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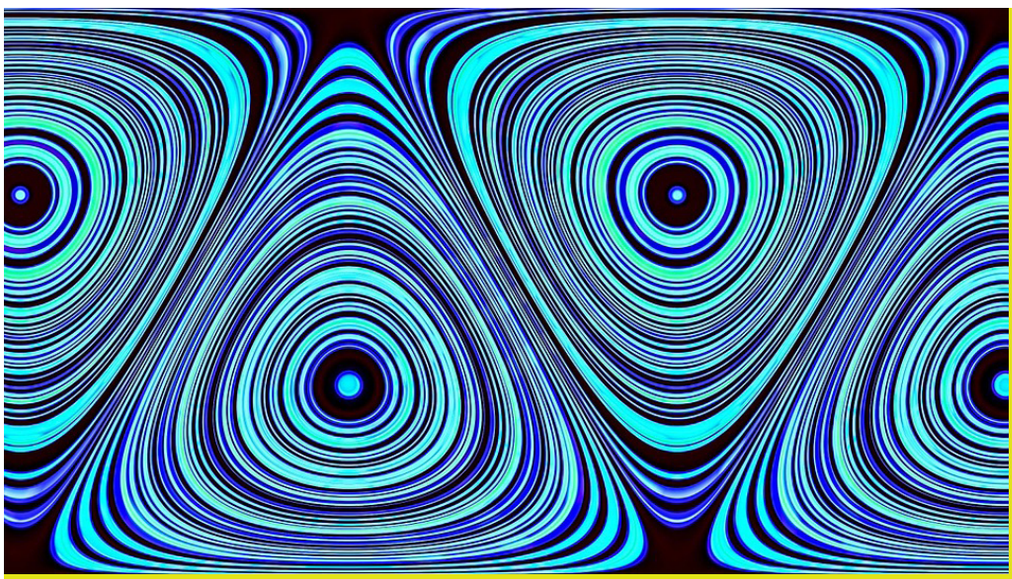

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RESEARCH ARTICLE

ADAPTATION OF THE QUALITY OF SIBLING EXPERIENCE SCALE FOR INDIVIDUALS WITH SPECIAL NEEDS SIBLINGS INTO TURKISH: A METHODOLOGICAL STUDY

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ABSTRACT

Purpose: The relationship between siblings, which is a lifelong bond, affects both the individual with special needs and their healthy sibling in many ways. This study was conducted to do the Turkish validity and reliability study of the Siblings' Experience Quality Scale (SEQS).
Material and Methods: The study was carried out with individuals who were aged over 18 and had a sibling enrolled in the Guidance and Research Center of a province. Shapiro-Wilk normality test, Pearson correlation analysis, content validity index, Cronbach's Alpha coefficient, McDonald's omega test, confirmatory factor analysis, and t-test were used in data analysis.
Results: The healthy siblings of 171 students with special needs voluntarily participated in the study. The scale explained 54.8% of the total variance. The omega reliability coefficient value for the overall scale was determined as 0.81. As a result of CFA, the fit indices were found as X2=247.893, df=128, X2/df=1.93, RMSEA=0.074, GFI=0.86, IFI=0.85, NFI=0.73, TLI=0.81, CFI=0.84, and AGFI=0.819.
Conclusion: The SEQS was proven to be a valid and reliable measurement tool in the Turkish population.

Keywords: children, special needs, sibling, relationships, validity, reliability

INTRODUCTION

The relationship between siblings during childhood, adolescence, and adulthood is affected by love-closeness, hostility-conflict, and rivalry-parental favoritism (1,2). In the literature, it has been reported that both emotional and instrumental support (material support and time allocation) is higher in the relationships of adults with their siblings. The bond between sibling relationships directly affects the family structure and the relationship level of the adult sibling in cases where one of the siblings has special needs (3).

As a result of the extension of life expectancy at birth, increase in chronic diseases, or habits acquired throughout life, the level of disability is growing globally. Disability affects not only the individual, but also their family, close environment, and society in which they live. According to the report of the World Health Organization, more than one billion people have some type of disability (approximately 15% of the world population) and almost everyone is likely to have some type of disability at some point in their life (4). In Turkey, the rate of the population with at least one disability is 6.9% according to 2011 data (5).

