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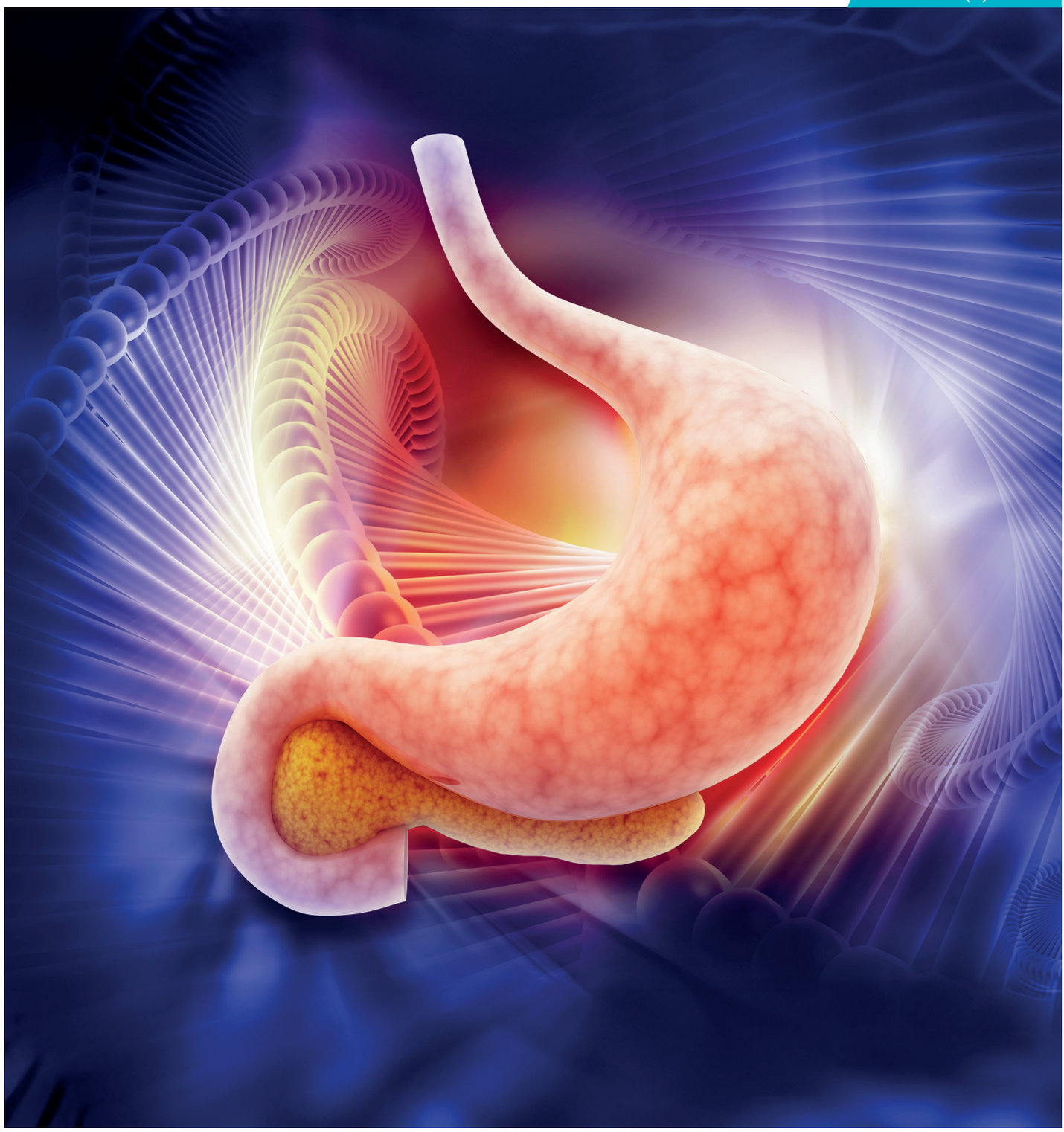

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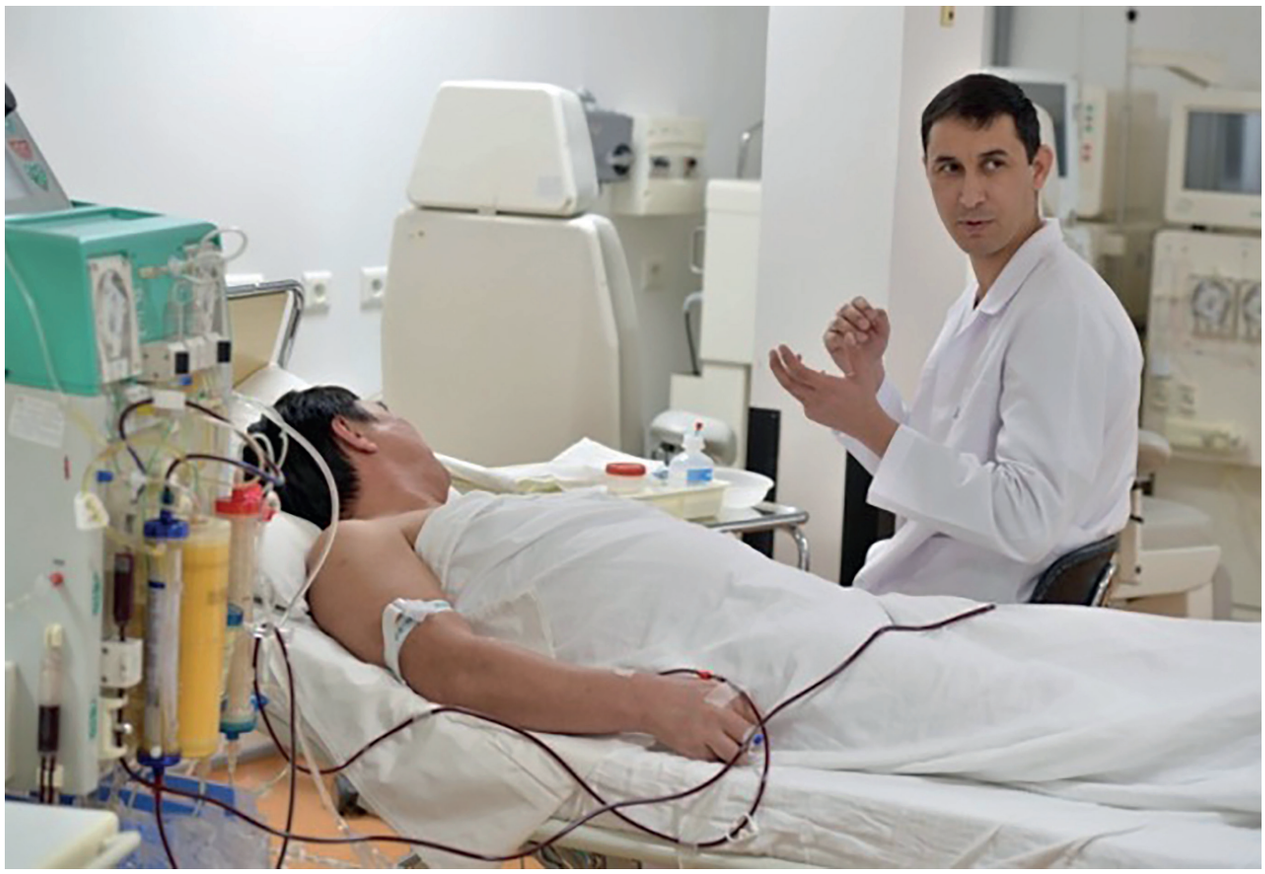

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Worsening air pollution an unfamiliar cause of low vitamin d levels: A systematic literature review

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Abstract

Air pollution is widely recognized as a future biohazard, yet its direct effects on human health, particularly in relation to bone health and vitamin D levels, are inadequately understood. While the detrimental impact on respiratory and cardiovascular health is well-documented, the correlation with vitamin D deficiency remains weak.

To explore the relationship between air pollution and vitamin D levels, an extensive search of scientific literature was conducted. This comprehensive search encompassed databases such as PubMed, Cochrane Library, and DOAJ, while also examining the bibliographies of relevant articles. The inclusion criteria focused on studies that specifically investigated the association between air pollution and vitamin D levels, while excluding systematic reviews, case reports, editor's letters, and studies lacking pertinent explanations or causative factors. Each included study underwent rigorous evaluation of its methodological quality, with data extraction performed to assess the reliability and robustness of the evidence for each research question.

The majority of studies have consistently demonstrated a negative correlation between ambient air pollution and vitamin D levels in humans. Several mechanisms have been proposed to explain this relationship, including reduced exposure to sunlight due to increased air pollution, production of reactive oxygen particles, and inflammation leading to decreased vitamin D synthesis. Moreover, certain pollutants, such as particulate matter and nitrogen dioxide, have been found to have a more pronounced impact on vitamin D levels. Variables such as age, gender, season, and geographical location may also influence the association between air pollution and vitamin D levels. Lifestyle factors, including indoor confinement and pre-existing vitamin D deficiency, may exacerbate the detrimental effects of air pollution.

In conclusion, air pollution detrimentally affects vitamin D levels primarily through increased body inflammation and the generation of free radicals. The presence of elevated levels of particulate matter and nitrogen dioxide further compounds this effect. Additionally, decreased cutaneous production of activated vitamin D, resulting from reduced ultraviolet B radiation penetration due to decreased Air Quality Index, contributes to the negative impact. Studies suggest that the intake of exogenous vitamin D supplements and adherence to a calcium-rich diet may confer benefits to individuals residing in highly polluted areas. To address this issue, public health policies should emphasize outdoor exposure to sunlight, promote healthy dietary choices, and reduce overall exposure to pollutants.

Key words: air pollution, vitamin D levels, dietary supplements, and free radicals

Introduction

The activated form of vitamin D plays a crucial role in the musculoskeletal system. It influences bone health by promoting the absorption of calcium and phosphorus from the intestine, maintaining calcium balance through the kidney, and regulating the release of parathyroid hormone [1].

Vitamin D deficiency can lead to decreased bone mineral density, osteomalacia, and an increased risk of fractures, making it particularly concerning

during childhood and adolescence when skeletal growth and development are critical [2, 3, 4]. Additionally, vitamin D is essential for immune regulation and cell growth [5].

Interestingly, the activated form of vitamin D is expressed in various tissues, including neurons, glial cells, epithelial cells, prostate cells, keratinocytes, and macrophages. This local expression contributes to the regulation of genes involved in cell proliferation and differentiation [6]. Epidemiological evidence suggests that vitamin D deficiency is linked to the pathogenesis and progression of chronic diseases and autoimmune disorders, such as cancer, multiple sclerosis, and rheumatoid arthritis [7].

In pregnant women, vitamin D insufficiency has been associated with pre-eclampsia, gestational diabetes mellitus, fetal development limitations, and preterm birth [8]. The primary sources of vitamin D for humans are sunlight exposure and dietary consumption [9].

While there is no consensus on the precise levels that define vitamin D deficiency and sufficiency, a level of 30nmol/L is generally considered protective of bone health, with levels below this considered insufficient. Studies have also classified serum 25(OH) D levels of 20 ng/ml as deficiency, 20 to 30 ng/ml as insufficiency, 30 to 60 ng/ml as sufficiency, and >150 ng/ml as intoxication [1].

Vitamin D deficiency is recognized as a global health concern [10]. Although the exact global burden of vitamin D deficiency is uncertain, research suggests that it is prevalent in many countries, particularly in the Middle East, India, China, and Mongolia [11]. For instance, studies have reported high prevalence rates of vitamin D deficiency in India (61%), Iran (86%), and Turkey (51%) [12].

In recent years, urban areas have experienced an increase in air pollution, primarily from sources such as motor vehicle emissions, industrial combustion, and heating [13]. In 2016, 95% of the world's population resided in regions where ambient particulate matter 2.5 m (PM2.5) levels exceeded the World Health Organization's (WHO) guidelines of 10g/m3 [14]. It is hypothesized that high levels of air pollutants, including ozone, particulate matter PM2.5, and sulfur dioxide, can reduce the cutaneous synthesis of pre-vitamin D3 by effectively absorbing UVB photons [15]. Moreover, high concentrations of air pollutants and dust in the atmosphere reduce visibility and limit exposure to the sun's ultraviolet radiation [16].

Vitamin D deficiency can negatively impact regular inflammatory mediators, potentially harming the skeleton [17]. Furthermore, exposure to high levels of air pollution may increase bone turnover markers, such as osteocalcin and C-terminal telopeptide of type I collagen (CTx) [18].

In this systematic review, we aim to explore the correlation between air pollution and vitamin D deficiency. We will also investigate factors such as race, gender, and age that may influence this correlation, and propose potential solutions to address this issue.

Material and methods

A systematic search was conducted on PubMed, Cochrane Library, and DOAJ using the terms 'Vitamin D' and 'Air Pollution.' References from primary and review articles and major orthopaedic texts were cross-referenced to identify additional reports that met the inclusion criteria but were not identified by the initial search. All articles published up until December 2022, including online articles, were included.

Inclusion requirements

The study included original articles on Vitamin D and Air pollution.

Exclusion criteria

Excluded were systematic reviews, case reports, and letters to the editor. Excluded were studies in which the association was discussed without cause or relevant explanation.

Extraction of data, analysis, and results

The articles included in the study were independently evaluated by two authors. One reviewer extracted the data, which was then reviewed by the second author. Additionally, data were extracted and reviewed independently. Prior to proceeding, disagreements were resolved.

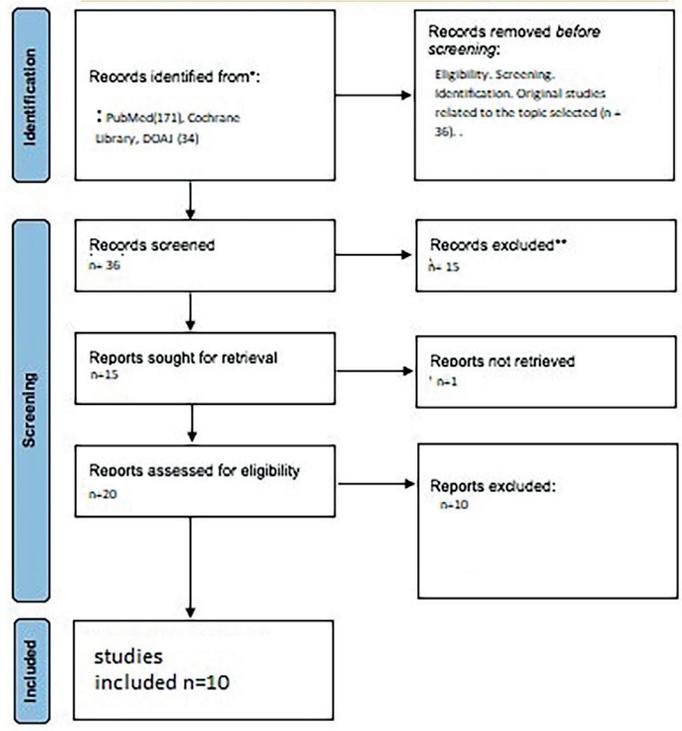

Figure 1 - Identification of studies via databases and registers

The authors then compiled and analyzed the data, attempting to answer the following questions:

1. Is there a connection between vitamin D and ambient air pollution, and if so, what is the nature of this connection?
2. Exist additional factors that influence this relationship and vitamin D levels?
3. How can we avoid this issue?

Results

Research conducted by Feizabad et al. [19] in 2017 suggests that air pollution reduces the amount of UVB radiation reaching the Earth's surface, thereby impacting vitamin D levels. Similarly, He et al. [20] found a negative correlation between air quality index (AQI) and vitamin D levels in China in 2020. Another study by Yan Zhao et al. [21] in China in 2019 revealed that a decrease in daily net radiation mediates the negative association between particulate air pollution (PM10 and PM2.5) and vitamin D levels in expectant women. This study also highlighted that UVB absorption primarily drives this association.

Furthermore, Aggarwal et al. [16] found that children living in areas with higher air pollution levels had a higher incidence of vitamin D deficiency, rickets, and elevated levels of PTH and ALP. They indirectly measured the reduced amount

of UVB radiation reaching the Earth's surface in more polluted regions using haze sensors.

In a study comparing the vitamin D status of healthy women aged 20 to 55 in Tehran (a more polluted area) and Ghazvinian (a less polluted area), Farhad Hosseinpanah et al. observed that ground UVB radiation, measured using a haze meter, served as a proxy for air pollution. The study showed that the mean standard deviation of serum 25-hydroxy vitamin D was significantly higher in Ghazvinian women (18±11) compared to Tehranian women (13±7). The prevalence of vitamin D levels below 10 ng/ml and between 10 and 20 ng/ml was higher in the Tehranian group (36% and 54%) compared to the American group (31% and 32%). Additionally, secondary hyperparathyroidism was more prevalent among Tehranian women (47% vs. 32%) [4].

Manicourt DH conducted a Belgian study in 2008 involving postmenopausal women, where urban tropospheric ozone levels were used as a proxy for air pollution as it is the most common urban air pollutant and absorbs solar radiation. The study revealed that urban tropospheric ozone levels were three times higher than rural levels (80.4±18.2 g/m3 versus 27±10 g/m3). Despite urban residents having nearly 1.3 times more sun exposure than rural residents, more urban residents had insufficient vitamin D levels (32 out of 38 versus 18 out of 47 with 25-hydroxyvitamin D ≥ 75 nmol) [22].

A pilot investigation by Calderón-Garcidueas L et al. [23] involved 20 children from Mexico City and 15 controls. The study found that 6-year-olds in Mexico City had higher IL-6 levels, vitamin D deficiency, and spent less time outdoors. However, it is worth noting that adolescents in more polluted areas tend to spend less time outdoors, which could be a confounding variable. Their investigations in Mexico City showed that lifelong exposure to a polluted environment, including above-standard concentrations of particulate matter 2.5, is associated with significant systemic inflammation and immune dysregulation. There are further studies like the studies by Minna Pekkinen et al that emphasize that in adolescents with similar dietary intake and similar physical activity level the vitamin D levels and BMD levels can vary hence the association with other factors like air pollution need to be evaluated [24-27].

In a cross-sectional study of 100 children, Roya Kelishadi et al. [28] discovered an independent inverse association between Air Quality Index and 25-hydroxy (OH) vitamin D levels. This finding explains the high prevalence of vitamin D deficiency among infants in Isfahan, Iran. The study concluded that the dietary vitamin D intake alone could not account for the extremely low serum 25(OH)D levels. As a result, air pollution should be considered a contributing factor to the etiology of hypovitaminosis D in regions with abundant sunlight.

In a study involving 375 mothers in France, Nour Baz et al. [29] used the atmospheric dispersion modelling system (ADMS-Urban) pollution model, which combined data on traffic conditions, topography, meteorology, and background pollution at the mother's home address to asses particulate matter less than 10 μm in diameter and nitrogen dioxide during pregnancy. Cord blood samples were then collected at birth and were analyzed for levels of 25(OH)D. It was found that maternal exposure to ambient urban levels of Nitrogen Dioxide (NO2) and PM10 strongly predicted low vitamin D status in infants. The correlation was particularly significant during the third trimester. The associations were significant in almost every trimester of gestation but were strongest for the last trimester exposure window. The impact of air pollution exposure during the last trimester of gestation is all the more important because maternal supply of vitamin D declines gradually in pregnancy and reaches its lowest level in the third trimester. Their observation that cord blood serum 25(OH)D concentration varied with the seasons higher levels in summer when sun exposure is the highest as compared to other seasons and then their d model was adjusted for this parameter.

However, a review article by M. Barati et al. [30], which analyzed 35 articles, found no association between the prevalence of vitamin D deficiency and various air pollution parameters, including the number of days with reduced visibility due to dust.

Taken together, these studies demonstrate a correlation between air pollution and diminished vitamin D levels, potentially leading to vitamin D deficiency and rickets in children. Air pollution reduces the amount of UVB radiation reaching the Earth's surface, which primarily contributes to this relationship. Regions with higher air pollution levels also tend to have a higher prevalence of vitamin D deficiency. Additionally, expectant women exposed to NO2 and PM10 are more likely to give birth to infants with low vitamin D levels.

In a 2017 investigation conducted by Elham Feizabad and colleagues, the focus was primarily on adolescents (325 middle and high school students were studied). During the study period, detailed daily data on air pollution were obtained from archived data collected by Tehran Air Quality Control Company (AQCC). Serum levels of calcium, phosphorus, parathyroid hormone (PTH), bone-specific alkaline phosphatase, 25(OH) vitamin D, osteocalcin, cross-linked C-telopeptide (CTX), total protein, albumin, and creatinine were obtained from the study group. The study observed that males had higher levels of vitamin D in both polluted and unpolluted areas, while the prevalence of vitamin D deficiency was twice as common in girls. However, the difference in vitamin D levels between polluted and unpolluted areas was significantly greater in males, suggesting that they are more susceptible to the effects of air pollution (55.7.1 vs 9%), serum vitamin D levels in the polluted area were significantly lower than levels in the non-polluted area (22.4±1.23 ng/ml vs 34.25±1.71 ng/ ml) [19].

A 2020 study conducted in China by He et al. revealed that in subtropical regions, young people and females tended to have lower levels of vitamin D despite similar environmental conditions [20]. For younger age groups, even though their vitamin D intake was higher, the prevalence of vitamin D deficiency remained high due to spending less time outdoors and more time indoors. Other studies have also suggested that excluding the elderly population from taking supplements would lead to an even higher prevalence of vitamin D deficiency [31]. In females, the use of sunscreen and cosmetics contributed to reduced UV exposure, while the prevalence of vitamin D deficiency was lower in the summer [20, 32].

In a study published in China in 2019 by Yan Zhao et al. [21], it was found that exposure to particulate air pollution during the third trimester of pregnancy had a greater impact on vitamin D status. Similar results were observed in a French study by Nour Baz et al. [29]. Since 80% of intrauterine bone mineralization occurs during the third trimester and vitamin D plays a crucial role in this process [33], the fetal demand for vitamin D increases, potentially leading to vitamin D depletion in the maternal blood supply [34]. Therefore, there is a need for vitamin D supplementation during the third trimester, especially in polluted areas.

In a study conducted in Tehran in 2017, Feizabad discovered that adolescents with calcium intakes exceeding 5,000 mg per week showed significant improvements in markers of bone turnover [19]. A study by Agarwal et al. [16] recommended administering a single, large dose of oral vitamin D supplementation at the beginning of winter to protect infants from vitamin D deficiency.

A pilot study conducted on six-year-olds in Mexico City by Calderón-Garcidueas et al. [23] concluded that pediatricians should evaluate the bone health of children living in highly polluted megacities and communities with high levels of particulate matter, taking into account systemic inflammation and deficiencies in vitamin D and calcium in the diet.

Conclusion

Air pollution negatively impacts vitamin D levels primarily due to increased body inflammation, elevated levels of particulate matter nitrogen dioxide, and reduced production of activated vitamin D in the skin as a result of decreased air quality index (AQI) and reduced penetration of UV B radiation. According to various studies, the consumption of exogenous vitamin D supplements and a diet rich in calcium may be beneficial for individuals residing in highly polluted areas. To address this issue, public health policies should emphasize the importance of outdoor sunlight exposure, promote a healthy diet, and reduce exposure to pollutants.

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The effect of corticosteroid use in septic shock on secondary infection frequency, microorganism species, morbidity, and mortality

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Abstract

Aim: We aimed to examine the effect of corticosteroid treatment in vasopressor-refractory septic shock on secondary infections, microorganism species, survival, and length of hospital stay.

Material and methods: In this observational study, the records of 108 septic shock patients admitted to the intensive care unit (ICU) were reviewed. Patients were divided into two groups: the corticosteroid group (Group S, n=60) and the non-corticosteroid group (Group S-0, n=48). The results of three cultures [blood, endotracheal aspirate (eta), urine, wound] taken after ICU admission were recorded. The groups were compared in terms of demographic characteristics, culture growth rates and microorganisms, length of hospital stay, and survival rates.

Results: The hospital (p=0.043) and ICU stay (p=0.035) were longer in Group S. There was no significant difference between the groups in terms of survival (p>0.05). The growth rate of the first urine culture was significantly higher in Group S-0 than in Group S (p=0.018), but there was no difference in terms of microorganism species (p>0.05). There was no significant difference in growth rates and microorganism species in blood, eta and wound cultures (p>0.05), but increase in growth rates were observed in the 2nd and 3rd eta and, wound cultures compared to first culture in Group S (p<0.05).

Conclusion: There was no difference between the patients who received and did not receive corticosteroid treatment in septic shock in terms of culture growth rates, growing microorganism species and mortality; however, the frequency of growth in eta and wound cultures increased and the length of hospital stay was longer in patients who received corticosteroids.

Key words: septic shock, corticosteroid, secondary infection, mortality, morbidity

Introduction

Sepsis, a condition characterized by organ dysfunction resulting from an irregular response of the host to the infectious agent, requires immediate initiation of antibiotic therapy and administration of at least 30 mL/kg of intravenous crystalloid fluid support and colloid albumin within the first 3 hours from diagnosis [1]. However, in more severe cases, despite fluid support, septic shock may develop, which necessitates vasopressor therapy. Septic shock is defined as persistence hypotension requiring vasopressor therapy to maintain a mean arterial pressure (MAP) 65 mmHg or higher and a serum lactate level greater than >2 mmol/L despite adequate fluid resuscitation. Corticosteroid therapy is recommended as additional treatment in cases of vasopressor-refractory hypotension or the need for an increase in vasopressor dose in septic shock [1-3].

Glucocorticoids are steroid hormones secreted from the adrenal glands in a daily rhythm or during stress. They are both naturally produced in the body and synthetically manufactured. Cortisol is the most important glucocorticoid secreted in humans. Cortisol

is the primary corticosteroid released from the adrenal cortex. Critical illness can affect cortisol levels and function. The use of corticosteroids in septic shock is based on the possibility of developing a relative adrenal insufficiency due to impaired cortisol secretion and function, which needs to be replaced. The aim of corticosteroid therapy in septic shock is to correct the hypothalamic-pituitary-adrenal axis and to target clinical hemodynamic improvement [4]. Glucocorticoids also reduce inducible nitric oxide and prevent endogenous vasodilation, contributing to the vasopressor response produced by catecholamines. Furthermore, in septic shock, the administration of corticosteroids aims to reduce pro-inflammatory cytokines by taking advantage of their immunosuppressive and anti-inflammatory properties [5]. After binding to glucocorticoid receptors, a conformational change occurs in the receptor. Glucocorticoids inhibit transcription factors that regulate proinflammatory mediators. Their another important effects are inhibition of phospholipase A2, which is responsible for production of inflammatory mediators and suppressing genes responsible for expression of pro-inflammatory cytokines and various interleukins [6].

The recommended synthetic corticosteroid dosage in septic shock is hydrocortisone (200 mg/day) [2,3]. However, if hydrocortisone is not available, an equivalent dose of methylprednisolone can be administered. There is no definitive evidence showing that any corticosteroid drug or treatment (bolus or infusion) is more effective than the others in reducing mortality in septic shock [7].

In the literature, there are studies linking the use of corticosteroids in septic shock to the development of multisystemic adverse effects, as well as secondary infections that may arise due to immunosuppression [3,4]. Systemic corticosteroid therapy is associated with an increase risk of bacterial, viral, and fungal infections due to its dose-dependent inhibitory effects on phagocyte function. Also, intensity of therapy and several patient-specific factors such as older age, lower functional status and concomitant immunosupressive therapy influence infection risk. However, some studies have shown that the use of corticosteroids in septic shock does not affect the development of secondary infections [8,9]. Since there are different results in the literature regarding whether the use of corticosteroids in septic shock increases the incidence of secondary infections, the primary objective of our study was to determine whether corticosteroid therapy in septic shock increases the incidence of secondary infections and effects the microorganisms that grow in cultures. The secondary objective was to examine whether corticosteroid therapy influences the length of hospital stay and survival.

Material and methods

After obtaining approval from the Hospital Ethics Committee (E2-22-2188/2022), the hospital records of 150 adult patients over the age of 18 who had been hospitalized in the ICU with a diagnosis of septic shock between January 2016 and December 2017 were retrospectively reviewed. Patients who had been hospitalized for more than a week, who had no previous history of corticosteroid or other immunosuppressive treatment were included in the study. Patients who had stayed in the ICU for less than one week and those who had previously received immunosuppressive or corticosteroid therapy were excluded from the study.

Septic shock diagnoses and the recommendation of corticosteroid therapy were evaluated using the 2016 sepsis guidelines. Patients who received vasopressor therapy due to septic shock and did not achieve an average arterial pressure of 65 mmHg or systolic arterial pressure above 90 mmHg despite fluid and vasopressor therapy, were evaluated in terms of administering corticosteroid treatment (recommendation of guideline: 200 mg/day IV hydrocortisone or 40 mg/day IV methylprednisolone infusion). Corticosteroid doses were reduced when their vasopressor requirements decreased, and discontinued about total 7-10 days. Patients who included the study were divided into two groups: patients in septic shock who were given corticosteroid therapy (the corticosteroid group- Group S) and who were not given corticosteroid therapy (the non-corticosteroid group- Group S-0).

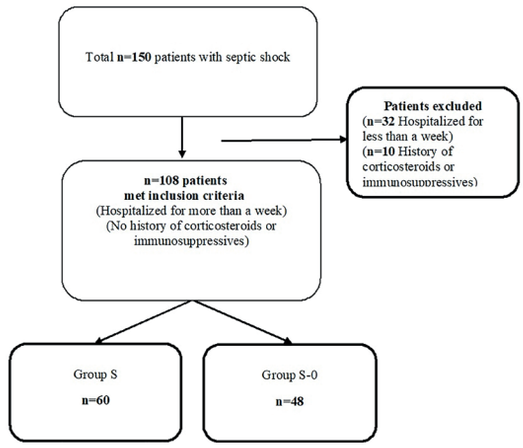
The demographic characteristics, comorbidities, length of ICU and hospital stay, and survival status (death or discharge) of the patients were retrospectively analyzed from the hospital records. The first three culture results obtained within the 28-day period following ICU admission were retrospectively recorded in chronological order. Microorganisms growing in cultures obtained from peripheral blood, blood obtained from a central venous catheter (referred to as the catheter culture), endotracheal aspirate (eta), urine, and wound (taken from decubitus ulcers or surgical incision areas of patients) were screened. Cultures taken from other body fluids (stool, pleural fluid, ascites, cerebrospinal fluid, rectal swabs) were excluded from the study as they were of limited quantity and not homogeneously distributed. The first cultures obtained from the patients were evaluated as cultures examined to determine the microorganisms causing infection, as they were taken upon admission of patients diagnosed with septic shock. The second and third cultures were taken during patients' hospitalization, upon the development of new fever, increase in acute phase reactants, and worsening clinical course. The first cultures obtained from the patients were labelled as blood-1, urine-1, catheter-1, wound-1, eta-1, the second cultures were labelled as blood-2, urine-2, catheter-2, wound-2, eta-2, and the third cultures were labelled as blood-3, urine-3, catheter-3, wound-3, eta-3. Microorganisms growing in these cultures were recorded. The patients' antimicrobial treatments were prescribed in collaboration with an infectious disease specialist, and the daily assessment of clinical and laboratory findings, as well as antibiotic susceptibility tests, was conducted. The potential side effects associated with corticosteroids, such as hypernatremia, hyperglycaemia, gastrointestinal bleeding, and cardiac events, were also recorded.

The data analysis was performed using IBM SPSS 23.0 (IBM. Corp. released 2015. IBM SPSS Statistics for windows, Version 23.0. Armonk, NY: IBM Corp.) statistical software package. Power analysis was conducted using G*Power 3.1.9.2 (Faul, F., Erdfelder, E., Buchner, A., & Lang, A.G., 2014. Germany: University of Kiel) statistical software package, and the power was determined to be 0.98, with n1=48, n2=60, α=0.05, and effect size d=0.8. Descriptive statistical methods such as frequency, percentage, mean, and standard deviation were used to evaluate the study data, as well as Pearson chi-square, Yates chi-square, and Fisher exact tests were used to compare qualitative data depending on the situation. The conformity of the data to normal distribution was evaluated by Kolmogorov-Smirnov test. Independent samples T test (independent samples T test) was used to evaluate the normally distributed quantitative data. Inter-temporal comparisons of cultures were performed by McNemar's Test. Statistically significance level of $p<0.05$ was considered.

Results

Among 150 patients, ultimately, 108 patients who met the inclusion criteria were included in the study. It was observed that the number of patients who were given steroid treatment was

Figure 1 - Flowchart of the Study



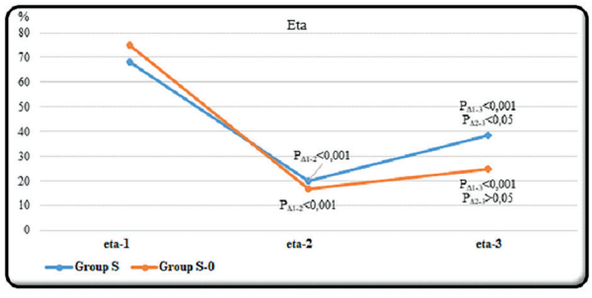
n=60 (Group S), and the number of patients who were not given was n=48 (Group S-0). The diagram of the study is presented in Figure 1. The mean age was significantly lower in Group S (68.1±18.1 years) than Group S-0 (76.3±12.5 years) (Table 1) (p=0.007). When comorbidities were examined, no significant differences were found between the groups in terms of cardiovascular disease, malignancy, and cerebrovascular disease (p>0.05) (Table 1). The length of hospital and ICU stay for patients in Group S (34.2±20.4 and 30.0±20.1 days) was found to be significantly longer than that in Group S-0 (27.5±20.2 and 23.0±13.7 days) (p=0.043, p=0.035). However, there was no significant difference between Group S (68.3%, n=41) and Group S-0 (75%, n=36) in terms of survival (p>0.05) (Table 1).

