

The short, easy-to-understand, and self-reported AIQ, which is suitable for the age and developmental level of children, can be used to determine insomnia. The AIQ cutoff score can be used to identify adolescents with insomnia during routine assessments of healthy adolescents. It is important to adapt the AIQ to different countries, languages, and cultures. The aim of this study was to assess the validity and reliability of AIQ on Turkish adolescents.

For content validity, CVR was calculated for each item of the questionnaire by taking the opinions of researchers who are experts in their fields. In line with expert opinions, the minimum CVR values vary according to the number of experts, but it is expected that the CVR value collected from experts will be higher than 0.50[13]. The CVR values of the AIQ Turkish version were determined to be higher than 0.875. Therefore, it can be said that the content validity of the AIQ was ensured.

It should be determined whether there are latent factors that should be done first in questionnaire development and adaptation studies[11]. EFA was used to determine the construct validity of the AIQ Turkish version. In this study for EFA, the concordance of the correlation coefficients between the variables was evaluated with BTS ($p<0.05$), and the sample adequacy was evaluated with the KMO test (KMO coefficient=0.77). The KMO must be higher than 0.50 for the adequacy of the sample size. Whether the questionnaire is suitable for factor analysis is evaluated by BTS significance ($p<0.05$). When the p-value is <0.05 for BTS, it is accepted that the questionnaire is relevant to the EFA[11]. It was determined that the AIQ Turkish version explained 71.76% of the total variance and a three-factor structure was obtained in the EFA. While the EFA was 69.21% and the factor loads were between 0.50 and 0.90 in the original questionnaire[6], it was determined that the factor loads of the AIQ Turkish version items were between 0.53 and 0.96 in this study. These values are acceptable ranges[11].

In this study, the three sub-dimension structure of AIQ was confirmed according to CFA. CFA was conducted according to various fit indices such as χ^2/df, RMSEA with its 90%CI, CFI, IFI, NFI, and TLI[10,14-18]. When the model fit index, CFI, NFI, IFI, TLI, and RMSEA values of the AIQ Turkish version are examined, it is observed that the model is at an acceptable level[16-18]. The χ^2/df ratio can be used as a measure of fit, and a ratio less than 5 is considered a good fit. The desired χ^2/df value is below 3, and this value is found to be 2.86 in this study. The other fit indices scores were CFI=0.966, IFI=0.966, TLI=0.956, NFI=0.949, and RMSEA=0.084 in this study. χ^2/df, NFI, CFI, IFI, and TLI values show a perfect fit, while the RMSEA value shows an acceptable fit. The CFA results of this study are accepted as excellent values according to the Measurement Models Fit Index and Accepted Values[19-21]. The fit indices in the original AIQ were RMSEA=0.097, CFI=0.92, and TLI=0.90[6].

The relationship between the CASQ and the AIQ Turkish version score was examined using the Pearson product-moment correlation analysis, and the CRV was examined by calculating the correlation coefficients. It was determined that there was a high level of positive ($r=0.634$, $p<0.001$) significant correlation between the AIQ and the CASQ scores.

The Cronbach's α value for the total AIQ score was found to be 0.82 in this study. The Cronbach's α values of factors 1, 2, and 3 which are sub-dimensions of AIQ were found to be 0.90, 0.83, and 0.93, respectively. These results show that the AIQ Turkish version is highly reliable[21]. In the original AIQ study, while Cronbach's α of AIQ was found to be 0.91, the sub-dimensions of factors 1, 2, and 3 of AIQ Cronbach's α were found to be 0.87, 0.79, and 0.89, respectively[6]. The Cronbach's α of the AIQ Danish version was 0.88. The sub-dimensions of factors 1, 2, and 3 of Cronbach's α values in the AIQ Danish version were found to be 0.87, 0.84, and 0.73, respectively[22]. Besides, ICC was checked for internal consistency analysis of AIQ. While the ICC for the AIQ Turkish version total score was specified as 0.822 in this study, the ICC for the AIQ Danish version was 0.890[22].

In the AIQ Turkish version retest, it was not found between the test and retest with the paired t-test analysis. A high level of positive correlation was found because of the PC analysis between the AIQ score and the AIQ retest score ($r=0.675$, $p<0.001$). According to the results of the AIQ which were performed by three different research groups in three different countries including this study, this questionnaire is an important tool for measuring insomnia in adolescents and

can be used safely to test the clinical insomnia status[6,22]. However, further research is needed to determine all the features of the AIQ and to reveal its use in different countries and cultures.

CONCLUSION

The findings of this study showed that AIQ is a valid and reliable tool for evaluating insomnia in Turkish adolescents aged 11–18 years. AIQ is a brief, practical, self-reported, age-appropriate, easily applicable, valid, and reliable tool in Turkish.

AUTHORS' CONTRIBUTIONS

PT: Conceptualization, Data curation, Formal Analysis, Writing – original draft, Writing – review & editing. **EE:** Conceptualization, Data curation, Formal Analysis, Writing – original draft, Writing – review & editing.

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ORIGINAL ARTICLE

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Levosimendan: efficacy and safety in pediatric heart failure treatment

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SUMMARY

OBJECTIVE: The objective of this study was to assess the effectiveness and safety of levosimendan as an alternative treatment for pediatric patients with decompensated heart failure unresponsive to conventional inotropes and to emphasize its role in enhancing cardiovascular stability.

METHODS: A total of 15 pediatric patients with decompensated heart failure, stemming from acute fulminant myocarditis (53.3%) and post-congenital heart disease surgery complications (46.7%), received levosimendan. The evaluation focused on adverse effects, respiratory support requirements, and concurrent inotropic medication use during levosimendan treatment. Key cardiovascular parameters were assessed at 0, 6, 12, and 24 h post-levosimendan infusion.

RESULTS: Levosimendan administration significantly improved key cardiovascular metrics. Left ventricular ejection fraction increased notably from 45±14.8% to 58±15.6% at 24 h (p<0.001). Systolic and diastolic blood pressures rose significantly, with systolic increasing from 79 (68–90) to 98 (89–109) mmHg and diastolic from 47 (40–57) to 66 (54–76) mmHg by 24 h (p<0.001). Heart rate decreased from 162 (111–175) to 132 (99–148) bpm (p=0.02), and lactate levels significantly decreased from 4.15 (2.3–6.5) to 1.85 (0.8–2.6) mmol/L within 6 h (p<0.001).

CONCLUSION: Levosimendan demonstrates its significance in managing pediatric heart failure, indicating its safety and potential to enhance cardiac outcomes by reducing reliance on traditional inotropes.

KEYWORDS: Cardiac inotropism. Heart Failure. Levosimendan. Low cardiac output syndrome. Myocarditis. Pediatrics.

INTRODUCTION

Heart failure (HF) is a complex clinical syndrome characterized by structural or functional cardiac abnormalities that impair the heart's ability to fill with or eject blood. This deficiency leads to inadequate tissue perfusion and an inability to meet the body's metabolic demands[1]. Acute HF is distinguished by symptoms of congestion, reduced blood flow, tachycardia, and hypotension, often resulting from rapid changes in cardiac structure or function over minutes to hours[2]. In pediatric populations, HF's etiology significantly differs from adults, predominantly due to congenital heart disease, postoperative reperfusion injury, and severe myocarditis[3].

Low cardiac output syndrome (LCOS) describes a state of reduced cardiac output resulting from transient myocardial dysfunction, commonly observed in severe sepsis, myocarditis, and various cardiomyopathies, and as a significant complication after cardiac surgery[4]. LCOS is identified by a constellation of laboratory and clinical signs, including elevated blood lactate levels, low central venous oxygen saturation, decreased urine output, reduced left ventricular ejection fraction (LVEF), and an increased need for inotropic support[5].

The ongoing research for an optimal inotropic agent to treat acute decompensated HF highlights the limitations of current therapies in reducing symptoms and morbidity. With its unique mechanism of enhancing myocardial contractility without increasing myocardial oxygen demand, levosimendan has shown promise in cases of decompensated HF refractory to standard therapy[6]. By sensitizing myocardial cells to calcium at the systolic phase while preserving diastolic function and preventing cellular damage through controlling intracellular calcium influx, levosimendan improves ventricular function[7].

Although studies have shown efficacy in improving cardiac function in the pediatric intensive care unit (PICU), in the treatment of severe HF, and in the management of acute fulminant myocarditis and postoperative cardiac conditions, there are no established guidelines[8]. Conventional inotropic therapies are sometimes inadequate despite high doses. New treatment modalities and guidelines are needed for decompensated HF in children. At this stage, levosimendan is a very promising agent that may replace some of the agents in the treatment modalities. This study evaluates the efficacy and safety of levosimendan in pediatric patients with LCOS and decompensated HF following

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cardiac surgery or acute myocarditis. The therapeutic benefit of levosimendan in improving cardiac output and overall cardiovascular stability in this vulnerable population will be assessed.

METHODS

Study design and population

A retrospective analysis was conducted, examining clinical records of pediatric patients administered levosimendan from May 1, 2017, to July 1, 2019, in the PICU at Health Sciences University, Kayseri City Hospital. Approval was obtained from the Ethics Committee of Erciyes University for the use of patient data, and informed consent was secured from legal guardians.

A total of 15 pediatric individuals who were diagnosed with decompensated HF, post-acute myocarditis, or following cardiac surgery and were treated with levosimendan were included in this study. Exclusion criteria involved patients with chronic comorbidities.

Data collection method

Patient records were retrospectively reviewed for diagnostic specifics, etiology of HF, prior medication use, results of physical examinations, laboratory data, arterial blood gas and lactate levels, levosimendan dosage, and vital signs (including blood pressure and heart rate) during treatment, as well as urine output.

Therapeutic approach

Before levosimendan administration, epinephrine and milrinone were used as standard inotropic therapies. Levosimendan was introduced in cases of persistent low cardiac output despite high-dose inotropic therapy, characterized by tachycardia, elevated lactate levels, reduced ejection fraction, and low systolic pressure. Administration began 48–96 h after acute HF onset, with a loading dose of 12 pg/kg over 1 h, followed by a continuous infusion of 0.1 pg/kg/min for 24 h, unless adverse effects necessitated dosage modification. Treatment was carried out in the PICU, with continuous arterial monitoring and rhythm surveillance for real-time tracking of blood pressure and heart rate.

Vasoactive inotropic score calculation

The VIS was calculated using the following equation: [dopamine (μg/kg/min)]+[dobutamine (μg/kg/min)]+[100 × epinephrine (μg/kg/min)]+[10 × milrinone (μg/kg/min)]+[10,000 × vasopressin (U/kg/min)]+[100 × norepinephrine (μg/kg/min)]. Data were collected at baseline (T0) and at 6, 12, and 24 h (T6, T12, and T24) following the initiation of the levosimendan infusion, as extracted from the ICU database.