Some studies have shown that sibling relationships with warm and supportive bonds create a buffer against low self-esteem, depression, and loneliness against low parent or peer support and that individuals with such relationships tend to establish close relationships during adolescence and adulthood (6,7). According to the results of a study, it was reported that the mental health of the healthy sibling directly affected the health of the sibling with special needs (8). The presence of a child with a disability in the family affects relationships within the family and causes changes in the family balance. Although all family members are affected by each other, it is an undeniable reality that siblings are also exposed to these influences. The expectation that siblings should first accept the child with a disability can lead to feelings of guilt in siblings, feeling ashamed of the unhealthy sibling's behavior or appearance and avoiding communication with him/her, fear of having the same disorder, jealousy or anger due to receiving less attention, or pressure to be very successful to compensate for what the unhealthy sibling cannot do (9,10). The relationship between siblings is very important, especially in terms of the health of the sibling with special needs, and the social relationships he/she will establish in the future. Knowledge of how having a sibling with special needs affects the other healthy sibling is important for nurses to carry out planned nursing interventions while maintaining holistic care. In the Turkish literature, there are a limited number of measurement tools that measure the sibling relationships of individuals with special needs (11). There is a need for an up-to-date, valid, and reliable measurement tool in this regard. Therefore, this study aims to adapt the Siblings' Experience Quality Scale (SEQS) into Turkish and conduct its validity and reliability analyses.

MATERIAL AND METHODS

Research Type

This is a methodological, descriptive, and cross-sectional study that was conducted to perform the Turkish validity and reliability study of the SEQS, which measures the sibling relationships of healthy individuals who are aged over 18 and have a sibling with special needs.

Ethical Considerations

For study, Maltepe University Scientific Research Ethics Committee permission was obtained (Date: 13.11.2020, Decision No: 2020/14-01). The institutional permission of the Kocaeli Provincial Directorate of National Education (Issue: 99332089/605.01/17684827). The present study followed the principles outlined in the Declaration of Helsinki for Human Studies.

Population and Sample

In this study, data were collected from healthy individuals who were aged over 18 and had a sibling who had special needs and were enrolled in the Guidance and Research Center in a province between December 2020 and May 2021. The informed consent of the participants were obtained verbally. The scale used in the study consisted of 23 questions. The permission of the author who developed the scale was obtained via e-mail. To collect healthy data in the study, the sample size was determined based on the rule that a sample size of about 5-10 times the number of items on the scale should be reached, which is recommended in validity and reliability studies (12). In the literature, it has been reported that a sample size of up to 100 is insufficient in scale development studies, moderate up to 200, good up to 300, very good up to 500, and excellent up to 1000 individuals (13). A total of 171 healthy siblings who agreed to participate in the study were included in the sample. According to this result, it can be said that the sample size is medium. The sample size was taken as 7.4 times the average number of items.

Inclusion criteria: 1) Having a sibling with special needs enrolled in the Guidance and Research Center, 2) Being aged over 18-64, 3) Voluntary participation in the study and submitting consent for participation.

Exclusion criteria: 1) Disagreeing to participate in the study, 2) Not knowing Turkish.

Data Collection Tools and Characteristics

The study data were collected by using a socio-demographic characteristics form, the Siblings' Experience Quality Scale (SEQS), and the Attitude Scale for Disabled Sibling (ASDS).

The Socio-demographic Characteristics Form: This form, which was created by the researchers following a review of the literature, consists of questions, such as gender, age, and special needs, about children with special needs and their healthy siblings (14,15).