Table 1 Comparison of demographic and general characteristics of patients in groups

Variables	Groups		p value
	Group S (n=60)	Group S-0 (n=48)	
Age (year)	68.1±18.1	76.3±12.5	**0.007**
Length of ICU stay (day)	30.0±20.1	23.0±13.7	**0.035**
Length of hospital stay (day)	34.2±20.4	27.5±20.2	**0.043**
Renal Disease	13 (21.7)	7 (14.6)	0.456
Cardiovascular Disease	31 (51.7)	28 (58.3)	0.619
Malignancy	5 (8.3)	8 (16.7)	0.305
Cerebrovascular Disease	17 (28.3)	12 (25.0)	0.865
Lung Disease	20 (33.3)	5 (10.4)	**0.010**
Diabetes mellitus	5 (8.3)	13 (27.1)	**0.019**
Survival Death Discharge	41 (68.3) 19 (31.7)	36 (75.0) 12 (25.0)	0.584

Data are presented as mean ± SD and [n (%)]. p<0.05 is statistically significant. Group S: corticosteroid group, Group S-0: non-corticosteroid group

Figure 2 - Intra-group comparison of eta cultures



Data are presented as mean ± SD and [n (%)]. P<0.05 is statistically significant. Group S: corticosteroid group, Group S-0: non-corticosteroid group. $P_{\Delta1-2}$: Comparison between 1st and 2nd cultures, $P_{\Delta2-3}$: Comparison between 2nd and 3rd cultures, $P_{\Delta1-3}$: Comparison between 1st and 3rd cultures

Table 2 Comparison of eta cultures of patients in groups

Cultures		Groups		p value
		Group S (n=60)	Group S-0 (n=48)	
Eta-1	**No growth**	19 (%31,7)	12 (%25,0)	0.584
	Growth	41 (%68.3)	36 (%75)	
	A.baumannii	*11 (%18.3)*	*12 (%25)*	
	P. aeruginosa	*6 (%10)*	*8 (%16.7)*	
	Klebsiella spp.	*5 (%8.3)*	*8 (%16.7)*	
	Candida spp.	*9 (%15)*	*3 (%6.3)*	
	Other	*5 (%8.3)*	*2 (%4.2)*	
	MRSS	*2 (%3.3)*	*1 (%2.1)*	
	S. marcescens	--	*2 (%4.2)*	
	Enterococcus spp.	*1 (%1.7)*	--	
	E.coli	*1 (%1.7)*	--	
	Aspergillus	*1 (%1.7)*	--	
Eta-2	**No growth**	48 (%80,0)	40 (%83,3)	0.846
	Growth	12 (%20,0)	8 (%16,7)	
	A.baumannii	*4 (%6.7)*	*4 (%8.3)*	
	P. aeruginosa	*2 (%3.3)*	*2 (%4.2)*	
	Klebsiella spp.	*4 (%6.7)*	--	
	Candida spp.	--	*2 (%4.2)*	
	E.coli	*1 (%1.7)*	--	
	Other	*1 (%1.7)*	--	
Eta-3	**No growth**	37 (%61,7)	36 (%75,0)	0.206
	Growth	23 (%38,3)	12 (%25,0)	
	A.baumannii	*6 (%10)*	*6 (%12.5)*	
	P. aeruginosa	*6 (%10)*	*2 (%4.2)*	
	Candida spp	*5 (%8.3)*	*3 (%6.3)*	
	MRSS	*2 (%3.3)*	*1 (%2.1)*	
	Klebsiella spp.	*2 (%3.3)*	--	
	E.coli	*1 (%1.7)*	--	
	Other	*1 (%1.7)*	--	

Data are presented as mean ± SD and [n (%)]. P<0.05 is statistically significant. Group S: corticosteroid group, Group S-0: non-corticosteroid group. Eta: endotracheal aspirate. spp: species, MRSS: methicillin-resistant Staphylococcus species, E. coli: Escherichia coli, A.baumannii: Acinetobacter baumannii, P. aeruginosa: Pseudomonas aeruginosa, S. marcescens: Serratia marcescens

The microorganisms that grew in the cultures of eta-1, eta-2, and eta-3 of the patients and their distribution among the groups are presented in Table 2. There was no significant difference between the two groups in terms of culture growth rates and isolated microorganism species (p>0.05). When intra-group comparisons of eta cultures were evaluated, a significant decrease in the growth of eta-2 cultures was determined compared to eta-1 in both groups (PΔ1-2<0.001, Figure 2). However, while there was an increase in both eta-2 and eta-3 culture growth in both groups, a statistically significant increase in eta-3 culture growth compared to eta-2 was observed in Group S (PΔ2-3<0.05, Figure 2). Comparison between eta-1 and eta-3 in both groups revealed a significant increase in eta-3 culture growth compared to eta-1 (PΔ1-3<0.001, Figure 2).

There was no statistically significant difference between the two groups in terms of culture growth rates and identified microorganism species in both blood-1, blood-2, and blood-3 cultures, as well as central venous catheter cultures (catheter-1, catheter-2, and catheter-3) (p>0.05, Table III). In addition, when intra-group comparisons of blood cultures and central venous catheter cultures were evaluated, a significant decrease was observed between the 1st, 2nd and 3rd culture growths in both groups (p<0.05 - p<0.001). Although there was a decrease in the 3rd culture growth compared to the 2nd culture, it was not statistically significant (p>0.05).

Table 3 Comparison of blood and central venous catheter cultures of patients in groups

Cultures		Groups		p value
		Group S (n=60)	Group S-0 (n=48)	
Blood-1	**No growth**	32 (%53,3)	22 (%45,8)	0.561
	Growth	28 (%46.7)	26 (%54.2)	
	MRSS	*15 (%25)*	*11 (%22.9)*	
	A.baumannii	*4 (%6.7)*	*3 (%6.3)*	
	Klebsiella spp.	*3 (%5)*	*3 (%6.3)*	
	Enterococcus spp.	*3 (%5)*	*3 (%6.3)*	
	P. aeruginosa	*1 (%1.7)*	*3 (%6.3)*	
	Candida spp.	*1 (%1.7)*	*1 (%2.1)*	
	Other	*1 (%1.7)*	*1 (%2.1)*	
	E.coli	--	*1 (%2.1)*	
Blood -2	**No growth**	46 (%76,7)	37 (%77,1)	0.959
	Growth	14 (%23,3)	11 (%22,9)	
	MRSS	*7 (%11.7)*	*2 (%4.2)*	
	A.baumannii	*2 (%3.3)*	*3 (%6.3)*	
	Enterococcus spp..	*1 (%1.7)*	*4 (%8.3)*	
	P. aeruginosa	*1 (%1.7)*	*2 (%4.2)*	
	Klebsiella spp.	*2 (%3.3)*	--	
	E.coli	*1 (%1.7)*	--	
Blood -3	**No growth**	52 (%86.7)	40 (%83.3)	0.832
	Growth	8 (%13.3)	8 (%16.7)	
	MRSS	--	*5 (%10.4)*	
	Enterococcus spp.	*2 (%3.3)*	*2 (%4.2)*	
	A.baumannii	*2 (%3.3)*	--	
	Other	*2 (%3.3)*	*1 (%2.1)*	
	Klebsiella spp.	*1 (%1.7)*	--	
	Candida spp.	*1 (%1.7)*	--	
Cathater-1	**No growth**	45 (%75)	36 (%75)	1.000
	Growth	15 (%25)	12 (%25)	
	MRSS	*9 (%15)*	*3 (%6.3)*	
	A.baumannii	*3 (%5)*	*5 (%10.4)*	
	Klebsiella spp.	*2 (%3.3)*	*1 (%2.1)*	
	E.coli	*1 (%1.7)*	*1 (%2.1)*	
	Candida spp.	--	*1 (%2.1)*	
	Other	--	*1 (%2.1)*	
Cathater-2	**No growth**	56 (%93.3)	45 (%93.8)	1.000
	Growth	4 (%6.7)	3 (%6.3)	
	A.baumannii	*2 (%3.3)*	*1 (%2.1)*	
	Candida spp.	*1 (%1.7)*	*1 (%2.1)*	
	Klebsiella spp.	--	*1 (%2.1)*	
	MRSS	*1 (%1.7)*	--	
Cathater-3	**No growth**	59 (%98.3)	48 (%100)	1.000
	Growth	1 (%1.7)	--	
	P. aeruginosa	*1 (%1.7)*	--	

Data are presented as mean ± SD and [n (%)]. p<0.05 is statistically significant. Group S: corticosteroid group, Group S-0: non-corticosteroid group. Eta: endotracheal aspirate, spp: species, MRSS: methicillin-resistant Staphylococcus species, E. coli: Escherichia coli, A.baumannii: Acinetobacter baumannii, P. aeruginosa: Pseudomonas aeruginosa

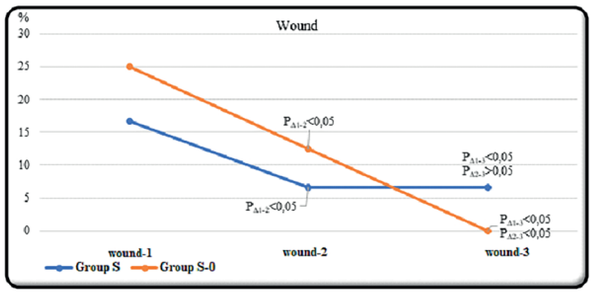
Table 4 Comparison of urine and wound cultures of patients in groups

Cultures		Groups		p value
		Group S (n=60)	Group S-0 (n=48)	
Urine-1	**No growth**	41 (%68.3)	21 (%43.8)	**0.018**
	Growth	19 (%31.7)	27 (%56.3)	
	Candida spp.	*7 (%11.7)*	*12 (%25)*	
	P. aeruginosa	*4 (%6.7)*	*5 (%10.4)*	
	E.coli	*3 (%5)*	*2 (%4.2)*	
	Klebsiella spp.	*2 (%3.3)*	*2 (%4.2)*	
	A.baumannii	*1 (%1.7)*	*2 (%4.2)*	
	Enterococcus spp.	--	*3 (%6.3)*	
	P.mirabilis	*2 (%3.3)*	--	
	Other	--	*1 (%2.1)*	
Urine-2	**No growth**	49 (%81.7)	36 (%75)	0.546
	Growth	11 (%18.3)	12 (%25)	
	Candida spp.	*6 (%10.0)*	*2 (%4.2)*	
	E.coli	*2 (%3.3)*	*4 (%8.3)*	
	A.baumannii	*2 (%3.3)*	*2 (%4.2)*	
	P. aeruginosa	--	*3 (%6.3)*	
	Klebsiella spp.	--	*1 (%2.1)*	
	Enterococcus spp.	*1 (%17)*	--	
Urine-3	**No growth**	57 (%95)	45 (%93,8)	1.000
	Growth	3 (%5)	3 (%6.3)	
	Candida spp.	*1 (%1.7)*	*1 (%2.1)*	
	P. aeruginosa	*1 (%1.7)*	--	
	Klebsiella spp.	--	*1 (%2.1)*	
	A.baumannii	*1 (%1.7)*	--	
	E.coli	--	*1 (%2.1)*	
Wound-1	**No growth**	50 (%83.3)	36 (%75)	0.408
	Growth	10 (%16.7)	12 (%25)	
	P. aeruginosa	*3 (%5)*	*4 (%8.3)*	
	A.baumannii	*3 (%5)*	*3 (%6.3)*	
	Klebsiella spp.	*2 (%3.3)*	*2 (%4.2)*	
	E.coli	*1 (%1.7)*	*1 (%2.1)*	
	Enterococcus spp.	--	*1 (%2.1)*	
	MRSS	*1 (%1.7)*	--	
	P.mirabilis	--	*1 (%2.1)*	
Wound-2	**No growth**	56 (%93.3)	42 (%87.5)	0.334
	Growth	4 (%6.7)	6 (%12.5)	
	A.baumannii	*3 (%5)*	*4 (%8.3)*	
	P. aeruginosa	--	*1 (%2.1)*	
	MRSS	--	*1 (%2.1)*	
	P.mirabilis	*1 (%1.7)*	--	
Wound-3	**No growth**	56 (%93.3)	48 (%100)	0.127
	Growth	4 (%6.7)	--	
	A.baumannii	*2 (%3,3)*	--	
	E.coli	*1 (%1,7)*	--	
	P.mirabilis	*1 (%1,7)*	--	

Data are presented as mean ± SD and [n (%)]. p<0.05 is statistically significant. Group S: corticosteroid group, Group S-0: non-corticosteroid group. Eta: endotracheal aspirate, spp: species, MRSS: methicillin-resistant Staphylococcus species, E. coli: Escherichia coli, A.baumannii: Acinetobacter baumannii, P. aeruginosa: Pseudomonas aeruginosa

The microorganisms and their distribution in patients' urine-1, urine-2, and urine-3 cultures are given in Table IV. In the first urine culture taken with the diagnosis of septic shock, the growth rate was significantly higher in Group S-0 compared to Group S ($p<0.05$). However, in patients' urine-2 and urine-3 cultures, no significant difference was observed between the groups in terms of growth rates and microorganisms that grew ($p>0.05$). When intra-group comparisons were evaluated in urine cultures, a decrease in growth was observed in urine-2 and urine-3 cultures compared to urine-1 cultures in both groups. Additionally, there was also a decrease in growth in urine-3 culture compared to urine-2 culture in both groups. The decrease in urine culture growth in urine-2 compared to urine-1, and in urine-3 compared to urine-2, was statistically significant in Group S-0 ($p<0.05$), but not in Group S. The decrease in culture growth between urine-1 and urine-3 was statistically significant in both groups (Group S-$p<0.05$, Group S-0-$p<0.001$).

Figure 3 - Intra-group comparison of wound cultures



Data are presented as mean ± SD and [n (%)]. p<0.05 is statistically significant. Group S: corticosteroid group, Grup S-0: non-corticosteroid group. PΔ1-2: Comparison between 1st and 2nd cultures, PΔ2-3: Comparison between 2nd and 3rd cultures, PΔ1-3: Comparison between 1st and 3rd cultures

The microorganisms and their distribution in patients' wound-1, wound-2, and wound-3 cultures are given in Table IV. No significant difference was observed between the groups in terms of the microorganism species and the growth rates grown in the wound cultures of the patients (p>0.05). When comparing the intra-group wound cultures (Figure 3), a statistically significant decrease in growth rate was observed in both groups in wound-2 and wound-3 cultures compared to wound-1 culture. However, the decrease in growth rate in wound-3 culture compared to wound-2 culture was only significant in Group S-0 (p<0.05).

The records of patients in both groups were reviewed, and no cases of hypernatremia, hyperglycaemia, gastrointestinal bleeding, or cardiac events were observed in any patient.

Discussion

In this study, primarily, we found that in septic shock patients who were given corticosteroids, there was a significant increase in eta culture growth and a slight increase in wound culture growth which did not reach a statistically significant level in the 3rd cultures compared to the 2nd culture. According to these findings, when corticosteroids are used in recommended doses and duration in septic shock, they slightly increase the rate of secondary infections but do not create a significant difference in terms of the species of microorganisms that grow. Secondarily, despite longer hospital and ICU stay durations, we observed no increase in hospital mortality in patients receiving corticosteroids compared to those who did not.

The use of corticosteroids in septic shock has been studied in many studies in the literature and even presented as a recommendation in sepsis guidelines. In the previous 2017 sepsis management guidelines, IV hydrocortisone treatment (200 mg/day) was recommended in case hemodynamic did not improve despite adequate fluid resuscitation and vasopressor therapy [10]. However, in the latest guidelines, hydrocortisone therapy is recommended with moderate quality of evidence (weak recommendation; moderate quality of evidence) in cases of septic shock despite adequate fluid therapy, when the dose of norepinephrine or epinephrine is ≥0.25 mcg/kg/min for at least 4 hours [2]. Corticosteroid treatment is widely used in the management of lung diseases, including obstructive pulmonary disease and interstitial lung disease [11]. In our retrospective study, we found that corticosteroid treatment was significantly less likely to be administered to elderly and diabetic patients, and significantly more likely to be administered to patients with lung disease. The reason for this could be due to the inclusion of septic shock patients in the intensive care unit between 2016-2017 in our study and the lack of clear guidelines at that time regarding when to initiate corticosteroid therapy in vasopressor-resistant septic shock. Additionally, clinicians may have been more hesitant to initiate corticosteroid treatment in elderly and diabetic patients due to concerns about potential side effects, while being more willing to do so in patients with lung disease. Our patients receiving corticosteroid therapy were younger in age and had a lower prevalence of diabetes. Despite this, we observed a higher rate of growth in their ETA cultures. We are of the opinion that the lower rates of corticosteroid treatment in patients with advanced age or diabetes might have influenced our study results.

In a study of patients with pneumonia, corticosteroid treatment was found to have no effect on mortality but was associated with prolonged hospital stay [12]. A comprehensive review on the use of corticosteroids in pneumonia showed that in a serious community-acquired pneumonia patient population, corticosteroid treatment reduced mortality, shortened treatment and hospital stays, prevented the development of shock and respiratory failure that were not present in admission to hospital however in non-severe community-acquired pneumonia, corticosteroid treatment had no effect on mortality but reduced morbidity [13]. In a study of critically ill patients with ARDS and severe sepsis treated with corticosteroids, it was found that corticosteroid treatment improved oxygenation but had no effect on mortality [14].

There are studies in the literature that demonstrate the use of corticosteroids in septic shock reduces shock duration, decreases the need for mechanical ventilation, reduces mortality, and shortens hospital and intensive care unit stays [3,5,15,16,17]. However, in a study of infants with diaphragmatic hernia who received corticosteroids for refractory shock requiring vasopressor treatment, longer hospital stay, more days on mechanical ventilation, and higher mortality rates were observed [8]. Additionally, a smaller study showed that the use of hydrocortisone in septic shock increased mortality [18]. However, in meta-analyses it was reported that the use of corticosteroids did not affect short-term mortality but provided a mild decrease in long-term mortality and shortened hospital and ICU stays [9,15]. In our study, the length of stay for those who were received corticosteroids were longer than those who were not received, but there was no significant difference in terms of hospital mortality between groups. However, in our study, we only examined patients' hospital outcomes as mortality, and did not evaluate long-term mortality. We believe that the main reason for prolonged length of stay in corticosteroid-treated patients was the slight increase in secondary infections in these patients. Additionally, although not proven with tests, we believe that corticosteroid treatment may have increased muscle weakness and prolonged the ICU and hospital discharge.

Secondary infections are known as one of the adverse effects of corticosteroid treatment [3,4,7,19]. Corticosteroid therapy may cause iatrogenic immunosuppression, which can provide a ground for opportunistic and resistant microorganisms and fungi to become infectious agents. However, in a meta-analysis that included randomized controlled trials conducted in children and adults examining the use of steroids in sepsis, it was reported that corticosteroid use probably does not cause superinfection (RR 1.06, 95% CI 0.95 to 1.19; 5356 participants; 25 studies; moderate certainty evidence) [3]. Another review on the use of steroids in pneumonia treatment has also indicated that steroid treatment did not cause superinfection [13]. In a study conducted on infants in shock who received hydrocortisone treatment, no difference was observed between patients who received steroid treatment and those who did not in terms of developing secondary bacterial sepsis [8]. A meta-analysis evaluating corticosteroid use in sepsis also showed no effect on superinfections [9]. In a study comparing hydrocortisone

and fludrocortisone treatment in septic shock to placebo, it was observed that the use of steroids did not increase the risk of superinfection [17]. In a study on sepsis patients receiving corticosteroid treatment, the risk of developing infection in the long term was found to be 5 times higher than in those not receiving corticosteroid treatment [20]. The use of corticosteroids is known to be one of the risk factors for Candida colonization [21]. In a study on malignant patients in septic shock treated with hydrocortisone, approximately 23% of the secondary infections encountered were recorded as fungal infections [19]. Moreover, in the same study, the mortality rate of patients who had secondary infections was found to be higher than those who did not [19]. Another study examining complicated urinary tract infections caused by Pseudomonas revealed that the incidence of such infections was associated with corticosteroid therapy [22]. In our study, the initial cultures obtained from the patients were taken at the time of sepsis diagnosis, therefore we did not consider the difference in the initial urine culture results between the group receiving corticosteroids and the group not receiving corticosteroids because of corticosteroid use. The number and rate of growth, species that growth in subsequent urine cultures taken during hospitalization after corticosteroid use did not differ from those in the group not receiving corticosteroids.

Bloodstream infections, often catheter-related, can also be secondary to the transfer of infection sources from other areas into the bloodstream. The most common cause of these infections are Gram-positive microorganisms [23]. Central catheters become colonized within 1-3 days after placement, and it is known that the agents in the biofilm layer are often Gram-positive and Gram-negative microorganisms, as well as Candida species [24]. In a study conducted in newborns, the most common growth observed in blood cultures was methicillin-resistant coagulase-negative staphylococci, but no association was found between the growth and steroid use [25]. In another study, coagulase-negative staphylococci and Klebsiella were the most isolated microorganisms in hospital-acquired infections, and the use of antenatal corticosteroids was found to be effective in the development of infection in these patients [26]. In a study of patients with Acinetobacter bacteraemia mortality was associated with corticosteroid therapy [27]. In a study, it has been demonstrated that the growth of Pseudomonas in the blood of patients with haematological malignancy is associated with the use of corticosteroids [28]. Although steroid use is considered a risk factor for invasive candidiasis, a study evaluating the risk factors for candidemia reported that corticosteroid therapy was not related with candidemia [29]. In our study, no significant difference was observed in the growth of microorganisms between patients who received corticosteroid treatment and those who did not, in both catheter and blood cultures. MRSS was the most cultured microorganism in blood cultures, consistent with the literature. Gram-negative microorganisms and Candida species were also found in both central venous catheter and peripheral venous blood cultures. The second and third cultures were obtained upon the development of new fever, increase in acute phase reactants, and deterioration in clinical status during hospitalization. In both corticosteroid-treated and untreated patients, a decrease in microbial growth was observed in the second and third cultures obtained from the patients.

Nosocomial pathogens isolated from the respiratory tract are mostly Gram-negative and Gram-positive microorganisms, and their growth can also be polymicrobial [13]. It is known that corticosteroid treatment is a risk factor for Aspergillus infection [30]. In our study, Gram-negative microorganisms were the most isolated from patient cultures, followed by Candida species and Gram-positive microorganisms. Aspergillus growth was detected in only one of our patients and was not associated with corticosteroid treatment as it was observed only in the first culture. Studies suggest that corticosteroid treatment may contribute to the development of resistant Gram-negative microorganisms such as Acinetobacter, Klebsiella, and Pseudomonas [19,31-33]. A study on patients with community-acquired pneumonia who received corticosteroid treatment found higher rates of nosocomial infection compared to those who did not receive corticosteroid treatment [12].

In a study on ventilator-associated pneumonia, Gram-negative microorganisms were the most isolated, and corticosteroid use was evaluated as a risk factor associated with mortality [34]. In a meta-analysis examining risk factors for resistant klebsiella growth, corticosteroid use was found to be one of the risk factors [35]. The microorganisms isolated in our study were consistent with other studies; however, the increase in growth observed in the third culture compared to the second culture in patients who received corticosteroids suggested an increased risk of secondary infection associated with corticosteroids.

Pressure ulcers are a significant source of infection in intensive care unit patients. Gram-positive and Gram-negative microorganisms are frequently isolated in soft tissue infections. The incidence of Gram-negative microorganisms has increased in surgical site infections and diabetic foot wounds of hospitalized patients [36]. In our study, wound cultures were mainly obtained from pressure ulcers, and less frequently from surgical incision sites. It was observed that pressure ulcers in patients with limited mobilization were also a source of septic shock. Moreover, in our clinically severe patients with multiple risk factors for pressure ulcer development, the microorganisms in the wounds were also predominantly Gram-negative, consistent with the literature. There was no difference in the rates of wound culture growth and types of microorganisms isolated between patients who received corticosteroids and those who did not. However, while the rate of wound culture growth decreased in patients not receiving corticosteroids, we observed that the rate of growth did not decrease and even slightly increased in the third wound cultures of corticosteroid-receiving patients, suggesting the presence of secondary infections in these patients. In our study, Acinetobacter was the most isolated microorganism in all cultures evaluated, and the other isolated microorganisms were predominantly Gram-negative bacteria. However, steroid use did not cause any differences in the types of microorganisms isolated in all cultures.

In septic shock, in addition to secondary infections, adverse effects such as hyperglycaemia gastrointestinal bleeding, and cardiac events may also be observed due to corticosteroid use [11]. It is believed that major side effects caused by corticosteroids are associated with prolonged use and high doses [2,4,9]. In our study, no major side effect related to corticosteroid use was recorded in patients receiving corticosteroids. This may be due to the low dose, infusion form, and short duration of corticosteroid administration. It has also been reported that the use of corticosteroids in septic shock does not cause any significant side effects [16,17].

Limitations of our study include its retrospective design, small sample size, heterogeneity in the distribution of patient characteristics (age, disease, etc.) and lack of examination of long-term outcomes. Only three cultures (including one baseline culture) from our patients within a 28-day period were evaluated. We believe that monitoring patients' infection symptoms and cultures for a longer period and examining post-hospital mortality could change our study results. Alongside its limitations, we think that out study is valuable in that it shows

that there may be an increase in culture growth as a result of 28-day follow-up even in patients who were given corticosteroid treatment at recommended dose and duration in septic shock. Furthermore, standardizing factors other than corticosteroids and conducting prospective studies with more homogeneous and larger patient groups may yield different results.

In conclusion, in our retrospective study of patients with septic shock who were administered low-dose corticosteroid therapy for replacement purposes and adjusted according to their vasoactive needs, we found that corticosteroids partially increased the risk of secondary infection and prolonged hospital and ICU stays but did not affect the type of microorganisms or mortality rates. Therefore, we believe that the use of corticosteroids in vasoactive treatment-resistant septic shock should be re-evaluated through randomized controlled trials with a larger number of patients, considering the potential benefits and secondary infection risks.

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Identifying potential risk factors associated with gastrointestinal tract cancers: A case-control study in Turkey

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Abstract

Objective: Gastrointestinal cancers have different risk factors. However, it was clearly thought that the risk factors of these cancers should be determined by a case-control studies. The study aims to determine the potential risk factors associated with gastrointestinal cancers with a case-control study design.

Material and methods: This case-control study was conducted with a total of 620 people applied to Department of General Surgery of two hospital in Turkey. The case group consisted of 310 patients diagnosed with gastrointestinal tract cancers. The control group consisted of 310 subjects without any history of cancer including gastrointestinal cancers. The data were collected with the Patient Identification Form including socio-demographic characteristics, and risk factors for gastrointestinal tract cancers.

Results: The mean age of the case and control groups were 58.9±12.9 and 50.0±10.0, respectively. Although there was statistically significant differences in several factors compared, multivariate analysis identified male gender (OR=1.729, p=0.02), higher age (OR=1.068, p<0.001), low body mass index (OR=1.110, p<0.001), high number of children (OR=1.563, p<0.001), cancer history in the family (OR=4.444, p<0.001) and the presence of other chronic diseases (OR=6.314, p<0.001) as risk factors. Mostly vegetable consumption (OR=2.923, p<0.001) was also found to be a lower risk factor for gastrointestinal cancers.

Conclusion: According to this study; age, gender, body mass index, number of children, family history of cancer, chronic diseases were among risk factors for gastrointestinal tract cancers. Vegetable consumption was an important factor to decrease gastrointestinal cancers.

Key words: cancer, gastrointestinal tract, nursing, risk factors

Introduction

Cancer is still a major cause of morbidity and mortality worldwide [1-3], as well as the second leading cause of death. It is estimated that 9.6 million people died because of cancer in 2018, and one out of every six deaths is related to cancer [4]. Due to the global increase in average life expectancy and human population, 20.3 million new cancer cases are expected by 2030 [1].

Gastrointestinal tract cancers originate from the esophagus, stomach, pancreas, hepatobiliary system, small intestine, large intestine, rectum, and anus [3,5]. According to the World Health Organization in 2018, colorectal cancers (1.80 million cases) are the third most common cancers and stomach cancers (1.03 million cases) are the sixth [4]. Based on Turkey Statistical Institute 2018 data; stomach cancer ranks third (8.2%) and colon cancer ranks fourth (7.8%) [6].

Though the incidence of gastrointestinal tract cancers rapidly increases, survival rates for these cancer are high thanks to early diagnosis, effective medical and surgical approaches. Early diagnosis is so important to decrease the incidence of cancer risk. Therefore, it is essential to detect risk factors for gastrointestinal cancers to decrease morbidity and mortality rates [1,7-9].

Several cancer types have specific risk factors such as geographic, race, cultural and socioeconomic characteristics, diet, environmental factors, genetic and familial predisposition [1,8,10,11]. Approximately one-third of deaths due to cancer are most commonly caused by high body mass index, lack of physical

activity, low fruit and vegetable intake, smoking and alcohol consumption [4]. To our knowledge, there are no studies exploring risk factors for gastrointestinal tract cancers except for histopathological identification. In this context, the study was aimed at identifying potential risk factors associated with gastrointestinal tract cancers.

Material and methods

Study design and sample size

This case-control study was conducted with 620 people who applied to Department of General Surgery of two hospitals in Istanbul, Turkey between May-December 2017. The case group consisted of 310 patients diagnosed with colon or stomach located gastrointestinal tract cancers who admitted to the Departments of General Surgery. The control group was consisted of 310 subjects who visited to outpatient clinics of the Departments of General Surgery without having a GI problems/ diagnosis, and any type of cancer history. Patients aged 18 years and older who met the inclusion criteria were included in the sample both in the intervention and control groups. The phase of the gastrointestinal cancer was determined by clinical and histopathological evaluation. Participants who have Phase I or II cancer admit inpatient clinics because of bleeding, having a biopsy, or an emergency etc. and participants who have Phase III or IV cancer admit inpatient clinics to have surgery. For this, the number of participants who has Phase III or IV cancer was admitted the hospital more in this study.

Power analysis was performed to determine the sample size of the study. The power of the test was calculated with G*Power 3.1 program. It was aimed to reach at least 176 people in two groups; 88 people in each group with a 5% significance level and 0.5 effect size in order to exceed 95% value.

Data collection tools

The participants were informed about the aim of the study. Then, data were collected by face to face interview method from patients who voluntarily participated in the study. Informed consent was obtained from all study participants. Each interview lasted about 10-15 min. The data were collected with the Patient Identification Form containing a total of 32 items prepared by the researchers as a result of the literature review [2,12-17]. This form included socio-demographic characteristics, and risk factors for gastrointestinal tract cancers such as gender, age, body mass index (BMI), marital status, number of children, alcohol use, smoking, exercise, family medical history, chronic diseases, eating and drinking habits.

Data analysis

Statistical analysis was carried out using the SPSS software package (Statistical Package for Social Sciences, version 22.0, SPSS Inc., IBM Corporation, Armonk, New York, USA). Data were analyzed with Shapiro-Wilk test to check the normality of distribution, and Leneve test was used to evaluate the homogeneity of variances assumption. Independent-samples T-test was used in comparison of independent groups, while Monte Carlo simulation technique was used in Mann-Whitney U test. The comparison of categorical variables was performed with Chi-squared or Binomial tests, and Monte Carlo simulation technique was used in Pearson chi-squared and Fisher's exact tests. Multiple logistic regression test was used to evaluate the cause and effect relation of descriptive variables with categorical variables in dichotomous and multinomial categories. Quantitative data were summarized as mean±standard deviation and categorical data with descriptive statistics (number and percentage). P-value less than 0.05 was accepted as statistically significant.

Ethical statement

The study protocol was approved by the Ethics Committee of a university in Istanbul Turkey (Dated: 06.01.2016 Numbered:10840098-604.01.01-E.319) and complied with the guidelines of the Helsinki Declaration. Informed consent was obtained from all study participants.

Results

Diagnostic factors in medical characteristics of the case group are summarized in Table 1. Accordingly, a statistically significant portion of the patients had more than one symptom, where abdominal pain (n=79, 25.5%) followed by nausea and vomiting (n=50, 16.1%) were the most frequently reported ones (p<0.001). Most of the case group visited a practitioner right after their first symptom (n=180, 58.1%, p<0.001). General surgery was the first practitioner visited by most of the study patients (n=142, 45.8%), followed by internal medicine (n=108, 34.8%) (p<0.001).

Table 1 Diagnostic factors in medical characteristics of the case group (n=310)

		Case group (n = 310)	p value
Clinical manifestation			
	Bleeding	21 (6.7)	< 0.001[a]
	Abdominal pain	79 (25.5)	
	Abdominal swelling	10 (3.2)	
	Nausea and vomiting	50 (16.1)	
	Constipation	43 (13.9)	
	Multiple complaints	107 (34.5)	
How soon you visited a practitioner after your first symptom?			
	Right after	180 (58.1)	< 0.001[a]
	In a few weeks	30 (9.7)	
	After a month	23 (7.4)	
	More than a month	77 (24.8)	
Which practitioner you visited first?			
	General surgeon	142 (45.8)	< 0.001[a]
	Gastroenterology	60 (19.4)	
	Internal medicine	108 (34.8)	
Phase			
	Phase I	7 (2.2)	< 0.001[a]
	Phase II	24 (8.1)	
	Phase III	75 (24.0)	
	Phase IV	204 (65.7)	
Tested for helicobacter pylori			< 0.001[b]
	Yes	300 (96.8)	
	No	10 (3.2)	
Helicobacter pylori detected			< 0.001[b]
	Yes	50 (16.7)	
	No	250 (83.3)	
Gastrointestinal tumor location			
	Stomach	87 (28.1)	< 0.001[b]
	Colon	223 (71.9)	

Data are given as n (%). Statistical analysis was performed with [a] Chi-squared test, or [b] Binomial test.