Statistical analyses

Data were analyzed using SPSS for Windows (Version 22.0) and Sigma Stat (Version 3.1). The Shapiro-Wilk test was applied to determine the distributional characteristics of all variables. Parameters with normal distribution were reported as mean±SD, while those with non-normal distribution were presented as median (interquartile range: 25th–75th percentile). For intra-group comparisons, parametric variables were analyzed using the paired-sample t-test, and non-parametric variables were assessed via the Wilcoxon test. The Friedman test was employed for intergroup comparisons of non-parametric data, including hemodynamic and blood gas values and echocardiographic parameters. A p-value of <0.05 was considered statistically significant.

RESULTS

Patient demographics and heart failure etiology

The study encompassed 15 pediatric patients treated with levosimendan for HF. The detailed characteristics of HF are presented in Table 1. Acute fulminant myocarditis was identified as the primary etiology in 53.7% (8 patients), while the remaining 46.7% (7 patients) had postoperative complications from congenital heart surgeries. The surgeries included ventricular septal defect repair in 33.3% (5 patients), atrioventricular septal defect repair in 6.7% (1 patient), and tetralogy of Fallot repair in 6.7% (1 patient). There was one fatality on the seventh postoperative day in a patient with tetralogy of Fallot, and all other patients survived.

Respiratory support and inotropic therapy during levosimendan treatment

Data on respiratory support and inotropic therapies during levosimendan administration are detailed in Table 2.

Table 1. Patient characteristics and etiology in the administration of levosimendan.

Patients (numbers)	15
Age, months	20 (2–156 min–max.)
Length, cm	76 (54–158 min–max.)
Weight, kg	11.2 (4.2–62 min–max.)
Male/female, n	7/8
Etiology of heart failure	
Acute fulminant myocarditis	8 (53.4%)
Postoperative cardiac surgery	7 (46.7%)

Mechanical ventilation was required for 66.7% (10 patients), while high-flow oxygen was provided to 33.3% (5 patients).

Concomitant inotropic therapy included a combination of epinephrine and milrinone in 66.6% (10 patients), epinephrine, milrinone, and dopamine in 13.3% (2 patients), and epinephrine, milrinone, and norepinephrine in 20% (3 patients).

Analysis of hemodynamic, blood gas, and echocardiographic parameters

Significant improvements were observed in several parameters following levosimendan infusion. LVEF increased from 45±14.8% at baseline to 58±15.6% at 24 h (p<0.001, Table 3). Systolic pressure showed significant increases at 12 h [91 (83–101) mmHg] and 24 h [98 (89–109) mmHg] compared with baseline [79 (68–90) mmHg] (p<0.001). Diastolic pressure also increased significantly at 24 h [66 (54–76) mmHg] versus baseline [47 (40–57) mmHg] (p<0.001). Heart rate decreased from 162 (111–175) at baseline to 132 (99–148) at 24 h (p=0.02). Lactate levels decreased significantly from 6 h [1.85 (0.8–2.6) mmol/L] compared with baseline [4.15 (2.3–6.5) mmol/L] (p<0.001, Table 3). No significant changes were observed in partial arterial carbon dioxide pressure ($PaCO_2$), urine output, and vasoactive inotropic score (p=0.39, p=0.09, and p=0.11, respectively). A summary of hemodynamic parameters after a 24-h levosimendan infusion is presented in Table 3.

Table 2. Overview of respiratory support and concomitant inotropic medications during levosimendan administration.

Patients (n=15)	n (%)
Respiratory support therapy	
Mechanically ventilated	10 (66.7%)
High flow oxygen	5 (33.3%)
Concomitant inotropes	
Epinephrine + milrinone	10 (66.6%)
Epinephrine + milrinone + dopamine	2 (13.3%)
Epinephrine + milrinone + norepinephrine	3 (20%)

DISCUSSION

The results of this investigation have highlighted the efficacy of levosimendan in improving cardiac function in pediatric patients with HF, particularly following acute fulminant myocarditis and post-operative congenital heart disease scenarios. Initiated with the aim of exploring the role of levosimendan in a pediatric setting, this study has supported the hypothesis that levosimendan can significantly improve heart rate, systolic and diastolic pressure, pH, and lactate levels, thereby improving cardiac function. Such findings reflect the growing interest in identifying and optimizing treatment strategies that can improve clinical outcomes in this vulnerable population, positioning levosimendan as a potential linchpin in the management of pediatric HF.

In reviewing the strengths of this study, it is important to highlight the novel insights it provides into the use of levosimendan in children. Despite well-documented efficacy in adult patients, pediatric-specific evidence remains relatively scarce. The study fills this gap by demonstrating the safety and therapeutic benefits of levosimendan in children, a cohort previously underrepresented in HF research. Notably, levosimendan was administered predominantly in cases where conventional inotropic support failed to maintain stable hemodynamics, highlighting its utility in challenging clinical situations.

Table 3. Changes in hemodynamic, blood gas, and echocardiographic parameters during 24-h levosimendan infusion.

	Before 0 h (n=15)	6 h (n=15)	12 h (n=15)	24 h (n=15)	p
Heart rate (bpm)	162 (111–175)	139 (114–163)	127 (104–151)	132 (99–148)[a]	**0.02**
Systolic pressure (mmHg)	79 (68–90)	89 (82–100)	91 (83–101)[b]	98 (89–109)[a]	**<0.001**
Diastolic pressure (mmHg)	47 (40–57)	55 (47–65)	61 (53–67)	66 (54–76)[a]	**<0.001**
pH	7.36 (7.29–7.44)	7.4 (7.35–7.43)	7.46 (7.39–7.50)[b]	7.45 (7.38–7.48)	**0.04**
Lactate (mmol/L)	4.15 (2.3–6.5)	1.85 (0.8–2.6)[c]	1.0 (0.8–1.85)	1.04 (0.8–1.45)	**<0.001**
pCO_2 (mmHg)	44 (29–49)	45 (36–48)	39 (33–42)	40 (35–46)	0.39
Urine output (mL/kg/h)	3.2 (1.8–5.07)	_	_	4.2 (2.7–5.2)	**0.09**
LV ejection fraction, %	45±14.8	_	_	58±15.6[a]	**<0.001**
Vasoactive inotropic score	60 (40–90)			55 (40–80)	0.11

Data are presented as mean ± SD or as median (25th–75th percentile), as appropriate. Notations a, b, and c indicate statistically significant differences: (a) between baseline and 24 h, (b) between baseline and 12 h, and (c) between baseline and 6 h, respectively. Statistically significant p-values are highlighted in bold.

The safety and efficacy of levosimendan for HF treatment have been well-established in adults, prompting its consideration for pediatric applications[9]. The adult-centric evidence base contrasts with the relatively sparse pediatric data, particularly for children with specific heart conditions such as cardiomyopathy or those experiencing LCOS when alternative inotropic treatments prove inadequate. Levosimendan's pediatric use shines in its capacity to support children through various challenging conditions, including post-cardiac surgery, cardiomyopathy, and HF, as demonstrated in a study with 27 children[10]. Our research adds to this growing evidence by highlighting levosimendan's effectiveness in pediatric patients afflicted with acute fulminant myocarditis and those facing postoperative complications following surgeries for congenital heart defects such as ventricular septal defects, atrioventricular septal defects, and tetralogy of Fallot, who exhibited low cardiac output in spite of intensive inotropic therapy.

The acknowledgment of levosimendan's potential to defer the need for mechanical assistive devices in pediatric cases of cardiomyopathy was documented in a survey where 89% of responding clinicians reported positive outcomes[11]. This aligns with our observations, showing levosimendan as a crucial stabilizer of hemodynamics, circumventing the immediate need for mechanical interventions. Furthermore, a meta-analysis encompassing 1,036 patients, both pediatric and adult, underscored levosimendan's ability to effectively lower serum lactate levels and bolster cardiac function[12], mirroring our findings among children with acute fulminant myocarditis and postoperative congenital heart diseases.

In detailing the effects of levosimendan post-congenital heart surgery, one study noted its advantages in 64 children, including reduced lactate levels, improved cardiac output, and maintained vasoactive inotropic scores[13]. Another piece of research found diminished inotropic support requirements, better ejection fraction, and decreased lactate levels following levosimendan treatment in 15 children, specifically addressing LCOS following cardiac surgeries[14]. These outcomes resonate with our study, which observed significant improvements in cardiac output parameters and lactate levels, pointing to enhanced myocardial contractility and tissue oxygenation in children with diagnoses of acute fulminant myocarditis and post-surgical complications from congenital heart repairs.

Contrasting insights emerge on renal function and urine output. Some studies suggest renal benefits from levosimendan[15], yet our investigation did not reveal notable changes in urine output, implying that the synergistic effect of inotropes and inodilators, like milrinone, was likely instrumental in preserving renal perfusion across our patient group, particularly those recovering from congenital heart surgeries.

The respiratory advantages of levosimendan, particularly in adult cohorts with challenges in weaning from mechanical ventilation, have been documented[16]. Our findings, showing beneficial impacts on pH and lactate levels without significant alterations in blood carbon dioxide levels, hint at a targeted improvement in metabolic efficiency and potentially respiratory muscle functionality, especially relevant for children with acute fulminant myocarditis and those recovering from surgical interventions for congenital heart defects.

Addressing the limitations of our study is critical to contextualizing its contributions. The retrospective design and modest sample size of 15 patients may limit the extrapolation of our findings to the broader pediatric population. Furthermore, the etiological diversity within our cohort, ranging from acute fulminant myocarditis to post-cardiac surgery conditions, requires a cautious interpretation of the results. Such heterogeneity highlights the complexity of pediatric HF and underscores the need for further research tailored to different patient subgroups.

Considering the wider implications of levosimendan use, it is clear that this study adds to the existing literature by providing pediatric-specific data, thereby filling a critical gap. As demonstrated in our cohort, the safety and efficacy of levosimendan suggest its potential to reduce reliance on traditional inotropes, potentially mitigating associated risks and improving patient outcomes. This finding is invaluable to clinicians navigating the complex landscape of pediatric HF management and offers a glimmer of hope for improved therapeutic strategies.

In conclusion, this study represents a significant advance in our understanding of the role of levosimendan in the management of pediatric HF. By demonstrating its beneficial effects on key cardiac parameters and its safety profile, the study not only adds to the growing body of evidence supporting the use of levosimendan in children but also highlights the need for further research. Future studies, ideally with larger sample sizes and prospective designs, are essential to fully delineate the efficacy of levosimendan and optimize its use in pediatric HF, ultimately aiming to improve the quality of care and outcomes for this vulnerable patient population.