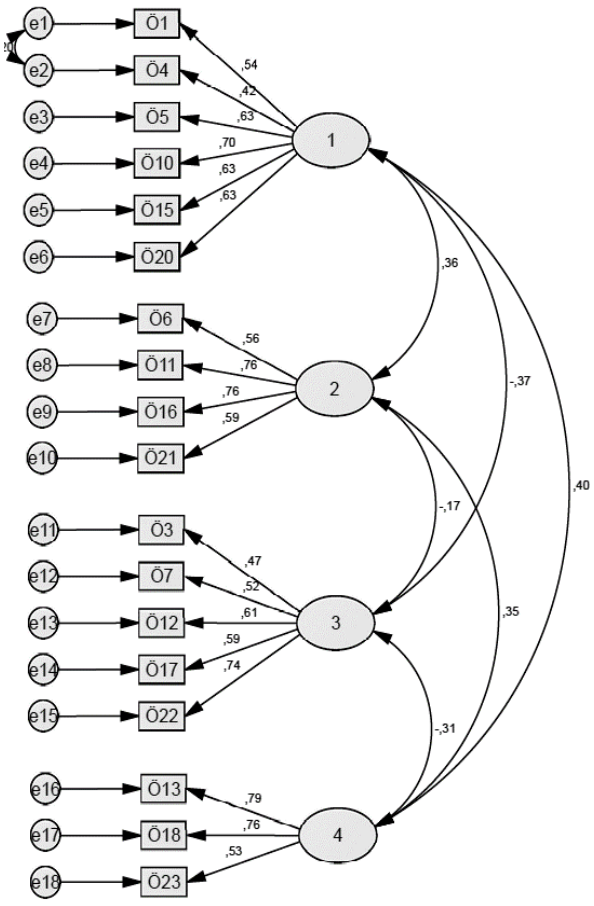

Figure 1. Confirmatory factor analysis of the Siblings' Experience Quality Scale-Adult Form for individuals with siblings with special needs

The Siblings' Experience Quality Scale (SEQS):
This scale was developed by Sommantico et al. (2020) to measure sibling relationships of healthy children with siblings with disabilities and chronic and mental illnesses. It consists of 23 items evaluated on a 7-point Likert-type scale with options ranging from 1 (strongly disagree) to 7 (strongly agree). The scale has five subscales, namely, closeness (items 3,7,12,17 and 22); conflict (items 1,5,10,15 and 20); jealousy (items 4,8,13,18 and 23); self-marginalization (items 9,14 and 19); worry (items 2,6,11,16 and 21). The closeness subscale involves sibling relationships based on friendship, love, knowledge, and sincerity; the conflict subscale refers to feelings such as fight, enmity, or envy towards the sibling; the jealousy subscale is about the presence of feelings such as jealousy and rivalry between siblings and the perception of biased love towards siblings by the parents; the self-marginalization subscale is about the difficulty in expressing needs and wishes and making parents exhausted; the worry subscale is about worrying about the health and future life of the sibling with special needs. In the evaluation of the scale, the total mean score of each subscale is calculated. A high score obtained from a subscale of the scale indicates that the related relationship is at a higher level. The reliability coefficients (α) of the original scale were found as .78 for closeness, .88 for conflict, .87 for jealousy, .74 for self-marginalization, and .88 for worry. According to confirmatory factor analysis, the goodness of fit indices were found as $\chi 2/df = 1.98$; RMSEA (Root Mean Square Error of approximation) = 0.047 [.033–.061]; CFI (Comparative Fit Index) = 0.92; TLI = 0.91; SRMR = 0.063. The original scale is suitable for siblings aged 18-69 (15).

Attitude towards Sibling with Disability Scale (ASDS):
This scale was developed by Küçüker (1997). It has a 4-point Likert-type structure and consists of 28 questions and 4 subscales. The subscales are feelings and thoughts about living with a disabled sibling (1,4,6,8,9,10,11,12,14,17,18,19,20,21,24,27), feeling sad and worried regarding the (current and future) situation of the disabled sibling (2,3,13,16,25,26), and thoughts about the characteristics of the disabled sibling (5,7,15,22,23,28). The items expressing a positive attitude on the scale are evaluated from 4 to 1 with options varying between "totally agree" to "totally disagree", and the items expressing a negative attitude are evaluated from 1 to 4 with options varying from "totally agree" to "totally disagree". The minimum and maximum scores that can be obtained from the scale vary between 28 and 112, respectively, and high scores indicate positive attitudes and low scores indicate negative ones. Cronbach's alpha coefficient values of the subscales were found as .84 for the attitude towards the disabled sibling subscale, .81 for the living with the disabled sibling subscale, .70 for the status of the disabled sibling subscale, and .73 for the characteristics of the disabled sibling subscale (11).