A statistically significant number of gastrointestinal cancer cases in this study were diagnosed at phase IV (n=204, 65.7%, p<0.001). The location of gastrointestinal tract cancers in the patients involved in this study was colon (n=223, 71.9%) and stomach (n=87, 28.1%).

Table 2 summarizes the comparison of the demographic characteristics of the case group with the control group with respect to gender, age, BMI, marital status and number of children, alcohol abuse and current smoking habits, exercising, family medical history, presence of other chronic diseases, and common eating and drinking habits. There was a statistically significant male dominance in the case group (n=209, 67.5%) compared to the control group (n=168, 54.2%) (p=0.001). The mean age of the case group diagnosed with gastrointestinal cancers was significantly higher (58.9±12.9 and 50.0±10.0 years, respectively, p<0.001), and, contrarily, the mean BMI (25.2±4.7) was significantly lower than the control group (27.0±3.5, p<0.001). Most of the subjects in both groups were married with children and lived in a nuclear family (Table 2). Smoking, alcohol abuse or exercising habits of the case and control groups were comparable without any statistically significant difference (Table 2, p>0.05). The family history of cancer and the presence of other chronic diseases other than gastrointestinal cancers were significantly higher in the case group compared to the control group (Table 2, p<0.001 for both). There was a statistically significant difference between the two groups with respect to eating habits, except the regular cola consumption (Table 2). The control group consumed either no meat (n=92, 27.7%) or eat meat once a week (n=102, 32.3%); whereas the case group consumed meat once (n=92, 29.7%) or twice (n=120, 38.7%) weekly (p<0.001). Adding salt to most meals was significantly higher in the case group (p<0.001), and margarine was more commonly used in cooking in the case group (n=70, 22.6%). Another interesting observation was that vegetables were the mostly consumed in the control group (n=192, 61.9%), whereas the patients involved in the study preferred both vegetables and meet (n=100, 32.3%).

The risk factors for gastrointestinal cancers were analyzed by multiple logistic regression test (Table 3). Accordingly, in multivariate analysis, the gender male (odds ratio [95% confidence interval], 1.729 [1.090-2.745], p=0.02), higher age (1.068 [1.045-1.091], p<0.001), low BMI (1.110 [1.050-1.174], p< 0.001), occupation as housewife (4.200 [1.484-11.890], p=0.007), number of children (1.563 [1.277-1.912], p< 0.001),

Table 2 Comparison of demographic characteristics of the case group with the control group (n=620)

Demographic characteristics		Control group (n = 310)	Case group (n = 310)	p value
Gender (Women / Men)		142 (45.8) / 168 (54.2)	101 (32.5) / 209 (67.5)	0.001[a]
Age		50.0±10.0	58.9±12.9	<0.001[d]
BMI		27.0±3.5	25.2±4.7	<0.001[d]
Education	Primary school	49 (15.9)	119 (38.8)	<0.001[b]
	Secondary school	12 (3.8)	31 (10)	
	High school	132 (42.5)	100 (32.3)	
	Higher education	117 (38.8)	60 (19.4)	
Marital status (Single / Married)		36 (11.6) / 274 (88.4)	47 (15.1) / 263 (84.9)	0.519[a]
Occupation	Workman	95 (30.6)	35 (11.3)	<0.001[b]
	Civil servant	85 (27.4)	15 (4.8)	
	Self-employed	49 (15.9)	98 (31.6)	
	Housewife	11 (3.5)	62 (20.0)	
	Retired	70 (22.6)	100 (32.3)	
Children (Number of children)		2 (4-0)	3 (5-0)	<0.001[c]
Family type (Nuclear / Extended)		277 (89.4) / 33 (10.6)	232 (74.8) / 78 (25.2)	<0.001[a]
Smoking (No / Yes)		240 (77.4) / 70 (22.6)	238 (76.8) / 72 (23.2)	0.924[a]
Alcohol use (No / Yes)		253 (81.6) / 57 (18.4)	265 (85.5) / 45 (14.5)	0.233[a]
First degree relative with any cancer (No / Yes)		250 (80.6) / 60 (19.4)	150 (48.4) / 160 (51.6)	<0.001[a]
Chronical disease (No / Yes)		260 (83.9) / 50 (16.1)	140 (45.2) / 170 (54.8)	<0.001[a]
Exercise (No / Yes)		218 (70.3) / 92 (29.7)	221 (71.3) / 89 (28.7)	0.860[a]
Weekly meat consumption	None	92 (27.7)	58 (19.4)	<0.001[b]
	1	102 (32.3)	92 (29.7)	
	2	67 (22.3)	120 (38.7)	
	3 and more	49 (16.5)	40 (12.2)	
Add salt to most meals (No / Yes)		275 (88.7) / 35 (11.3)	233 (75.2) / 77 (24.8)	<0.001[a]
Gallstone (No / Yes)		267 (86.1) / 43 (13.9)	249 (80.3) / 61 (19.7)	0.067[a]
Common oil type in cooking	Olive oil	142 (45.2)	100 (32.3)	<0.001[b]
	Vegetable oil	108 (35.5)	90 (29)	
	Butter	31 (9.7)	25 (16.1)	
	Margarine	29 (9.7)	70 (22.6)	
Mostly consumed food group	Vegetable	192 (61.9)	129 (41.6)	<0.001[b]
	Meat	68 (22.0)	81 (26.1)	
	Both	50 (16.1)	100 (32.3)	
Cola (No / Yes)		280 (90.3) / 30 (9.7)	290 (93.5) / 20 (6.5)	0.184[a]
Tea (Number of cups)		3 (5-1)	2 (10-1)	0.017[c]

Data are given as n (%), mean ± standard deviation (SD) or median (max-min). Statistical analysis was performed with [a] Fisher's exact test, [b] Pearson chi-squared test, [c] Mann-Whitney U test, or [d] Independent T-test. BMI, body mass index.

Table 3 Multiple logistic regression of risk factors in patients with gastrointestinal system tumors

	p value	Odds ratio	95% Confidence Interval	
			Lower	Upper
Gender (Male)	0.020	1.729	1.090	2.745
Age	<0.001	1.068	1.045	1.091
BMI	<0.001	1.110	1.050	1.174
Occupation (Civil servant)	<0.001	0.088	0.031	0.244
Occupation (Housewife)	0.007	4.200	1.484	11.890
Number of children	<0.001	1.563	1.277	1.912
First degree relative with any cancer (Yes)	<0.001	4.444	2.674	7.386
Chronical disease (Yes)	<0.001	6.314	3.708	10.752
Mostly consumed food group (Vegetable)	<0.001	2.923	1.646	5.192

BMI, body mass index.

cancer history in the family (4.444 [2.674-7.386], p<0.001) and the occurrence of other chronic diseases (6.314 [3.708-10.752], p<0.001) were identified as the diagnostic factors for colon and stomach located gastrointestinal cancers. Occupation as civil servant (0.088 [0.031-0.244], p<0.001) and mostly consuming vegetables (2.923 [1.646-5.192], p<0.001) were found to be the factors associated with lower risk for colon and stomach located gastrointestinal cancers (Table 3).

Discussion

The aim of this study was to provide data for potential risk factors that are associated with colon or stomach located gastrointestinal tract cancers. Data to identify potential risk factors were collected from 310 patients diagnosed with gastrointestinal cancers and compared with 310 randomly selected volunteer healthy subjects. Accordingly, the diagnostic factors for gastrointestinal cancers were identified as multiple nonspecific symptoms in clinical manifestation, gender (male), age, education level, occupation, BMI, no helicobacter pylori, number of children, cancer history in the family and the presence of other chronic diseases. Gastrointestinal tract cancers including stomach and colon cancer are known to have a higher incidence rate in males with an increasing cumulative risk up to age 75 years [1,15,18]. Consistent with the literature, in this study, the statistical comparison of the study patients with the healthy subjects indicated a striking dominancy of male patients (67.5% in the case group and 54.2% in the control group) diagnosed with gastrointestinal cancers, and the mean age of patients was 58.9±12.9 years and significantly higher than that of the healthy subjects (50.0±10.0 years). The most common presenting symptom was abdominal pain (25.5%), where most of the study patients suffered from multiple symptoms including bleeding (34.5%). Regarding other factors considered for better diagnosis of gastrointestinal cancers, the data obtained from this study mostly coincided with the literature. Improved sanitation and eradication strategies are known to effectively reduce the incidence of gastric cancer caused by helicobacter pylori [8,12,13,19]; similarly, in this study, a statistically significant proportion of the study patients were tested negative for helicobacter pylori. BMI and education level of the study patients were shown to be negatively related to gastrointestinal cancer occurrence. The incidence of gastrointestinal cancers increased with the increase in the number of children and other chronic diseases. Icli et al. (2011) in their study with 253 Turkish patients with gastric cancer, on the other hand, reported no considerable difference between the groups with respect to three types of chronic diseases, diabetes, hypertension, and arteriosclerotic hearth disease, namely [13]. There are, however, other studies in the literature showing a positive correlation between diabetes and colon cancer [10]. The occupation of the patient is also an important factor for cancer development, since a high stress environment is a well-known risk factor for many cancer types [8,12,16,17]. In this study occupation of a housewife, that is a stay-home women/mother, appeared to have a very high risk with respect to the incidence of gastrointestinal cancers. The results obtained in this study also indicated cancer history in the family as one of the diagnostic factors for gastrointestinal cancer. It is important to note that there is no study in the literature evaluating the correlation of a family medical history on cancer with the occurrence of gastrointestinal cancer, which can be an important factor for early diagnosis. Rinzivillo et al. (2015) have recently showed that family history of colorectal and breast cancer to be risk factors for the development of small intestine neuroendocrine cancers [20]. Although known as significant risk factors for many cancer types [8,11,21,22], in this study, smoking and alcohol habits of both the study patients and healthy subjects were similar without any statistically significant difference; therefore, there was no relationship between smoking or alcohol abuse and the occurrence of gastrointestinal cancers.

Dietary and lifestyle factors are known to be risk factors for the occurrence of many cancer types including gastrointestinal cancers [2,8,11,23-25]. There are many reports in literature identifying high level of salt consumption as a risk factor for cancer development, especially for gastric cancer [2,8,10]. Total meet intake is another important factor for cancer development, since heme iron present in meet can damage DNA and is the main precursor for important carcinogens such as endogenous nitrosamine and free radicals [2,8,12,15,21,26,27]. Vegetables are rich in vitamin C and fiber were shown to have a protective role against cancer in many studies [10,12,14,16,17,26-29]. Studies for the carcinogenic effect of soda and tea consumption, on the other hand, are inconclusive and appear to be dependent on the dosing/frequency and carcinogenic and anti-carcinogenic chemicals present in the food and beverages [8,17,26]. The effect of physical activity, i.e., exercising, is also not clear, since there are contradictory reports in the literature proving either positive or negative effect on the occurrence of cancer [8,11,16]. With its antiangiogenic effect using olive oil for cooking was, on the other hand, reported to be beneficial for cancer [11,15,17]. In this study with 310 patients with stomach and colon located gastrointestinal cancers and 310 healthy subjects, eating meet at most once a week, adding less or no excessive salt to meals, using olive or vegetable oil, but not margarine, for cooking, and preferring vegetables as the main course were significantly more common in the healthy subjects than in the study patients. However, multiple logistic regression tests indicated only the consumption of fewer vegetables or preferring both meat and vegetables for the main course as significant risk factors.

Limitations

There are some limitations in this study. Firstly, the research cannot be generalized to all samples. Lastly, our sample was consisted mostly of people who has colorectal tumors, and the risk factors may differ according to the localization of the tumor. Our suggestion is to plan studies who investigate the risk factors for both colorectal and stomach tumors separately.

Conclusion

This study on investigation of potential risk factors for gastrointestinal cancers evaluated diagnostic factors such as helicobacter pylori and the presence of other chronic diseases, and also explored risk factors such as eating and cooking habits, to provide a broad range of factors to be considered in gastrointestinal cancer patients. The determination of diagnostic factors such as low BMI will enable the identification of asymptomatic patients and early diagnosis of gastrointestinal cancer for better treatment outcomes. In addition, the identification of more vegetable consumption as an important factor for reducing risk of gastrointestinal cancer development is important for self-care measures of the patients. Nurses should also provide counseling to the patients on healthy diet and identify risk factors for gastrointestinal cancer.

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The experiences in the self-management of gestational diabetes: A qualitative study based on pender's health promotion model

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Abstract

Aim: Cultural differences, facilitators or barriers affect lifestyle changes in women with GDM. This descriptive qualitative study examined experiences in self-management of Gestational Diabetes Mellitus (GDM) through Pender's Health Promotion Model (HPM) of the nursing theories that develop cultural care models.

Material and methods: The qualitative descriptive research was designed based on a deductive qualitative content analysis, within the framework of the Health Promotion Model. A total of 23 women, who were diagnosed with GDM, participated in semi-structured interviews. Data were analyzed a deductive qualitative directed content analysis.

Results: Three themes that reflected "Behavior-specific cognitions and affect" component of HPM were obtained: perceptual changes, emotional changes, and changes in the support system. Sub-themes such as believing in the benefit of implementation and succeeding, monitoring the implementation, positive support were determined as the facilitating sub-themes. The difficulty of implementations, serious dimension in treatment, believing in failing, fear, stress, sadness, confusion and negative support were identified as barriers in GDM self-management.

Conclusion: This study highlighted on the value of the intervention based on Pender's Model shall guide healthcare professionals in improving and to perform individualized GDM self-management. Also, the educational based on nursing models can increase self-efficacy and motivation in women.

Key words: gestational diabetes mellitus, health promotion, nursing care, qualitative study, content analysis

Introduction

Gestational Diabetes Mellitus (GDM) is defined as diabetes that occurs for the first time in the second or third trimester of pregnancy [1]. International Diabetes Federation (IDF) is reported (2019) that 129.5 million pregnant women worldwide were affected by hyperglycemia as the 83.6% of these pregnant women were diagnosed with GDM [2]. The prevalence of gestational diabetes was estimated to be 7.7% in Turkey (2019). The prevalence of diabetes in different regions of Turkey varies from 5.1% to 17.6% [3].

The diabetes foundations in the world recommend medical treatment, medical nutrition therapy, exercise, and applying health behavior changes for the proper management of gestational diabetes in antepartum and postpartum periods. This treatment planning positively influences mother and baby's health in antenatal and postnatal periods [1,4]. The pregnancy period is particularly important in acquiring health behaviors that affects women's entire life. Because it offers enough time for change in behaviors and facilitates implementation to the process through consideration of the baby's health.

However, literature in our country and the World indicates that women with GDM still face problems in initiating and maintaining health behavior changes despite the education and supervision provided in antepartum and postpartum periods [5-8]. The main reason for this situation is that although women mostly

consult health personnel as a source of information since the diagnosis of GDM, a significant part of them also benefit from different sources. In addition to the mass media, it has been determined that family elders and friends are used as a source of information in health [9].

Background

The other studies in the world focus on the life experiences that play an important role in increasing or decreasing self-efficacy of women with gestational diabetes and facilitators and barriers factors [10,11]. It is stated that at the beginning of facilitator factors, caring about the health of the baby and evaluating it as an opportunity to reduce the risk of Type 2 DM will have a facilitating effect for lifestyle change [12,13]. Similarly, it is stated that receiving social and professional support will contribute positively to the lifestyle changes of pregnant women [13,14].

In the different nations, it is seen that there are many barriers' factors in the management of GDM. Studies conducted in developed countries reported nutritional regulation of the meals and having no time for blood glucose measurements as factors that prevent GDM management [12,13]. Additional problems were reported as experiencing anxiety and pain in the blood glucose monitoring [15], and physical and social limitations in exercise planning [13]. The studies conducted with South Asian women in Australia and women of Mexican origin in a United States border region stated that women did not prefer diet, believing that eating plan has a negative impact on the babies' development [10,12]. Similarly, another study revealed that although considering a healthy diet, women with GDM were socially isolated since they had to eat alone [12,16]. It was also stated that inadequate social and professional support during the treatment negatively affected the process [13,17].

Current study

Due to the difficulties in obtaining and maintaining health-promoting behaviors, some behavior change theories and models are used by researchers as guidelines [18,19]. Pender's Health Promotion Model is one of the comprehensive models used to implement health promotion behaviors and is used frequently [20]. This model is a guide to evaluate the complex biological-psychological processes of individuals to improve their health behaviors and reveals their decisions about their health promoting behaviors [18,21]. In this direction, it is thought that this model shall contribute to the self-management of GDM, develop culture-oriented nursing care, individualize it and increase effectiveness. Therefore, the aim of this study was to elaborate GDM self-management experiences among pregnant women with GDM through Pender's Health Promotion Model.

Theoretical framework

The Health Promotion Model (HPM) developed by Nola Pender aims to form the basis for structuring nursing initiatives and develop and maintain a health behavior. Pender's HPM consists of components that include individual characteristics and experiences, behavior-specific cognitions and affect, and behavioral outcomes [22,23].

Individual characteristics and experiences

It is emphasized that behaviors are affected by individual characteristics and experiences. Experiences are explained as events that leave an impression/effect on someone. Individual characteristics are classified as biological, psychological and socio-cultural. The biological features include age, gender, number of births given, while psychological features include self-motivation and perceived health status. Ethnic group, education, and socioeconomic features are included in the socio-cultural features (Figure 1a) [22,23].

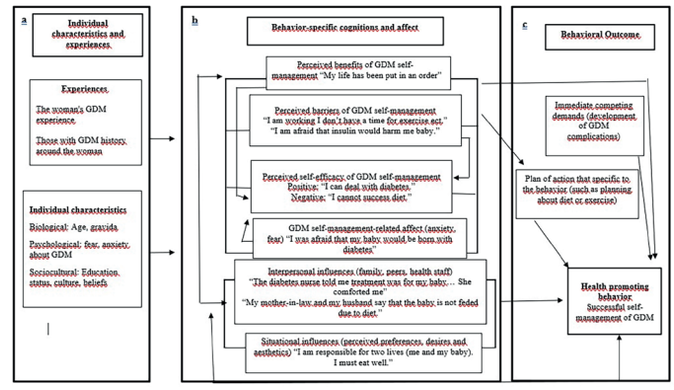

Figure 1 - The Experiences in GDM Self-Management Based on Pender's Health Promotion Model. a) The component presents examples of individual characteristics and experiences. b) In the area Behavior-specific cognitions and affect in showing examples of facilitators and barriers factors in GDM management. c) It is the part that shows whether GDM is successfully managed. According to the results in this area, re-planning is done to obtain or maintain health behavior.

Behavior-specific cognitions and affect

These parts affect the motivation required to gain and maintain behaviors. It makes up a significant component and includes Perceived Benefits of Action, Perceived Barriers to Action, Perceived Self-Efficacy, Activity-Related Affect, Interpersonal Influences and Situational Influences (Figure 1b) [22,23].

Perceived benefits of action

It should be easier to gain a behavior if an individual believes that it is beneficial. For example, if a pregnant woman believes that GDM management practices (such as diet, exercise, blood glucose monitoring etc.) is going to reduce the risks for herself and her baby, she will make an effort for the implementations (Figure 1b) [12].

Perceived barriers to action

If insulin therapy used in the treatment of GDM is perceived as a bad condition or a serious dimension of treatment, a woman may experience difficulties in carrying out the insulin treatment properly and regularly (Figure 1b) [17].

Perceived self-efficacy

Perceived self-efficacy is the self-belief that a woman has the capability and power to apply practices in GDM management and health promotion behaviors (Figure 1b) [24].

Activity-related affect

Emotions that occur before, during and after the behavior affect individuals perceived self-efficacy and the continuation or repetition of a behavior. Repeating insulin therapy and blood glucose measurement used in GDM management at certain intervals during the day may develop negative feelings in woman and adversely affect treatment engagement (Figure 1b) [15].

Interpersonal influences (family, peers, health staff)

The support given to an individual by the individuals around for gaining the desired behaviors. The positive support (for housework, child care, nutrition, exercise) provided by the spouse, family and friends for the pregnant woman with GDM during the treatment process is a factor that increases her implementation and success (Figure 1b) [13,17].

Situational influences

Factors such as perceived preferences, desires and aesthetics may affect GDM management in both ways. Some examples of situational effects are the beliefs such as "The diet treatment prevents the growth of the baby." and "A pregnant woman eats more." (Figure 1b) [10].

Behavioral outcome

The outcome of whether a woman with GDM can perform the desired behaviors during disease management and achieve behavioral change. Immediate competing demands (development of GDM complications) or whether the individual has made an action plan specific to the desired behavior (such as planning about diet or exercise) affects behavioral output. When making an action plan, individual characteristics, experiences, behavior-specific cognitions, and their affect are very important for women to succeed. As the engagement in the action plan increases, the change in health-promoting behavior remains for a long time (Figure 1c) [22,23].

Material and methods

Study design

This study was performed deductively using directed qualitative content analysis according to Hsieh and Shannon's approach [25]. The analysis was guided by HPM as a framework in order to assume that experiences are formed in human consciousness, and it was selected to better understand the experiences of pregnant women with GDM with in their circumstances. The study report was also prepared in parallel to the Consolidated Criteria for Reporting Qualitative Research (COREQ) [26].

Participant selection

Data were collected between January 2018 and January 2019 in the obstetric outpatient clinics of two university hospitals in Turkey. The pregnant women were selected through the purposeful sampling method. All pregnant women with GDM who met the inclusion criteria were invited to participate in the study. Informed consent (both orally and in writing) was obtained from those who were interested in the study, and were informed about the aims and methods. They were assured that the recorded interviews would remain confidential.

The in-depth interviews with the participants were continued until the data saturation point (the answers/opinions start to repeat each other) was reached [27]. The interviews were conducted with 23 women. The inclusion criteria were as follows: being 18 years old and over, diagnosed with GDM and being in the 36th gestational week or over (Pregnant women who diagnosed with GDM at the 28th gestational week were expected to experience at least 8 weeks of treatment.). Exclusion criteria were having type 1 or type 2 diabetes, any psychiatric diagnosis, vision, hearing, perception and physical obstacles.

Data collection

Semi-structured interview technique was used. After obtaining their consent, we decided on a mutually convenient time and venue for the interview. The interviews were conducted in a suitable and quiet interview rooms with good lighting in the hospital's relevant unit. All of the participants were interviewed individually and in-person. The interviews were made by the same researcher (who had taken qualitative research courses during PhD and she is research assistant at the obstetrics and gynecology nursing department in X University), and the same voice recorder and interview form were used. Before interviews the introduction form that included sociodemographic and obstetric data such as age, educational status of the pregnant woman, educational status of the husband, occupation, gestational week, obstetrical story, and current treatment was performed. Subsequently, interviews were conducted with a semi-structured interview form prepared based on HPM (Table 1). Semi-structured interview form was evaluated by three experts with PhD degrees who previously used the HPM. During the interview process, additional questions were asked according to the responses from women. Each interview was audio-recorded that lasted between 28 to 43 minutes.

Table 1 Semi-Structured Interview Questions

Interview questions of individuals	*Components of Pender's Health Promotion Model*
What did you feel when this disease was diagnosed?	Behavior-specific cognitions
What has changed in your life since you were diagnosed with this disease?	Behavior-specific experiences, Activity-related affect
Could you please describe your life with gestational diabetes?	Behavior-specific experiences, Activity-related affect, Perceived self-efficacy
How do you feel about the treatment/exercise diet?	Behavior-specific cognitions and affect
How do you feel when your blood sugar is measured several times a day or insulin injection is applied?	Behavior-specific cognitions and affect
What barriers do you face in managing your gestational diabetes?	Perceived barriers to action, Interpersonal influences, Situational influences
What facilitators do you face in managing your gestational diabetes?	Perceived benefits of action, Interpersonal influences, Situational influences
How do you evaluate yourself in Gestational Diabetes Mellitus management?	Perceived self-efficacy

Data analysis

According to the recommendations of Hsieh and Shannon, directed content analysis was made [25]. First, the researcher transcribed each recorded interview separately. Secondly, transcripts re-read several times by the researchers, it was ensured that the participants' experiences were understood as a whole. Thirdly, highlighted areas were coded using the predetermined categorization. Subsequently, that the codes were examined in terms of their relations with each other and the themes and subthemes were determined. The identified themes were discussed in the context of the 'Behavior-Specific Concepts and Effects' component of Pender's Health Promotion Model.'

Rigor

The qualitative data reliability was ensured through certain procedures such as credibility, dependability, transferability, and verifiability [28].

In terms of credibility, the interviews were made by the same coder (the first coder who had taken qualitative research courses during PhD), and the same voice recorder and interview form were used. The interviews were analyzed by the two coders independently. No contact was made between the interviewees and the interviewer before the interviews. The in-depth interviews with the participants were continued until the data saturation point was reached. It was ensured that results were presented to an expert to provide dependability and by frequent meetings of the authors to discuss data analysis. Inclusion criteria and purposive sampling method were used to ensure transferability. The list of themes and quotations were sent back to available eleven women (others refused to interview because they had no time) on the mail to represent the true meanings. Confirmations were gained after the interviews. This way was ensured to enhance verifiability.

Results

The average age (±SD) of participants was 32.3 (±6.3). Of the pregnant women, 30.4% had GDM in a previous pregnancy. Furthermore, 56.5% of them were receiving insulin treatment. The majority of participants were housewives (69.6%) (Table 2).

The thematic analysis revealed fifty-three codes and eleven sub-themes. Three themes, which reflected “Behavior-specific cognitions and affect” component of Pender’s HPM, were obtained from the coded data (Table 3).

Theme 1: Perceptual changes

The theme ‘Perceptual changes’ reflects Perceived Benefits of Action, Perceived Self-Efficacy, Perceived Barriers to Action and Perceived Self-Efficacy, which are the factors of ‘Behavior-specific cognitions and affect’ component.

Sub-theme: Believing in the benefit of implementation and succeeding: The participants expressed that they applied blood glucose monitoring, diet, insulin treatments and physical activity because they believed that it was beneficial. Also, women, who believe they can be successful, expressed that they were more in control of behavioral change.

Table 2 Demographic Characteristics of Participants

Participant no	Age	Education status	Occupation	Educational status of spouse	Gestational week	Gravida	GDM history	Current treatment
P1	33	Primary School	Housewife	Primary School	37	3	No	Insulin
P2	33	High School	Housewife	High School	36	3	No	Diet
P3	43	Middle school	Worker	University	36	2	No	Insulin
P4	37	Middle school	Housewife	Middle school	38	6	Yes	Insulin
P5	45	University	Worker	High School	36	7	No	Insulin
P6	32	High School	Housewife	Middle school	38	4	No	Diet
P7	45	Primary School	Housewife	Middle school	36	3	Yes	Insulin
P8	24	High School	Housewife	High School	36	1	No	Insulin
P9	26	High School	Housewife	High School	37	1	No	Diet
P10	28	Middle school	Housewife	High School	38	1	No	Diet
P11	28	Middle school	Worker	Middle school	36	3	Yes	Insulin
P12	35	Primary School	Worker	Primary School	37	2	No	Insulin
P13	30	Middle school	Housewife	Middle school	38	2	Yes	Diet
P14	28	University	Worker	High School	38	2	No	Diet
P15	36	Primary School	Housewife	Middle school	36	3	Yes	Insulin
P16	22	High School	Housewife	High School	36	1	No	Insulin
P17	32	High School	Housewife	High School	37	3	Yes	Diet
P18	28	University	Worker	High School	39	1	No	Diet
P19	29	Primary School	Housewife	Primary School	36	2	No	Insulin
P20	38	High School	Housewife	University	37	4	No	Diet
P21	25	High School	Worker	High School	37	1	No	Diet
P22	34	Primary School	Housewife	Middle school	36	2	Yes	Insulin
P23	32	Primary School	Housewife	Primary School	38	3	No	Insulin

Table 3 Themes, Sub-Themes, Codes and "Behavior-Specific Cognitions and Affect" Component of Pender's Health Promotion Model

Theme	Sub-theme	Code	"Behavior-specific cognitions and affect" component of HPM
Perceptual changes	Believing in the benefit of application and succeeding	-Thinking about the baby's health -Turning it into a habit -Thinking that it's temporary -Taking precautions due to diabetes in the family history	Perceived Benefits of Action and Perceived Self-Efficacy
	Monitoring the application	-Considering it as a duty -Turning it into a habit -Thinking that the physician will control -Providing experience -Organizing life	
	Difficulty of applications	-Feeling bad -Finding applications boring -Boredom -Despair -Hurting needles -Feeling lazy -Difficulty in living under control	Perceived Barriers to Action
		-Feeling of hunger -Thinking that prohibitions are attractive -Weakness -Difficulty in timing main and snack meals -Having different responsibilities -Preparing separate meals for everyone in the house	Perceived Barriers to Action
	Serious dimension in treatment	-Having prohibited food and drinks on the table -Ignoring the risk of diabetes -Thinking that the treatment is getting serious	
		-Being happy when seeing the desired results -Belief in living with the current situation	
	Belief in failing	-Failure to balance -Inability to plan a meal -To giving up quickly	Perceived Self-Efficacy
Emotional changes	Fear	-Fear of being persistent -Fear of transmitting diabetes to the baby -Fear of losing the baby -Fear that insulin will become addictive -Not being satisfied with the given training	Activity-Related Affect and Situational influences
	Stress	-Having ongoing and different responsibilities -Difficulty in living a regular life -Having diabetes in the family history	
	Sadness	-Despair -Crying for reaction -Refusing to communicate -Frustration	
	Confuse	-Wonder -Confusion	
		-Perception of overfeeding pregnant women in the society -The thought that the baby cannot be fed during the diet	
Changes in the support system	Positive support (family, spouse, close environment and health professional)	-Getting the support of the husband -Getting the support of the kids -Getting the support of the mother-in-law -Positive approaches of the diabetes nurses	Interpersonal Influences
	Negative support (family, spouse, close environment and health professional)	-Receiving no support from the husband -Husband's inaccurate knowledge -Hearing startling expressions from the physician	

"... I have to do it even if there are obstacles. I have an obligation to do it on time... If I do not have a diet, my baby is in danger. I believe that I will cope with diabetes." (P4, Housewife, Insulin treatment)

Sub-theme: Control of the implementations: Pregnant women stated that they performed treatment implementations more quickly when checked by a physician or diabetes nurse. They stated that their lives were regulated in this way.

"How can I describe this feeling? I think of the implementations like a homework given by the teacher. I measure my blood sugar every day... At first, I felt bad, but afterwards, it became a habit, I feel better. My life has been put in an order." (P14, Worker, Diet treatment)

Sub-theme: Difficulty of implementations: Practices such as diet, exercise, insulin etc. caused negative perceptions in women. Most women stated that they had several responsibilities in their daily practices such as housework, child care, and working life.

"I cannot promise to exercise, it is not possible, I don't have time, the person who is already working never has time, but of course I can adapt to a diet. However, preparing food according to my own diet plan is a challenge. You are cooking a different meal for your partner, and a different one for your children..." (P3, Worker, Insulin treatment)

"For example, when I have a guests, go to a bazaar or

somewhere else… sometimes I have difficulty in measuring it on time. Besides, I cannot go out with my other child for exercises due to the cold weather." (P19, Housewife, Insulin treatment)

Sub-theme: Serious dimension in treatment: Some women believed that insulin was insistent, and spouses also had inadequate knowledge about insulin treatment. They also mentioned that they started to think that treatment became serious when the insulin treatment was started.

"...When I learned that I must start insulin treatment, I cried on the way to the hospital... I felt so bad. I thought it was a more serious stage. My husband was concerned that the insulin might be harmful for our baby. I started insulin treatment, but I could not cope with it, and ultimately quit." (P22, Housewife, Insulin treatment)

Sub-theme: Belief in failing: Women who experienced GDM management difficulties reported that they felt unsuccessful and quickly gave up. *"I tried to plan my meals. But I could not… I am a person who gives up very quickly... I am anxious because I cannot comply with the treatment. I cannot, I cannot success… I'm angry with myself, why can't I? Why I can't be patient for my baby?" (P20, Housewife, Diet treatment)*

Theme 2: Emotional changes

The theme 'Emotional changes' reflects Activity-Related Affect and Situational influences, which are the factors of 'Behavior-specific cognitions and affect' component.

Sub-theme: Fear: "Fear" was defined as the most common feeling after being diagnosed with GDM. Women primarily get concerned about harming their babies. During the treatment process, they stated that they were afraid that the baby would suffer malnutrition during the diet and that insulin would harm the baby.

"The physician and the nurse told me, but I am not satisfied... They said, "You will use insulin for 3 months". But I did not want to use insulin. Maybe if they said that insulin was not going to necessary after birth, I would have accepted. When the physician said, "we would increase the dose", I gave up... I thought it would be permanent, I was quite afraid... I was afraid that my baby would be born with diabetes..." (P1, Housewife, Insulin treatment)

Sub-theme: Stress: Women state that they are experiencing stress in health behavior changes and GDM complications.