ETHICAL ASPECTS

This study was conducted in accordance with the ethical standards outlined by the responsible committee for human experimentation and in compliance with the Helsinki Declaration.

Institutional Ethical Committee approval was obtained, and informed consent was secured from all participants involved in the research.

AUTHORS' CONTRIBUTIONS

MAD: Data curation, Writing – original draft. **MY:** Data curation. **MA:** Writing – review & editing.

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ORIGINAL ARTICLE

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The role of serum adropin in determining the clinical outcomes of patients with traumatic brain injury: a case-control study

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SUMMARY

OBJECTIVE: It has been determined that adropin has a role in tissue healing. This study aimed to determine the effects of head trauma on the tissues and blood levels of patients admitted to the emergency department.

METHODS: The study group was divided into two to compare the adropin level in healthy individuals and patients with head trauma. Blood tests from patients and healthy volunteers were compared using the adropin kit. Adropin levels, Glasgow Coma Scale, and revised scores of trauma patients were recorded and analyzed.

RESULTS: All patients in the trauma group had significantly higher adropin levels than the control group. Among these patients, the adropin level of the discharged patients was higher than the others. In addition, patients with high Glasgow Coma Scale and normal blood pressure were found to have higher adropin levels than the others.

CONCLUSION: Although adropin cannot make a sharp distinction in determining the prognosis, the increase in its level in trauma patients shows that it triggers a protective mechanism.

KEYWORDS: Adropin protein. Brain injury. Emergency room.

INTRODUCTION

Adropin is a peptide hormone encoded by the Energy Homeostasis Associated (Enho) gene located on chromosome 9 in the central nervous system (CNS), heart, kidney, liver, pancreas, and human umbilical vein[1]. Studies show that it modulates endothelial nitric oxide synthase (eNOS) expression and provides cytoprotective and vasculoprotective effects[2,3]. Studies have shown that the level of adropin is low during acute ischemia, increases gradually, and can be an alternative to troponin, especially in the follow-up of myocardial infarction[4]. It is thought that diseases such as diabetes and vasculitis may develop in their absence[5,6].

Studies at the molecular and cellular levels in recent years have shown that adropin, as a biochemical parameter, plays an essential role in the pathogenesis and progression of CNS diseases (stroke, bipolar disorder, schizophrenia, schizophrenia, bipolar disorder, Alzheimer's, Parkinson's, and Huntington's diseases) (one). In animal studies, it has been shown that adropin regulates locomotor activity and plays a role in the development of the cerebellum. It does this through the neuronal recognition molecule 3 (NB3/Notch) cascade as a membrane-bound protein[7,8]. In ischemia, it activates vascular endothelial growth factor receptor 2 (VEGFR2), and apoptosis occurs due to cascade activation. Thus, the width of the ischemia area in the brain increases in direct proportion to the level of adropin in the blood[9].

Head trauma is a type of injury that can be fatal. Considering the intensity of the emergency department (ED), new strategies are needed to facilitate patient follow-up and predict mortality, especially in the ED, which is the first place of admission for head trauma patients. This study indicated that adropin, whose various effects were discovered and popularized daily, could also be used in the follow-up of trauma patients. In addition, it will be discussed whether the findings obtained in the study affect the determination of medical priority in this patient group.

METHODS

Ethical approval

This study was approved by the Local Ethics Committee (2018-14/8.5.2018). Written and verbal consent was obtained from all patients or their relatives who participated in the study.

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Study type

The is a single-center, prospective, randomized, controlled study.

Study sample

All male and female patients over 18 years who came to the ED due to isolated head trauma between June 1 and August 30, 2018, and healthy volunteers without a history of trauma or any other complaint were included in the study.

Patients with multiple traumas who did not want to consent and were under 18 years old or the healthy population were excluded from the study.

Data collection

Written and verbal consent was obtained from the patients and healthy volunteers who volunteered to participate in the study, and they were divided into two groups: patient and control. The patients were stabilized hemodynamically, and 3-5 mL of blood samples were taken into the biochemistry tube at any time from healthy adult individuals in the control group in the early post-traumatic period (within the 6 h). Blood samples were centrifuged at 3,000 rpm for 5 min and kept at 80°C according to the manufacturer's protocol. Serum human adropin was determined using commercial enzyme-linked immunosorbent assay (ELISA) kits (BT-LAB, China). Serum samples were not diluted with those kits, and the standards were serially diluted from starting concentrations of 640 ng/L down to 40 ng/L for adropin in the sample diluent supplied with the kit. The intra- and inter-assay coefficient of variation for the assays is<10%.

Age, gender, vital signs, and trauma scores of all patients and volunteers in the study, as well as trauma scores [Glasgow Coma Scale (GCS) and Revised Trauma Score (RTS)], trauma mechanism, brain computed tomography (CT) findings, serum adropin levels, and clinical results (exitus, hospitalization, and discharge) were noted. Patients with trauma were divided into three groups: I (GCS 3-8), II (GCS 9-13), and III (GCS 14-15), according to GCS scoring, and serum adropin levels were compared between the groups.

Sample size and statistical method

In the descriptive statistics of the data, mean, standard deviation, median minimum, maximum, frequency, and ratio values were used. The distribution of variables was measured using the Kolmogorov-Smirnov test. The Kruskal-Wallis and Mann-Whitney U tests were used to analyze independent quantitative data. The chi-square test was used to analyze independent qualitative data, and the Fisher's test was used when the chi-square test conditions were not met. The effect level and cutoff value were investigated with the ROC curve. The SPSS 28.0 program was used in the analysis.

RESULTS

In the study, 58 (32.2%) were female, and 122 (67.8%) were male, of which 118 (65.5%) were in the patient group and 62 (34.5%) were in the control group. The mean age was 46.3±18.4 years in the control group and 44.8±18.8 years in the patient group (p>0.05). Adropin levels increased approximately two times in the patient group compared with the control group (p<0.05). A total of 26 patients (22.0%) were hospitalized, and 4 (15.3%) of these patients died (Table 1).

All patients in the trauma group had significantly higher adropin levels than the control group. Among these patients,

Table 1. Demographic data and adropin levels of the groups in the study.

	Mean±SD/n (%)			
	Healthy group	Discharged	Inpatient	p
Age	46.3±18.4	45.4±19.9	42.5±14.1	0.748[k]
Gender				
F	24-38.7%	30-32.6%	4-15.4%	
M	38-61.3%	62-67.4%	22-84.6%	0.101x[2]
Adropin Level	148.1±182.0	222.5±292.4	180.8±217.2	**0.001**[k]
Adropin ≤80	36-58.1%	24-26.1%	6-23.1%	
>80	26-41.9%	68-73.9%	20-76.9%	**0.000x**[2]
GCS I	-	-	9-34.6%	
II	-	-	11-42.3%	**0.000x**[2]
III	63-100%	92-100%	6-23.1%	

GCS: Glasgow Coma Scale; k: Kruskal-Wallis (Mann-Whitney U test); X^2: chi-square test.
Significant values (P<0.05) are marked in bold.

the discharged patients' adropin level was higher than the others (Figure 1).

The adropin cutoff level in the patient group in the study was accepted as 80 ng/l and divided into two groups. No pathology was detected in the CT results of 92 patients (77.9%). Subarachnoid bleeding was observed at the highest rate (53.8%) in patients with pathological CT results. Adropin level was high in most patients with normal or abnormal CT results. In addition, patients with high GCS and normal blood pressure had higher adropin levels than the others (Table 2).

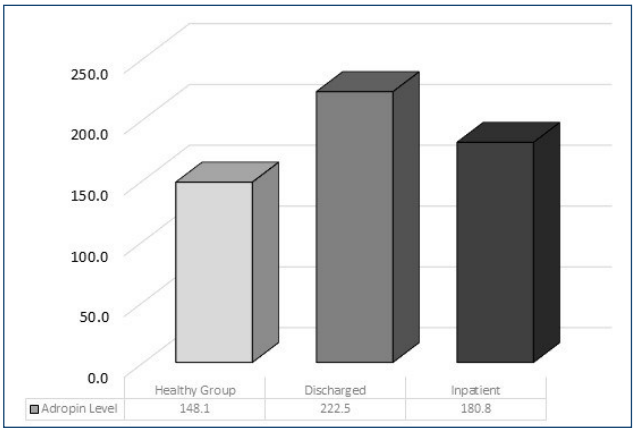

Figure 1. Comparison of adropin levels between groups.

DISCUSSION

Most of the studies on adropin are on its effects on endocrine or neurological diseases. This study showed that the level of adropin increased in trauma patients.

There was no difference in the mean age of the patients included in the study and healthy volunteers (p>0.05). Thus, the early inflammatory response after trauma, which is observed more rapidly in the elderly population than in young people, was not likely to trigger secondary brain damage[10]. Accordingly, the significantly higher adropin level in the patient group from the two physiologically homogeneous groups compared with the other group is strongly evident that adropin can be used in post-traumatic follow-up.

Cell necrosis that develops after hypoxia-induced vasospasm is held responsible for the pathophysiology of brain injury. This situation is like ischemic stroke, but in stroke, cerebral blood flow must decrease to deficient levels (5–8.5 mL/100 mL/min). In traumatic brain injury, necrosis develops after higher blood flow (15 mL/100 mL/min), which explains the higher adropin level observed in ischemic stroke in a shorter time[9,11]. In this study, the fact that the adropin level in the patient group was higher than the healthy group indicates that the adropin level increased with the hypoxic damage caused by the trauma. In addition, although the level of adropin increases in the acute period of traumatic brain injury, it can be predicted to

Table 2. Results in groups according to adropin levels.

		Adropin ≤ 80				Adropin > 80				p	
		Mean±SD n (%)			Median	Mean±SD n (%)			Median		
BT*	Normal	21		80.8%		71		77.2%		0.696	X^2
	Abnormal	5		19.2%		21		22.8%			
Epidural		1		3.8%		4		4.3%		1.000	X^2
Intraparenchymal hemorrhage		0		0.0%		1		1.1%		1.000	X^2
Subdural		1		3.8%		5		5.4%		1.000	X^2
SAH		4		15.4%		10		10.9%		0.530	X^2
Contusion		2		7.7%		3		3.3%		0.304	X^2
Linear fracture		3		11.5%		5		5.4%		0.372	X^2
Deplete fracture		0		0.0%		1		1.1%		1.000	X^2
Others		0		0.0%		2		2.2%		1.000	X^2
SBP		131.4	±	18.5	133.0	124.1	±	16.0	122.0	***0.018***	x^m
DBP		80.0	±	11.9	80.0	73.1	±	10.4	70.0	***0.003***	x^m
RTS		11.9	±	0.4	12.0	11.8	±	0.7	12.0	0.699	x^m

*More than one CT is found in the same patient. x^m: Mann-Whitney U test; X^2: chi-square test (Fisher's test). CT: computed tomography; SAH: subarachnoid hemorrhage; GCS: Glasgow Coma Scale; Others: diffuse axonal injury, extra-axial hemorrhage; SBP: systolic blood pressure; DBP: diastolic blood pressure; RTS: Revised Trauma Score.
Significant values (P<0.05) are marked in italics.

continue to increase during the post-traumatic tissue edema, degeneration, and regeneration process in long-term follow-up for patients for recovery.