Steps of the Study
Expert opinion stage: The adaptation study was initiated by obtaining the permission of the author, Sommantico, who developed the scale, via e-mail. The English form of the scale was translated into

Table 1. Explanatory Factor Analysis (EFA) (n=171)

	Subscales			
Items	**Closeness**	**Conflict**	**Jealousy**	**Worry**
m3	.53			
m7	.72			
m12	.73			
m17	.60			
m22	.74			
m1		.74		
m4		.46		
m5		.68		
m10		.73		
m15		.63		
m20		.66		
m13			.84	
m18			81	
m23			.64	
m6				.69
m11				.74
m16				.81
m21				.72

Turkish by two academics who are expert linguists and fluent in English and Turkish, and the two translations were integrated into a single form by the researchers. The Turkish form was translated back into English by two experts with good command of the two languages. After back translation, the Turkish version of the scale was found to be close to the original form of the scale. Then, the translations were prepared for expert opinion. According to the literature, at least three experts should be consulted to determine the content validity of a scale (16–18). A total of 10 experts, including four faculty members working in the field of public health nursing, five faculty members working in the field of child health and diseases nursing, and one faculty member working in the field of internal medicine nursing, were consulted. The experts were given the draft form of the scale, and they were asked to rate each item by using a score between 1 and 4 (1=not appropriate at all, 4=completely appropriate) to evaluate their suitability. Scores were evaluated by using the content validity index. After expert opinions were obtained, the Turkish form of the scale was finalized by making necessary changes.

Pilot application: The form was piloted to 20 siblings (17,18). After the application, it was found that the items did not need any change, and the siblings involved in the pilot application.

Reliability calculations: Pearson correlation analysis was used for the item-total score analysis of the scale and its subscales. Cronbach's alpha coefficient was calculated to determine the internal consistency of the scale and the subscales (16–18). Reliability was calculated by using McDonald's Omega coefficient for the total scale and the subscales (19).

Validity calculations: Explanatory factor analysis was employed to determine the item-factor relationship, and confirmatory factor analysis was used to determine whether the items and subscales explained the original structure of the scale (16–18).

Data Analysis

In the analysis of the data, percentages and mean scores were employed for descriptive statistics. The statistical analyses also included the Shapiro-Wilk test for testing the normality of the data, content validity index for the analysis of the inter-rater reliability, Pearson correlation analysis for the item-

Table 2. Reliability analysis of the scale and sub-scale scores (n=171)

Subscale	*Cronbach α*	*M ± SD*	*Min-Max*	*McDonald's ω*
Closeness	.72	30.24±5.59	11-35	.77
Conflict	.76	15.50±7.44	6-42	.73
Jealousy	.71	7.18±4.94	3-21	.74
Worry	.76	14.61±6.76	4-28	.77
Total Scale	.80	67.55±13.58	41-121	.81

Table 3. Item-total score correlations of the subscales (n=171)

Subscales	*Items*	Corrected Item-subscale score correlations *(r)**
Closeness	3	.38
	7	.46
	12	.50
	17	.45
	22	.58
Conflict	1	.50
	4	.37
	5	.53
	10	.58
	15	.53
	20	.53
Jealousy	13	.59
	18	.53
	23	.50
Worry	6	.49
	11	.59
	16	.62
	21	.51

*p<0.001

total score analysis of the scale and subscales, Cronbach's Alpha coefficient for determining the internal consistency of the scale and subscales, Davis technique for content validity, explanatory factor analysis for determining item-factor correlation, omega coefficient for the total scale and subscale reliability, confirmatory factor analysis for determining whether items and subscales explained the original structure of the scale, the ASDS for determining the relationships between the factors of the scale and parallel forms reliability, Pearson correlation analysis for the correlation between the factors of the scale, and t-test for known group comparisons. The margin of error was set at p=0.05. The analyses were conducted on SPSS 24.0, AMOS 24.0, and Jamovi 2.2.2 software packages.

RESULTS

Of the participants in the study, 62% were female (n=106), 50.9% had a university level education (n=87), 33.3% had high school education (n=57), and 15.8% (n=27) had secondary school education. The mean age of the siblings was determined to be 23.94±5.01 (min: 19-, max: 43). Of the siblings with special needs, 54.4% (n=93) were male, 64.3% had a mental disability (n=110), 18.1% had an emotional disability (n=31), and 17.5 had a physical disability (n=30).