"My physician said that if my blood glucose is too high, there is a risk of transmitting that to the baby and that I have to pay attention from the start. I almost fainted when they told me that my baby could be larger, so the birth could be challenging. I had a hysterical fit. I am quite stressed. Thus, my blood sugar level is always measured high in the tests. I cannot soothe myself." (P17, Housewife, Diet treatment)

Sub-theme: Sadness: Some women expressed sadness after receiving the GDM diagnosis.

"I'm sad… Because I wasn't expecting it. I had diabetes in my first pregnancy, but I thought this would not happen in this pregnancy. I am not comfortable in my pregnancies. It's hard for me to inject that needle. I am sad." (P11, Worker, Insulin treatment)

Sub-theme: Confuse: Women expressed confusion expressions such as "What will I do? How will I cope with it?"

"I was confused. I have a lot of questions… Why did this happen? How is the process works? Will it be treated? Different questions in my mind... first about the baby, and the pregnancy period. I had questions about myself. How long can I endure after all?" (P10, Housewife, Diet treatment)

Theme 3: Changes in The Support System

The theme 'Changes in the support system' reflects Interpersonal Influences, which is the factor of 'Behavior-specific cognitions and affect' component.

Sub-theme: Positive support: The pregnant women expressed that her spouse and child provided the highest level of support, encouraging diet treatment and exercise. It was also expressed that the participants who communicated positively with the diabetes nurse, better adapted to the insulin treatment process.

"... she (the diabetes nurse) told me it was for my baby. She explained that the insulin was not going to get transferred to my baby… She expressed that maybe after the pregnancy, diabetes will be cured and there will be no need for insulin. She comforted me a little. My husband has been a solid supporter in this period. He had the diet with me. He kept reminding me to measure my blood sugar." (P10, Housewife, Diet treatment)

Sub-theme: Negative support: Some women's support systems were negatively affected from certain factors such as having inaccurate information in the family, negative expressions uttered by the healthcare professionals, and negative communication with them.

"A close friend also had diabetes. She shared her experience, which got me even more concerned. Her baby was quite large and she had to use insulin after birth. Also, physician and nurse said that I was going to use it for three months (insulin treatment) and they did not give any other explanation. But I did not want to use it... My husband said that we didn't want to use insulin. We were anxious that it would be permanent." (P2, Housewife, Diet treatment)

Discussion

This study revealed that the experiences, perceptions, feelings of pregnant women and their support systems affected the health behavior changes in GDM management. Moreover, nursing theories assume that behavioral changes do not occur or old behaviors are maintained due to the lack of necessary care and education. Therefore, identifying the problems through nursing theories and planning appropriate education and care programs are considered substantial to ensure and maintain behavioral change in GDM self-management. The discussion section of this study presents the themes separately.

Perceptual changes

The expressions showed that some participants easily adapted to the health behavior changes as they perceived the benefits. Moreover, it has been revealed that the belief of making behavior changes can motivate women. Women have individual responsibilities to manage the GDM, and the first of the responsibilities is to protect their infants [10]. It was reported that pregnant women in Thailand tolerated the finger-piercing pain for their babies [15]. On the other hand, Carolan-Olah et al., (2017) reported that women are motivated by avoiding foods that they are prohibited for babies and believing that they protect their babies' health [12]. The motivation of the individuals who have the energy to succeed is increasing. Protecting the baby's health is the basis of required belief and motivation for a successful process [12]. Pregnant women take responsibility for caring themselves to provide a healthy start for their baby. It was stated that women search for the knowledge to reach the level of optimal health for themselves and their babies, and control and plan their daily lives. It is essential to raise awareness in pregnant women to contribute their self-confidence and motivate them

for the health behavior changes, particularly in the antepartum period. Furthermore, it is also necessary that health staff further increase the energy of pregnant women, who already have certain motivation level, and should embrace a strengthening approach for those, who need motivation.

It was expressed that some women have the motherhood role and different responsibilities in their lives which create difficulties in treatment compliance. Previous study reported that cooking two different sets of meals for children and spouses, which was very time-consuming, and was the biggest challenge [17]. Moreover, it was reported that women with GDM were negatively affected by multiple responsibilities such as shopping, cooking, looking after other children, and coping with occupational responsibilities [13,16]. Therefore, similar concerns may be experienced in different societies worldwide. It was mainly observed that women keep themselves in the background and prioritize the preferences of their spouse and child, like in the cooking case. The possible motives behind this behavior might be the fact that motherhood role-model used by pregnant women to motivate themselves in some cultures, as women devote themselves to their spouse and children in Turkish culture. Furthermore, some participants believed that insulin was permanent, and spouses opposed insulin treatment. It was also determined that pregnant women and their relatives, who were misinformed that insulin treatment was permanent, refused the treatment.

Similarly, the transition to insulin treatment in non-Western ethnic minority pregnant women in Denmark made women think their situation was critical and that the baby's health was in great danger [17]. In study was conducted by Hjelm et al. (2018), it was expressed that most women expressed worries about not being able to live a normal life and the need for insulin injections [11]. These misunderstandings should be resolved in women who have different cultural backgrounds. It can be effective to emphasize that every treatment performed is purposeful to protect the health of both the mother and the baby.

Emotional changes

The study participants experienced certain emotional changes such as fear, stress, sadness, and confusion. In a qualitative study conducted in different countries (United Kingdom, Thailand and South Asian), it was stated that women experienced feelings such as sadness, shock, frustration, fear, tearfulness, insecurity, and confusion after GDM diagnosis [15]. Among the fears that women focus on were well-being of babies and their future health status [10,12,29]. It was shown that women from different cultures experienced similar emotions after the GDM diagnosis. These emotional reactions can affect women's motivation levels.

Some participants stated that they could not comply with the diet treatment because they thought that their babies' growth would be hindered, as in other studies [10,12]. This indicates that women are confused on this matter. In our culture, fatter babies are considered by some as healthy and cute. Since the increase in the mother's weight during pregnancy reflects the growth of the baby, women gain more weight in pregnancy which is accepted as a positive development for some rural parts of our society. This is usually explained by the social norms, summarized by the often-used expression 'eating for two' [30].

Changes in the support system

The findings suggest that having adequate positive support (social support and professional support) was a significant advantage in balancing daily life and managing the GDM process in pregnancy. In the study conducted by Carolan-Olah et al. (2017) on Australian pregnant women with GDM, it was found that the spouses and the immediate environment encourage women and provide emotional support for exercise, diet, and insulin therapy [12]. Other studies also emphasized that women needed social support and professional support provided by the health professionals [11,13,17]. Social support usually makes life more manageable for women.

However, this study revealed that family members with a traditional approach made it difficult for women to adapt to treatment. Certain misbeliefs such as "the baby cannot receive adequate nutrition" and "the mother will be addicted to insulin" showed that social support was incorrectly applied. Also, in the study on women with different ethnic, it was expressed that felt that the time with the dietician was limited, not personalized enough [13]. Therefore, it is crucial to detect the support level of each pregnant woman. If the spouse or family has inadequate GDM management knowledge, or if they govern the process from a traditional viewpoint, the treatment process may be impeded. It should not be forgotten that social support provided by conscious family members makes it easier for women to take responsibility and manage the GDM process. However, an overprotective approach might complicate self-management for pregnant women. The excessive concern and dissatisfaction of family members might cause the women to lose self-confidence, feel sick, give rise to excessive attachment to family, and discourage them in performing their daily activities. Therefore, it is critical to inform the families about the over-protective approach that is often encountered in Turkey.

Limitations of the study

Some limitations are unavoidable, when interpreting the findings from the qualitative studies. As the study has a qualitative nature, the findings cannot be generalized to all women with GDM. Also, the measures taken to support the robustness of this qualitative study, it constitutes an important step for further studies on GDM management due to the cross-sectional nature of the interviews.

Conclusion

In this study, the researchers focused on women's experiences during all GDM self-management stages.

It was found that when the practices in GDM management were perceived as beneficial for the baby and the mother, they easily adapted to the treatment. However, based on the expressions of some participants, it was determined that GDM management has a significant effect on women's life. It was defined that having an occupation, another child, and intense housework had negative effects on GDM management. Despite the barriers, some women displayed a great effort to protect the health of both their babies and themselves.

As Turkish society has a patriarchal system, it was stated that women are under more pressure about governing their health and that of their babies. It was observed that particularly those, who did not have social and professional support, could not succeed in GDM management.

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A retrospective CT based comparative analysis of available screw pathways to determine optimal iliac screw trajectory

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Abstract

Introduction: The lumbo-sacral stabilization using iliac screw is gaining popularity in such cases of long multi segment lumbar constructs. Iliac screws help to achieve strong spinopelvic fixation, augments and protects sacral screws. However, there is a great variability found in literature for iliac screw fixation in terms of trajectory, screw length and screw diameter. Also, to the best of our knowledge, there is lacunae in current literature regarding the optimal pathway, screw length and screw diameter in the Indian population. Hence, we planned the study with the aim to analyze the available screw pathways to determine optimal iliac screw trajectory, screw length and diameter for the Indian population.

Material and methods: This was a tertiary center-based retrospective study. One hundred pelvic CT scans of patients in 18-70 years age, who underwent abdominal CT on Siemens 256-slice dual source CT scanner for various indications were evaluated. Subsequently, 4 iliac screw trajectories were assessed by connecting the points given below using double oblique reformats on which the lengths and narrowest zones of these trajectories were measured. Path A: Posterior Superior Iliac Spine (PSIS) to Anterior Superior Iliac Spine (AIIS); Path B: point between PSIS and posterior inferior iliac spine (PIIS) to Anterior Inferior iliac spine (AIIS); Path C: iliac crest intersection point (CLIC) point to Upper acetabulum; Path D: CLIC point to acetabular center.

Results: Statistically significant difference was found in the lengths of various pathways. Path A (PSIS to AIIS) was found to be the longest (mean 13 cm). The second longest path in our study was path C (CLIC point to Upper acetabulum). The narrowest widths of each path were not found to have any statistically significant difference.

Conclusion: Iliac screw fixation is of paramount importance for lumbosacral stabilization. Of the studied paths, trajectory from posterior-superior iliac spine to Antero-inferior iliac spine has the longest passage length and is the most optimal path for the Indian population. In case additional screws are required, the trajectory from CLIC point to Upper acetabulum provides the second largest screw passage.

Key words: iliac screw trajectory, lumbo-sacral fixation, iliac screw, spinal fixation

Introduction

Lumbo-sacral stabilization using iliac screws has gained popularity as an effective technique for achieving strong spinopelvic fixation and protecting sacral screws in cases of long multi-segment lumbar construct [1,2]. However, the literature exhibits considerable variability regarding the optimal trajectory, screw length, and diameter for iliac screw fixation [3-7]. These variations pose challenges for surgeons in selecting the most appropriate parameters, potentially leading to suboptimal outcomes and complications [8-10]. Moreover, there is a notable gap in the current literature regarding the optimal iliac screw pathway, length, and diameter specifically for the Indian population [11].

The Indian population exhibits distinct anatomical characteristics compared to other populations, including differences in pelvic morphology, bone density, and body habitus [12]. These unique features necessitate a thorough understanding of the optimal iliac screw parameters tailored to the Indian population. However, the existing literature predominantly comprises studies conducted in non-Indian populations, limiting their applicability and relevance to the Indian context [13].

The lack of specific research addressing the optimal iliac screw parameters in the Indian population creates a significant gap in knowledge and hampers evidence-based decision-making for surgeons performing lumbo-sacral stabilization procedures [3,4]. This gap can lead to suboptimal outcomes, including inadequate screw purchase, increased risk of screw loosening or failure, and compromised biomechanical stability of the construct.

Hence, the aim of our study is to analyze available screw pathways and determine the optimal iliac screw trajectory, length, and diameter for the Indian population. By conducting a comprehensive analysis of 100 pelvic CT scans, we intend to provide valuable insights into the most effective and appropriate iliac screw parameters for use in lumbo-sacral stabilization procedures in the Indian population.

The findings of our study will have several implications. Surgeons performing lumbo-sacral stabilization surgeries in India can utilize the recommended optimal iliac screw parameters to enhance surgical planning, improve screw placement accuracy, and optimize biomechanical stability. This, in turn, can lead to better clinical outcomes, reduced complications, and improved patient satisfaction.

Moreover, our study will contribute to the existing literature by filling the current knowledge gap regarding the optimal iliac screw parameters specific to the Indian population. This will expand the understanding of iliac screw fixation techniques and provide valuable evidence for future research and advancements in the field of lumbo-sacral stabilization.

Material and methods

This was a tertiary center-based retrospective study. Inclusion criteria were as follows:

1. Patients between the ages of 18 and 70 years.
2. Pelvic CT scans performed on single machine in radiology department - Siemens 256-slice dual-source CT scanner.
3. Patients who underwent abdominal CT for various indications.

Exclusion criteria were as follows:

1. Patients with pelvic deformities: Patients with pre-existing pelvic deformities, such as congenital abnormalities, scoliosis, or other structural anomalies affecting the pelvis, will be excluded. Including such cases could introduce confounding variables and affect the generalizability of the study.
2. Patients with pelvic trauma: Individuals who have undergone pelvic trauma, such as fractures or dislocations, will be excluded. Pelvic trauma can result in significant anatomical changes and alterations in the bone structure, which could impact the trajectory and feasibility of iliac screw fixation. Excluding these cases will ensure that the study focuses on the normal pelvic population.
3. Patients with pelvic tumors: Patients diagnosed with any pelvic tumors, such as primary bone tumors or metastatic lesions, will be excluded. Pelvic tumors can cause structural changes, bone destruction, or pathologic fractures, making the anatomy unsuitable for standard iliac screw fixation. These cases would not represent the normal pelvises targeted by the study.

Based on sample size calculation, One hundred pelvic CT scans of patients in 18-70 years age (mean age 34 years) who underwent abdominal CT on Siemens 256-slice dual source CT scanner for various indications were evaluated. There were 43 female and 57 male pelvises. In order to avoid selection bias, we included all the last 100 scans done on single machine in radiology department that met our inclusion criterion. Orthogonal axial, coronal and sagittal multi planar reformat (MPR) in the bone window and volume rendered technique (VRT) images were viewed alongside on screen in a 2 x 2 format. First, the chaotic line - iliac crest intersection point (CLIC point) was determined by extrapolating the chaotic line (drawn from iliopubic eminence to the anterior most point of the auricular surface of the sacrum) to the posterior iliac crest (Figure 1).

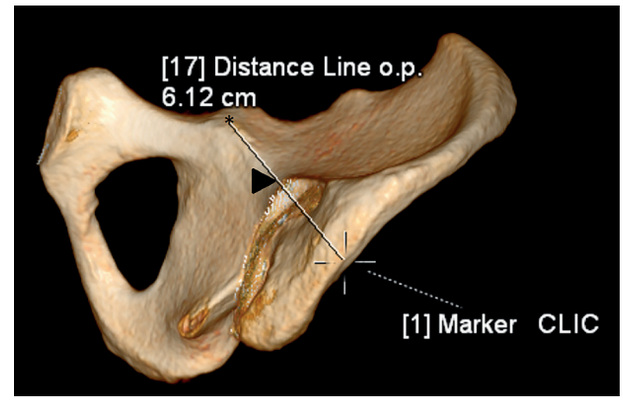

Figure 1 - Volume rendered CT image depicting the chaotic line drawn from iliopubic eminence (*) to the anterior most point of the auricular surface of the sacrum (black arrowhead). Further extrapolation of chaotic line to posterior iliac crest to determine the CLIC point.

The CLIC point, Anterior inferior iliac Spine (AIIS), Posterior superior iliac spine (PSIS), a point between PSIS and posterior inferior iliac spine (PIIS), upper edge of acetabulum and the acetabular center were marked on the VRT image. Subsequently, 4 iliac screw trajectories were assessed by connecting the points as given below to obtain their double oblique reformats. The lengths of these paths, as well as the width of the two narrowest points of cancellous bone were determined. To check the inter- and intraclass correlation coefficient of the measurements, two independent senior residents of Radiology department and Radiology consultant reviewed all data. The measurements were taken three times 2 weeks apart by each of the observer and the means of measured values were used as the final value [1,2]. The four trajectories assessed were as follows:

Path A: PSIS to AIIS (Figure 2-4).
Path B: Point between PSIS and PIIS to AIIS (Figure 5-7).
Path C: CLIC point to upper acetabulum (Figure 8-10).
Path D: CLIC point to acetabular centre (Figure 11-13).

Statistical analysis

Sample size estimation was done based on study of Liu et al. To calculate the sample size for our study with an effect size of 2 m , an alpha level of 0.05, and a power of 80%, we used the following sample size formula.

$n = 2 * [(Z\alpha/2 + Z\beta)^2 * \sigma^2] / \delta^2$

Where:

n = Sample size per group

$Z\alpha/2$ = Critical value for the desired alpha level (two-tailed test)

$Z\beta$ = Critical value for the desired power level

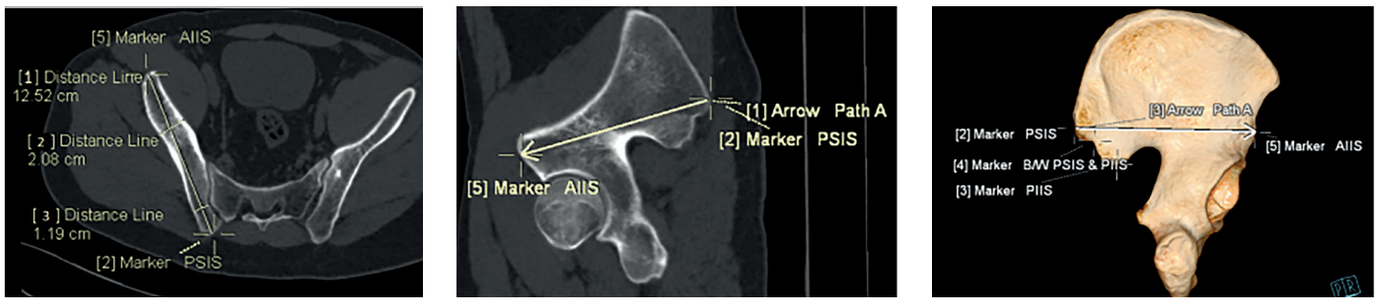

Figure 2a - **Path A [PSIS to AIIS].** Double oblique reformatted CT image showing Path A trajectory; where [1] represents its length, while [2] and [3] represent width of its narrowest zones, [2] being anterior to [3].
Figure 2b. image showing Path A trajectory in sagittal oblique reformatted image.
Figure 2c. image showing Path A trajectory in Volume rendered CT image.

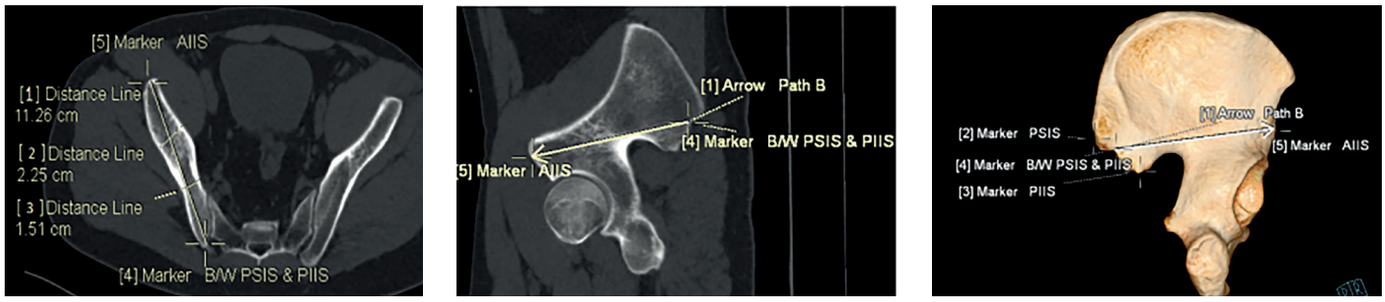

Figure 3a - **Path B [Point between PSIS and PIIS to AIIS].** Double oblique reformatted CT image showing Path B trajectory; where [1] represents its length, while [2] and [3] represent width of its narrowest zones, [2] being anterior to [3].
Figure 3b. Image showing Path B trajectory in sagittal oblique reformatted image.
Figure 3c. Image showing Path B trajectory in volume rendered CT image.

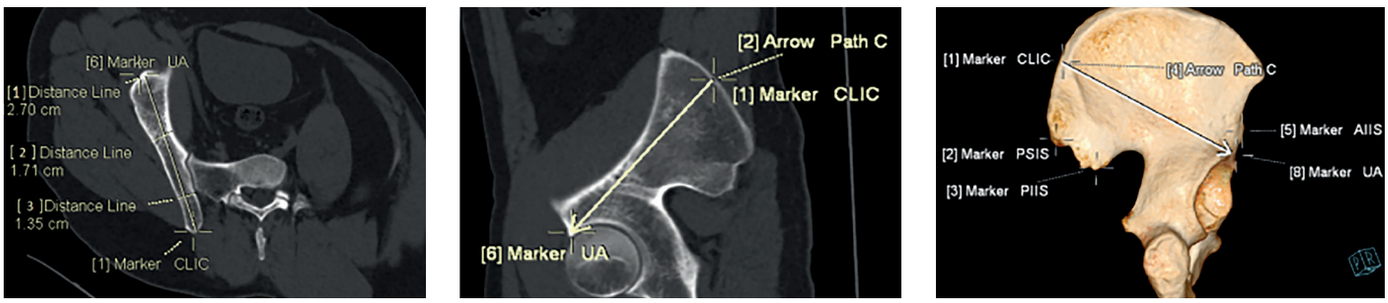

Figure 4a - **Path C [CLIC point to upper acetabulum (UA)].** Double oblique reformatted CT image showing Path C trajectory; where [1] represents its length, while [2] and [3] represent width of its narrowest zones, [2] being anterior to [3].
Figure 4b. Image showing Path C trajectory in sagittal oblique reformatted image.
Figure 4c. Image showing Path C trajectory in volume rendered CT image.

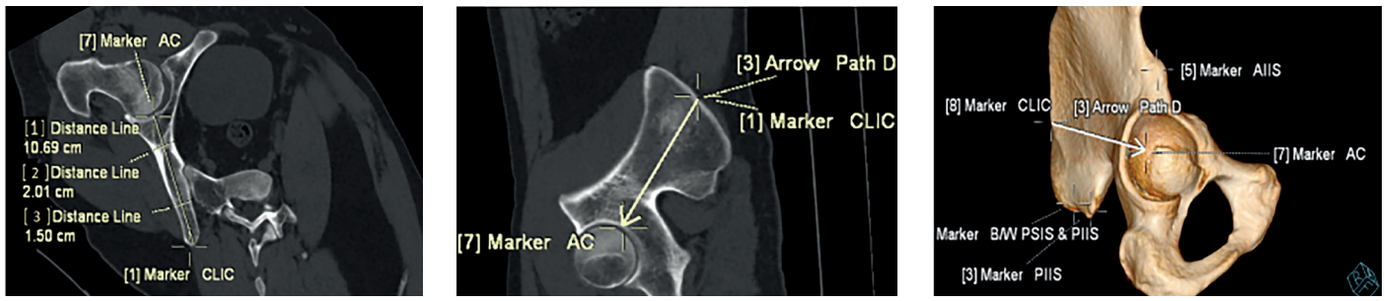

Figure 5a - **Path D [CLIC point to acetabular centre (AC)].** Double oblique reformatted CT image showing Path D trajectory; where [1] represents its length, while [2] and [3] represent width of its narrowest zones, [2] being anterior to [3].
Figure 5b. Image showing Path D trajectory in sagittal oblique reformatted image.
Figure 5c. Image showing Path D trajectory in volume rendered CT image.

σ = Standard deviation of the outcome variable (assumed to be equal for both groups)

δ = Effect size (mean difference)

Substituting these values into the formula we got n=89.8 which is approximately 89. A sample size of 100 was therefore appropriate. SPSS 20.0 software was used for the study. The Shapiro-Wilk test was used for assessing the normality of data. The measured data was within normal distribution and was presented as mean ± standard variation. Independent group t test was used to compare various paths with acceptable alpha error of 5%. The correlation of variables was tested in both groups using Pearson correlation coefficient. p value of less than .05 was considered statistically significant.

Results

Table 1 shows the descriptive analysis of lengths and breadths of each path. All measured paths showed excellent inter- and intraclass agreement. The intra- and interclass correlation coefficients were 0.9 and 0.92 for path A, 0.93 and 0.95 for path B, 0.91 and 0.9 for path C and 0.94 and 0.95 for path D. The lengths of various pathways were found to be statistically significantly different as illustrated in Table 2. Path A (PSIS to AIIS) was found to be the longest (mean 13 cm).The second longest path in our study was path C (CLIC point to Upper acetabulum). The narrowest width of each path were not found to have any statistically significant differences as shown in Table 2. The mean widths of all of the above paths were over 12 mm, thus allowing the thickest iliac screw to be placed comfortably. Thus, Path A (PSIS to AIIS) and path C (CLIC point to Upper acetabulum) were found to be superior to the other paths.

Table 1 The lengths and width of various pathways. Width A and Width B are two narrowest zones in the described paths; width A being anterior to width B.

		N	Mean	Std. Deviation	Std. Error
LENGTH (cm)	PSIS TO AIIS	100	13.0033	.73129	.07313
	BETWEEN PSIS AND PIIS TO AIIS	100	11.8924	.98000	.09800
	CLIC TO UPPER ACETABULUM	100	12.5876	.95383	.09538
	CLIC TO ACETABULAR CENTRE	100	10.9858	.63057	.06306
	Total	400	12.1173	1.13186	.05659
WIDTH A (cm)	PSIS TO AIIS	100	1.6615	.34227	.03423
	BETWEEN PSIS AND PIIS TO AIIS	100	1.6597	.32572	.03257
	CLIC TO UPPER ACETABULUM	100	1.7580	.29424	.02942
	CLIC TO ACETABULAR CENTRE	100	1.6532	.29444	.02944
	Total	400	1.6831	.31665	.01583
WIDTH B (cm)	PSIS TO AIIS	100	1.2382	.27163	.02716
	BETWEEN PSIS AND PIIS TO AIIS	100	1.2544	.35489	.03549
	CLIC TO UPPER ACETABULUM	100	1.2244	.21538	.02154
	CLIC TO ACETABULAR CENTRE	100	1.2880	.24567	.02457
	total	400	1.2512	.27678	.01384

Table 2 The statistical comparison of length and width means of various pathways with p value. Width A and Width B are the two narrowest zones in the described paths.

Dependent Variable	From	To	Std. Error	Sig.
LENGTH	PSIS TO AIIS	BETWEEN PSIS AND PIIS TO AIIS	.11838	.000
		CLIC TO UPPER ACETABULUM	.11838	.003
		CLIC TO ACETABULAR CENTRE	.11838	.000
	BETWEEN PSIS AND PIIS TO AIIS	PSIS TO AIIS	.11838	.000
		CLIC TO UPPER ACETABULUM	.11838	.000
		CLIC TO ACETABULAR CENTRE	.11838	.000
	CLIC TO UPPER ACETABULUM	PSIS TO AIIS	.11838	.003
		BETWEEN PSIS AND PIIS TO AIIS	.11838	.000
		CLIC TO ACETABULAR CENTRE	.11838	.000
	CLIC TO ACETABULAR CENTRE	PSIS TO AIIS	.11838	.000
		BETWEEN PSIS AND PIIS TO AIIS	.11838	.000
		CLIC TO UPPER ACETABULUM	.11838	.000
WIDTH A	PSIS TO AIIS	BETWEEN PSIS AND PIIS TO AIIS	.04453	.968
		CLIC TO UPPER ACETABULUM	.04453	.031
		CLIC TO ACETABULAR CENTRE	.04453	.852
	BETWEEN PSIS AND PIIS TO AIIS	PSIS TO AIIS	.04453	.968
		CLIC TO UPPER ACETABULUM	.04453	.028
		CLIC TO ACETABULAR CENTRE	.04453	.884
	CLIC TO UPPER ACETABULUM	PSIS TO AIIS	.04453	.031
		BETWEEN PSIS AND PIIS TO AIIS	.04453	.028
		CLIC TO ACETABULAR CENTRE	.04453	.019
	CLIC TO ACETABULAR CENTRE	PSIS TO AIIS	.04453	.852
		BETWEEN PSIS AND PIIS TO AIIS	.04453	.884
		CLIC TO UPPER ACETABULUM	.04453	.019
WIDTH B	PSIS TO AIIS	BETWEEN PSIS AND PIIS TO AIIS	.03915	.679
		CLIC TO UPPER ACETABULUM	.03915	.725
		CLIC TO ACETABULAR CENTRE	.03915	.204
	BETWEEN PSIS AND PIIS TO AIIS	PSIS TO AIIS	.03915	.679
		CLIC TO UPPER ACETABULUM	.03915	.444
		CLIC TO ACETABULAR CENTRE	.03915	.391
	CLIC TO UPPER ACETABULUM	PSIS TO AIIS	.03915	.725
		BETWEEN PSIS AND PIIS TO AIIS	.03915	.444
		CLIC TO ACETABULAR CENTRE	.03915	.105
	CLIC TO ACETABULAR CENTRE	PSIS TO AIIS	.03915	.204
		BETWEEN PSIS AND PIIS TO AIIS	.03915	.391
		CLIC TO UPPER ACETABULUM	.03915	.105

Discussion

Our study to the best of our knowledge is the first study in Indian patients that measures the lengths of iliac screws trajectories. Due to morphometric differences among pelvis of western and Indian sub-continent, the study is of great clinical significance. Further, the other strength of the study is that we have defined two paths large and wide enough to insert widest iliac screws. This may be important in conditions where two screws are required for enhanced stability or in cases where due to surgical error, the primary path is breached and screw insertion is not possible.

The non-union and pseudoarthrosis rates have been found to be significantly higher when lumbo-sacral fixations are not augmented by iliac screw fixation [1-2]. McCord et al. compared 10 different lumbosacral fixation models and found that iliac screw model had largest capacity to bear high loads [7]. The insertion of these screws are difficult and have a steep learning curve. The accuracy of placement is of paramount importance due to close vicinity of various vital structures. Further, the literature does not support uniform entry site [1,2,11,12]. Liu et al. studied iliac screw paths in Chinese patients and found paths PSIS to AIIS and CLIC to upper edge of acetabulum to have similar lengths [1]. However, we found these lengths to be significantly different in our study. Further, Liu et al. found that PSIS to ASIS was thicker than other paths. Our study, however, showed no significant difference in width of these paths. Another important difference was the mean width in the Chinese and Indian pelvis of the iliac screw corridors. The mean width in their study for the PSIS to AIIS path was 17.3 mm; whereas the Indian pelvises had a narrower corridor of 13 mm. Above differences are probably due to differences in the pelvic morphology of the Chinese and Indian populations. This further highlights the importance of studies in various ethnic groups.

Similar to findings of Yilmaz et al. [2] who studied screw trajectories in American pelvises, we also found PSIS to AIIS to be longest screw pathway. However, they recommended path connecting a point between PSIS to PIIS to AIIS to be the second best path and recommended its usage when PSIS to AIIS trajectory could not be used. In contrast, we found CLIC to upper acetabulum to be longer compared to the above trajectory [12.5 vs 11.8 cm (p=.000)]. Also, another point of note is that the PSIS to AIIS corridor width was 16 mm in their study as compared to our study in which it was 13mm.

We believe that our study would help surgeons in their decision making to choose the optimal iliac screw trajectory. Regardless of the chosen trajectory, careful preoperative planning and intraoperative fluoroscopy is crucial for optimal screw placement.

Our study has few limitations. The study is of retrospective nature and is based on radiological assessment. Its true intraoperative correlation with respect to length and width of screw requires further study. Biomechanical and clinical studies could be performed to evaluate strength of various screw trajectories.

Conclusion

Iliac screw fixation is of paramount importance for lumbosacral stabilization. Of the studied paths, the trajectory from posterior-superior iliac spine to Antero-inferior iliac spine has the longest passage length and is the most optimal path for the Indian population. In case additional screws are required, trajectory from chaotic line - iliac crest intersection point (CLIC) point to Upper acetabulum provides the second largest screw passage.

Disclosures: There is no conflict of interest for all authors.

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Determining incontinence awareness, attitude, and frequency in female students enrolled in the Faculty of Health Sciences

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Abstract

Aim: This study was conducted to determine urinary incontinence awareness, attitude, and frequency among female students studying at a faculty of health sciences.

Material and methods: This cross-sectional study was conducted with 458 female students enrolled in a university's faculty of health sciences between February 2023 and April 2023. The data of the study were collected using a "Personal Information Form" and an "Incontinence Awareness and Attitude Scale" prepared by the researchers. Descriptive statistics, independent t-test, and ANOVA test were used to evaluate the data.

Results: The mean age of the students was 21.17±1.70, and only 2% had been diagnosed with urinary incontinence during their lifetime. The mean scores of the students in the sub-dimensions of incontinence awareness were found as follows: factors affecting acceptance of incontinence as a health problem 32.98±5.13, health motivation 7.15±2.51, coping with urinary incontinence 17.25±5.77, limitation 10.54±3.38, and fear of urinary leakage 11.47±4.34.

Conclusion: The mean score of female students on the urinary incontinence awareness scale, factors preventing acceptance of incontinence as a health problem, coping with urinary incontinence, health motivation, and limitation sub-dimensions were found to be at a good level.