Axonal damage after head trauma cannot be detected on imaging. The patient is followed up when neurological symptoms or bleeding are seen on CT. Apart from these, it may not show any symptoms in the acute period. The ED follow-up of patients in this group may be affected. A study found that endothelial proliferation and angiogenesis increased in mice injected with adropin after vascular injury[3]. In this study, the result was normal in most patients who underwent CT, but the adropin level was high, and the adropin level was high in most patients with pathology on CT. This demonstrates the promoting role of adropin in healing tissue damage regardless of trauma severity.

Studies have shown that adropin level is decreased in hypertensive patients compared with normotensive patients. It has been stated that these patients are prone to risks that may develop after endothelial dysfunction, and the endothelial protective effect of adropin has been emphasized[12,13]. In this study, adropin level was higher in normotensive patients than in hypertensive patients.

CONCLUSION

Most of the patients in the study were discharged because of high GCS levels, and most had high adropin levels. According to this, although adropin cannot make a sharp distinction in determining the prognosis, the increase in its level in trauma patients shows that it triggers a protective mechanism. In addition, the clinician may consider discharging the patient with a markedly high (200 ng/L) adropin level.

This study observed that the adropin level was higher in the patient group with high GCS. We think that in this patient group with a good prognosis and discharged from the ED, the adropin level can be taken at regular intervals during the outpatient control, and the tissue healing process after trauma and the complications that may develop can be followed up.

Limitations

There are some limitations in the study. Only the adropin level taken in the ED follow-up was measured. The adropin levels in the hospitalized patients' service or intensive care follow-up could not be determined. According to the result of this study, although it is predicted that the blood level will increase more significantly in long-term patient follow-up, long-term studies are needed to determine the time to return to the average level.

AUTHORS' CONTRIBUTIONS

ÖT: Conceptualization, Funding acquisition, Writing – original draft, Writing – review & editing. **OG:** Formal Analysis, Writing – original draft, Writing – review & editing. **İD:** Data curation, Writing – original draft, Writing – review & editing. **ÖS:** Conceptualization, Writing – review & editing. **MY:** Data curation. **OD:** Data curation. **EÖA:** Data curation.

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ORIGINAL ARTICLE

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Validity of the Brazilian online version of the Sexual Desire Inventory 2

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SUMMARY

The Sexual Desire Inventory 2 is a self-report instrument for assessing sexual desire in men and women. In Brazil, there is no validated sexual desire self-report for the adult population.

Objective: The aim of this study was to determine the evidence of validity for the content and construct of the Brazilian online version of the Sexual Desire Inventory 2.

Methods: This was a cross-sectional study with Brazilian men and women. The sample size was calculated using the criterion of more than 20 participants per item. The invitation to participate in the study was conducted online by the platform Survey Monkey®. The Sexual Desire Inventory 2 was evaluated for content, construct, reliability, and invariance.

Results: A total of 818 female and male adults participated in the study. The two-dimensional factorial solution represented 71% of the total variance explained by the model, and the factorial loads of the model were ≥0.40; commonalities presented values ≥0.23. Reliability was measured by the coefficients of Cronbach's alpha with a total score of 0.87, McDonald's of 0.87, Omega, and greatest lower bound with a total score of 0.95. The metric invariance was tested for the sex variables ΔCFI (comparative fit index) and ΔRMSEA (root mean square error of approximation) with a total score of 0.01.

Conclusion: The analyses indicate evidence of robust validity in the Brazilian online version of the Sexual Desire Inventory 2.

KEYWORDS: Libido. Reproducibility of results. Psychological tests. Psychometric. Sexual health.

INTRODUCTION

The World Association of Sexual Health recently adopted sexual pleasure, defined as "the physical and/or psychological satisfaction and enjoyment derived from shared or solitary erotic experiences, including thoughts, fantasies, dreams, emotions, and feelings," as the cornerstone of sexual health[1].

In Brazil, two studies showed that the most relevant problem is low sexual desire[2,3]; because of the Hypoactive Sexual Desire Disorder has been associated with biological and psychological causes[4], validated instruments of measurement are essential to adequately assess sexual desire in the population[5] by determining the prevalence of estimates and showing the evidence of the problem. However, there are no validated online instruments to measure sexual desire or the construct of sexual desire in Brazil[6]. In addition, the measurement of the evaluation of sexual desire through the use of multi-domain instruments of sexual function is feasible. However, it may not be adequate to evaluate the construct of sexual desire[7] because it can potentially compromise some of its psychometric properties[8,9]. The Sexual Desire Inventory 2 (SDI-2)[10] is a measuring instrument that has been adapted to other cultures[11-13] and has now been culturally adapted and validated for the Brazilian population. Therefore, the present study aimed to demonstrate evidence of the validity of the Brazilian online version of the SDI-2.

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METHODS

Study design

This was a cross-sectional study conducted between May and October 2018, with Brazilian men and women, to determine the evidence of validity for the content and construct of the Brazilian online version of the SDI-2.

Participants and procedures

Participants were selected based on the following inclusion criteria: women and men over 18 years of age, literate, and capable of understanding the content of the SDI-2. The sample size was calculated using the criterion of more than 20 participants per item in the SDI-2[8].

The invitation to participate in the study was conducted online by sending a URL (uniform resource locator) link made available through the social networks Facebook® and Twitter® and by e-mail invitations. The link directed users to the invitation to participate in the study and, subsequently, to the platform Survey Monkey, where participants had access to the Informed Consent Terms (TCLE).

Exploratory factor analysis

The adequacy of the correlation matrix was evaluated through Bartlett's statistic and Kaiser-Meyer-Olkin (KMO) tests and analyzed using the polychoric correlation and considering the amplitude of the scale from 0 to 8[8].

For the dimensionality testing, parallel analysis was applied through the optimal implementation of parallel analysis. In addition, the UNICo (one-dimensional congruence) >0.95; the ECV (explained common variance) >0.85; or the MIREAL (mean of item residual absolute loading) <0.30[8] was used to confirm if the model was unidimensional or multidimensional.

The robust unweighted least squares was used for data extraction, associated with a bootstrap (n=5,000) and the direct oblimin rotation. The two-dimensional model was adopted as the initial model and as the original instrument. Factorial solutions were evaluated by factorial saturation >0.40, with total explained variance >60%, and commonalities >0.40[8].

Pratt's importance measures[14] were used as a way of complementing the factorial solution. This method helps to solve three difficulties of interpretation that arise in oblique models. First, it integrates the information between the standard and structure coefficients. Second, it restores horizontal and vertical addition properties while allowing factors to be oblique. Third, it solves, in part, the traditional problem of rules to evaluate the meaning of the relationship between the observed variable and the factor[8].

The confirmatory factor analysis (CFA) was evaluated by the factorial model index adjustments, the root mean square error of approximation (RMSEA) ≤0.006, the non-normed fit index (NNFI; Tucker & Lewis) >0.95, the comparative fit index (CFI) >0.95, the goodness-of-fit index (GFI) >0.95, and the adjusted goodness-of-fit index (AGFI) >0.95[15].

Reliability, quality, and replicability of the factorial solution

Reliability was evaluated by the coefficients of Cronbach's alpha, the greatest lower bound (GLB), and McDonald's Omega.

The quality of the factorial solution and replicability of the model were tested by the generalized H (GH) index, and the quality and effectiveness of estimates of factors' scores were calculated by the factor determinacy index (FDI) and the ORION marginal reliability[8].

Invariance

The metric invariance was tested with the ΔCFI and ΔRMSEA between a sample of men and women. The difference between models should not be greater than 0.01 for ΔCFI and 0.015 for ΔRMSEA[16].

Study approval by the University Institutional Review Board was obtained prior to commencing the study (CAAE number 79325517.2.0000.5393). Additionally, all participants signed an online free and informed consent form according to Resolution 466/12 of the Brazilian National Council of Health.

Measures

Sociodemographic characteristics

A structured sociodemographic and clinical questionnaire comprising 12 questions and including personal data such as date of birth, country of residence, sex, marital status, education, occupation, race, history of chronic illness, religion, relationship length, sexual preference, and frequency of sexual activity was used.

Sexual Desire Inventory 2

The Brazilian version of the Sexual Desire Inventory 2 was applied to determine evidence of validity. The cultural adaptation of the instrument, which preceded the present validation study, has been previously reported in detail. The Brazilian version of the Sexual Desire Inventory 2 includes 14 items: 4 of them with scores ranging from 0 to 7 and related to the frequency of desire, and the remaining 10 items are answered on a scale with scores ranging from 0 to 8. The scores from items 1 through 8 are added to obtain the sexual desire score in a

relationship, while scores from items 9 through 11 are added to obtain the solitary sexual desire score. SDI-2 scores range from 0 to 112[10].

Data analysis

The statistical analyses were performed using the FACTOR software version 10.8.04 with a statistical power of 95% and a significance index of 0.05, and the IBM SPSS AMOS software version 22.0 with a statistical power of 95% and a significance index of 0.05. The descriptive statistical analyses of the sociodemographic variables were performed, and the minimum and maximum frequencies and percentages were calculated. Measurements of central tendency and dispersion were calculated for the variable of age.

RESULTS

A total of 960 participants were recruited, of whom 818 agreed to participate. Out of these, 142 participants were excluded due to the incomplete filling of collection instruments, and the final sample comprised 818 subjects. Of note, 65.8% (n=538) were women and 34.2% (n=280) were men. Table 1 shows the sociodemographic characteristics of the study participants.

Table 1. Descriptive characteristics of the respondents (n=818).

Characteristics	min–max	n (%)
Chronic disease		
No		672 (82.2)
Yes		146 (17.8)
Religion (active participation)		
No		521 (63.7)
Yes		297 (36.3)
Relationship		
No		301 (36.8)
Yes		517 (63.2)
Sexual preference		
By women		187 (22.9)
By men		487 (59.5)
For men and women		130 (15.9)
Rather not answer		14 (1.7)
Sexual activity		
Two or three times a month		147 (18.0)
Twice a week		136 (16.6)
More than once a day		13 (1.6)
Not once		111 (13.6)
Three or four times a week		119 (14.5)
Once a month		130 (15.9)
Once a day		24 (2.9)
Once a week		138 (16.9)

Construct validity

The suitability of the sample pointed to a KMO=0.85 and Bartlett's statistics value of 74.7 ($p<0.010$), indicating the good factorability of the data. The analysis of dimensionality performed by the robust parallel analysis indicated the existence of two dimensions. The complementary indicators for dimensionality also indicated a multidimensional model with UNICo=0.873; ECV=0.675, and MIREAL=0.383.