Content Validity

The item-based content validity index was found to be between 0.99 and 1.00, and the scale-based content validity index was determined as 0.99.

Construct Validity of the Scale

Explanatory Factor Analysis (EFA)

As a result of the explanatory factor analysis (EFA), the Kaiser-Meyer-Olkin (KMO) coefficient was determined as .759, and the Bartlett test as X2=906.695. The original version of the scale consists of five subscales. As a result of EFA, the Turkish form of the scale was determined to consist of four subscales. The scale explained 54.8% of the total variance. The rate of the total variance explained by the subscales was as follows: closeness, 10.3%; conflict, 24.03%; jealousy, 8.6%; worry, 11.7%. Five items (2, 8, 9, 14, and 19) on the original scale were removed from the Turkish form of the scale with the approval of the author who developed the scale due to their low factor loads.

The factor loads of the items of the subscales were found to range between .53 and .74 for closeness, .46 and .74 for conflict, .64 and .84 for jealousy, and .69 and .81 for worry.

Confirmatory Factor Analysis (CFA)

As a result of CFA, the fit indices were found as X2=247.893, df=128, X2/df=1.93, RMSEA=0.074, GFI=0.86, IFI=0.85, NFI=0.73, TLI=0.81, CFI=0.84, and AGFI=0.819. It was determined that the factor loads of the items of the subscales varied between .47 and .74 for closeness, .42 and .70 for conflict, .53 and .79 for jealousy, and .56 and .76 for worry. Item 4 was on the jealousy subscale in the original form of the scale, but it was determined to be on the conflict subscale in the Turkish version of the scale (Figure 1).

Reliability Analysis

Cronbach's alpha values of the subscales of the scale were found to be 0.76 for conflict, 0.76 for worry, 0.71 for closeness, and 0.71 for jealousy. As a result of the split-half analysis of the scale, Cronbach's alpha values of the first and second halves were determined as .50 and .51, respectively. Spearman-Brown and Guttman Split-Half coefficients were both found to be .71. McDonald's omega coefficient was calculated as 0.77 for worry, 0.77 for closeness, 0.74 for jealousy, and 0.73 for conflict. The total alpha value of the scale was calculated as 0.80 and the omega value as 0.81. It was determined that the item-total score correlations of the subscales ranged between .37 and .62 (Table 3).

It was found that there was a significant correlation between the closeness and conflict subscales of the SEQS and the total score of the ASDS ($p<0.001$) (Table 4).

Table 4. Correlations between the total score of ASDS and the subscales of the SEQS

Subscales	ASDS total score (*r*)*
Closeness	0.315*
Conflict	-0.234*
Jealousy	-0.143
Worry	-0.117

*p<0.001

DISCUSSION

Content Validity of the Scale

It is stated in the literature that an item-and scale-based content validity index of greater than 0.80 shows a high level of agreement between experts (20,21). In the analysis, the content validity index was found above 0.80. As a result, it was determined that the items on the Turkish form of the scale adequately represented the desired area.

Construct Validity of the Scale

In the literature, it has been stated that the Barlett Sphericity test value should be statistically significant and the KMO value should be at least 0.60 to perform factor analysis (20,21). As a result of the analysis, it was found that the KMO value was greater than 0.60 and $p<0.05$ according to the Barlett Sphericity test result. According to this result, factor analysis could be performed (20–23). In exploratory factor analysis, the eigenvalue is accepted as 1 and above in determining the number of factors (22,24). The Turkish version of the scale consisted of four subscales, and these four subscales explained 54.8% of the total variance. In the original form of the scale, the subscales explained 66.3% of the total variance (15). The total explained variance in our study was greater than 50%, and it was close to the explained variance in the original form of the scale, both of which indicated that the scale was a valid measurement tool. This result also supported the construct validity of the scale. In the literature, it is emphasized that the minimum factor load should be 0.30 and above, and the items below this value should be removed from the scale when determining

which factor the items should belong to (20–23). While the original scale had 5 factors, our study revealed a 4-factor structure. The five items (2, 8, 9, 14, and 19) on the original form of the scale were found to have low factor loadings and were found not to be compatible with the factor in the original scale sub-dimension in our study. In studies conducted on cross-cultural validity and reliability in the literature, it has been reported that these items can be removed by informing the author(s) of the original scale. After the Turkish translation, it is thought that these items do not culturally explain the original sub-dimension, so these items were removed from the Turkish form of the scale with the approval of the author who developed the scale (Sample item-I usually feel that I shouldn't worry my parents.). In our study, factor loads of the items on the four subscales were found to be greater than 0.46. This finding was similar to the findings of the original form of the scale (15). All these results showed that the scale had a strong factor structure.