Key words: attitude, awareness, student, urinary incontinence

Introduction

Urinary incontinence (UI) is simply defined as the loss of bladder control or involuntary leakage of urine [1]. UI is a common problem that is often underreported due to its embarrassing nature and associated social stigma. Urinary incontinence can have a significant impact on an individual's quality of life, but it can be significantly improved with proper evaluation, treatment, and management [2].

The presence of embarrassment and denial about the existence of incontinence, the hope for spontaneous recovery, and the fear of treatment make it difficult to determine the prevalence of urinary incontinence in society [3]. The prevalence of UI worldwide ranges from 19% to 88%, while in Turkey, it ranges from 20.5% to 68.8% [4-6]. The reasons for this variability are attributed to differences in the definition of urinary incontinence, studies being conducted on different groups, and the use of different data collection methods. Additionally, the prevalence of urinary incontinence varies depending on ethnic origin and race [3].

Studies have shown that awareness of urinary incontinence is low and, individuals try to cope with the problem themselves instead of seeking professional support when they experience it [7]. This situation makes urinary incontinence a more complex issue and negatively affects individuals' daily lives, leading to a decrease in their quality of life [8].

UI is not only a medical problem for women but also one of the long-standing health issues that affects women physically, hygienically, psycho-socially, economically, and sexually [9]. Urinary incontinence is common among women, and there are various effective treatment options for the most common types of UI (stress, urge, and mixed), including lifestyle and behavioral therapy, medication, and minimally invasive procedures. Most women recover with treatment [10]. Identifying the underlying cause of incontinence is critical to providing appropriate treatment [1]. In evaluating patients, priority should be given to the quality of life, prevention of depressive symptoms, evaluation of feelings of loneliness, improvement of social relationship quality, and strengthening of existing social network structures [11].

Aim: This study was designed to determine awareness, attitude, and frequency of incontinence among female students in the Faculty of Health Sciences.

Material and methods

Design

This cross-sectional study was conducted between February and April 2023 with the aim of determining the awareness, attitude, and frequency of incontinence among female students studying in the Faculty of Health Sciences.

Population and dataset

The universe of the study consists of female students who are enrolled in the Faculty of Health Sciences at Karabük University (N=1900). The sample size of the research was determined using the known sample calculation method. It was calculated that a minimum of 320 participants should be reached with a 95% confidence level and a 5% margin of error. However, in order to exceed the target population number, 458 participants were included in the study.

Data collection tools

The data of the study were collected using a "Personal Information Form" and "Incontinence Awareness and Attitude Scale" prepared by the researchers based on a literature review.

Personal information form

The form prepared by researchers based on literature review includes 14 questions that query the socio-demographic characteristics of participants and their status of having urinary incontinence diagnosis.

Incontinence awareness and attitude scale

The urinary incontinence awareness scale, developed by Avci et al. in 2022 to measure individuals' awareness of urinary leakage, consists of 26 items [12]. The scale consists of five sub-dimensions: factors that prevent accepting it as a health problem, coping with urinary incontinence, health motivation, restriction, and fear of urinary leakage. Responses given to each statement on the scale are in a 5-point Likert-type format. The scores obtained from the sub-dimensions are as follows: for the factors that prevent accepting it as a health problem sub-dimension, the minimum score is 8, and the maximum is 40; for the health motivation sub-dimension, the minimum is 5, and the maximum is 25; for the coping with urinary incontinence sub-dimension, the minimum is 6, and the maximum is 30; for the restriction sub-dimension, the minimum is 3, and the maximum is 15; for the fear of urinary leakage sub-dimension, the minimum is 4, and the maximum is 20. There is no total score for the scale. Obtaining high scores from the sub-dimensions of factors that prevent accepting it as a health problem, restriction, and fear of urinary leakage indicate that the individual accepts urinary incontinence as a health problem and does not experience restriction or fear of urinary leakage. Obtaining low scores from the health motivation and coping with urinary incontinence sub-dimensions indicate that the individual has better health motivation and coping with urinary incontinence. The Cronbach's alpha values of the scale's sub-dimensions in the validity and reliability study are as follows: factors that prevent accepting it as a health problem: 0.87, health motivation: 0.92, coping with urinary incontinence: 0.86, restriction: 0.79, fear of urinary leakage: 0.60. In this study, the Cronbach's alpha values of the scale's sub-dimensions are as follows: factors that prevent accepting it as a health problem: 0.75, health motivation: 0.70, coping with urinary incontinence: 0.87, restriction: 0.85, fear of urinary leakage: 0.81. They are within acceptable limits in the literature [13]. The interpretation of the incontinence awareness scale is made as poor, moderate, and good over the median score.

Statistical analysis

Statistical analyses were performed using the SPSS 23.0 software package. It was observed that the Skewness and Kurtosis values of the data remained within the range of +2.0/-2.0, indicating a normal distribution [14]. Continuous data obtained from the study were summarized as mean and standard deviation, while categorical data were summarized as percentage distributions. Independent t-test and ANOVA tests were used for the comparison of means. Multiple comparisons were evaluated using the Bonferroni method. The obtained data were evaluated at a confidence interval of 95%, and a significance level of $p<0.05$ was considered statistically significant.

Ethical aspect of the study

Prior to the study, approval was obtained from the Non-Interventional Clinical Research Ethics Committee of a university (Date: 27.02.2023 and Number: E-77192459-050.99-224247). Necessary permissions were also obtained for the measurement tools to be used in the study. Participants were provided with information about the purpose of the study in accordance with the Helsinki Declaration and were invited to participate voluntarily, with their written consent obtained.

Results

Table 1 Sociodemographic characteristics of the students (n = 458)

Sociodemographic Characteristics	$\bar{X} \pm SS$	Min.max	Mand
Age	21.17±1.70	18-26	21
		n	%
Marital status	Married	19	4.1
	Single	439	95.9
Department	Midwifery	195	42.6
	Nursing	44	9.6
	Child Development	112	24.5
	Physiotherapy and Rehabilitation	107	23.4
Class	1	94	20.5
	2	150	32.8
	3	95	20.7
	4	119	26.0
Revenue status	Income equals expense	224	48.9
	Income is more than expense	67	14.6
	Income less than expense	167	36.5
Place of residence	Homestay	233	50.9
	Dormitory	158	34.5
	Student house	67	14.6
Smoking status	Where	71	15.5
	No	387	84.5
Alcohol status	Where	53	11.6
	No	405	88.4
Chronic disease status	Where	38	8.3
	No	420	91.7
Diagnosis of urinary incontinence	Where	9	2.0
	No	449	98.0

$\bar{X}$:Ortalama, SS:Standart sapma, Min-max: Minimum-maximum, Med:Median

When the socio-demographic characteristics of the students were examined, it was observed that they had an average age of 21.17±1.70 years. Furthermore, 95.9% of the students were single, 42.6% were studying in the midwifery department, and 32.8% were in their second year of study. In terms of lifestyle habits, 15.5% of the participants used cigarettes, while the majority (88.4%) did not consume alcohol. Additionally, 8.3% of the students reported having a chronic disease. Interestingly, only 2% of the participants had been diagnosed with urinary incontinence throughout their lifetime.

When the sub-dimension scores of students' incontinence awareness were examined, it was seen that the total score average of factors affecting the acceptance of incontinence as a health problem was 32.98±5.13, the health motivation score average was 7.15±2.51, the score average for coping with urinary incontinence was 17.25±5.77, the restriction score average was 10.54±3.38, and the fear of urinary leakage score average was 11.47±4.34.

Looking at the results, it can be seen that female students studying in health sciences have good levels of awareness regarding urinary incontinence, as indicated by the scores on the Urinary Incontinence Awareness Scale. The factors that prevent the acceptance of urinary incontinence as a health problem, coping with urinary incontinence, health motivation, and restriction sub-dimensions also show good levels within the scope of the study. However, the sub-dimension of fear of urinary leakage is at a moderate level.

Table 2 Students' Incontinence Awareness Sub-Dimensions Score Averages (n=458)

Incontinence awareness sub-dimensions	$\bar{X}$ ± SS	With (Min.max)
Factors that prevent its acceptance as a health problem	32.98±5.13	34 (17-40)
Health motivation	7.15±2.51	6 (5-17)
Coping with urinary incontinence	17.25±5.77	17 (6-30)
Restriction	10.54±3.38	11 (3-15)
Urinary incontinence fear water	11.47±4.34	11 (4-20)

$\bar{X}$: Ortalama, SS:Standart sapma, Min-max: Minimum-maximum, Med:Median

Table 3 Comparison of the mean scores of urinary incontinence awareness sub-dimensions according to the socio-demographic characteristics of the students (n=458)

	Factors that prevent its acceptance as a health problem	Health motivation	Coping with urinary incontinence	Kısıtlanma	Fear of urinary incontinence
Part					
Midwifery	33.28±5.57	7.28±2.50	16.91±5.96	10.24±3.31	11.02±4,16
Nursing	33.00±4.52	7.06±2.39	17.09±6.02	10.43±3.57	11.61±4.82
Child development	32.83±4.79	6.78±2.08	17.40±5.92	11.19±3.48	12.06±4.39
Physiotherapy and rehabilitation	32.60±4.90	7.35±2.95	17.79±5.17	10.46±3.25	11.63±4.37
Statistics	F=0.450	F=1.215	F=0.573	F=1.960	F=1.462
	p>0.05	p>0.05	p>0.05	p>0.05	p>0.05
Class					
First1	32.37±4.91	7.43±2.49	16.90±5.17	10.79±3.46	11.96±4.33
Second2	32.74±5.38	7.04±2.27	18.01±5.96	10.73±3.18	11.80±4.06
Third3	31.89±5.38	7.42±2.90	17.30±6.48	10.22±3.73	11.36±4.42
Fourth4	34.65±4.37	6.86±2.47	16.53±5.33	10.36±3.26	10.75±4.57
Statistics	F=6.396	F=1.366	F=1.596	F=0.727	F=1.809
	P<0.001*	p>0.05	p>0.05	p>0.05	p>0.05
Income status					
Equal	33.00±5.38	7.22±2.58	17.25±5.98	10.79±3.38	11.91±4.20
Much	33.50±4.29	6.70±1.92	17.52±6.46	10.38±3.22	11.05±4.40
Low	32.76±5.11	7.25±2.61	17.15±5.22	10.26±3.42	11.05±4.46
Statistics	**F=0.507**	F=1.295	F=0.096	F=1.261	F=2.252
	p>0.05	p>0.05	p>0.05	p>0.05	p>0.05
Place of residencei					
Homestay	32.97±5.11	6.94±2.22	16.87±5.92	10.64±3.53	11.84±4.37
Dormitory	33.14±4.95	7.06±2.19	17.68±5.21	10.37±3.31	11.08±4.04
Student house	32.65±5.65	8.11±3.71	17.56±6.51	10.61±3.00	11.11±4.79
Statistics	F=0.213	**F=5.972**	F=1.035	F=0.315	F=1.723
	p>0005	**p<0.05***	p>0.05	p>0.05	p>0.05
Cigarette					
Yes	33.21±5.07	7.18±2.60	19.08±6.15	10.14±3.58	11.67±4.47
No	32.94±5.15	7.15±2.50	16.91±5.65	10.62±3.34	11.43±4.32
Statistics	t=0.396	t=0.094	**t=2.925**	t=-1.099	t=0.422
	p>0.05	p>0.05	**p<0.05***	p>0.05	p>0.05
Alcohol					
Yes	33.15±5.14	7.54±2.70	18.30±5.98	10.09±3.44	11.43±4.70
No	32.96±5.14	7.10±2.48	17.11±5.74	10.60±3.37	11.48±4.30
Statistics	t=0.244	t=1.201	t=1.403	t=-1.034	t=-0.075
	p>0.05	p>0.05	p>0.05	p>0.05	p>0.05
Kronik disease					
Yes	33.89±4.81	7.02±2.50	17.73±6.30	10.76±3.25	12.55±4.63
No	32.90±5.16	7.16±2.51	17.21±5.73	10.52±3.39	11.37±4.30
Statistics	t=1.136	t=-0.335	t=0.536	t=0.413	t=1.598
	p>0.05	p>0.05	p>0.05	p>0.05	p>0.05

* = p<0.05, t = t test in independent groups, F = One-way analysis of variance
** Benferroni = 4>1, 4>2, 4>3

According to the results, there was a statistically significant difference in the mean scores of the sub-dimension of factors preventing the acceptance of urinary incontinence as a health problem among students according to their class level ($p<0.001$). Bonferroni post-hoc analysis was conducted to determine the specific groups between which the difference existed. The analysis revealed that the difference was specifically between the 4th class and the other classes.

In addition, there was a statistically significant difference in the mean scores of the sub-dimension of health motivation among students according to their place of residence ($p<0.05$). Further analysis was conducted to determine which groups the difference was between, and the results showed that the difference was between students living in dormitories and others.

There was also a statistically significant difference in the mean scores of the sub-dimension of coping with urinary incontinence according to the students' smoking status ($p<0.05$).

Discussion

Research in the literature indicates that Urinary Incontinence (UI) is generally not a life-threatening condition, but it can directly impact women's social life and mental health [16-18]. Studies have shown that women with UI complaints experience a lack of self-confidence, are prone to social isolation, and suffer from high levels of anxiety [18]. These findings highlight that UI not only affects physical health but also significantly impacts women's psychosocial well-being.

In this study aimed to determine the awareness, attitudes, and prevalence of incontinence among female students studying at the Faculty of Health Sciences, the prevalence of incontinence was found to be 2%. In the study conducted by Öz Yıldırım et al. (2020), the prevalence of incontinence among students was found to be 28.8%, while in the study conducted by Durukan et al. (2015) with women living in Mersin, the prevalence of incontinence among women was 21.3%. In a study conducted to determine the frequency and risk factors of urinary incontinence in women who applied to Family Health Centers, the prevalence of incontinence was found to be 37.2% (Kılıç, 2016), and in the study conducted by Ghafouri et al. (2014) in Qatar, the prevalence of urinary incontinence in women was found to be 20.7% [3,8,16,17]. Age, obesity, race, obstetric history, chronic constipation, and urinary tract infections have been identified as risk factors for UI [19]. In this current study, the lower frequency of incontinence may be attributed to the low average age of the students and their high awareness as health science students.

In Kılıç's (2016) study, no relationship was found between smoking and UI. In this study, a significant difference was found in the mean scores of the coping with urinary incontinence subscale based on the students' smoking status ($p<0.05$).

In this study, it was observed that students perceived urinary incontinence as a health problem, had good health motivation, and coped well with incontinence without experiencing any restriction fears, but they had a moderate fear of urine leakage. In the study conducted by Öz Yıldırım et al. (2020), it was found that the awareness scale, factors that hinder acceptance of urinary incontinence as a health problem, coping with urinary incontinence, and fear of urine leakage subscales were moderate, while the health motivation subscale was poor, and the restriction subscale was good [8].

A statistically significant difference was found in the mean scores of the subscale of factors that hinder acceptance of urinary incontinence as a health problem based on the students' study year ($p<0.001$). The higher mean scores of fourth-year students compared to other students suggest that awareness increases with the level of education. In addition, the health motivation subscale score of students living in dormitories, which is a crowded environment, was found to be lower than that of students living with their families or in a hostel. Living in a crowded environment like a dormitory may cause a decrease in health motivation.

In conclusion, it was observed that female students studying in health sciences accepted urinary incontinence as a health problem, had good health motivation, and coped well with incontinence without experiencing any restriction fears, but they had a moderate fear of urine leakage.

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Unraveling the interplay: Exploring the relationship between children's obesity, sleep disorders, depressive symptoms, and age dynamics

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Abstract

Objective: This study explores the relationships between sleep disorders, depressive symptoms, and obesity among children of different ages.

Material and methods: The data for this study were collected from 163 Turkish children aged 7 to 10 and 11 to 15 years (mean age=9.4, SD=1.9) who were attending well-child visits. Parents completed an online questionnaire on sociodemographic data, while children completed the Children Depression Inventory and The Children's Sleep Habits Questionnaire.

Results: The regression model for the 7-10 years group included sleep resistance, sleep onset delay, nighttime awakenings, and daytime sleepiness, and explained 33.8% of the variance in Children Depression Inventory scores (R^2=0.338, F=9.779, p<0.001). For the 11-15 age group, the regression model included obesity status, Children's Sleep Habits Questionnaire scores, sleep onset delay, sleep-related anxiety, and sleep-disordered breathing, and explained 80.3% of the variance in Children Depression Inventory scores (R^2=0.803, F=28.489, p<0.001).

Conclusion: Overall, the results of this study emphasize the significance of addressing sleep disorders, obesity, and depressive symptoms in children, particularly in adolescents, to promote better mental health outcomes.

Keywords: adolescent, children, depressive, sleep, obesity

Introduction

Sleep disorders and obesity are two prominent public health concerns with significant impacts on the well-being of children and adolescents worldwide [1]. Over the past years, the prevalence of both conditions has been steadily rising, posing potential adverse effects on physical and mental health, academic performance, and overall quality of life among this vulnerable population. As these issues continue to grow, there is a pressing need to comprehend the intricate relationship between sleep patterns and weight-related factors to develop effective preventive measures and interventions.

Psychological and sentimental problems are becoming increasingly prevalent, disabling, and recurrent in the younger population [2]. While depression is more prevalent among adolescents, unaddressed psychological problems in children and adolescents can lead to poor academic performance, impaired social functioning, and substance abuse [3,4]. Furthermore, depression may persist into adulthood, increasing the risk of suicide, which ranks as the second most avoidable cause of mortality among young individuals [5].

A meta-analysis involving 143 603 children found strong evidence indicating that obese female children have a substantially greater likelihood of being at risk of experiencing depression in contrast to their normal-weight counterparts, and this risk endures into adulthood. As such, healthcare providers should take into account the potential for depression symptoms when screening obese female children [5].

Despite the evident significance of addressing sleep and weight-related issues, our understanding of the underlying mechanisms and mediators linking these conditions remains limited. Thus, unraveling the complex interplay between sleep, obesity, and mental health is imperative for the development of comprehensive interventions targeting these intertwined

problems. This study seeks to investigate the potential mediating role of depressive symptoms in the association between sleep disorders and obesity among children and adolescents. By elucidating the mechanisms through which these factors interact, we aim to contribute valuable insights to inform public health strategies and support the design of tailored interventions for this vulnerable population.

Material and methods

This study aimed to include both obese and normal-weight children who were seeking routine health check-ups. To be eligible for the study, children needed to be between the ages of 6 and 18 years and have no known medical or psychiatric conditions that could affect their sleep or weight. To assess depressive symptoms, we planned to use the Beck Depression Scale, which has been validated for use in children of this age group. Therefore, we included children aged 6 years and above in our study. Other inclusion criteria were the completion of an online questionnaire that we provided, being obese or normal-weight based on body mass index (BMI) measurements, and having no known chronic medical or psychiatric conditions that could affect sleep or weight. Children who met these criteria were recruited from the pediatric outpatient clinic at Başkent University between March 1, 2023, and July 1, 2023. Parents of eligible children were provided with detailed information about the study and asked to provide written informed consent before their child's participation. Ethical approval for the study was obtained from the institutional review board at Başkent University (project number: KA 23/206) before its commencement.

We determined that a sample size of 156 participants would be sufficient for conducting one sample proportion testing, with a power of 90% and a type I error of 0.05, assuming an effect size of 0.10. To obtain the maximum sample size, we chose a probability of success of 0.50. All statistical tests were interpreted using a significance level of 0.05. We performed statistical analyses using SPSS v25.0 software (SPSS Inc., IBM, USA).

All parent-child pairs completed an online questionnaire, which encompassed parent sociodemographic factors, children's weight and height measurements, as well as scales such as the Children's Depression Inventory and The Children's Sleep Habits Questionnaire.

Measures

Obesity or normal weight

This study utilized the BMI (Body Mass Index) percentile to categorize the children as either obese or non-obese. The participants' BMI was calculated by dividing their weight in kilograms by the square of their height in meters. The children's height and weight were measured to compute their body mass index (BMI), which was determined using BMI percentile charts developed based on the reference values for Turkish children [6]. The study included children who were of normal weight (between the 5th and 85th percentile) and those who were obese (above the 95th percentile).

Children's depression inventory

We used the Children's Depression Inventory (CDI), developed by Kovacs (1985), to assess the levels of depression in the children [7]. The scale comprises 27 items, with scores ranging from 0 to 54. Higher scores on the CDI indicate a greater degree of depression [8]. In our study, Cronbach's alpha coefficient for the scale was 0.89.

The children's sleep habits questionnaire

We used the Children's Sleep Habits Questionnaire (CSHQ) to assess the sleep patterns of the children in our study. The CSHQ was originally developed by Owens et al. (2000) to investigate sleep issues in preschool and school-aged children, with the aim of identifying high-risk situations for sleep problems rather than diagnosing sleep disorders [9]. The scale was adapted to Turkish in 2010 by Fis et al., who conducted a validity and reliability study (Cronbach's alpha value = 0.78) [10]. Parents retrospectively completed the scale by evaluating their child's sleep habits over the previous week. The scale consists of eight subscales, including bedtime resistance, sleep onset delay, sleep duration, sleep anxiety, night waking, parasomnias, sleep-disordered breathing, and daytime sleepiness. Based on the scores obtained from the Sleep Habits Questionnaire, we divided the children into two groups: Sleep Group I (≤ 41 points) and Sleep Group II (> 41 points). This study's results showed that 64.6% (n=62) of the children were classified as belonging to Sleep Group II. In our study, Cronbach's alpha coefficient for the scale was 0.75.

Statistical analysis

Statistical analyses were conducted using IBM SPSS Statistics version 25.0 (IBM Corp., Armonk, NY, USA). Qualitative data were summarized using numbers and percentages, while quantitative data were described using mean and standard deviation. The normality of distribution was assessed using the Kolmogorov-Smirnov test. To compare the two continuous groups, the Mann-Whitney U test was employed. For categorical variables, the Pearson chi-square test, continuity correction chi-square test, and Fisher's exact test were performed. Multiple linear regression analysis was conducted to examine the factors influencing children's BECK depression across different age groups. The level of statistical significance was set at $p<0.05$ for all analyses.

Results

A total of 497 children were initially assessed in the pediatric clinic. Since the Children's Depression Inventory was only validated for children aged 7 and above, 290 children under the age of 7 were excluded from the study. This resulted in a final sample of 207 children who were included in the subsequent analysis. Among them, 35 children were categorized as underweight (BMI percentile < 5%), and 9 children declined to participate in the survey.

Additionally, the study involved 163 parents, comprising both parents of normal-weight and obese children. Of the included children, 55.8% (n=91) were obese, while 44.2% (n=72) were of normal weight. The gender distribution among obese and normal-weight children was found to be similar (p=0.145). Notably, there were no children aged 15 or above among the applicants. Consequently, the children were divided into two age groups: 7-10 years and 11-15 years. When these age groups were analyzed separately, it was observed that the proportion of girls was 65% in the normal-weight group, whereas it was only 30% in the obese group (p=0.016).

Regarding the sociodemographic characteristics of the parents, the majority (89.5%) were married, with mothers having an average age of 40.5±4.3 and fathers having an average age of 45.4±4.8. Additionally, a significant number (74.3%) of parents reported having a monthly income of at least three times the minimum wage. Moreover, 76% of mothers and 75% of fathers had attained at least a bachelor's degree.

Table 1 Comparison of Sociodemographic Characteristics, Depressive Symptoms, and Sleep Habits Between Obese and Normal Weight Children Across Different Age Groups.

	7-10 years (n=122)			11-15 years(n=41)		
	Normal (n=59)	Obese (n=63)	p	Normal (n=13)	Obese (n=28)	p-values
Parenteral Characteristic						
Marital status n (%)*						
Married	53 (90)	55 (87)	0.878[b]	11 (85)	25 (89)	0.645[c]
Divorced	6 (10)	8 (13)		2 (15)	3 (11)	
Income level n (%)*						
<Minimal wage	3 (5)	-		-	-	0.987[b]
Minimal wage -3x	15 (25)	25 (40)	0.064[a]	6 (46)	13 (46)	
>Minimal wage 3x	41 (70)	38 (60)		7 (54)	15 (54)	
Educational level n (%)*						
Mother						
High school and below	15 (25)	8 (13)	0.118[b]	5 (39)	11 (39)	1.000[b]
License and graduate	44 (75)	55 (87)		8 (61)	17 (61)	
Father						
High school and below	16 (27)	9 (14)	0.126[b]	7 (54)	9 (32)	0.326[b]
License and graduate	43 (73)	54 (86)		6 (46)	19 (68)	
Employment status n (%)*						
Mother						
Not working	16 (27)	15 (24)	0.833[b]	5 (39)	11 (39)	1.000[b]
Working	43 (73)	48 (76)		8 (61)	17 (61)	
Father						
Not working	3 (5%)	3 (5%)	1.000[c]	-	-	
Working	56 (95%)	60 (95%)		13 (100)	28 (100)	-
Age of the parents (M±SD)						
Mother	39.44±6.07	38.86±4.16	-	41.38±5.08	42.54±5.17	-
Fathers	41.37±6.42	41.89±3.98	-	48.54±3.91	44.96±4.75	-
BMI	17.84 ±2.26	21.35±4.18	-	18.02±1.97	21.02±5.51	-
Children's Variables						
Gender n (%)*						
Female	39 (66)	28 (44)	**0.016**[a]	6 (46)	15 (54)	0.915[b]
Male	20 (34)	35 (56)		7 (54)	13 (46)	
Scores of the Children on the Scale (M±SD)						
CDI Score	6.42±3.61	8.76±7.67	0.611[U]	8.92±8.11	13.14±9.37	0.112[U]
CSHQ Total and Subscales Score						
Total	47.86±6.09	47.76±4.92	0.813[U]	47.69±5.17	48.82±5.04	0.692[U]
Bedtime resistance	9.54±1.50	9.87±1.56	0.259[U]	9.23±1.59	9.57±1.62	0.653[U]
Sleep onset delay	1.94±0.22	1.92±0.27	0.527[U]	1.69±0.48	1.75±0.44	0.702[U]
Sleep duration	6.57±0.83	6.53±0.88	0.703[U]	6.53±0.88	6.36±1.22	0.615[U]
Sleep anxiety	7.17±2.66	6.41±2.19	0.171[U]	4.92±1.98	5.61±1.93	0.102[U]
Night waking	4.03±1.26	3.88±1.15	0.547[U]	3.77±0.93	4.21±0.96	0.165[U]
Parasomnias	7.98±0.97	8.27±1.31	0.374[U]	9.92±2.18	8.32±1.81	0.015[U]
Sleep-disordered breathing	3.29±0.79	3.29±0.68	0.637[U]	3.31±1.11	3.25±0.80	0.828[U]
Daytime sleepiness	11.08±2.70	11.16±2.31	0.963[U]	10.92±2.81	12.53±2.32	0.092[U]

n: number, a: Pearson X^2 test, b: Continuity correction X^2 test, c: Fisher's exact test, U: Mann-Whitney U test.
*: column percentage, $p<0.05$ is statistically significant. CSHQ: The Children's Sleep Habits Questionnaire,
CDI: The Children's Depression Inventory.

Table 1 presents the sociodemographic data, gender distribution, CDI, and CSQI scores of both obese and normal-weight children aged 7-10 years and 11-15 years. Sociodemographic information, depressive scores, and total sleep scores of parents were found to be similar between obese and normal-weight children in both age groups ($p>0.05$). Analyzing the data separately for each age group, it was determined that 51.6% of the overall sample population was classified as obese, and this percentage increased to 68.2% among the 11-15 age group. Although obese children in both age groups scored higher on the Child Depression Inventory (CDI) and Children's Sleep Habits Questionnaire (CSHQ), no statistically significant difference was observed between the depressive symptoms of obese and normal-weight children within each age group ($p>0.05$; Table 1).

Regression models for both age groups revealed no multicollinearity issues based on the VIF values and no autocorrelation according to Durbin Watson d statistics. The regression equations estimating depressive symptom levels for ages 7-10 years and 11-15 years, respectively, were as follows:

- CDI = 2.515 - 0.939x1 + 1.705x2 - 4.601x3 + 1.544x4 + 2.523x5 + 1.413x6
- CDI = -15.377 + 4.470x1 + 1.155x2 - 12.462x3 - 1.404x4 - 2.186x5

The regression model for factors affecting the CDI in 7-10-year-olds was significant ($F=9.779$, $p<0.001$). The regression coefficients for total sleep, bedtime resistance, sleep onset delay, night waking, parasomnias, and daytime sleepiness were significant, explaining 33.8% of the variance in depressive symptom levels. Specifically, a 1-point increase in night waking

Table 2 Multiple linear regression

(CDI 7-10 years)	*b*	*S(b)*	*VIF*	*t*	*p*
CSHQ Total (X_1)	-0.939	0.262	9.525	-3.590	<0.001
Sleep onset delay (X_2)	1.705	0.479	2.483	3.563	0.001
Bedtime resistance (X_3)	-4.601	2.057	1.203	-2.237	0.027
Night waking (X_4)	1.544	0.624	2.586	2.477	0.015
Parasomnia (X_5)	2.523	0.594	2.201	4.246	<0.001
Daytime sleepiness (X_6)	1.413	0.318	2.912	4.443	<0.001
R^2=0.338 (F=9.779 p<0.001) d=0.617					
(CDI 11-15 years)	*b*	*S(b)*	*VIF*	*t*	*p*
Obese-normal (X_1)	4.470	1.480	1.040	3.020	0.005
CSHQ (X_2)	1.155	0.194	2.039	5.966	<0.001
Bedtime resistance (X_3 (X_4)	-12.462	1.650	1.172	-7.552	<0.001
Sleep anxiety (X_4)	-1.404	0.435	1.535	-3.229	0.003
Sleep-ordered breathing (X_5)	-2.186	0.956	1.566	-2.287	0.028
R^2=0.803 (F=28.489 p<0.001) d=1.149					

VIF: Variance Inflation Factor

was associated with a 1.544 increase in the CDI score. Similarly, the regression model for factors affecting the CDI in 11-15-year-olds was also significant (F=28.489, p<0.001). Regression coefficients for obesity status, total sleep, sleep onset delay, sleep anxiety, and sleep-disordered breathing were significant, explaining 80.3% of the variance in depressive symptom levels. Depressive scores were 4.47 points higher in obese versus normal-weight children.

Moderate correlations were observed between BECK depression and sleep scales in 7-10-year-olds (r=0.384) and 11-15-year-olds (r=0.512) (p<0.001).

Discussion

It is important to identify depressive symptoms in children and recognize associated factors. This study shows that in particular, in the adolescent age group, depressive symptoms are associated with sleep habits, bedtime resistance, sleep anxiety, breathing difficulties during sleep, and obesity. In the 7-10 years group, although sleep habits were associated with depressive symptoms, obesity was not found to be related.

The present study sheds light on the significant link between disrupted sleep habits and depression among adolescents, without any notable disparities between genders. A study conducted by Goldstone and colleagues, involving a substantial national sample of more than 11,000 participants aged 9-10 years in the United States [11], further supports these findings. The researchers utilized parent reports to assess sleep disturbance and sleep duration, while mental health was evaluated using the Child Behavior Checklist. The outcomes of their investigation revealed that higher levels of sleep disturbance and shorter sleep duration were correlated with an increase in mental health symptoms, and notably, sleep disturbance emerged as a predictor of future mental health issues, especially depression. These compelling findings underscore the potential of leveraging proper sleep patterns as a preventive measure against depression during early adolescence. An intriguing aspect noted in their study, which contrasts with our own, was the significant interaction between sleep disturbance and gender. This interaction indicated that the association between sleep disturbance and depression was more pronounced among girls compared to boys. Such nuanced gender differences emphasize the need for tailored approaches when addressing sleep-related interventions for adolescents.

There is a relationship between depressive symptoms and sleep disturbances in children [12]. Many studies have shown that sleep problems can contribute to the onset of depression or increase the severity of existing depression. Additionally, depression itself can lead to sleep problems. Sleep problems can lead to the emergence of depressive symptoms in children. For example, symptoms such as insomnia, fatigue, and difficulty concentrating can affect a child's social and school life and lead to depressive symptoms. The disturbances in the circadian rhythm of sleep and wakefulness were related to depression in adolescents [13]. On the other hand, depression can cause sleep problems [14]. Depressive symptoms in children can reduce sleep quality and lead to difficulty falling and staying asleep. Therefore, if both sleep problems and depressive symptoms are present in children, it is important to consider that these two conditions are related. Treating sleep problems can also help with treating depression, and similarly, treating depression can improve sleep problems.

The current study found obese children scored higher in depression in both the 7-10 and 11-15 age groups, but the link between obesity and depression was only significant among adolescents. In contrast to our findings, Moreno et al. (2021) discovered that later sleep midpoints were linked to greater BMI increases during summer [15]. Additionally, another study found that obesity was linked to increased sleep difficulties and lower Pediatric Quality of Life scores [16]. While children with more depressive symptoms had more sleep difficulties, this was not linked to the degree of obesity. As a result, it was concluded that obese children and adolescents require support to improve their sleep quality, quality of life, and depressive symptoms.

Our study demonstrated that disturbed sleep habits varied according to age groups, with adolescents having higher scores in the parasomnia subgroup. This finding is consistent with a study by Lewien et al. (2020) who also found that sleep habit disorders differed according to age groups [17]. However, unlike their study, we did not observe a difference in disturbed sleep habits based on the socioeconomic status of the parents. This discrepancy may be due to the relatively small sample size of our study or the homogeneity of our sample. Our findings highlight the importance of evaluating sleep habits and addressing sleep disturbances in children and adolescents, particularly those with depressive symptoms. Further research with larger and more diverse samples is needed to confirm and expand upon our findings.