The two-dimensional factorial solution represented 71% of the total variance explained by the two-dimensional model. The configuration was defined as Factor 1 (responsive sexual desire interpreted as sexual desire in the relationship) retaining items 1, 2, 3, 4, 5, 6, 7, 8, and 9, and Factor 2 (related to spontaneous sexual desire interpreted as solitary sexual desire) retaining items 10, 11, 12, and 13. Table 2 presents the values of factorial loads, commonalities, and Pratt's measures. Table 3 presents the adjustment index values observed in the one- and two-factor models of the CFA.

The factorial loads of the model were ≥0.40, and commonalities presented values ≥0.23. The technique of Pratt's measures reaffirmed the alignment of items in two factors, corroborating the solution proposed in the factorial analysis.

Reliability, quality, and replicability of the factorial solution

Reliability was evaluated by the values of the Cronbach's alpha coefficient for the instrument, with a total score of 0.87; for the subscale of desire in a relationship, 0.84; and for the subscale of solitary desire, 0.91. The McDonald's Omega coefficient value was 0.87, and the GLB coefficient value was 0.95.

The stability of the Brazilian version of the SDI-2 was evaluated through the GH index, with a value of 0.90 for the subscale of solitary desire and a value of 0.93 for the subscale of sexual desire in a relationship. The quality and effectiveness of estimates were evaluated through the FDI, which indicated the values of 0.95 and 0.96, and through the ORION marginal reliability, which indicated the values of 0.90 and 0.93 for the first and second factors, respectively. All indicators were above the stipulated minimum limits.

Table 2. Standardized factor loadings, communalities (h2), and confirmed factorial solutions from the exploratory factorial analysis.

Item number of the Sexual Desire Inventory 2 (SDI-2)	Número do item do Inventário de Desejo Sexual 2 (IDS-2)	Factor loading		Communalities	Pratt's measure	
		F1	F2	h2	F1	F2
1	1	0.64	0.04	0.43	0.42	
2	2	0.62	0.12	0.45	0.41	
3	3	0.81	-0.05	0.64	0.64	
4	4	0.44	0.14	0.25	0.21	
5	5	0.40	0.16	0.23	0.18	
6	6	0.57	-0.12	0.30	0.30	
7	7	0.86	0.01	0.73	0.73	
8	8	0.65	0.03	0.43	0.43	
9	9	0.80	-0.00	0.64	0.64	
10	10	0.12	0.74	0.63		0.58
11	11	-0.00	0.91	0.83		0.83
12	12	-0.06	0.92	0.81		0.81
13	13	0.01	0.88	0.79		0.79

F1: dyadic sexual desire; F2: solitary sexual desire; h2: communalities; Pratt's importance measures; $p<0.05$.

Table 3. Summary of goodness-of-fit statistics for Sexual Desire Inventory 2.

Model CFA	X^2	df	X^2/df		RSMEA	NNFI	CFI	GFI	AGFI
Two factors	339.133	53	6.39		0.121	0.91	0.939	0.979	0.968
Metric invariance across sex for SDI-2	**Model**	**n**	**X^2**	**Df**	**X^2/df**	**CFI**	**RMSEA**	**ΔCFI**	**ΔRMSEA**
Female	Two factor	538	217.078	53	4.09	0.941	0.123	0.001	0.013
Male	Two factor	280	145.469	53	2.74	0.94	0.11		

CFA: confirmatory factor analysis; RMSEA: root mean square error of approximation; NNFI: non-normed fit index; CFI: comparative fit index; GFI: goodness-of-fit index; AGFI: adjusted goodness-of-fit index.

Invariance

The metric invariance (Table 3) showed stability between the models for the female and male genders. The ΔCFI and ΔRMSEA resulted in 0.01, that is, within limits established in the literature.

DISCUSSION

The present study aimed to demonstrate evidence of the validity of the online version of the SDI-2 instrument. Furthermore, the increase in the validation of measurement instruments has impacted new proposals for cultural adaptation and/or validations of online versions, which brings multiple advantages[9,17].

One study showed that web-based data collection does not statistically increase or decrease the consistency of responses, nor does it compromise the integrity of the test, and it is a suitable alternative to more traditional methods[18].

Corroborating the results found in the present study, some validation studies have demonstrated adequate results using different psychometric techniques[19-21]. The choice of techniques applied in this study aimed at increasing the accuracy and consistency of analyses[8,16,22,23].

CONCLUSION

The online version of the SDI-2 is a self-report that presents satisfactory, and at first, stable, construct validity evidence with a final model composed of 14 items and divided into two dimensions.

Future studies using the Brazilian online version of the SDI-2 may be essential to estimate the prevalence of sexual desire disorder in men and women and to identify effective interventions that promote sexual health and well-being in the Brazilian adult population.

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AUTHORS' CONTRIBUTIONS

DCR: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Methodology, Visualization, Writing – original draft, Writing – review & editing. **MFT:** Conceptualization, Data curation, Formal Analysis, Funding acquisition, Methodology, Visualization, Writing – original draft, Writing – review & editing. **LHA:** Conceptualization, Data curation, Formal Analysis, Funding acquisition, Methodology, Visualization, Writing – original draft, Writing – review & editing. DTS: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Methodology, Visualization, Writing – original draft, Writing – review & editing. **FR:** Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **LASL:** Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **RAA:** Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **LCN:** Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

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ORIGINAL ARTICLE

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Cogent integration of inflammatory biomarkers and perioperative complications of thyroid surgery in thyroidology

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SUMMARY

OBJECTIVE: Back to the sources, postoperative nausea and vomiting, hypo- and hypertension, heart rate alterations, and hypoxemia due to laryngospasm might be considered perioperative complications.

METHODS: This cross-sectional study was conducted at an Education and Research Hospital between January 2018 and June 2023. The study included a total of 437 cases of thyroid surgery. The demographic data such as age, sex, co-morbidities of the instances, hypotension, hypertension, bradycardia, hypoxemia, and postoperative nausea and vomiting, as well as laboratory data were obtained and analyzed.

RESULTS: Of 437 cases, 334 (76%) were females and 103 (24%) were males, with a mean age of 51.83±11.91 years and 55.32±11.87 years, respectively. No statistical significance was realized between the complications, co-morbid diseases, and age. Notably, no liaison between the complications after awakening from the anesthesia and preoperative laboratory parameters was discerned. However, a high but no significant relationship was revealed between the platelet-to-lymphocyte ratio (P/L) in cases with hypoxemia and hypotension. Finally, no significance between laboratory values, bradycardia, hypertension, and postoperative nausea and vomiting was distinguished.

CONCLUSION: We postulate that the so-called inflammatory biomarkers measured at the time of preoperative examination in the blood count concept selectively do not enrich for anticipating complications that arise in the perioperative echelon.

KEYWORDS: Inflammation. Biomarker. Pathology. Thyroid gland. Thyroidology. Thyroidologists.

INTRODUCTION

Thyroidectomy is the most common endocrine surgery that is carried out globally. Though thyroid surgery is considered to have low morbidity and mortality, it is still essential to predict and attenuate relevant perioperative complications. Postoperative nausea and vomiting (poNV), hypotension and hypertension, heart rate alterations, and hypoxemia due to laryngospasm might be considered perioperative complications. Moreover, increased mean platelet volume (MPV) value has been enunciated to be associated with situations such as atherosclerosis, cardiac diseases, and obstructive sleep apnea disorders[1]. Some authors propounded a liaison between MPV values and hypertension as well as the development of preeclampsia during pregnancy[2,3]. The undulations in blood pressure and heart rate during the perioperative period more frequently emerge in cases with cardiac comorbidities. However, augmented MPV was raised in cardiovascular disorders, cerebral stroke, respiratory diseases, chronic renal failure, diabetes, and various carcinomas[4].

Inhaled anesthetics, nitrous oxide, and opioids can be counted as risk factors for poNV, which is a frequent complication of general anesthesia that leads to discomfort for the patient. poNV remains a common issue for many cases, particularly those regarded as high risks, such as women, non-smokers, and individuals with a history of motion sickness, despite the numerous recommendations and strategies aimed at reducing it[5]. Studies suggesting that platelet-to-lymphocyte ratio (P/L) and neutrophil-to-lymphocyte ratio (N/L) could serve as indicators for poNV risk often draw on the idea that inflammation[6-9] may play a role in its development. Inflammation can influence various physiological processes, including those related to the gastrointestinal system and vomiting behavior[6]. Many studies revealed that P/L and N/L are essential indicators of systemic inflammation, and their values with mortality, morbidity, prognosis, and surgical complications have been enunciated[6-11]. This study aimed to investigate whether preoperative MPV, P/L, or N/L was an indicator of complications and identify its relationship with the need for treatment.

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METHODS

Study design

This study was conducted according to the declaration of Helsinki. This cross-sectional study was conducted at the Giresun Education and Research Hospital, Giresun, Turkey, between January 2018 and June 2023. The study incorporated a total of 437 cases who had undergone thyroid surgery. The inclusion criteria were (i) being over 18 years old, (ii) possessing an American Society of Anesthesiologists Physical Status I–III, and (iii) undergoing thyroid surgery. The demographic data such as age, sex, and co-morbidities of the cases were obtained. We examined anesthesia follow-up papers and recovery room records for hypotension, hypertension, bradycardia, hypoxemia, and poNV.

Laboratory parameters

All the cases were examined preoperatively with blood count samples. The relevant white blood cells, neutrophils, lymphocytes, platelets, hemoglobin, MPV, N/L, and P/L were recorded meticulously.

Statistical analysis

The sample size of our study was calculated using a one-way analysis of variance (ANOVA) experimental design, taking power (power of the test) 0.80, effect size 0.35, and type-1 error 0.05, and it was determined as "a total of 96 samples/patients with a minimum of 24 samples in each group." The study used Kolmogorov-Smirnov (n>50) and skewness-kurtosis tests to check whether the measurements were continuous. The descriptive statistics of this study were expressed as mean, standard deviation, minimum, maximum, number (n), and percent (%). Independent t-test and one-way ANOVA were used to compare the measurements according to the categorical groups. Kruskal-Wallis and Mann-Whitney U tests compared the non-normally distributed data. Furthermore, we used the Duncan test to identify different groups following variance analysis. We calculated Pearson correlation coefficients to determine the relationship between measurements. Moreover, we used the chi-square (Fisher's exact) test to determine the relationships between categorical variables. Statistical significance level (a) was taken as 5% (95% confidence interval) in the calculations, and the SPSS (IBM for Windows, v.26) statistical package was used for analysis. Binary logistic regression analysis was applied to describe the effect of "MPV, P/L, and N/L" on "the presence of hypoxia."

RESULTS

In all, 437 patients undergoing thyroid surgery were included in the analysis. The patients' characteristics and postoperative complications are presented in Table 1. As such, 334 (76%) were females and 103 (24%) were males. The mean age was 51.83±11.91 years in females and 55.32±11.87 years in males. There was no significant relationship between the age and gender of the patients included in the study (p>0.001). The mean ages of the female and male groups were similar. The demographics and clinical characteristics of the matched patient pairs are summarized in Table 1.

When the relationship between systemic diseases and patient characteristics was examined, a significant association was found between hypertension and age (p<0.001). The cases with hypertension had a noticeably higher age. No statistically significant difference between sex and systemic diseases was recognized, whereas the frequency of COPD was higher in males. In addition, no considerable difference (p>0.05) in the comparison of other systemic diseases and laboratory values was raised (Table 2). A weak significance was revealed between age and platelets (PLT), hemoglobin (Hgb), and MPV. There was no significant difference between sex and laboratory parameters, but WBC was significantly higher in males only. The overall incidence of the complications was 69%. Patients who developed hypotension had a significantly higher average age. However, no significant relationship was recognized between complications and other systemic diseases and age.

The association between complications occurring after awakening from anesthesia and preoperative laboratory parameters, a high P/L in cases with hypoxemia and hypotension, emerged without any significance (Table 3). Finally, there is no statistical significance in laboratory values for bradycardia, hypertension, and poNV.

Table 1. The demographic and categorical characteristics.

		n	%
Sex	Female	334	76
	Male	103	24
Diabetes mellitus		33	7.6
Hypertension		135	30.9
Cardiac diseases		7	1.6
COPD		8	1.8
Hypothyroidism		264	60.4
Complications	Desaturation	151	34.7
	Bradycardia	53	12.2
	Hypotension	35	8
	Hypertension	3	0.7
	PONV	188	43.2

poNV: postoperative nausea and vomiting.

DISCUSSION

About 310 million patients undergo surgery worldwide each year[11]. Postoperative complications, such as infectious and cardiopulmonary, occur in up to 20% of patients[12]. They increase treatment costs and cause a decrease in life expectancy and quality of life. Bleeding, laryngeal nerve injury, laryngomalacia, and sore throat are complications expected after thyroid surgery. Hence, the patient should be extubated carefully due to the possibility of bleeding. This study investigated the relationship between preoperative blood count parameters and perioperative complications, and statistically significant findings were obtained. It was found that chronic obstructive pulmonary disease (COPD) is more prevalent in male patients, and those who develop hypotension tend to be older. A higher P/L was recognized in cases experiencing hypoxemia and hypotension without significance. This study evaluated the role of N/L, P/L, and MPV as predictive tools for early complications during awakening from anesthesia in thyroidectomy. Laryngospasm, hypertension, hypotension, bradycardia, and poNV are essential complications that

Table 2. The association between comorbidities and adverse events.

		Desaturation	Bradycardia	Hypotension	Hypertension	pONV
Diabetes mellitus (n=33)	Yes (n)	10	3	5	0	13
	p-values	0.716[a]	1.000[a]	0.170[a]	1.000[a]	0.781[a]
Hypertension (n=135)	Yes (n)	43	16	7	1	61
	p-values	0.400[a]	1.000[a]	0.200[a]	1.000[a]	0.579[a]
Cardiac diseases (n=7)	Yes (n)	1	1	0	2	0
	p-values	0.689[a]	0.205[a]	1.000[a]	1.000[a]	1.000[a]
COPD (n=8)	Yes (n)	3	3	2	0	3
	p-values	1.000[a]	1.000[a]	0.130[a]	1.000[a]	1.000[a]
Hypothyroidism (n=264)	Yes (n)	94	107	19	39	2
	p-values	0.577[a]	0.053[a]	0.549[a]	1.000[a]	0.187[a]

[a]Chi-square independence tests. COPD: chronic obstructive pulmonary disease. poNV: postoperative nausea and vomiting.

Table 3. The association between laboratory parameters and anesthetic complications.

		Desaturation	Bradycardia	Hypotension	Hypertension	pONV
Leukocyte	Mean±SD	7529 (2.30)	7.133 (1.88)	7.590 (1.96)	9.576 (2.95)	7.379 (2.1)
	p-value	0.992[b]	0.235[b]	0.671[b]	0.102[b]	0.292[b]
Neutrophil	Mean±SD	4480 (1.85)	4135 (1.55)	4.329 (1.57)	6003 (3.63)	4391 (1.7)
	p-value	0.970[b]	0.075[b]	0.860[b]	0.484[b]	0.154[b]
Platelets	Mean±SD	257.33 (66.7)	246.37 (56.7)	265.28 (70.5)	352.3 (10.4)	264.27 (60)
	p-value	0.103[b]	0.038*[b]	0.969[b]	0.015*[b]	0.356[b]
Lymphocyte	Mean±SD	2328 (0.71)	2220 (0.61)	2599 (0.73)	2803 (0.5)	2282 (0.6)
	p-value	0.485[b]	0.412[b]	0.006**[b]	0.129[b]	0.412[b]
Hemoglobin	Mean±SD	13.51 (1.5)	13.71 (1.3)	13.91 (1.2)	12.80 (0.45)	13.50 (1.52)
	p-value	0.784[b]	0.149[b]	0.062[b]	0.262[b]	0.881[b]
MPV	Mean±SD	9.75 (1.04)	9.51 (1.10)	9.50 (1.04)	10.03 (0.1)	9.54 (1.1)
	p-value	0.028*[b]	0.414[b]	0.825[b]	0.312[b]	0.196[b]
N/L	Mean±SD	2.07 (1.01)	1.99 (0.94)	1.79 (0.74)	2.33 (1.82)	2.07 (1.0)
	p-value	0.838[b]	0.460[b]	0.115[b]	0.704[b]	0.413[b]
P/L	Mean±SD	118.57 (45.9)	117.83 (38.3)	107.30 (33.9)	128.55 (23.9)	124.15 (42)
	p-value	0.008**[b]	0.418[b]	0.006**[b]	0.532[b]	0.514[b]

[b]Mann-Whitney U test (*p<0.05; **p<0.01). poNV: postoperative nausea and vomiting.

may occur during the postoperative period. However, early detection, rapid intervention, and standardized care are crucial for successful management. Laryngospasm can become an anesthetic emergency that happens during the induction, maintenance, and emergence phases of general anesthesia. It usually manifests with the sign of inspiratory stridor, which may progress to complete obstruction, increased respiratory effort, and oxygen desaturation with or without bradycardia. This condition more frequently happens in pediatric cases, whereas its incidence was reported to be 0.78–0.94% in adults[13]. Triggering factors for that phenomenon are the inappropriate depth of anesthesia, frequent suction catheter, inhalational-induced irritation, secretion, airway stimulation, endotracheal intubation, and upper respiratory tract infection. Furthermore, tonsillectomy, adenoidectomy, appendectomy, and thyroidectomy can be considered surgical factors for laryngospasm. It can also cause negative pulmonary pressure edema (NPPE) with a significant negative intrathoracic pressure generated by forced inspiration against an obstructed airway. NPPE is a rare but life-threatening complication of general anesthesia[14]. This study revealed that P/L values were significantly high in cases with hypoxia. Nevertheless, this has led us to think the inflammation process may contribute to early postoperative complications. As such, we postulate that the so-called P/R value might guide estimating postoperative laryngospasm. Therefore, evaluating the association between inflammatory biomarkers and early postoperative anesthesia complications in a prospective-randomized trial would be appropriate. Herein, poNV is a frequent complication of general anesthesia, with a frequency of approximately 30% in the general population, and causes discomfort to the patient, which may be an obstacle to early recovery[15]. Therefore, numerous studies have focused on risk factors such as inhaled anesthetics, nitrous oxide, and opioids to be able to attenuate poNV. It might be more stressful than postoperative pain for the patients, which makes it crucial to predict and manage poNV for the comfort of the patients. In a study, it was stated that there is a relationship between the P/L and hyperemesis gravidarum[16]. A Turkish study investigated the liaison between N/L and poNV in cases who underwent maxillofacial surgery and finally reported that poNV risk augmented significantly in patients with a higher N/L. The authors claimed that antiemetic prophylaxis could be given according to the N/L value by stating that it might indicate poNV[17]. Nevertheless, this study indicates no significant association between poNV ratios and N/L values.

Cardiovascular complications, particularly hypertension, are a dime in a dozen during tracheal extubation. Patients with cardiac co-morbidities are vulnerable to hyper- or hypotension and heart rate abnormalities. Preoperative identification of high-risk individuals and appropriate perioperative management can attenuate cardiovascular risk. Furthermore, the N/L and P/L were also found to be an indicator of the prognosis in cardiovascular diseases, malignancies, and chronic inflammatory diseases[18]. Elevated MPV is associated with an increased incidence of hypertension independent of other risk factors, which suggests that platelet activity may play a role in hypertension incidence. Moreover, the P/L values were high in the patients with hypotension without a significant difference between P/L and hypotension.

We postulate that the so-called inflammatory biomarkers might not be practical for use in forecasting cardiac and respiratory complications and poNV in the perioperative period. Although many studies have shown the interrelation between these complications and biomarkers, the discrepant cutoff values in each clinical situation make these biomarkers impractical to apply in the clinical practice of the providers, such as in thyroidology, which is a crucial and pivotal field, interconnecting many organ systems of human being[18-20]. Actually, thyroidologists are defined as the "first string" players in awareness efforts globally by the 2023 American Thyroid Association[21-23].

Limitations

The retrospective design of this study imposes limitations on our ability to analyze the extent of saturation drops following laryngospasm and bronchospasm, whether hypertensive patients had regular blood pressure before, and all other parameters influencing these relevant complications.

CONCLUSION

NLR, P/L, and MPV measured during preoperative examination are insufficient for predicting complications occurring in the perioperative period. To universally accept these biomarkers as predictors of risk factors, prospective studies with larger sample sizes are required, and efforts should be made to minimize conditions by thyroid providers, in particular, that can affect blood cell counts. This issue merits further investigation. Thyroidologists are defined as the "first string" players in awareness efforts globally by the American Thyroid Association in 2023. Herewith, without which, not, no overlook thyroid diseases to opt for "thyroid health" purposes.