Conclusion

Preventive measures are vital for children's health. Assessing sleep habits in obese or visibly depressed children can help implement preventive measures. We thought this study contributed to scientific understanding. However, our study has limitations. Firstly, wider research covering the 11-18 age range is needed due to hormonal changes and weight gain during adolescence. Secondly, participant selection should consider social status and other influencing factors. Additionally, the small sample size limits generalizability and statistical power. The study relied on the CDI scale without a psychiatric diagnosis. Nonetheless, this research is valuable, showing that obesity and sleep habits predict depressive symptoms in adolescents with affluent parents.

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How fixation affects the results of lymph node immunophenotyping by flow cytometry

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Abstract

Aim: Flow cytometric diagnosis of lymphoma and leukemia is of high clinical and research importance. However, performing flow cytometry analysis on the day of biopsy might be of challenge due to several reasons, including late sample delivery, problems of preparing the reliable panel for immunophenotyping based on other diagnostic studies, etc. This problem could be partially solved if cell suspension could be fixed and stained on another day or after several days after standard FFPE (formalin-fixed and paraffin-embedded) procedure.

Material and methods: Addressing this issue, we compared staining of live lymphocytes in suspension obtained from lymph node biopsies and same specimens fixed using 2-4%-paraformaldehyde, 1-3%-glyoxal, and 0.1-1% glutaraldehyde with subsequent immunostaining on the next day or later.

Results: Staining after fixation could be partially representative only after paraformaldehyde fixation for 20 min and subsequent storage of cell suspension in phosphate-buffer saline within not more than 3 days. Probes stained after fixation always shows lower stain index compared to staining of live cells.

Conclusion: Staining after fixation cannot be used for determining of the percentage of CD45-positive cells and for testing B-cell lymphomas since antigens against light chains of IgG cannot be properly detected in fixed specimens.

Key words: flow cytometry, immunophenotyping, cell suspension, lymph node, sample fixation, B cells, T cells, lymphoma

Introduction

Nowadays, flow cytometry (FC) is one of the major clinical tools in immunophenotyping patients with hematological and lymphoproliferative disorders. Meanwhile, performing flow cytometry analysis on clinical samples upon receiving them might be a challenge for some laboratories. Moreover, researchers or doctors might decide on performing additional studies based on the result of morphological or immunohistochemical studies later, which also requires preservation of biological specimens.

A comprehensive review on the problems appearing throughout the process of preparing a single cell suspension from solid tissues for prolonged storage prior to flow cytometry analysis was recently published [1].

Chemical fixation is a routinely used procedure aimed at maintaining cell or tissue architecture and antigenicity [2]. There is a wide range of fixators, mixtures of different fixators, and procedures, where each laboratory adapts their own protocol for achieving the optimal staining results [2,3]. The choice of the fixative depends on its maximum fixation ability without adverse effect on antigenicity thus providing long-term storage and reliable results of immunophenotyping post fixation. Apart from losing antigenicity, fixation can cause autofluorescence and nonspecific fluorescence [4,5]. In flow cytometry, researchers generally suggest staining live cells first and then preserve them by fixation known as post fixation procedure [4-6]. A variety of studies has been performed to preserve mainly liquid forms of human samples (whole blood, bone marrow and cerebrospinal fluid) using different fixatives and methods [4-7]. However, no data is available on chemical preservation of cells isolated from lymphatic nodes.

Formaldehyde or paraformaldehyde (PFA) solutions are common fixatives routinely used in flow cytometry and in diagnostic pathology [8]. It has been suggested that short fixation of cells from solid tissues

might prevent their degradation, while not affecting light scatter characteristics [9]. Alcohol fixation is another type of fixators successfully used for intracellular staining in flow cytometry [10], but it results in cell clumping and loss of light scatter thus it is completely unacceptable for cell fixation and storage [11]. Less popular among fixators is glyoxal-based solutions, which is nowadays positioned as alternative fixator to paraformaldehyde in immunostaining [12,13]. Authors emphasize glyoxal's advantages including safeness and easiness in handling, better preservation of cell fine structure and more intense immunostaining without antigen retrieval. However, no information was published on preservation of surface antigens in cell suspension using glyoxal solution. Since glyoxal was introduced as a fixative more recently than formalin and show at the microscopic level better tissue preservation [12,14], we decided that it is important to test this fixative along with formalin and glutaraldehyde. In this study, we approbated two different concentrations of glyoxal – 1% and 3% for fixation of cells in suspension. To the best of our knowledge, no data has been previously published on assessing the effect of fixation with glyoxal on cells isolated from solid tissues.

Neither, we found any papers related to such multi-parametric analysis of the effect of fixation imposed on the main lymphocytic antigens. Developing easy, safe, and fast method of chemical fixation allowing prolonged storage without deterioration of FC results might be very useful for clinical laboratories and field research. In this study, we suggest fixation of unlabeled cells first following with antibodies staining a few days later. This will benefit both clinicians and researchers by extending the range of panels used and experiments conducted on a single sample.

Aim of the study

In the current study, we used live cells in suspension obtained from the lymph node biopsy for immediate multicolor immunostaining and then stained and analyzed PFA, glyoxal, and glutaraldehyde fixed samples to compare the staining and expression of lymphocyte specific antigens post fixation. The research objectives were the following:

Firstly, to approbate widely used (PFA, glutaraldehyde) and recently suggested (glyoxal) chemical fixators aiming to preserve lymphoid cell morphology (reflected as light scatter properties) and antigenicity (antibody staining) in suspension stored for at least three-days at +4ºC.

Secondly, to test different concentrations of PFA (2-4%) and glyoxal (1-3%) identifying the optimal working concentrations.

Thirdly, to compare the percentage of positive cells, mean fluorescence intensity, and stain index between live and fixed cells, as a measure to analyze the effect of fixation posed in cell surface antigen expression.

Material and methods

Sample collection

In this study, lymph node biopsies with suspected lymphoma were provided by the National Research Oncology and Transplantology Center and delivered to the laboratory for the analysis within an hour. Adult patients with clinically evident lymphoma with excision biopsy recommended at the National Research Oncology and Transplantology center were included in this study upon informed consent. Sample group contained 29 patients, among which there were 15 women and 14 men, median age 49.

Approval №9 dated 21.06.2021 was obtained from the Ethical Committee of National Research Oncology and Transplantology Center. Written informed consent was obtained from the patients. Flow cytometric analysis was performed on the same day of biopsy on live cells in suspension isolated from solid tissue.

Cell suspension preparation for immunostaining

One third of biopsy was disaggregated by either or both mechanical and manual methods providing sufficient cell recovery to perform flow cytometric analysis. The manual tissue disaggregation method was adapted by our laboratory with some minor changes [15]. The specimen was hold in place with forceps and multiply perforated using a 10-ml syringe with a 21-gauge needle. Approximately 10 ml of phosphate buffered saline (PBS) containing 0.1% sodium azide was repeatedly injected into the tissue until complete tissue dissociation. For better cell preservation no vortexing of biopsy tissue used at any time of obtaining cell suspension. Finally, cell suspension was filtered through a 40-μm cell strainer to get rid of any connective tissue that can occasionally appear. After centrifugation (500 g for 5 min) cell pellets were resuspended in sufficient amount of RPMI-1640 medium and collected into one tube, labelled MD (manual disaggregation).

Cells were treated with erythrocyte lysing solution containing ammonium chloride for 10 min, then washed twice with cold PBS (5 min). Mechanical disaggregation was used when poor cell yield came from manual disaggregation, or when (i) biopsy was very small; (ii) contained mainly connective tissue, and (iii) had a rigid structure. The number of cells were then counted using hemocytometer and cell viability accessed through staining with Trypan blue. Cell suspension has been then split into two parts: one stained immediately with fluorophore-conjugated antibodies, and another fixed using PFA, glyoxal, or glutaraldehyde and stored at 4°C for 1-5 days until further staining and analysis.

Flow cytometric analysis of live/unfixed cells

Panels for analysis were composed depending on patient's initial diagnosis and accounting for fluorophores compatibility. Immunostaining of cells included the following monoclonal antibodies from BD (Becton Dickinson, Franklin Lakes, NJ, USA), BioLegend (San Diego, California, USA) and Abcam (Cambridge, United Kingdom) companies against: common leukocyte antigen (CD45-APC-Cy7 clone 2D1), B-cell antigens (CD19-FITC clone HIB19, CD19-BV711 clone SJ25C1, CD20-BV421 clone 2H7, CD22-BV510 clone HIB22, CD23-APC clone EBVCS-5, Ig light chain κ-FITC clone TB28-2, Ig light chain λ-PE clone MHL-38, and CD10-BV421 clone HI10a), and T-cell antigens (CD3-PE-Cy7 clone SK7, CD5-PE clone UCHT2, CD5-BV510 clone HIT2, CD4-FITC clone OKT4, CD8-Alexa Fluor 647 clone SK1).

The same antibodies were used through the whole study. Two extended 8-color panels were used for all patients, namely general lymphoid panel and B-cell panel. General panel (including anti-CD45, anti-CD19, anti-CD3, anti-CD5, anti-CD10, anti-CD38, anti-CD4, and anti-CD8) was used to detect any most common aberrations in surface antigen expression among B- and T-lymphocytes in lymphomas. B-cell panel (including anti-CD45, anti-CD19, anti-CD20, anti-CD22, anti-CD23, as well as anti-kappa and anti-lambda light chains of immunoglobulins) was used to identify light chain restriction and changes in B-cell antigens expression if any. FC studies might require the inclusion of additional markers (extended

T-cell panel, intracellular staining panel, etc.) [16], which is out of scope of this study.

Cells before staining were washed three times with PBS and then the cell number was adjusted to roughly $1*10^6$ cells/100 µl of PBS containing 1% of BSA and incubated on ice for 30 min. For each panel we mixed the corresponding antibodies first and then added 100 µl of washed cell suspension. Staining was performed on ice for 20 minutes. After incubation, cells were rinsed in PBS and resuspended in 500 µl of fresh PBS ready for flow cytometric analysis.

Stained cells were analyzed using FACS Aria II cell sorter with FACS Diva software version 8.0 equipped with 405 nm (violet), 488 nm (blue), 561 nm (yellow-green), and 638 nm (red) lasers using the following channel/bandpass filter combinations: BV421 (450/50), BV510 (530/30), BV711 (730/45), FITC (530/30), PE (586/15), PE-Cy7 (780/60), Alexa Fluor 647 (660/20), APC-Cy7 (780/60). (Becton Dickinson, Franklin Lakes, NJ, USA). For each sample analyzed, 100 000 events were collected. The acquired data were analyzed using FlowJo software v10.2. Gating was carried out as following: A. Forward (FSC-Area) versus side scatter (SSC-Area); B. FSC-Height/FSC-Width (to exclude doublets); and C. SSC-Area/CD45 (to gate leukocytes).

Fixators

We used freshly (the same day) prepared 4% and 2% w/v paraformaldehyde (PFA), a 3% and 1% v/v glyoxal solutions, and 0.1% and 1% glutaraldehyde solutions. PFA solution was prepared by dissolving 1 g of powder PFA, 96% (Alfa Aesar, A11313) in 25 ml of PBS. Paraformaldehyde was slowly dissolved in PBS on a heated magnetic stirrer, then solution was cooled down, filtered, and stored at +4°C until use. Similarly, a 2% PFA solution was prepared by dissolving 0.5 g of stock powdered PFA in 25 ml PBS. Glyoxal solution was made by mixing the following components: 2.835 ml ddH_2O, 0.789 ml ethanol (96%), 0.313 ml glyoxal (40% stock solution, Sigma Aldrich), and 0.03 ml acetic acid. The pH of the solution was adjusted between 4 and 5 by adding dropwise about 0.12 ml of 1M NaOH. The solution was also stored at +4°C until use. A 1% glyoxal solution was prepared by diluting initially prepared 3% glyoxal with water. Glutaraldehyde in two concentrations (0.1% and 1%) was prepared by diluting the corresponding amount of stock glutaraldehyde (25% stock, AppliChem, A5252) in phosphate-buffer saline.

Cell fixation and flow cytometric analysis

The second tube of suspension cells was equally divided into tubes and washed once with PBS before fixation. The supernatant was then discarded and pellets were resuspended in the corresponding fixative. Primarily we employed 4% (final conc.) PFA, without washing and leaving tubes at +4°C for prolonged storage (several months). Prior to staining 200 µl of paraformaldehyde stored cells was washed twice with PBS (5 min, 300 g) and stained following the protocol as for live cells.

The second method was cell fixation in 4% and 2% PFA in PBS for 20 mins on ice. In this case, fixed cells were washed twice with PBS (400g, 5 min), the resuspended in sufficient amount of PBS, and left at +4°C.

The fixation with 3% and 1% v/v glyoxal solutions was performed on ice for 10 min, followed the double wash in PBS and leaving samples in PBS at +4°C.

The fixation with 0.1% and 1% glutaraldehyde solution was performed on ice for 20 min, followed the double wash in PBS and leaving samples in PBS at +4°C.

For staining fixed cells in suspension on day 1-5, 200 µl of suspension was washed once with PBS, and then stained with the cocktail of antibodies.

Data processing and analysis

The analysis of fixed cells by staining with antibodies was performed usually within 5 days after fixation; some samples were analyzed one week and one month after fixation (data not shown). Staining effectiveness of fixed specimens was assessed through the calculations of the stain index (SI), as well as comparison of the percentage of positive cells and MFI (median fluorescence intensity) of a given population between live/unfixed and fixed cells. For quantitative analysis, the stain index was calculated using the following equation: SI = (Median positive - Median negative)/ (2×rSD negative) as described elsewhere [17-20].

All bar graphs represented mean values, and all error bars denote the standard error of the mean. All statistical analysis was performed in GraphPad Prism 8.0 software (GraphPad, San Diego, CA, USA). Differences between means were compared using paired t-test, where the significance was identified by P-value: $p<0.05$.

Results

In the present study, we assessed the extent to which fixation before immunostaining affects the surface antigen expression of lymphocytes from lymph node biopsies by flow cytometry. The effect of different fixatives was analyzed through comparison of stain index (SI), MFI and percentages of positive cells between live and fixed specimens. The results show that cell fixation prior to immunostaining and subsequent cell storage results in

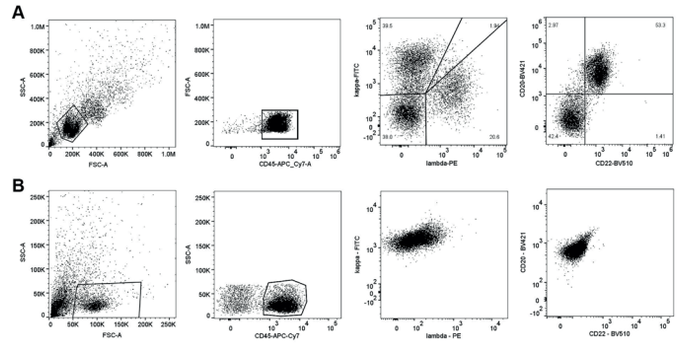

Figure 1 - (A) light scatter characteristics and results of immunophenotyping of live cells; (B) light scatter characteristics and results of immunephenotyping of cells stored in 4% PFA for a month.

a decrease in SI incompatible with the one demonstrated by live cells, yet to the different extent.

Prolonged storage (more than 24 h) of suspended cells from lymph nodes in 4% PFA did not provide satisfactory staining results. Fixation resulted in the loss of the majority of surface antigens (Figure 1). Leaving samples for one hour or overnight in PBS to get rid of excess PFA did not make any improvements in antigen expression. Moreover, staining with two or more fluorophore-conjugated antibodies resulted in high autofluorescence without possibility to differentiate between populations.

0.1% and 1% glutaraldehyde fixative resulted in changes in the light scatter and showed worse surface antigens preservation – lower SI (in the case of CD45) or sometimes inability to

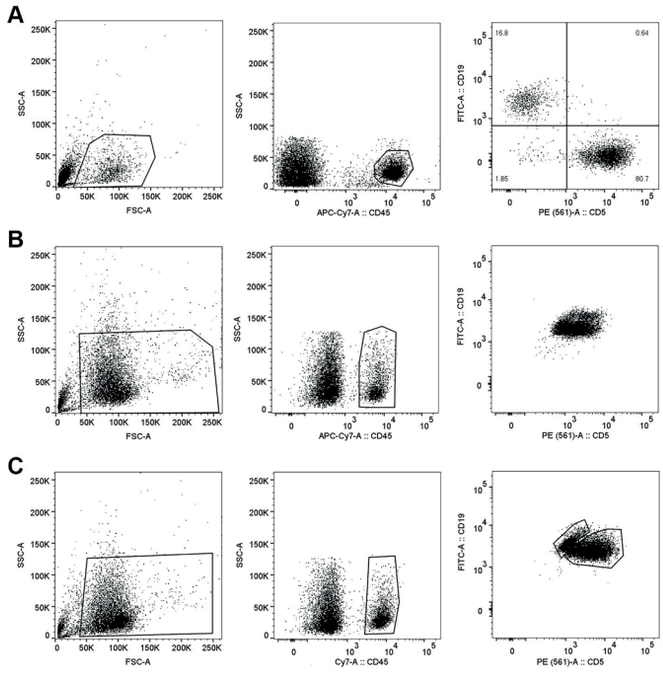

Figure 2 - The result of glutaraldehyde fixation. The upper line (A) shows light scatter characteristics and results of CD45, CD5 and CD19 immunostaining of live cells, the middle line (B) - after 1% glutaraldehyde fixation, and the bottom line (C) - after 0.1% glutaraldehyde fixation.

differentiate between positive and negative cell populations (CD19 and CD5) (Figure 2).

Thus, these two types of fixation procedures (leaving samples in 4% PFA, as well as glutaraldehyde at two concentrations) were excluded from the further study. We next used freshly prepared 2% and 4% PFA for cell fixation on ice for 20 minutes, washed off the fixative by PBS two-three times (5 min, 500 g) and left samples in PBS at 4ºC from 1 to 5 days before analysis. Similarly, we fixed samples with 1% and 3% glyoxal solution on ice for 10 minutes, washed off the fixative three times with PBS, and stored cells in PBS at 4ºC from 1 to 5 days.

The effect of fixation on light scatter parameters

Fixation of suspended lymphocytes caused significant changes in light scatter properties, resulting presumably in cell shrinkage and causing subsequent reduction of SSC and FSC values making gating more difficult.

Apparently, there is a loss of cell debris possessing the lowest light scattering values, where we can observe a single population of events with decreased SSC and FSC (Figure 3).

The decrease was apparent especially for SSC-A signal reflecting cell complexity and granularity. However, FSC-A was affected commensurately decreasing the cell size, which might be important in lymphoma evaluation and detection of large cells in case of large B-cell lymphomas. In fixed cells, the exclusion of cell debris is almost impossible, due to a single cell population observed in light scatter.

The least effect on light scatter properties of fixed cells was observed in samples left in 4% PFA without washing (Figure 3). However, this treatment dramatically decreased antigenicity of cells affecting staining with antibodies.

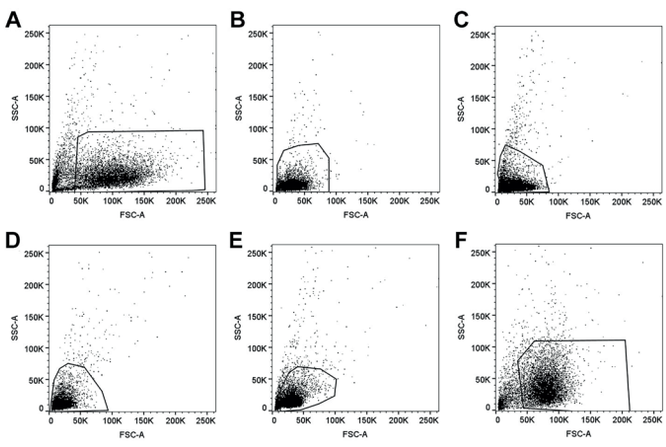

Figure 3 - Changes in light scatter properties of cell suspensions after different fixations. (A) – live cells; (B) – cells fixed with 4%-PFA, stored in PBS; (C) – cells fixed with 2%-PFA, stored in PBS; (D) – cells fixed with 1%-glyoxal, stored in PBS; (E) – cells fixed in 3%-glyoxal, stored in PBS; (F) – cell left in 4%-PFA for long-term storage.

Leukocyte common antigen (CD45)

The light scatter properties of cells derived from a lymph node biopsy is different from whole blood or PBMC and is often not as obvious for identifying lymphocytes. Thus, we employed the SSC-A/CD45 plot to isolate the lymphocyte population. The subsequent analysis of B- and T-cells was performed within the CD45 positive population only, and it was important to access the effect of fixation on this particular antigen (Figure 4).

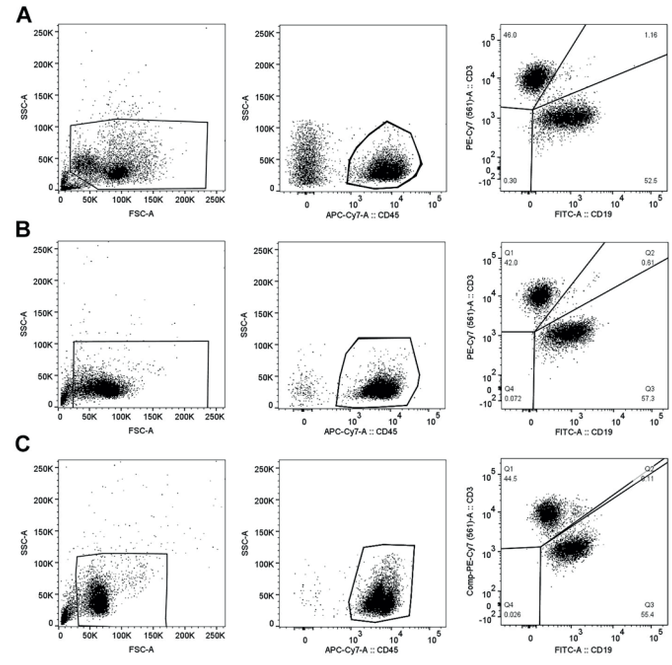

Figure 4 - Fixation with PFA and glyoxal show similar changes in antigen representation: (A) – light scatter and immunostaining (CD45/CD3/CD19) of live cells; (B) – cells, fixed in 4%-PFA, and stored in PBS before staining; (C) – cells, fixed in 3%-glyoxal, and stored in PBS before staining.

The percentage of CD45 positive cells as well as the SI values after fixation differed from live cells significantly for all treatments apart from the fixation with 3% glyoxal. Fixation resulted in an apparent increase in the percentage of CD45+ cells and decrease in SI. However, fixation did not pose any significant effect on MFI of CD45 positive cell population. On average, MFI was lower in fixed cells when compared to live cells (Figure 5). Generally, the side scatter characteristics of the lymphocytes gated through high CD45 expression and low side scatter were not markedly affected by fixation of cells in suspension.

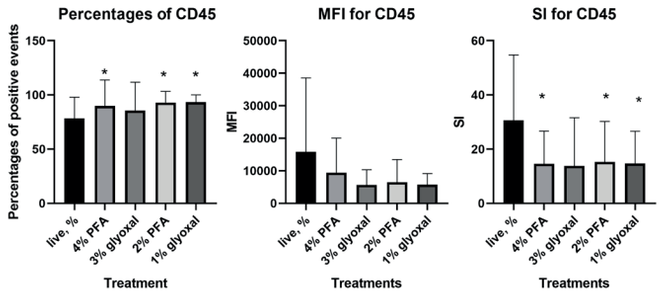

Figure 5 - The effect of different fixatives on percentage, MFI, and SI of CD45 positive cells. * - the difference from live cell staining is significant, p≤0.05.

B-cells specific antigens

The majority of lymphoma has B-cell origin, thus, it was important to analyze the effect of fixation on antigenicity of B-lymphocytes. In this study, we have assessed the effect of fixators on CD19 and CD20 antigens, as well as important in lymphoma evaluation analysis of kappa and lambda light chains of immunoglobulins.

The percentage of gated CD19-positive lymphocytes was not significantly altered with any of the variant procedures apart from 4% PFA (p=0.044). However, all types of fixation negatively affected SI and MFI of anti-CD19 stained cells (Figure 6).

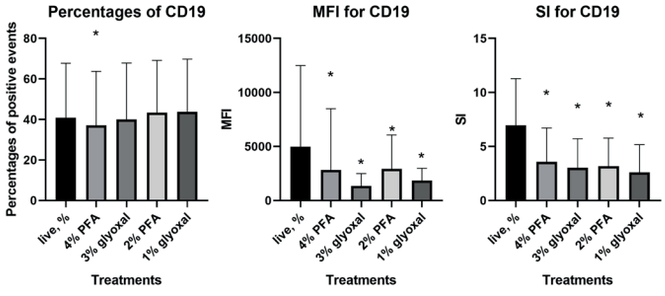

Figure 6 - The effect of different fixatives on percentage, MFI, and SI of CD19 positive cells. * - the difference from live cell staining is significant, p≤0.05.

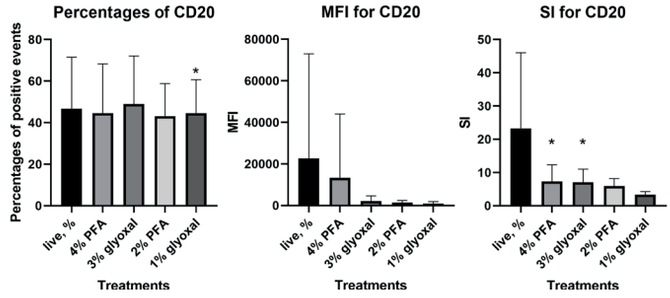

Figure 7 - The effect of different fixatives on percentage, MFI, and SI of CD20 positive cells, * - the difference from live cell staining is significant, p≤0.05.

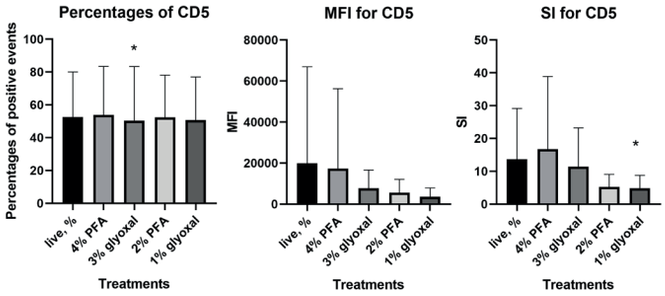

Figure 8 - The effect of different fixatives on percentage, MFI, and SI of CD5 positive cells, * - the difference from live cell staining is significant, p≤0.05.

B-cell specific CD19 antigen was better preserved in cells fixed in 4% PFA, washed and then stored in PBS at 4°C, when compared to continuous cell storage in the same fixative (data not shown). This was true for cells labelled with anti-CD19-FITC on either the next day post-fixation or 1 week after. Cells fixed with 4% PFA and stored in PBS for 1 month showed ten times decrease in SI values when compared to live cells. The result of fixation with 3% glyoxal was worse at preserving CD19 antigens over time when compared to 4% PFA.

Fixation caused changes in CD20 representation to a lesser extent when compared to other antigens. Nevertheless, we observed a decrease in SI values and in MFI, while the percentage of CD20 expressing cells in all samples was almost unaffected by fixation (Figure 7). It is important to mention that gating populations in fixed samples based on FSC/SSC is challenging and might be biased.

Evaluation of the expression of CD5 is important in lymphomas as it is a marker of normal T-cells and abnormal B-cells from CLL and mantle cell lymphoma. Based on the results obtained we can conclude that CD5 antigen is one of the least affected antigens, where no significant difference was found between live and fixed cells in any of the assessed parameters (Figure 8). However, similarly fixation caused gradual decrease in SI values for CD5 staining over time.

T-cells specific antigens

Flow cytometry plays an important role in evaluation of T-cell lymphoma. Thus, we assessed the expression of the main T-lymphocytic antigens (CD3, CD4 and CD8) by cells after fixation. There is only minor effect of fixation on antigenicity of T-cells, both the percentage of positively gated and MFI (Figure 9). Similarly as for all cell types analyzed in this study fixation negatively affected SI values of major T-cell antigens. CD3 was affected to a lesser extent (p=0.3), and CD4 and CD8 to a greater.

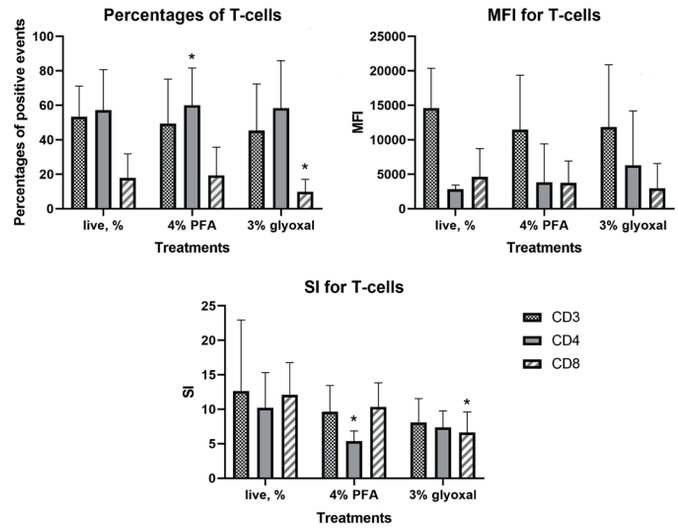

Figure 9 - The effect of different fixatives on percentage of positive cells, MFI, and SI for main T-cell antigens, * - the difference from live cell staining is significant, p≤0.05.

Discussion

We describe a dramatic effect of fixation on light scatter properties of suspended lymphocytes. Forward scatter/Side scatter-based dead cell discrimination is one of the possible and primary gating strategies applied in flow cytometry [21]. Light scattering of all cells after fixation became similar making it harder to discriminate lymphocytes from cell debris. The data

on the effect of fixation on light scatter properties of cells is in agreement with previously reported experiments [22,23]. Generally, fixation significantly affected cell distribution on SSC-A/FSC-A graph as well as the antibody binding efficiency for the majority of surface antigens [22,23]. Although, the data is variable, and fixation using 4% PFA before lysing red blood cells, might preserve whole blood light scattering profile and antigenicity of some leukocyte surface markers [24].

Despite the fact, that fixation is designed to stabilize biological samples close to the pattern demonstrated by live cells, and making further observations reliable, treating with the fixators cause cells to aggregate and shrink. Our results show that, fixation always leads to a substantial decrease in FSC-A values responsible for cell size. Cell morphology analyzed by atomic force microscopy and scanning ion conductance microscopy proofs that PFA (1-4%) fixation drastically change cell morphology through protein crosslinking when compared to live cells [25]. Recently glyoxal was described to be more efficient than PFA in preserving morphology of the attached cells and in cross-linking proteins [12]. However, we observed significant changes in light scatter properties of suspended cells subjected to glyoxal fixation.

Post-fixation procedure, where you first stain cell with fluorophores-conjugated antibodies and then fix them, is preferable by researchers as cell architecture change drastically during fixation, which might subsequently lead to false results [11,26]. PFA, when used prior staining, tend to interfere in the process of antibody-antigen complex formation by crosslinking with epitopes or their structural parts [26]. In previous work, stained lymphocytes fixed using 1% paraformaldehyde for 30 min following washing three times with HPSS and storing cells at 4°C retained its fluorescence properties for at least 2 weeks prior multicolor flow cytometry analysis [12]. Another study showed the possibility of one-week storage of cells fixed with 0.5-1% PFA and 0.05-0.1% glutaraldehyde prior to immunostaining of mouse T-lymphocytes [27]. On the contrary, fixation using 4% PFA is more effective for up to 10 days storage of mononuclear cells comparing to 1% PFA that only preserved cells for up to 3 days [28].

Cross-linkage formed during fixations with aldehydes partially explains loss of antigenicity or masking of surface epitopes, and subsequently decrease in the percentages of positively stained cells post-fixation [29]. However, our data suggests that alterations of cell surface caused by the action of fixators are very serious leading to the denaturation or even loss of surface epitopes available to antibodies.

Based on the previous research [6,30], the optimal concentration of PFA still needs to be identified, since generally used 4% PFA might not be the best working concentration for suspension cell fixation leading to permanent changes of surface antigens expression. However, in our studies reducing PFA concentration to 2% caused similar or even stronger reduction in SI values for some antigens. Overall, we did not find any substantial difference between fixation with 4% PFA or 2% PFA, and the same was true for two concentrations of glyoxal, 3% and 1%. Thus, in this study we mainly used 4% PFA and 3% glyoxal as fixators.

The fixation of leukocytes involving 2% PFA prior immunostaining, and without sample storage, considerably affects the consequent gating of T-lymphocytes, monocytes and basophils. This might lead to result falsifications and drawing a wrong conclusions, which is unacceptable for clinical samples [5]. It was also true for T-cell antigens CD4 and CD8 in our experiment, where fixation with both 4% PFA and 3% glyoxal resulted in a significant decrease in SI and the percentage of positive cells when compared to unfixed cells.

It turned out that washing cells after fixation and storing fixed cells in buffer is beneficial in both procedures of pre- and post-fixation [31]. Time of fixation might vary but generally, it does not exceed one hour [11,26]. In our experiments, the best results were achieved when fixation was performed for 20 min (with PFA) and 10 min (with glyoxal) on ice.

The feasibility of using glutaraldehyde even at low concentrations for the fixation of red blood cells is in doubt as it provides high autofluorescence and significantly reducing cell surface antigenicity [32]. Glutaraldehyde fixation at concentrations of 0.1% and 1% prior cell staining with antibodies did not show prominent results in antigen representation. Post staining fixation with glutaraldehyde resulted in high autofluorescence background, in accord with previous observations [33]. The same authors found that 1% PFA is the most appropriate fixator for stained cells, which can be stored for at least 1-week prior to analysis without significant changes in fluorescence. Another study performed on blood cell leukocytes (PBMC) and whole blood demonstrated the feasibility of fixation prior staining, however it resulted in a decrease in MFI (mean fluorescence intensities) for some antigens with little effect on percentages of positive cell [23].

We have noticed a decrease in positive cells number especially after fixation with glyoxal, where no cells were detected after 1-week storage at +4ºC. It has been found that long storage of fixed cells at +4ºC results in dramatic loss of positive cells when compared to storage in liquid nitrogen [34]. Prolonged storage of fixed cells by our method is in doubt as immunophenotyping can only be performed within 5 days without significantly compromising results. However, the analysis of some antigens might be of challenge. The fixation deteriorates immunostaining results by eradicating small aberrant populations and large cells [5,35]. The percentage of positive cells might increase or decrease depending on the clone and conjugate of the antibody used, which is not reflecting the real immune cell profile [5].

The expression of the common leukocyte antigen CD45 was stable after fixation of peripheral blood [4] or cryopreservation of suspension cells obtained from nodal biopsies [36], which has been also shown in our experiments. One study has shown long-term preservation of B-cell antigens when fixed chemically using a mixture of 2% formalin, enzyme inhibitors, glycine and gelatin [31]. This might suggest the usage of a mixture of fixators and other compounds for better antigen preservation. While, our experiments demonstrated dramatic changes in both SI and MFI of CD19 and CD20 antigens triggered by all types of fixation. This was supported by other study, where pre-fixation with PFA caused the reduction in staining intensity of CD19 in whole blood [24].

One of the problems arises with the fixation is a loss of negative populations and increased autofluorescence, which by use of the aldehyde fixators cannot be completely eliminated. Whole blood immunophenotyping is preferable with the use of fresh samples with no fixation of leukocytes, which reflects the true figure of cellular phenotype distribution [37]. Moreover, MFI tended to decrease by 10-30% for major leukocytes antigens after fixation with 1% paraformaldehyde [35]. Similarly, the study conducted on peripheral blood immunostaining demonstrated the worst preservation of MFI when blood samples were pre-fixed with both 1% and 4% PFA [30]. Our results on fixed lymphocytes isolated from fresh lymph node biopsies are in accord with these data.

Conclusion

The fixation of cells with 2% or 4% PFA for 20 min and then washing off the fixative and storing sample in PBS for up to 3 days partially retains cells antigenicity, although might cause the percentages changes while MFI and SI usually decreases. Other aldehyde fixatives give inappropriate results. The fixation with 4% PFA significantly increase the percentage of CD45 stained cells up to 95% or more. It also affects light scatter properties of cells. Fixation makes impossible to analyse large cells, which are important for detection of large cells lymphomas. Usually, the fixation causes slight increase in the number of CD19 positive B-cells, and slight reduction in the percentage of CD3 and CD5 positive T-cells. Some other B-cell antigens (CD10, CD22, CD23, and CD38) are strongly affected by fixation. Storing for more than 3 days results in the formation of the apparent CD19/CD5 double positive population, as well as the loss of CD10 and CD23 antigens, which is very important for lymphoma immunophenotyping. We recommend analyze fixed cells no later than three days after fixation.

Most papers describing chemical fixation of lymphoid cells in suspension were published in the last century, and used staining with only one-two antibodies available at that time. While in this study, we used antibody panels of eight antibodies when compared staining of live and fixed cells. This definitely complies with modern FC capabilities [16].

We approbated different concentrations of PFA (2%-4%) and glyoxal (1%-3%) aiming to retain compatible to live staining results for the most used lymphocytic antigens (CD45, CD19, CD20, CD3, CD5, CD4, CD8, kappa, lambda).

Even the best fixation affects representability of the staining to a lesser extend for antibodies CD45, CD19, CD20, CD3, and CD5, and to a greater extend for antibodies CD10, CD22, CD23, CD38, as well as antibodies against kappa and lambda light chains of immunoglobulins.

We tested the major clones of antibodies approved for lymphoma diagnostics. However, new monoclonal antibodies developed recently might give better results. Lymphomas are very heterogenic group of diseases, which falls into tens of separate clinical entities. Thus, comparison of staining live and post fixation samples might require more precise and individual assessment of each clinical case.

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The frequency of hypernatremia at presenting to the Emergency Department with acute bronchiolitis

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Abstract

Aim: Acute bronchiolitis is a common lower respiratory tract disease in children. In addition to its common clinical findings, it may also cause extrarenal water losses. Deficiency in total body water is a risk for hypernatremia. This study aimed to analyze the frequency of concomitant hypernatremia in children suffering from acute bronchiolitis.

Material and methods: This retrospective study was conducted with 1317 children who presented to the pediatric emergency department with acute bronchiolitis and whose sodium levels were measured from January 1, 2019, to March 1, 2020. The age, gender, and application season of the patients were recorded.

Results: Hypernatremia was detected in 193 (14.7%) patients, and hyponatremia was detected in 99 (7.5%). There was no statistical difference in age or gender between the hyponatremia, hypernatremia, and normonatremia groups ($p>0.05$). The frequency of hypernatremia was the highest in winter and spring ($p<0.05$).

Conclusion: Patients with acute bronchiolitis may be accompanied not only by hyponatremia but also by hypernatremia. To prevent dysnatremia in patients with acute bronchiolitis, each child's intravenous hydration regimen must be ordered to electrolyte levels.

Key words: hypernatremia, acute bronchiolitis, children, pediatric emergency

Introduction

Hypernatremia is defined as serum sodium (Na) concentration higher than 145 meq/ L [1]. It shows net water loss or excess sodium intake in the body. Hypernatremia is most often created by a loss of hypotonic fluids from renal or extrarenal [2]. When the plasma sodium level increases, thirst, and vasopressin release are important defense mechanisms against the development of hypernatremia. If water is available, it is possible to prevent hypernatremia by taking in water. However, in cases of inability to ask for water, such as infants, intubated patients, or patients with neurological deficits, hypernatremia will occur due to insufficient water intake [1-3].

Clinical findings such as fever, vomiting, hyperventilation, and sweating may cause hypernatremia secondary to extrarenal fluid loss [2,4]. Although these symptoms are common in acute bronchiolitis, there is a large literature on hyponatremia accompanying lower respiratory tract infections. It is generally accepted that this is caused by the release of non-osmotic antidiuretic hormone (ADH) in acute bronchiolitis patients [5-8].

However, we suspect that (I) an increase in extrarenal fluid loss in acute bronchiolitis symptoms, (II) immaturity of renal functions about the capacity to excrete excess sodium in infants, and (III) infancy inability to describe thirst, may predispose to hypernatremia in young children with acute bronchiolitis [1,3,4,9]. Since hypernatremia increases the risk of morbidity and mortality even at mild levels, it is important to recognize it in the early period and apply the essential treatment [3,10].

This study aimed to evaluate the frequency of hypernatremia in children under two who were diagnosed with acute bronchiolitis in the pediatric emergency department of a tertiary hospital.

Material and methods

This study was approved by the ethics committee of Ankara Research and Educational Hospital (No: E-20/ 371).

We retrospectively analyzed acute bronchiolitis patients who were admitted to the pediatric emergency department of Ankara Research and Educational Hospital from January 1, 2019, to March 1, 2020. Inclusion criteria were children under 2 years with a diagnosis of acute bronchiolitis and tested for sodium levels by the biochemical method. In addition, patients who applied to the emergency department for any reason in the last week were excluded from the study, due to the risk of receiving intravenous fluid therapy. To exclude patients who had electrolyte measurements after intravenous fluid intake, care was taken to allow a maximum of one hour between the acceptance of the examination and the laboratory acceptance of the examination. Sodium levels were analyzed using the indirect ISE method by the Roche Cobas 6000 analyzer (Roche Diagnostics, Barcelona, Spain).

The plasma sodium level of less than 135 mEq/L was considered hyponatremia. Hyponatremia patients were also divided into 3 subgroups according to their sodium levels mild (130-134 mEq/L), moderate (125-129 mEq/L), and severe (<125 mEq/L) [11]. Hypernatremia was defined as >145 mEq/L. They were also divided into 3 subgroups depending on sodium levels; mild (146-149 mEq/L), moderate (150-169 mEq/L), and severe (>169 mEq/L) [12]. Plasma potassium levels less than 3.5 mEq/L was noted hypokalemia (mild: 3-3.4 mEq/L; moderate: 2.5-2.9 mEq/L, and severe: <2.5 mEq/L) and higher than 5 mEq/L was noted hyperkalemia (mild: 5.1-6 mEq/L, moderate: 6.1-7 mEq/L, and severe : >7 mEq/L) [13].

CRP levels were analyzed by immunonephelometric method (BN 100 system, Dade-Behring, Germany). 5 mg/L was accepted as the reference for the CRP level [14].

Statistical analysis

For categorical variables, numbers and percentages were given as descriptive variables. Continuous variables were presented as mean± standard deviation and median (minimum-maximum). The Chi-square test and Fisher exact test were used to assess categorical variables. The normality of distribution for continuous variables was confirmed with the Kolmogorov-Smirnov test. To evaluate the correlations between measurements, Spearman Rank Correlation Coefficient was used. Multinomial logistic regression analysis was performed to determine significant predictors of serum sodium levels. In univariate analysis, variables significant at the p<0.25 level were entered in logistic regression analysis. A p-value of <0.05 was considered statistically significant and statistical analysis was performed using SPSS version 28.0 software for Windows (IBM Corp; Armonk, NY: 2021). The graphics were drawn in the JASP program (www.jasp-stats.org).

Results

From January 2019 to March 2020, a total of 1317 children with acute bronchiolitis had their serum sodium measured shortly after admission to the pediatric emergency. The distribution of hospital admissions by month is given in Figure 1. There were 525 (39.9%) girls, and 792 (60.1%) boys in the population. The study consisted of 1063 (80.7%) patients under one year old and 254 (19.3%) patients between 1-2 years old. Hyponatremia was detected among 99 (7.5%) and hypernatremia 193 (14.7%) patients (Table 1).

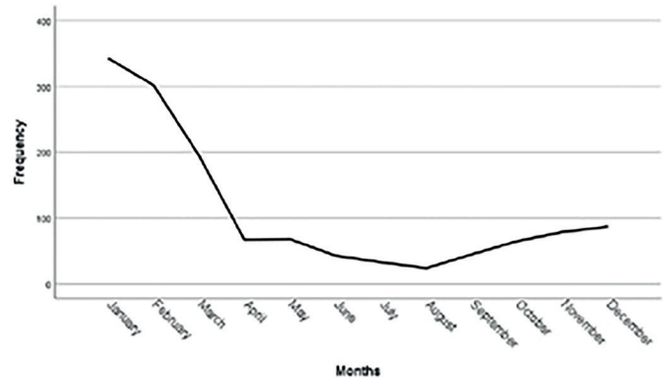

Figure 1 - Distribution of hospital admissions by months

When serum sodium levels were examined, 14.7% of the patients had hypernatremia and 7.5% had hyponatremia. Hyperkalemia was detected in 20.3% of the patients and hypokalemia was found in 8% of the patients. Hyperkalemia was detected in 22.3% (n=43) of the patients with hypernatremia. Hypokalemia was detected in 8.1% (n=8) of patients with hyponatremia. Both serum sodium and potassium levels were found to be normal in 71.4% of all patients. The sodium status of the patients was summarized according to age, gender, and seasonal variables (Table 2).

Table 1 Distribution of the variables

	n	%
Year (n=1351)		
2019	993	73.5%
2020	358	26.5%
Season (n=1351)		
Winter	732	54.2%
Spring	330	24.4%
Summer	100	7.4%
Autumn	189	14.0%
Sex (n=1351)		
Girl	537	39.7%
Boy	814	60.3%
Age (n=1351)		
0-1 years	1089	80.6%
1-2 years	262	19.4%
CRP (n=1324)		
≥5	708	53.5%
<5	616	46.5%
Sodium disorders (n=1317)		
normal	1025	77.8%
hyponatremia mild	96	7.3%
hyponatremia moderate	3	0.2%
hyponatremia severe	-	-
hypernatremia mild	90	6.8%
hypernatremia moderate	95	7.2%
hypernatremia severe	8	0.6%
Potassium disorders (n=1316)		
normal	944	71.7%
hypokalemia mild	97	7.4%
hypokalemia moderate	8	0.6%
hypokalemia severe	-	-
hyperkalemia mild	249	18.9%
hyperkalemia moderate	18	1.4%
hyperkalemia severe	-	-
Laboratory parameters	mean±SD	Median (min-max)
CRP (n=1324)	14.45±25.84	5.8 (0-338.1)
Chloride (n=780)	102.00±3.00	102 (92-114)
Sodium (n=1317)	140.00±7.00	139 (126-186)
Potassium (n=1316)	4.44±0.71	4.5 (2.7-6.4)

Table 2 Comparison of serum sodium levels for age, sex, and seasonal groups.

	normonatremia	hyponatremia			hypernatremia			p1	p2
		mild	moderate	severe	mild	moderate	severe		
Age groups									
0-1 age	827 (80.7%)	72 (75.0%)	2 (66.7%)	NA	76 (84.4%)	79 (83.2%)	7 (87.5%)	0.101	0.579
1-2 age	198 (19.3%)	24 (25.0%)	1 (33.3%)	NA	14 (15.6%)	16 (16.8%)	1 (12.5%)		
Sex									
Female	421 (41.1%)	29 (30.2%)	1 (33.3%)	NA	35 (38.9%)	37 (38.9%)	2 (25.0%)	0.169	0.390
Male	604 (58.9%)	67 (69.8%)	2 (66.7%)	NA	55 (61.1%)	58 (61.1%)	6 (75.0%)		
Season									
Winter	535 (52.2%)	55 (57.3%)	1 (33.3%)	NA	55 (61.1%)	68 (71.6%)	3 (37.5%)		
Spring	247 (24.1%)	26 (27.1%)	2 (66.7%)	NA	26 (28.9%)	20 (21.1%)	2 (25.0%)	0.001*	0.002*
Summer	85 (8.3%)	3 (3.1%)	NA	NA	2 (2.2%)	2 (2.1%)	2 (25.0%)		
Autumn	158 (15.4%)	12 (12.5%)	NA	NA	7 (7.8%)	5 (5.3%)	1 (12.5%)		

NA: Not available, p_1: comparison of sodium levels, p_2: Comparison of the severity of sodium disorders.*:p<0.05

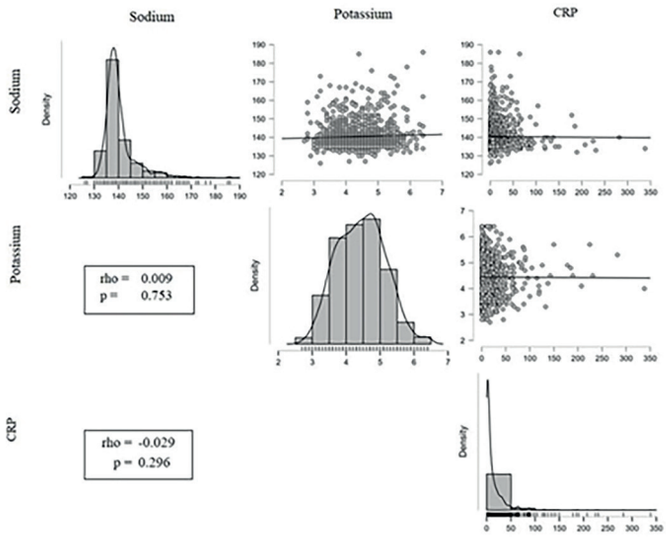

Figure 2 - Correlation between sodium and potassium

Although sodium disorders are seen more intensely in the 0-1 years group, the severity of sodium disorders does not show a statistically significant difference according to age groups (p=0.579). Although sodium disorders are more common in boys, the severity of sodium disorders does not differ significantly by gender (p=0.390). When sodium disorders are compared according to the seasons, sodium disorders are observed more frequently in winter and spring, and there is a significant relationship between the season and the severity of sodium disorders (p=0.002). While sodium disorders are more common in winter and spring, they are less common in summer and autumn.

We investigated the association between serum sodium levels and potassium, and CRP levels in children in a hospital in Ankara, Turkey. There was no significant correlation between serum sodium level and potassium and CRP levels (r=0.009; p=0.753 and r = -0.029; p=0.296 respectively) (Figure 2).

To determine the risk factors, multinomial logistic regression analysis was performed with age, gender, and seasonal variables, which were found to be significant as a result of univariate analysis. When the results of the multinomial logistic regression analysis are examined, it is seen that only the season

Table 3 Comparison of serum sodium levels for age, sex, and seasonal groups

			OR (%95 Cl)	p
hypernatremia	Age groups	0-1 years	1.28 (0.84-1.94)	0.250
		1-2 years	Ref	
	Sex	Girl	0.87 (0.63-1.19)	0.378
		Boy	Ref	
	Season	Autumn	1.20 (0.44-3.27)	0.723
		Winter	3.44 (1.47-8.06)	0.004*
		Spring	2.80 (1.16-6.78)	0.022*
		Summer	Ref	
hyponatremia	Age groups	0-1 years	0.72 (0.44-1.16)	0.176
		1-2 years	Ref	
	Sex	Female	0.61 (0.39-0.95)	0.030*
		Male	Ref	
	Season	Autumn	2.24 (0.61-8.17)	0.223
		Winter	3.1 (0.94-10.14)	0.062
		Spring	3.32 (0.98-11.22)	0.053
		Summer	Ref	

*: p<0.05, ref: reference category

is a significant factor in the detection of hypernatremia, while only the gender is significant in the detection of hyponatremia. Patients were 2.8 times more likely to have hypernatremia in the spring than in the summer (95% CI: 1.16-6.78, p=0.022). Patients were 3.4 times more likely to have hypernatremia in winter than in summer (95% CI: 1.47-8.06, p=0.004). The probability of hyponatremia in women is lower than in men (OR=0.61, 95% CI=0.39-0.95) (Table 3).

Discussion

In the current study, a total of 1317 infants with acute bronchiolitis, aged below two, were subjected to analysis. Among the study population, 193 (14.7%) had hypernatremia, and 99 (7.5%) had hyponatremia. While the majority of the hypernatremic children were moderate (n: 95, 49.2%), the majority of the hyponatremic children were mild (n: 96, 96.9%). Although not statistically significant, both hypernatremia and hyponatremia were detected more frequently in patients younger than one-year-old.

The frequency of hypernatremia was almost twice that of hyponatremia. Contrary to our results, many studies have reported that hyponatremia is the most common electrolyte disorder in lower respiratory tract infections in the literature. To our knowledge, this is the first time that a high frequency of hypernatremia has been reported in patients with acute bronchiolitis. Considering that even mild and moderate hypernatremia increases mortality and morbidity [3,10,15,16], we believe that the frequency of hypernatremia detected in this study is critical in child health.

The literature reports that hypernatremia is associated with undesirable outcomes, such as neurological changes (from lethargy and weakness to coma), decreased cardiac function, and impaired hepatic lactate clearance [3,17]. In this study, we did not aim to analyze the physiopathology of hypernatremia in acute bronchiolitis. However, we suggest that it may be related to malnutrition and insufficient drinking accompanying extrarenal fluid loss.

Fever, sweating, vomiting, increased respiratory secretions, and hyperventilation may cause hypotonic fluid loss in acute bronchiolitis patients [16]. This stimulates thirst. In this condition, drinking enough water will prevent hypernatremia [4,16]. If the children too young to express their thirst are not perceived by their parents and sufficient water intake is not provided, this may cause hypernatremia [18]. In our analysis, 83.9% of patients with hypernatremia were under the age of one. We believe that this difference is because children under one year are incapable of expressing their thirst.

In this study, the frequency of hypernatremia was affected by seasons. It was most often detected in winter and spring. In studies, conducted with the general patient population in the adult age group, hypernatremia was detected most frequently in the winter season, and it was reported that this might be due to the lack of attention to hydration during the winter months [10,19]. In addition, we assume that parents dressing their children in thick clothes to protect them from the cold might increase sweating and lead to hypernatremia.

This study has several limitations. First, although we demonstrated the frequency of hypernatremia due to its retrospective design, we did not have sufficient data to explain the causality. Second, sodium levels were not corrected according to serum glucose levels. Third, although patients who applied to a different hospital during the same disease period were excluded from the study due to possible intravenous fluid intake, we cannot be sure that these patients were completely excluded because the study was retrospective.

Conclusion

In conclusion, hypernatremia may accompany children with acute bronchiolitis. Therefore, patients with acute bronchiolitis should be investigated for electrolyte imbalances to personalize their fluid therapy. Which factors contributed to the risk of hypernatremia in acute bronchiolitis should be evaluated in further studies.

What is already known? Acute bronchiolitis infections in children may often be accompanied by hyponatremia.
What does this study add? Children with acute bronchiolitis are at risk not only for hyponatremia but also for hypernatremia.

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Conventional videolaryngoscope versus 3D printed videolaryngoscope

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Abstract

Background and objectives: In patients with Covid-19, using a video laryngoscope as an alternative to direct laryngoscopy is recommended to protect the intubator from infection and reduce intubation failures due to personal protective equipment. The cost of video laryngoscopes limits their availability in all healthcare institutions. The present study aimed to compare the efficacy and safety of 3D printed video laryngoscope and conventional video laryngoscope on intubation.

Material and methods: 30 ASA I-II patients who were not considered to have a difficult airway were included in the study after obtaining the ethics committee approval from Adnan Menderes University Clinical Research Ethics Committee. Patients were randomly divided into two groups, group 1 and group 2. After the induction of anesthesia under standard monitoring, the Cormack Lehane score was recorded by direct laryngoscopy in all patients. Patients in group 1 were intubated with a 3D-printed video laryngoscope. In contrast, patients in group 2 were intubated with a conventional video laryngoscope (STORZ C-mac videolaryngoscope). Intubation time, number of attempts, and hemodynamic values of patients with early postoperative complications were recorded. The data were recorded and statistically evaluated.

Results: There were no significant differences between the groups regarding demographic data, BMI, and hemodynamic data. The Cormack Lehane score was calculated as 1.6±0.51 in group 1 and 1.4±0.51 in group 2 (p=0.38). Intubation times of the groups were 32.6±18 s and 27.06±11.37 s, respectively (p=0.4). The number of intubation attempts was 1.2±0.63 in group 1 and 1±0.01 in group 2 (p=0.31). The image quality of the camera by the intubator, intubation conditions, and intubation satisfaction was similar in the two groups.

Conclusion: Comparing a 3D-printed videolaryngoscope with a conventional videolaryngoscope, no differences were observed in intubation times, number of intubation attempts, hemodynamic changes, and early postoperative complications. Intubation satisfaction values by the practitioner were found to be similar. It was concluded that the 3D-printed videolaryngoscope, which is cost-effective and easy to access, can be used instead of conventional videolaryngoscope in patients with a normal airway.

Key words: videolaryngoscope

Introduction

Endotracheal intubation is an aerosol-generating medical procedure that carries a high risk of pathogen transmission to the practitioner [1]. Anesthesiologists and intensivists are particularly at risk for infection transmissions [2]. Approximately 10% of healthcare workers who perform endotracheal intubation on patients with suspected or confirmed Covid-19 are reported to be infected with Covid-19 [3]. In patients infected with Covid-19, the use of video laryngoscopes instead of direct laryngoscopy is recommended to protect the intubator from infection and to reduce intubation failure due to personal protective equipment worn to prevent exposure to infection [4]. However, the cost of video laryngoscopes limits their availability in all health institutions. In clinics where the number of video laryngoscopes is inadequate, waiting for equipment for many simultaneous operations prevents effective use of the operating room. Also, this leads to disruption in the cleaning and disinfection steps of the video laryngoscope. Therefore, it was thought that 3D printed video laryngoscope, which is more readily available to clinics and has a lower cost, can be used. The

present study aimed to compare the efficacy and safety of 3D printed video laryngoscope and conventional video laryngoscope on intubation.

Material and methods

The present study was carried out prospectively with the approval of the Adnan Menderes University ethics committee (197/2020). A 3D printed videolaryngoscope was created by designing the handle from polylactic acid raw material, placing a wi-fi 1080P HD camera, and passing cytotoxicity, sensitization, irritation tests, and obtaining approval (Kırıkkale University Scientific and Technological Research Application and Research Center report number: 2022/000022-IVV-IRT-207). A total of 30 patients aged 18-65 years, ASA I-II, and undergoing elective surgery under general anesthesia were included in the study. Patients with difficult intubation (mallampathy III-IV, head-neck mobility limitation, kyphoscoliosis, undergoing or planned oral/neck surgery, prognathism and incisor spacing less than 2 cm), delayed gastric emptying (gastrointestinal malignancy, ileus), and gastroesophageal reflux patients and pregnant women were excluded. Intubations performed in three and/or more attempts were considered unsuccessful and were excluded from the study. Patients were informed preoperatively, and verbal and written informed consent was obtained. Demographic information (age, sex, height, weight), mallampathy scores, and the reason for anticipating difficult intubation, if any, were recorded. All patients included in the study were randomized, and the patients were divided into two groups. Group 1 was the 3D-printed video laryngoscope group, and Group 2 was the conventional video laryngoscope intubation. The patients were then taken to the operating room for the surgical procedure. Standard monitoring (heart rate, oxygen saturation, and non-invasive blood pressure) was performed. Anesthesia induction of all patients included in the study was achieved with intravenous (iv) lidocaine 1 mg/kg, propofol 2-3 mg/kg, and iv fentanyl 1µcg/kg. After ventilation with an appropriate face mask, iv-rocuronium 0.6 mg/kg was administered for neuromuscular blockade. After adequate mask ventilation, the glottic appearance of the patients in both groups was evaluated by direct laryngoscopy, and Cormack-Lehane (C&L) scores were recorded. The patients in group 1 were then intubated with a 3D-printed video laryngoscope. The patients in group 2 were intubated with pre-styled intubation tubes using a conventional video laryngoscope (STORZ C-MAC® videolaryngoscope). Intubation of patients in both groups was performed by anesthesia assistants with at least four (4) years of intubation experience and similar video laryngoscope experience. Correct placement of the endotracheal tube was confirmed by chest elevation and capnography. Patients were mechanically ventilated. The duration of intubation (from when the laryngoscope entered the oral cavity until the appearance of the end-tidal carbon dioxide wave), the number of intubation attempts, and complications (laceration, tooth damage, bleeding) were recorded. The camera's image quality, intubation conditions, and intubation satisfaction were evaluated by the person performing endotracheal intubation with a minimum score of 1 and a maximum score of 4. The recorded parameters are as follows; heart rate (HR), systolic arterial pressure (SAP), diastolic arterial pressure (DAP), mean arterial pressure (MAP), peripheral oxygen saturation (SpO_2), before induction (T0), immediately after induction (T1), immediately after intubation (T2) and at 1-minute intervals for 5 minutes (T3, T4, T5, T6, T7), while end-tidal carbon dioxide (ETCO2) immediately after intubation and at 1, 2, 3, 4, and 5 minutes after intubation. Patients were also examined at the sixth hour to evaluate for postoperative complications. The data were recorded and statistically analyzed.

Statistical analysis

The SPSS 25 for data evaluation (IBM Corp. Release 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.) statistical package program was utilized for the statistical evaluations. The variables are expressed using mean±standard deviation, percentile, and frequency values. The Kolmogorov-Smirnov test was performed to evaluate the homogeneity of the data. The Mann-Whitney U and chi-square test were used to analyze the data. The level of significance was set as $P<0.05$.

Results

The data of a total of 30 patients in group 1 and group 2 were analyzed. All 15 patients in group 1 were female; the mean age was 37.52±10.52 years. In group 2, 12 patients were female; the mean age was 34.6±13.83 years. The patients' body mass index was calculated as 23.97±5.07 kg/m2 in group 1 and 23.89±4.13 kg/m2 in group 2. No significant differences were found between the groups in terms of age and body mass index ($p>0.05$). There were no patients with a prediction of difficult intubation in both group. Intubation time was calculated as 32.6±18 s and 27.06±11.37 s in group 1 and group 2, respectively ($p=0.4$). The number of attempts was calculated as 1.2±0.63 in group 1 and 1±0.01 in group 2 ($p=0.31$). The Cormack-Lehane score was calculated as 1.6±0.51 and 1.4±0.51 in groups 1 and 2, respectively ($p=0.38$) (Table 1).

Table 1 Cormack- Lehane score and intubation result

	Group 1	Group 2	P
Cormack-Lehane Score	1.6±0,51	1.4±0.51	0.38
Intubation Time (seconds)	32.6±18	27.06±11.37	0.4
Number of Intubation Trials	1.2±0.63	1±0.01	0.31

Data are presented as mean±standard deviation

The patients' pulse rates before the anesthesia induction (T0) were 80.2±10.46 in group 1 and 85.73±17.78 in group 2. There were no significant differences between the groups according to pulse rate values before the induction of anesthesia ($p=0.72$). Instantaneous pulse rate values after anesthesia (T1) induction were 82.26±15.48 in group 1 and 91.4±18.41 in group 2 ($p=0.96$). After intubation (T2), the instantaneous pulse rate was 90.4±11.45 in group 1 and 94.2±18.87 in group 2. The groups had no significant differences in the instantaneous pulse rate values after intubation ($p=0.9$). Pulse rates measured at 1-minute-intervals for 5 minute after intubation (T3, T4, T5, T6, T7) were 91.13±13.10, 92.26±17.33, 92.06±17.70, 88.13±16.20, 86.26±17.04 in group 1 and 92.93±14.61, 95.13±14.09, 92.93±13.07, 92.66±9.04, 93.06±11.87 in group 2, respectively. Both groups had similar pulse rates at the 1st, 2nd, 3rd, 4th, and 5th minute after the intubation ($p>0.05$) (Table 2). The systolic blood pressure before the induction of anesthesia (T0) was 124.33±12.49 mmHg in group 1 and 125.53±22.41 mmHg in group 2 ($p=0.96$).

Table 2 Heart rate data

	Group 1	Group 2	P
T_0	80.2±10.46	85.73±17.78	0.72
T_1	82.26±15.48	91.4±18.41	0.96
T_2	90.4±11.45	94.2±18.87	0.9
T_3	91.13±13.10	92.93±14.61	0.9
T_4	92.26±17.33	95.13±14.09	0.77
T_5	92.06±17.70	92.93±13.07	0.8
T_6	88.13±16.20	92.66±9.04	0.44
T_7	86.26±17.04	93.06±11.87	0.11

Data are presented as mean±standard deviation

After the induction of anesthesia (T1), systolic blood pressure was 120.86±22.98 and 122.73±21.95 mmHg in groups 1 and 2, respectively (p=0.85). Systolic blood pressure measured immediately after intubation (T2) was 125.13±25.51 mmHg in group 1 and 117.06±13.95 mmHg in group 2. The systolic blood pressures measured at 1-minute-intervals for 5 minutes after intubation (T3, T4, T5, T6, T7) were 110.93±21.55, 104.8±23. 77, 111.93±28.83, 106.46±24.74, 103.2±21.19 mmHg in group 1 and 113.06±20.85, 113.28±42.03, 108.5±31.88, 108.71±18.46 and 103.78±14.59 mmHg in group 2, respectively. The groups had no significant differences according to the systolic blood pressures at the 1st, 2nd, 3rd, 4th, and 5th minute after the intubation (p>0.05) (Table 3).

Table 3 Systolic blood pressure result

	Group 1	Group 2	P
T_0	124.33±12.49	125.53±22.41	0.96
T_1	120.86±22.98	122.73±21.95	0.85
T_2	125.13±25.51	117.06±13,95	0.34
T_3	110.93±21.55	113.06±20.85	0.78
T_4	104.8±23.77	113.28±42.03	0.89
T_5	111,93±28.83	108.5±31.88	0.23
T_6	106.46±24.74	108.71±18.46	0.48
T_7	103.2±21.19	103.78±14.59	0.63

Data are presented as mean±standard deviation.

The diastolic blood pressure of the patients in group 1 before the induction of anesthesia (T0) was 72.73±8.81 mmHg and 70.4±14.88 mmHg in group 2 (p=0.95). After the induction of anesthesia (T1), diastolic blood pressure was 75.2±16.54 and 73.4±14.06 mmHg in groups 1 and 2, respectively (p=0.81). Diastolic blood pressure measured immediately after the intubation (T2) was 77.26±16.64 mm Hg in group 1 and 70.53±12.47 mm Hg in group 2 (p=0.23). Diastolic blood pressures at 1-minute-intervals for 5 minutes after the intubation (T3, T4, T5, T6, T7) were 69.46±13.75, 66.0±16.15, 67. 73±13.18, 64.86±16.79 and 62.93±17.19 mmHg in group 1 and 66.13±10.27, 66.35±19.21, 62.64±13.06, 64.78±11.11 and 60.64±7.67 mmHg in group 2, respectively. No significant differences were found in both groups' diastolic blood pressure values at the 1st, 2nd, 3rd, 4th, and 5th minutes after intubation (p>0.05) (Table 4). The mean arterial blood pressure before the induction of anesthesia (T0) was 93.53±10.23 mmHg in group 1 and 88.66±19.54 mmHg in group 2 (p=0.88). Mean arterial blood pressure after the induction of anesthesia (T1) was 91.46±15.17 and 92.8±16.33 mmHg in groups 1 and 2, respectively (p=0.74). Mean arterial blood pressure measured immediately after the intubation (T2) was 96.53±19.23 mmHg in group 1 and 89.13±14.66 mmHg in group 2 (p=0.31). Mean arterial pressures were calculated as 85.80±16.69, 81.26±18.39, 86.93±21.66, 81.40±18.65 and 79.53±16.80 mmHg in group 1 and 86.66±15.73, 85.42±24.16, 80.78±17.52, 83.71±11.64 and 79±9.58 mmHg in group 2 at 1-minute-intervals for 5 minutes (T3, T4, T5, T6, T7) after the intubation, respectively.