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AUTHORS' CONTRIBUTIONS

AB: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Resources, Validation, Visualization, Writing – original draft. **IS:** Investigation, Methodology, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **DS:** Investigation, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **FAB:** Methodology, Project administration, Validation, Visualization. **EC:** Investigation, Software, Validation, Visualization.

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ORIGINAL ARTICLE

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A new parameter in predicting contrast-induced nephropathy: Osaka prognostic score

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SUMMARY

OBJECTIVE: Nowadays, the frequency of complications is also increasing following the increasing frequency of coronary angiography and percutaneous coronary intervention. Contrast-induced nephropathy is one of the most common of these complications. This study aimed to investigate the relationship between the Osaka prognostic score, which has previously been shown to have prognostic importance in gastrointestinal malignancies, and the development of contrast-induced nephropathy.

METHODS: The study retrospectively examined the data of 1,498 patients who underwent coronary angiography and percutaneous coronary intervention due to acute coronary syndrome between 2018 and 2023. Demographic characteristics and laboratory findings were retrospectively collected from patients' charts and electronic medical records.

RESULTS: Osaka prognostic score (0.84±0.25 vs. 2.2±0.32, p<0.001) was higher in patients who developed contrast-induced nephropathy. Also, Osaka prognostic score [OR 2.161 95%CI (1.101–4.241), p<0.001] was found to be an independent risk factor along with age, diabetes mellitus, systolic pulmonary artery pressure, hemoglobin, hemoglobin, C-reactive protein, albumin, N-terminal brain natriuretic peptide, and systemic immune-inflammation index. The receiver operating characteristic curve showed that the optimal cutoff value of Osaka prognostic score to predict the development of contrast-induced nephropathy was 1.5, with a sensitivity of 83.4 and a specificity of 65.9% [area under the curve: 0.874 (95%CI: 0.850–0.897, p≤0.001)].

CONCLUSION: Osaka prognostic score may be an easily calculable, user-friendly, and useful parameter to predict the development of contrast-induced nephropathy in patients undergoing percutaneous coronary intervention after acute coronary syndromes.

KEYWORDS: Osaka prognostic score. Inflammation. Nutrition. Contrast-induced nephropathy. Acute coronary syndrome. Percutaneous coronary intervention.

INTRODUCTION

Acute coronary syndromes (ACS) are a common cause of mortality and morbidity today. Especially with increasing life expectancy, the frequency of ACS also increases[1]. Today's gold standard ACS diagnosis and treatment protocol is coronary angiography (CAG) and percutaneous coronary intervention (PCI)[2]. With the increasing frequency of ACS, the number of PCIs performed is also increasing. Although successful coronary revascularization has been achieved, contrast-induced nephropathy (CIN), which may occur in association with PCI, increases mortality and morbidity in patients and prolongs hospitalization, which can lead to poor outcomes[3]. Classically, CIN is defined as an increase in serum creatinine value after PCI by 5 mg/dL or more than 25% within 48–72 h[4].

The etiopathogenesis of CIN has a multicomponent structure. Although direct contrast agent-related renal cytotoxicity plays the leading role, local ischemia related to renal hypoperfusion (caused by decreased cardiac output), excessive activation of the immune system, and endogenous vasomotor imbalances that may occur as a result of ACS also contribute to the formation of CIN[5,6]. It is also known that poor nutritional status is directly related to CIN[7,8]. Osaka prognostic score (OPS) is a new marker based on inflammation and nutrition that has emerged recently and has been reported to have prognostic importance in gastrointestinal malignancies. OPS includes C-reactive protein (CRP), albumin, and lymphocyte count (TLC)[9]. It is known that all of these components are individual risk factors for CIN[10,11]. When all this information is evaluated, it is observed that low nutritional status and the presence of high inflammation increase CIN. OPS provides information on both parameters. For this reason, our study aimed to investigate the relationship between OPS and CIN that develops after PCI, which has not been investigated before.

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METHODS

Population

This study retrospectively examined the data of 1,498 patients admitted to the hospital with ACS and underwent PCI within 24 h between September 2018 and December 2023. The study was started after receiving approval from the local ethics committee. All steps of the study were planned and carried out following the directives of the Declaration of Helsinki. Patients with active infection, end-stage renal failure, use of nephrotoxic drugs in the last week, and use of contrast material for another reason in the last week, patients whose data could not be accessed for any reason, and patients with cardiogenic shock and any malignancy were excluded from the study. Patients with ST-elevation myocardial infarction (STEMI) were urgently taken immediately after diagnosis. Patients with non-STEMI (NSTEMI) were taken for CAG, and PCI was performed within 24 h at the latest. A nephrotropic, water-soluble, low osmolar, non-ionic contrast agent, Iohexol (300 mg iodine/mL; 672 mosml/kg of water; Omnipaque; GE Healthcare Inc., Marlborough, MA, USA) was used for angiography. Whole blood parameters were obtained from automatic hematology analysers (Symex XN-550 analyzer, Symex, Kobe, Japan), and biochemical data were obtained from biochemistry devices (Beckman Coulter Inc., Brea, New York, USA).

Definitions

Three variables are used to calculate the OPS: CRP (≤10.0 mg/L: 0 point and>10.0 mg/L: 1 point), albumin (≥3.5 g/dL: 0 point and<3.5 g/dL: 1 point), and TLC (≥1,600/μL: 0 point and<1,600/μL: 1 point). OPS was calculated as the sum of the scores from these parameters, thus resulting in four OPS-based groups: four groups (zero, one, two, and three). For Glasgow prognostic score (GPS), CRP (≤10.0 mg/L: 0 points and>10.0 mg/L: 1 point) and albumin values (≥3.5 g/dL: 0 points and<3.5 g/dL: 1 point) were used. Patients were grouped according to the scores from these parameters by receiving 0, 1, and 2 points[9]. The formula 10×albumin (g/dL)+0.005× TLC (per mm^3) was used to calculate the prognostic nutritional index (PNI)[12], and the formula platelet count*-neutrophil count/lymphocyte count was used to calculate the systemic immune-inflammatory index[13].

Statistical analysis

Categorical data are presented as numbers and percentages. For non-parametric data analysis, the chi-square test was used. All the variables obtained were examined with the Kolmogorov-Smirnov test for normality and the Levene test for homogeneity of variances before the significance tests were used. Normally distributed homogeneous data were evaluated with a t-test in independent groups and a Mann-Whitney U test for results that did not show normal distribution. Receiver operating characteristic (ROC) analysis was used to estimate the optimal cut-off value of OPS, GPS, and systemic immune-inflammation index (SII) in indicating CIN. Sensitivity, specificity, and area under the curve (AUC) were calculated. Logistic regression analysis was performed to determine values predicting CIN. The values that differed among these parameters for CIN were included in the univariate logistic regression analysis, and their significance was determined. Potential risk indicator parameters that were significant in the univariate logistic regression model were included in the multivariate logistic regression analysis (forced entry method). The analyses were performed with the IBM SPSS 23.0 statistical package program (IBM Corp., Armonk, NY, USA). A two-sided p<0.05 was considered significant.

RESULTS

The average age of the patients included in the study was 61.4±12.6 years. A total of 821 (54.8%) of the patients were male. When the demographic data of the patients were examined, it was determined that the patients who developed CIN were older (58.6±12.1 vs. 64.1±13.0, p<0.001) and were predominantly male [673 (54.1%) vs. 148 (57.8%), p=0.004]. Diabetes mellitus (DM), hypertension (HT), and heart failure (HF) were found to be more common in patients who developed CIN (p=0.001, p<0.001, and p=0.011, respectively). Other demographic data were found to be similar (Table 1).

When laboratory data were examined, in patients who developed CIN, hemoglobin, TLC, albumin, and PNI values were lower (p=0.040, p<0.001, p<0.001, and p=0.001, respectively). Systolic pulmonary artery pressure (sPAP), CRP, peak creatinine, N-terminal prohormone brain natriuretic peptide (NT-proBNP), OPS, GPS, and SII values were found to be higher (p=0.004, p = 0.009, p=0.003, p=0.013, p<0.001, p=0.025, and p=0.008, respectively) (Table 1). According to the univariate regression analysis, age, male gender, DM, HT, HF, sPAP, anterior MI, hemoglobin count, TLC, CRP, albumin, glucose, NT-proBNP, left anterior descending (LAD) as the infarct-related artery, OPS, GPS, PNI, and SII (p<0.001, p=0.001, p=0.001, p<0.001, p=0.011, p=0.003, p=0.001, p<0.001, p<0.001, p<0.001, p=0.001, p=0.001, p<0.001, p<0.001, p<0.001, p=0.004, p<0.001, and p=0.001, respectively) were found to be good prognostic factors in predicting CIN; as a result of multivariate analysis, age, DM, sPAP, anterior MI, hemoglobin count, TLC, CRP, albumin, Nt-proBNP,

Table 1. Baseline characteristics, laboratory results of all study patients, and patients with and without contrast-induced nephropathy.

	Non-CIN group, n=1242	CIN group, n=256	p-value
Demographics			
Age, years	58.6±12.1	64.1±13.0	**<0.001**
Male gender, n (%)	673 (54.1)	148 (57.8)	**0.004**
Diabetes mellitus, n (%)	381 (30.8)	160 (62.7)	**0.001**
Hypertension, n (%)	545 (44.0)	165 (64.5)	**<0.001**
Hyperlipidemia, n (%)	758 (61.0)	169 (66.0)	0.138
CAD, n (%)	474 (38.2)	108 (42.7)	0.203
HF, n (%)	920 (74.1)	209 (81.6)	**0.011**
Smoking, n (%)	381 (30.7)	81 (31.8)	0.211
BMI, kg/m^2	27.9±7.5	27.6±5.6	0.819
On admission, clinical characteristics			
Systolic blood pressure, mmHg	135.7±39.6	135.9±36.1	0.340
Heart rate, per minute	79.6±19.2	82.9±22.1	0.449
Left-ventricular ejection fraction (%)	42.9±10.5	40.3±10.6	0.542
sPAP, mmHg	35.0±7.8	39.2±8.4	**0.004**
MI type			
Anterior MI	300 (24.2)	114 (44.5)	**0.001**
Inferior MI	405 (32.6)	56 (21.9)	
NSTEMI	537 (43.2)	86 (33.6)	
Laboratory results			
Hemoglobin, g/dL	14.1±4.9	13.8±5.7	**0.040**
White blood cell count, cells/μL	10.5±4.9	10.8±5.6	0.732
Lymphocyte count, 10^9/L	1.6±0.3	1.5±0.2	**<0.001**
Platelet count, cells/μL	260.7±82.6	251.5±89.1	0.207
CRP, mg/L	7.5±3.2	12.4±2.8	**0.009**
Albumin, g/dL	3.7±0.5	3.2±0.6	**<0.001**
Admission blood glucose, mg/dL	161.8±85.2	187.6±96.7	**0.001**
Baseline creatinine, mg/dL	1.2±0.9	1.3±1.0	0.076
Peak creatinine, mg/dL	1.4±1.1	1.8±1.2	**0.003**
Peak creatinine kinase–myocardial band, ng/mL	70.8±21.2	117.6±18.8	0.390
Peak troponin, ng/L	10562±531	11538±413	0.574
NT-proBNP, pg/dL	2056±787	2935±766	**0.013**
Total cholesterol, mg/dL	185.3±23.9	171.1±22.4	0.223
TG, mg/dL	180.8±28.5	182.9±23.6	0.071
HDL, mg/dL	39.1±12.5	39.9±16.8	0.109
LDL, mg/dL	126.0±36.3	122.1±33.8	0.273
Angiographic and clinical data			
Multi-vessel stenosis (>50%), n (%)	360 (29.0)	61 (23.8)	0.109
LAD as the infarct-related artery, n (%)	608 (49.0)	163 (63.7)	**0.001**
Contrast volume, mL	274.1±61.0	273.2±58.2	0.764
Need for dialysis, n (%)	0 (0)	30 (11.7)	**<0.001**
Length of hospital stay, days	6.4±5.1	8.8±4.6	**0.012**
In-hospital mortality	34 (2.7)	24 (9.4)	**<0.001**
OPS	0.84±0.25	2.2±0.32	**<0.001**
GPS	0.42±0.15	1.46±0.13	**0.025**
PNI	44.9±4.7	39.1±6.4	**0.001**
SII	981.2±114.4	1177.9±111.8	**0.008**