Table 4 Diastolic blood pressure result

	Group 1	Group 2	P
T_0	72.73±8.81	70.4±14.88	0.95
T_1	75.2±16.54	73.4±14.06	0.81
T_2	77.26±16.64	70.53±12.47	0.23
T_3	69.46±13.75	66.13±10.27	0.67
T_4	66.0±16.15	66.35±19.21	0.77
T_5	67.73±13.18	62.64±13.06	0.35
T_6	64.86±16.79	64.78±11.11	0.6
T_7	62.93±17.19	60.64±7.67	1

Data are presented as mean±standard deviation.

No significant differences were found between the groups according to the mean arterial blood pressure values at the 1st, 2nd, 3rd, 4th, and 5th minutes after the intubation (p>0.05). Saturation values before the induction of anesthesia (T0), after induction (T1), and after intubation (T2) were 97.73±1.86, 98.6±1.5 and 98.4±1.68 in group 1 and 98.2±1.93, 98.53±1.54 and 98.26±1.38 in group 2, respectively. The saturation values measured at 1-minute-intervals for 5 minutes (T3, T4, T5, T6, T7) after the intubation were 97.73±1.83, 97.73±1.38, 98.06±1.22, 97.86±1.45, 97.86±1.5 in group 1; 98.13±1.35, 97.71±1.48, 97.64±1.54, 97.92±1.14 and 98±1.17 in group 2, respectively. No significant differences were found between the groups according to the saturation values at the 1st, 2nd, 3rd, 4th, and 5th minutes after the intubation (p>0.05) (Table 5). The end-tidal carbon dioxide values immediately after the intubation were 36.86±3.7 in group 1 and 35.35±3.17 in group 2 (p=0.11). Endtidal carbon dioxide (ETCO2) values at the 1st, 2nd, 3rd, 4th, and 5th minutes after intubation were 34.86±2.66, 33.86±3.71, 33.33±3.45, 33±3.31 and 33±2.8 in group 1 and 34.5±4.38, 33.5±4.1, 32.85±4.48, 32.07±4.28 and 31.85±4.48 in group 2, respectively. No significant differences were found between the groups according to ETCO2 values at the 1st, 2nd, 3rd, 4th, and 5th minutes after intubation (p>0.05). In the evaluation of intubation conditions in group 1, 1 point was

Table 5 Peripheral oxygen saturation result

	Group 1	Group 2	P
T_0	97.73±1.86	98.2±1.93	0.42
T_1	98.6±1.5	98.53±1.54	0.66
T_2	98.4±1.68	98.26±1.38	0.61
T_3	97.73±1.83	98.13±1.35	0.7
T_4	97.73±1.38	97.71±1.48	0.98
T_5	98.06±1.22	97.64±1.54	0.54
T_6	97.86±1.45	97.92±1.14	0.84
T_7	97.86±1.5	98±1.17	0.77

Data are presented as mean±standard deviation.

given in 1 (6.7%) case, 2 points were given in 2 cases (13.3%), 3 points were given in 7 (46.7%) cases, and 4 points were given in 4 (33.3%) cases for the evaluation of the application. In the evaluation of intubation conditions in group 2, 2 points were given in 3 (20%) cases, and 3 points were given in 12(80%) cases. In evaluating intubation conditions in groups, 2, 1, and 4 points were not given. No significant differences between the groups were found in the evaluation of intubation conditions (p=0.46). In the evaluation of intubation image quality in group 1, 1 point was given in 1 (6.7%) case, 2 points were given in 1 case (6.7%), 3 points were given in 7 (46.7%) cases, and 4 points were given in 6 (33.3%) cases. In the evaluation of intubation image quality in group 2, 1 point was given in 1 case (6.7%), 3 points were given in 13 (86.7%) cases, and 4 points were given in 1 (6.7%) case. In group 2, there were no applications in which 1 point was given to intubation image quality. The intubation image quality of both groups was similar (p=0.09). In the evaluation of intubation satisfaction lower than group 1, 1 point was given in 1 (6.7%) case, 2 points were given in 1 case (6.7%), 3 points were given in 9 (60%) cases, and 4 points are given in 4 (26.7%) cases. In the evaluation of intubation satisfaction in group 2, 1 point was given in 1 case (6.7%), 3 points were given in 13 (86.7%) cases, and 4 points were given in 1 (6.7%) case. In group 2, there were no applications in which intubation satisfaction was given 1 point. No significant differences were found in the groups' evaluation regarding intubation satisfaction (p=0.31). Early postoperative complications were not observed in both groups.

Discussion

Anesthesiologists who take an active role in aerosol-generating procedures such as endotracheal intubation are six times more at risk of coronavirus infection than other healthcare workers [5]. In patients infected with Covid-19, using a video laryngoscope as an alternative to direct laryngoscopy is recommended to protect the intubator from infection and reduce intubation failure due to personal protective equipment worn to prevent exposure to infection [4]. Also, aerosol cans used to reduce the risk of Covid-19 transmission during intubation may make it difficult to maneuver the laryngoscope. The present study evaluated and compared conventional video laryngoscope and 3D printed video laryngoscope. No differences were observed in intubation time, the number of intubation attempts, hemodynamic response, early postoperative complications, and satisfaction of the intubation practitioner.

In a randomized controlled trial comparing intubation with C MAC video laryngoscope and direct laryngoscopy using an aerosol can, it has been reported that video laryngoscope facilitated endotracheal intubation by providing direct visualization of the glottis [6]. Despite the advantages of the conventional video laryngoscope, its high cost makes it difficult to obtain in healthcare institutions. To the best of our knowledge, no studies evaluate the conventional video laryngoscope and the video laryngoscope developed with a 3D printer.

During endotracheal intubation, applying force to the tongue root with the laryngoscope causes sympathetic system activation and leads to hemodynamic changes. The hemodynamic response to intubation is well tolerated in healthy individuals. However, it may cause serious side effects in patients with limited coronary reserve [7]. Furthermore, a prolonged duration of laryngoscopy may exacerbate the intubation-induced hemodynamic response [8]. In that context, there are studies in the literature to evaluate the hemodynamic effects of intubation with different laryngoscope types. The present study included patients who underwent general anesthesia, aged 18-65 years, ASA1-2, and who were not considered to have a difficult airway, and hemodynamic response to intubation was evaluated employing four different laryngoscopes (Macintosh direct laryngoscope-classic laryngoscope, McCoy laryngoscope, McGrath video laryngoscope, and C-MAC video laryngoscope). Accordingly, heart rate, saturation, and systolic blood pressure were measured in the operating room at baseline (T0), immediately after induction (T1), immediately after intubation (T2), and for 5 minutes at 1-minute-intervals (T3, T4, T5, T6, T7). The number of intubation attempts did not differ between the groups. However, using the McGrath video laryngoscope has been reported to be advantageous in preventing cardiovascular stress response with fewer hemodynamic changes and shorter intubation time (7). In a study evaluating the hemodynamic response to intubation using a GlideScope video laryngoscope and a Macintosh direct laryngoscope, it has been reported that hemodynamic parameters were better preserved in the first three minutes after intubation when using the GlideScope video laryngoscope [9]. The present study showed no differences in intubation times between 3D printer video laryngoscope and conventional video laryngoscope. There was no difference in hemodynamic measurements (SAB, DAB, OAB, SpO2, ETCO2) during the pre-induction period, immediately after induction, immediately after intubation, and after 5 minutes with 1-minute intervals after intubation. This suggests that the 3D-printed video laryngoscope developed at a lower cost in the context of the present study has a similar hemodynamic effect to the conventional video laryngoscope.

Cormack-Lehane (C&L) scoring is used to grade the glottic appearance during direct laryngoscopy. So far, there is no definitive grading system for video laryngoscopes [10]. The present study recorded the C&L score by direct laryngoscopy employing a Macintosh laryngoscope in all patients before endotracheal intubation. The C&L score was significantly higher in the 3D-printed video laryngoscope group. However, the number of intubation attempts and duration were similar to the conventional video laryngoscope, with no significant differences. A 3D-printed video laryngoscope was thought to be as effective as a conventional video laryngoscope in providing a glottis view.

Following intubation, some complications including hoarseness, sore throat, and cough, can be seen in the early

postoperative period. Particularly, sore throat after endotracheal intubation is very common. Sex (female), young age, intubation with no neuromuscular blockades, pulmonary diseases, double lumen tube use, and high-cuff pressure may increase the incidence of sore throat [11]. The present study did not detect early postoperative complications using two video laryngoscopes. This was because the patients in both groups did not differ in age, sex, or comorbid diseases, using neuromuscular agents before intubation using a single lumen tube. Experienced anesthesiologists performed all intubations.

The present study evaluated the image quality and user satisfaction of the two video laryngoscopes by the person performing the intubation. The results in the present study showed no differences between the groups. Therefore, it was thought that the technical features of the 3D-printed video laryngoscope, including the ability to maneuver the laryngoscope and image quality, are similar to the conventional video laryngoscope.

Limitations

The present study had some limitations. The first was that the study included the ASA1-2 patient group in whom a difficult airway was not considered. This does not reflect the group of patients with severe comorbid diseases in whom a difficult airway was considered. Secondly, cuff pressure after intubation was not recorded, which would provide a better idea in evaluating early postoperative complications.

Conclusion

The present study compared a 3D-printed video laryngoscope with a conventional video laryngoscope. No differences were observed in intubation time, the number of intubation attempts, hemodynamic changes, and early postoperative complications related to intubation. Intubation satisfaction by the practitioner was found to be similar. It was thought that the 3D-printed video laryngoscope, which is cost-effective and has easy access, could be used as an alternative to conventional video laryngoscope in patients with normal airways. Nevertheless, prospective and multicase studies are needed for more definitive results.

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Case report of syphilitic hepatitis

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Abstract

Syphilitic hepatitis is a rare clinical presentation of syphilis and is easily misdiagnosed. However, clinical and histopathologic manifestations of Syphilitic Hepatitis can imitate other infectious and non-infectious diseases, and the diagnosis should be considered in patients with abnormal liver function tests. We present an unusual case of syphilitic hepatitis presenting with jaundice and abdominal bloating after treatment with benzylpenicillin, liver enzymes, and mucocutaneous findings improved.

Key words: syphilis, hepatitis, jaundice, liver enzyme

Introduction

Syphilis is a multisystem disease caused by the bacterium Treponema pallidum and can involve any organ of the body [1]. Syphilis infection is the least recognized etiological factor in liver dysfunction, and the prevalence of syphilitic hepatitis is about 0.2% to 3% of patients with syphilis [1-3]. The most common stage that causes abnormal liver enzymes is secondary Syphilis, and it is estimated that 3% of secondary syphilis cases can present as syphilitic hepatitis [4]. The prevalence is higher than reported since this pathology is often not detected. The more interesting is the appearance of patients with severe syphilitic hepatitis, which sheds light on the presence in modern practice of this variant of visceral Syphilis [3].

Case presentation

A 41-year-old man came to our clinic with an interesting medical history—the debut of the disease at the end of September 2021 with jaundice and abdominal bloating. There was no fever, abdominal pain, vomiting, stool disorders, fatigue, articular manifestations and skin rashes.

He started examination in a local hospital: in the biochemical analysis, increased values of serum total bilirubin (TBIL) 98.4 umol/L, direct bilirubin 33.2 umol/L, alanine aminotransferase (ALT) 175 U/L, aspartate aminotransferase (AST) 97 U/L, an alkaline phosphatase (ALP) 511 U/L and gamma-glutamyl transpeptidase (GGT) 104 U/L. Screening for viral hepatitis markers: antiHCV, HBsAg, antiHAV was negative. HIV and rapid plasma reagin test (RPR) were negative. Casual sexual relations are excluded; a regular partner is a spouse. There is no history of drug abuse, alcohol consumption and blood transfusion. An ultrasound of the abdomen and MRCP revealed hepatomegaly and mildly splenomegaly and were negative for biliary or pancreatic ductal dilation, lesions and ascites.

There were positive autoimmune markers: AMA-M2 +, M2-3E +, gp210 +, LKM-1+, LC-1 +, level of IgG and gamma-fraction were normal 14.26 g/L and 18.6%, respectively. He started therapy with UDCA (10mg/kg) in September and had asked to review after 1 month with the plan of performing a liver biopsy.

In October, he repeated testing for Treponema pallidum, and had positive results: increase of total Treponema pallidum IgG/IgM antibodies performed by Siemens ADVIA Centaur Syphilis treponemal assay - 20.6 COI, and FTA-ABS was also positive. Examination of the patient revealed multiple erythematous papules and plaques on the soft palate, on the trunk, upper and lower extremities, papules of the palm, soles and genitals, wide condylomas of the perianal region, bilateral inguinal scleradenitis. 21.10.21 hospitalized to dermatology department, started treatment with benzylpenicillin sodium salt 4 million units intramuscular per day for 20 days. During the treatment, patient became asymptomatic with a resolution of jaundice and mucocutaneous findings. In control biochemical results, there was normalization of TBIL from 60 to 10.8 umol/L, ALT from 110 to 50 U/L and AST from 80 to 35.1 U/L.

Figure 1 - Hyperpigmentation after erythematous scaly macules and papules on forearms and palms.

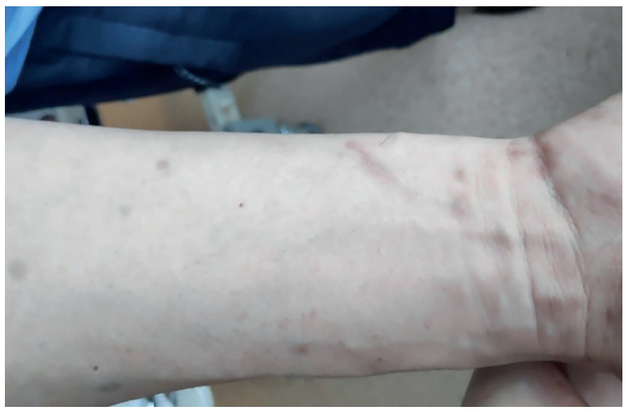
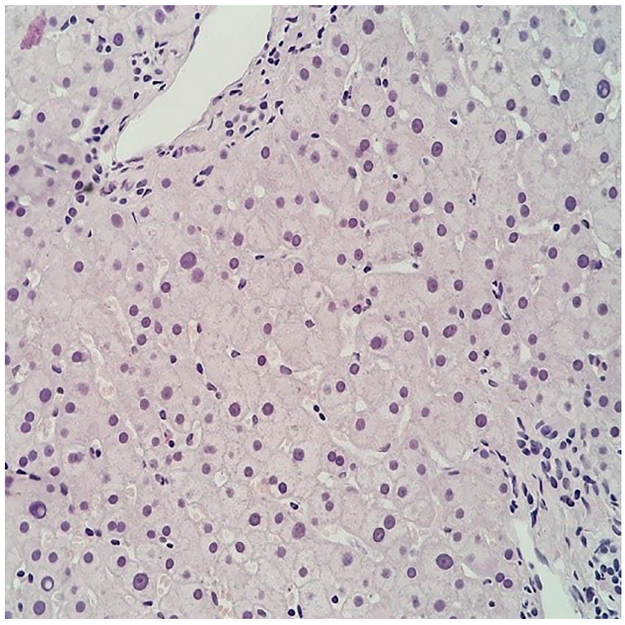


Figure 2 - Histopathological findings - mild lymphocytic infiltration of portal tracts (haematoxylin-eosin x40/0.55).



In January, he was hospitalized in our hepatology department; during examination on both forearms and palms, areas/spots of secondary hyperpigmentation (Figure 1). In addition, a control check of liver autoimmune markers also showed that previously positive markers had become negative, except weak positive anti-LKM-1.

A liver biopsy was performed after the normalization of biochemistry. It showed mild lymphocytic infiltration of portal tracts and early fibrosis around the portal tracts, the Knodell histology activity index 6/18, stage of fibrosis – 1 by K. Ishak (Figure 2).

Discussion

In a systematic review of SH, also liver damage occurred commonly in early Syphilis, with the most common symptoms: being rashes, fatigue or poor appetite, hepatomegaly and icterus [2]. In addition, the pattern of liver enzyme abnormalities is often cholestatic, with altered alkaline phosphatase (ALP) levels and mildly elevated transaminases and bilirubin [5]. The pathophysiology of SH is unknown, but the cholestatic pattern of injury is thought to have been caused by pericholangiolar inflammation [6,7].

There are no clear diagnostic criteria exist; however, Mullick et al. established 4 main criteria, such as the abnormal liver enzymes in a cholestatic pattern, serological evidence of Syphilis, exclusion of alternative causes of liver diseases, and improvement in liver enzymes with proper antibiotic therapy [6].

A previous review of performed liver biopsies showed non-specific histopathologic features, with the prevalence of the inflammatory cell infiltration of portal areas or hepatic lobules (87.2%), cholestasis and hepatocellular necrosis, in 49.0% 45.5% cases, respectively, and less common were granuloma with multinucleated giant cells and fibrosis, 20% and 18.2%, respectively [2]. In addition, immunohistochemical staining or silver stain can identify T. palladium [8].

Miyakawa et al. demonstrated that false-positive reactions to the PBC-specific anti-M2 AMA reactions could occur with hepatitis A, Syphilis, and rheumatoid arthritis [9]. In addition, Kern et al. showed a return to a negative anti-M2 AMA titer after recovery from Syphilis that was previously not reported [10].

In our case, we have typical cholestatic hepatitis with severe hyperbilirubinemia, positive serological tests for Syphilis, the presence of autoimmune antibodies, in particular, highly specific anti-M2 AMA, complete resolution of clinical manifestations and laboratory tests, and the disappearance of autoantibodies against the background of antimicrobial therapy. Skin rashes with positive antibodies to Syphilis appeared later than the liver reaction, and Baveja et al. described a similar case [7].

Conclusion

In conclusion, Syphilis remains a highly prevalent pathology, and any hospital admission requires testing. However, at the outpatient stage, there is no such alertness, and clinicians often overlook syphilis screening, in particular in the differential search algorithm for jaundice and cholestasis syndrome. One of the reasons for the omission in primary appointments is the mental aspect, which does not allow patients to reveal their sexual life, compared with Western countries thoroughly.

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Intracardiac foreign bodies: Diagnosis and management

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Abstract

Introduction: As a result of gunshot wounds due to a bullet or shrapnel entering the system of arteries or veins, direct wounds of the heart or vascular structures, as well as embolisms may occur. After entering one of the systemic veins, the bullet or shrapnel can enter the right heart or the pulmonary artery. Shrapnel embolism is a rare condition, usually asymptomatic and is detected accidentally during routine examinations. Due to the fact that the intensity of occurrence of this type of cases is quite rare, it was decided to present such clinical cases.

Material and methods: This article reports about 3 patients with intracardiac foreign body. All 3 patients took part in the battles during the Second Karabakh War and were hospitalized as a result of shrapnel wounds received in different parts of the body. In each of the patients, the lesion area was different, but as a result of venous migration, the movement of a foreign body (metal fragment) in the heart cavity was noted. In two patients, the foreign body was removed by open surgery (connection to a heart-lung machine) due to the presence of symptoms, but one patient was under observation due to the absence of symptoms.

Results: Bullet or shrapnel embolism is a very rare complication of gunshot wounds, occurring in about 0.3% of cases. These emboli often cause antegrade migration into the arterial system. However, in about 20% of cases, migration into the venous system can also occur. Venous embolism often results in foreign body migration into the right heart or the pulmonary artery. The diagnosis in these cases is often established by chance, but in some cases, arrhythmias and valve defects can occur due to exposure to a foreign body. The identification of such cases depends on the symptoms. A foreign body can be removed by an open method, as well as by invasive methods. In some cases, it is possible to keep the foreign body under control when it does not cause any symptoms.

In the clinical cases we mentioned, we also mentioned the management of cases of foreign bodies entering the venous system from peripheral zones of penetration.

Key words: cardiac trauma, intracardiac foreign body, foreign body migration

Introduction

The presence of foreign bodies in the heart resulting from migration is extremely rare, and there are very few reports on this. These foreign bodies can enter the heart either due to direct penetration during trauma or through intravenous migration during medical procedures. Foreign bodies typically found within the heart's cavities include bullet or splinter particles. Parts of the catheter or needle that have entered the venous system can also migrate and get into the cavities of the heart. The most widely used examination methods for detecting foreign bodies are X-ray examination methods [1]. Furthermore, more detailed information about cardiac foreign bodies can be obtained through computed tomography and echocardiography studies. This article presents information about cases of intracardiac foreign bodies that arose during the Second Karabakh War, which took place between September 27, 2020 and November 10, 2020. Timely diagnosis and appropriate treatment measures for such cases in wartime conditions are mentioned here.

Case presentation 1

The first clinical case relates to a 19-year-old patient who received multiple injuries as a result of a mine explosion in October 2020. So, after initial interventions, the patient was taken to the nearest medical center, and then transferred to a military hospital. A few days later, the patient was sent home to recover. According to the patient, about 5-6 days after the injury, there were minor pains and discomfort in the heart area. However, after about 2 months, his complaints intensified, arrhythmias began to be noted. For this reason, the patient was re-hospitalized and examinations were started.

During CT examination, a small metal shrapnel was found on the pericardium at the bottom right in the region of the cardiodiaphragmatic sinus (Figure 1). However, the exact localization of the shrapnel in the metal artifact has not been established. In addition, three small metal shrapnel were found on the right in the area of the shoulder joint.

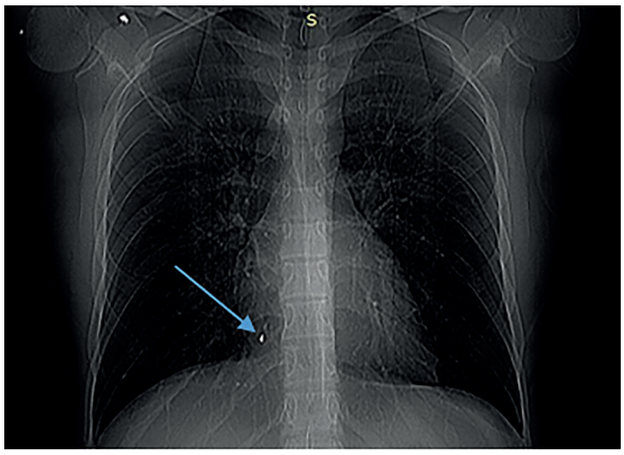

Figure 1 - Computed tomography showing a foreign body (indicated by a blue arrow).

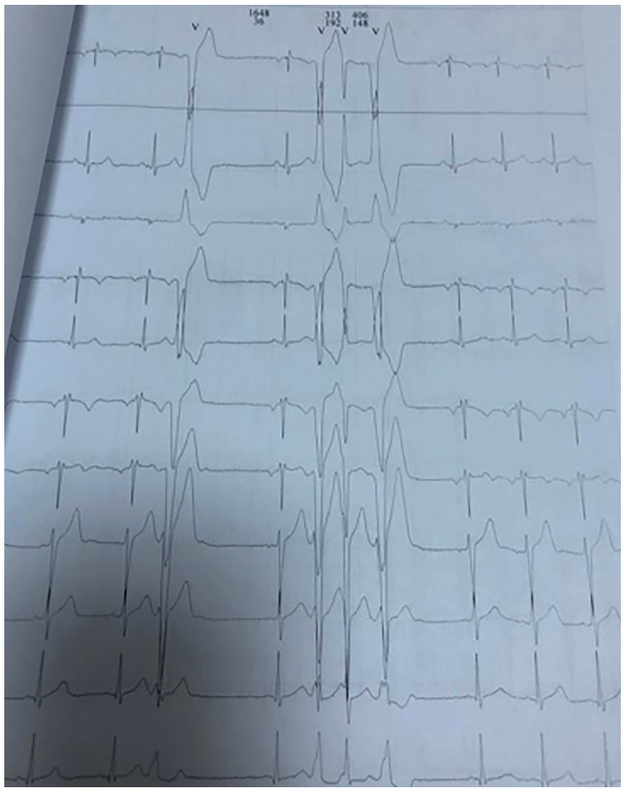

Figure 2 - Results of Holter examination of the patient (ventricular extrasystoles are noted, approximately 9-10 times per minute.

Since the patient had arrhythmias, a 24-hour Holter apparatus was connected and the presence of ventricular extrasystoles was determined approximately 9-10 times per minute (Figure 2).

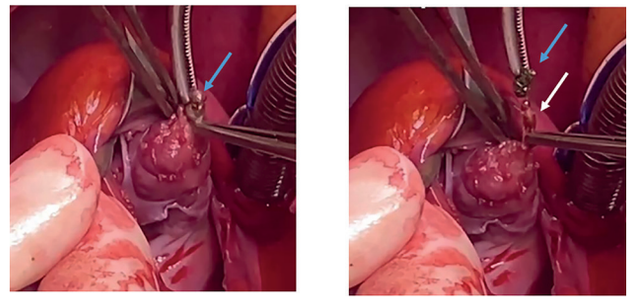

Figure 3 - Removal of an encapsulated foreign body in the right ventricle during surgery. A foreign body is indicated by a blue arrow. (A capsule with a foreign body is indicated by a white arrow).

Because of the patient's severe symptoms, the decision was made to surgically remove the foreign body. In order to avoid migration cases that may occur during the operation, it is considered advisable to perform the operation in a hybrid operating room. Median sternotomy was performed and the pericardium was examined, but no foreign body of the pericardium was found. For this reason, the heart was stopped and a right atriotomy was performed after appropriate aortic-bicaval cannulation.

During the examination of the right ventricle, an encapsulated metal shrapnel was found and removed behind the tricuspid valve flaps (Figure 3). The patient was discharged home in satisfactory condition, no complaints have been recorded yet.

Case presentation 2

The 25-year-old patient received numerous bodily injuries as a result of a mine explosion in battles during the Second Karabakh War and was hospitalized. During a CT scan of the patient, 2 shrapnel were found, one in the suprarenal region and the other around the heart. The patient underwent an X-ray examination in order to accurately determine the localization of the shrapnel (Figure 4) and it was found that the shrapnel is located inside the cavities of the heart due to the movement of the shrapnel along with the heart. In connection with the patient's complaints of rhythm disturbance, pain and discomfort in the chest, an open-heart operation with the removal of a shrapnel was planned. The operation was successfully performed, and the patient was discharged home in a state of recovery. To date, no complications have been noted in the patient.

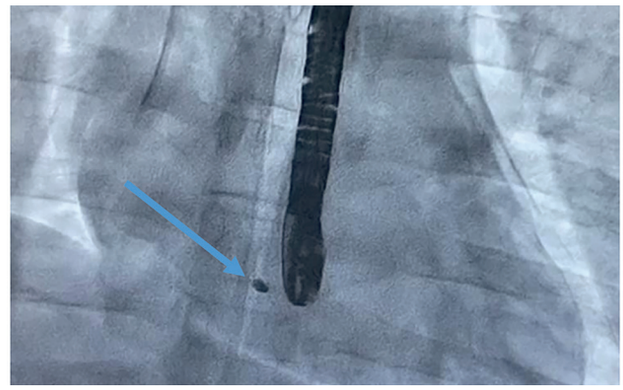

Figure 4 - An X-ray examination revealed a foreign body (indicated by a blue arrow).

Case presentation 3

A 30-year-old patient was hospitalized due to multiple injuries as a result of a mine explosion on the eve of the end of the Second Karabakh War (November 2020). Shrapnel wounds were found on many parts of the body, including the lower limbs and back. Initially, the patient was shown the necessary interventions, and the patient was sent home in a satisfactory condition.

Approximately 25 days after the injury, during a follow-up echocardiographic examination, suspicion arose regarding a foreign body (metal shrapnel) in the right ventricle of the heart. An X-ray examination of the patient was performed to determine the localization of the shrapnel (Figure 5). During the examination, it was confirmed that the metal shrapnel was inside the heart. In addition, foreign bodies were found in other parts of the patient's body (Figure 6). A decision was made to observe the patient due to the absence of complaints in the anamnesis, and also due to the fact that no pathology was detected during echocardiography and Holter examination.

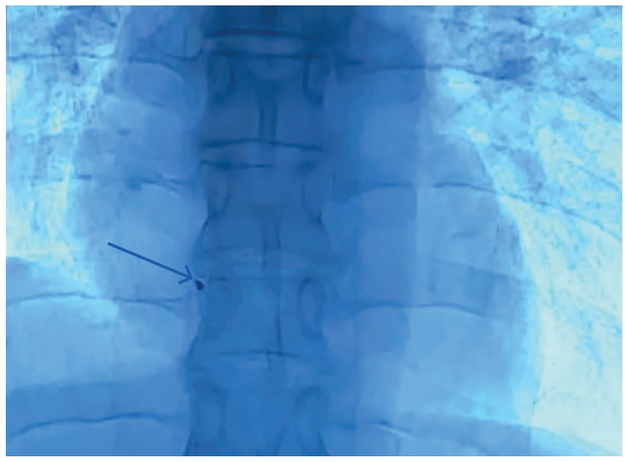

Figure 5 - X-ray examination shows a foreign body corresponding to the projection of the right ventricle of the heart (indicated by a blue arrow).

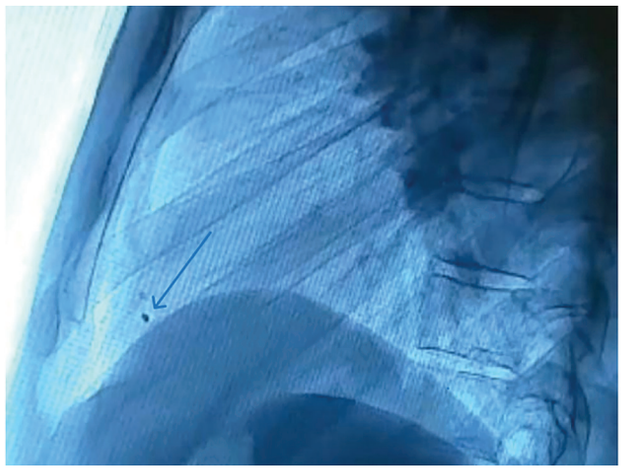

Figure 6 - X-ray examination shows a foreign body (metal shrapnel) in the right part of the chest (indicated by a blue arrow).

Discussion and conclusions

Bullet or shrapnel embolisms are rare complications of gunshot wounds and occur in 0.3% of cases [2]. Embolisms in most cases form anterograde migration in the arterial system, and in 20% of cases are detected in the venous system [3]. In 120 cases of venous embolism reported between 1900 and 1990, 83% of foreign bodies migrated to the right heart or the pulmonary artery, and only 4% remained in the peripheral veins [4].

As mentioned above, foreign bodies enter the heart as a result of direct penetration or as a result of migration into the venous system [5,6].

Patients with direct heart injuries are typically in critical condition. These patients may experience complications such as pericarditis, pulmonary or systemic embolization, arrhythmia, local infections, the formation of blood clots around a foreign body and local erosion of the surrounding bronchi or intracardiac structures [7,8].

Although X-ray and tomographic examinations are effective in these cases, echocardiographic examination is one of the most important methods of examination in determining the localization of intracardiac foreign bodies, as well as in determining the subsequent stages of treatment [9]. In such cases, a hyperechoic image is detected during ECHO examinations.

Treatment tactics in these patients varies depending on the size of the foreign body, its localization in the heart, the risk of infection, the risk of systemic embolization and the presence of arrhythmia. In some cases, surgical or transcatheter removal of the fragment may be required in these patients, and in asymptomatic patients, intervention may not be required.

The widely used mechanism of approach to the choice of therapeutic tactics is as follows [10]:

1. Symptomatic foreign bodies (if they cause infectious complications, arrhythmias or neurological problems) should be removed regardless of their localization.
2. It is necessary to remove foreign bodies that cause hemodynamic disorders, damage to intracardiac structures or serious problems with valves.
3. Asymptomatic foreign bodies should be removed if they are detected shortly after injury and it is suspected that they cause infectious complications, systemic embolization or erosion.
4. Asymptomatic cases of foreign bodies with a low probability of additional complications can be managed conservatively. This is especially true in cases where a foreign body has penetrated deeply into the myocardium or pericardium.

Conclusion

Our article reports about 3 patients with intracardiac foreign bodies. Each of these patients was offered an appropriate approach to the indicated therapeutic tactics. Thus, in 2 patients, due to the fact that an intracardiac foreign body caused critical symptoms, a surgical operation was performed and the foreign body was removed. In the postoperative period, both patients were treated with double antibiotic therapy, and after removal of a foreign body during surgery, the area was washed with antibiotic solutions. No complications were noted during the postoperative examination of each of these patients. Since the intracardiac foreign body in another patient did not cause any symptoms during the clinical and instrumental examination, it was decided not to remove the foreign body and leave the patient under observation.

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