CIN: contrast-induced nephropathy; CAD: coronary artery disease; HF: heart failure; BMI: body mass index; sPAP: systolic pulmonary artery pressure; MI: myocardial infarction; CRP: C-reactive protein; NT-proBNP: N-terminal prohormone brain natriuretic peptide; TG: triglycerides; HDL: high-density lipoprotein; LDL: low-density lipoprotein; LAD: left anterior descending artery; OPS: Osaka prognostic score; GPS: Glasgow prognostic score; PNI: prognostic nutritional index; SII: systemic immune-inflammation index. Bold values indicate statistically significant values.

OPS, and SII were found to be independent risk factors for the development of CIN (p=0.001, p=0.001, p=0.001, p=0.001, p=0.046, p<0.001, p<0.001, p=0.001, p=0.001, p<0.001, and p=0.001) (Table 2). To evaluate the significance of OPS in predicting CIN as a result of ROC analysis, the AUC was 0.874 (95%CI: 0.850–0.897, p<0.001) and the optimal cutoff value was 1.5 (83.4% sensitivity and 65.9% specificity) (Figure 1).

DISCUSSION

This is the first study to examine the relationship between OPS and CIN in the current literature. As a result of our study, OPS was found to be an independent predictor of CIN development in patients undergoing PCI after ACS.

As a result of previous studies, it is known that patients with decreased nutritional status and increased inflammatory activity have worse cardiac outcomes, especially CIN[6,8,10]. In our study, consistent with the literature, CIN was more common in patients with high OPS, GPS, and SII and low PNI. However, as a result of our study, CIN was also found to be more common in patients with advanced age, male individuals, DM, and HT. It is known that especially with increasing age, systemic inflammatory imbalance and activity increase more, and nutritional status weakens[14,15]. This may be an additional parameter

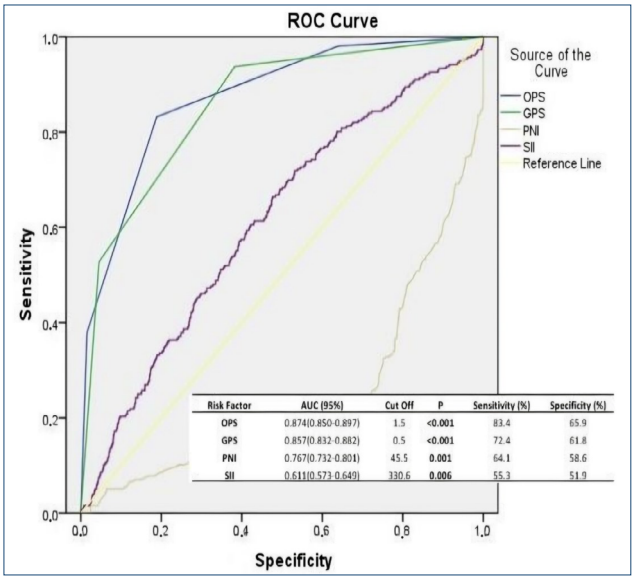

Figure 1. Receiver operating characteristics for Osaka prognostic score, Glasgow prognostic score, prognostic nutritional index, and systemic immune-inflammatory index.

Table 2. Univariate and multivariate analyses for the predictor of contrast-induced nephropathy.

	Univariate analysis	p-value	Multivariate analysis	p-value
	OR (95%CI)		OR (95%CI)	
Age	1.059 (1.047–1.070)	**<0.001**	1.046 (1.025–1.067)	**0.001**
Male gender	2.406 (1.817–3.186)	**0.001**	1.010 (0.610–1.1671)	0.970
Diabetes mellitus	3.793 (2.2863–5.024)	**0.001**	2.605 (1.612–4.210)	**0.001**
Hypertension	2.3112 (1.749–3.058)	**<0.001**	1.615 (0.985–2.650)	0.058
HF	1.556 (1.107–2.189)	**0.011**	1.659 (0.957–2.874)	0.071
sPAP	1.056 (1.041–1.072)	**0.003**	1.050 (1.026–1.074)	**0.001**
Anterior MI	3.008 (1.652–5.476)	**0.001**	2.521 (1.908–3.331)	**0.001**
Hemoglobin count	0.822 (0.774–0.873)	**<0.001**	0.913 (0.834–0.998)	**0.046**
Lymphocyte count	0.050 (0.024–0.103)	**<0.001**	0.109 (0.018–0.653)	**<0.001**
CRP	1.544 (1.465–1.628)	**<0.001**	1.479 (1.339–1.634)	**<0.001**
Albumin	0.167 (0.127–0.220)	**0.001**	0.256 (0.142–0.462)	**0.001**
Admission blood glucose	1.003 (1.002–1.004)	**0.001**	0.999 (0.997–1.002)	0.642
NT-proBNP	1.001 (1.001–1.002)	**<0.001**	1.001 (1.001–1.002)	**0.001**
LAD as the infarct-related artery	1.828 (1.384–2.413)	**<0.001**	0.942 (0.545–1.629)	0.832
OPS	8.128 (6.349–9.405)	**<0.001**	2.161 (1.101–4.241)	**<0.001**
GPS	5.441 (3.972–8.676)	**0.004**	1.098 (0.449–2.686)	0.838
PNI	0.825 (0.802–0.848)	**<0.001**	0.806 (0.782–1.004)	0.569
SII	1.002 (1.001–1.003)	**0.001**	1.001 (1.001–1.002)	**0.001**

CIN: contrast-induced nephropathy; DM: diabetes mellitus; HF: heart failure; sPAB: systolic pulmonary artery pressure; MI: myocardial infarction; CRP: C-reactive protein; NT-ProBNP: N-terminal brain natriuretic peptide; TG: triglycerides; HDL: high-density lipoprotein; LAD: left anterior descending artery; OPS: Osaka prognostic score; GPS: Glasgow prognostic score; PNI: prognostic nutritional index. Bold values indicate statistically significant values.

explaining why more CIN develops in patients with advanced age, which we found as a result of our study. Again, the fact that we found more CIN in male subjects is compatible with the literature and can be explained by the higher inflammatory response and lower nutritional status in men[16,17]. As another result of our study, CIN was higher in patients with DM and HT. Although DM and HT are direct risk factors for CIN, increased inflammation and decreased nutritional status, which are among the multifactorial etiologies of CIN, may have contributed to the development of CIN[18,19]. In our study, CIN was more common in patients with low hemoglobin levels. This finding has been shown many times before in the literature. Increased inflammation and poor nutritional status in patients with low hemoglobin values may also have played a role in the development of CIN[20].

Our study focuses on OPS, a combination of several parameters of CIN development's most well-known physiopathological components. These are CRP, albumin, and TLC, respectively. All of these components reflect the systemic inflammatory response[21]. Increased inflammatory response is also known to increase the development of CIN. OPS also has components that reflect nutritional status. Decreased nutritional status increases the development of CIN and also induces systemic inflammation[7,8]. Thus, using only OPS can obtain information about the patient's nutritional and systemic inflammatory status. These components, known to increase the development of CIN individually, can give more accurate results with a single score. It can easily increase the prediction of CIN development. Furthermore, a single score can also reveal the patient's preoperative nutritional status and systemic inflammation. In our study, nutritional and inflammatory parameters such as GPS, PNI, and SII were evaluated in addition to OPS. OPS proved to be a superior parameter in predicting the development of CIN by having a higher AUC area as a result of ROC analysis. This superiority of OPS over GPS may be due to the additional inclusion of TLC. Indeed, a direct association between TLC and CIN is known. OPS thus reflects the systemic increased inflammatory state better than GPS. PNI includes only albumin and TLC values. It does not contain CRP as in OPS. As a result of this situation, PNI, which has a more nutritional aspect, may neglect to reflect systemic inflammation a little more. The superiority of OPS in showing CIN in our study may be because it better shows increased systemic inflammation due to CRP. According to Ma et al.'s study, SII has recently been found to be a trendy CIN indicator[22]. However, the SII contains only simple blood parameters and provides no information on nutritional status. Therefore, it needs to be improved in one aspect compared with OPS. In our study, OPS was a better predictive parameter than SII.

The main limitations of our study are that it was retrospective and covered a limited geographical area. The fact that our study was single-centered is another area for improvement. In addition, long-term follow-up was not performed in these patients, laboratory values at presentation and at the time of CIN development were used as spots, and long-term results are unknown. Another limitation is that many scoring systems are known to predict the development of CIN, and these scoring systems and parameters were not used in our study. Future randomized controlled trials are needed to confirm the results of our study.

CONCLUSION

Our study found OPS as a parameter that may predict the development of CIN in patients undergoing PCI after ACS. OPS is an indicator that is relatively easy to calculate and its components are readily available in any healthcare institution. By using the OPS, patients with an exceptionally high risk for CIN development can be identified and treatment can be personalized to reduce the risk of CIN development.

AUTHORS' CONTRIBUTIONS

NBÖ: Conceptualization, Data curation, Formal Analysis, Methodology, Software, Validation, Writing – review & editing. **EA:** Conceptualization, Data curation, Methodology, Writing – review & editing.

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