According to the confirmatory factor analysis, factor loads of the four subscales ranged from .42 to .79 (Figure 1). All factor loads were greater than 0.30, most of the fit indices were greater than 0.80 (GFI=0.86, IFI=0.85, NFI=0.73, CFI=0.84), RMSEA was less than 0.080 (RMSEA=0.074), and X2/df was less than five (X2/df=1.93), all of which indicated that the items on each subscale adequately defined their own factor. The findings obtained were consistent with the findings of the original form of the scale (15). These results supported the construct validity of the scale and showed that an efficient evaluation could be made. It was seen that item 4 was on the jealousy subscale in the original scale. In the Turkish population, on the other hand, this item was under the conflict subscale, which is about hostility towards the sibling and jealousy between siblings. The explanatory and confirmatory factor analysis results of our study supported the construct validity of the scale and revealed that the scale was a valid tool.

Reliability Analysis of the Scale

Internal consistency analysis of the scale and its subscales

A Cronbach's alpha coefficient of between 0.60 and 0.80 indicates that the scale is quite reliable, and a value between 0.80 and 1.00 indicates high reliability (16,25,26). The results of this study showed that the omega reliability coefficient for the overall scale was 0.81, Cronbach's alpha was 0.80, and that Cronbach's alpha values of the subscales ranged between .71 and .76. These results were similar to those of the original form of the scale (15). The presence of moderate and low-level significant correlations between the measurement tool (ASDS), which was used in parallel forms reliability, and the closeness and conflict subscales indicated that Cronbach's alpha values of both halves obtained in the split-half method were greater than 0.50 and that there was a weak and significant correlation between the two halves. According to the results of this study, the scale can be used safely as a measurement tool.

Item-total score analysis of the scale and the subscales

Item-total score correlations should be greater than 0.20, as close to 1 as possible, and positive (17). According to the analyses, the item-subscale total correlations were found to be greater than 0.37. According to these results, it was found that the subscale total scores were highly correlated with each item, the subscales adequately represented the area to be measured, and that the subscale item reliability was high.

According to the results of our study, the highest relationship between siblings included closeness and the lowest level of relationship involved jealousy. This finding was consistent with the results of the original form of the scale (15).

Limitations

It can be said that the limitation of the study is the fact that the data collection process took place during the pandemic period, there was difficulty in reaching healthy siblings due to the absence of children with special needs in school, and therefore, no method was used in the selection of the sample. Since the study was conducted during the pandemic period, it may be useful to repeat this study and compare the results.

CONCLUSION

The original scale consisted of 5 subscales and 23 items, but some items (2, 8, 9, 14, and 19) in our study were removed in line with the permission of the author, who developed the scale since their factor loads were low although the factor structure of the scale had been controlled previously. In our study, the scale consisted of four subscales and 18 items. The results of the study indicated that the scale had validity and reliability in measuring sibling

relationships of individuals with siblings with special needs. The scale analyzed in this study can be used to measure the level of sibling relationships of individuals who have siblings with special needs in Turkey. It can also contribute to the development of future sibling relationships. At the end of this adaptation study, a scale development study suitable for Turkish culture can be conducted.

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Author Contributions: Study design: TO, GK, MB Data collection: TO Data analysis: MB, TO, GK Study supervision: TO, GK, MB Manuscript writing: TO, GK, MB Critical revisions for important intellectual content: MB, GK, TO

Conflict of Interests: No conflict of interest was declared by the authors.

Ethical Approval: Maltepe University Scientific Research Ethics Committee permission was obtained for the study (Date: 13.11.2020, Decision No: 2020/14-01). The institutional permission of the Kocaeli Provincial Directorate of National Education (Issue: 99332089/605.01/17684827) and the informed consent of the participants were obtained verbally. The permission of the author who developed the scale was obtained via e-mail. The present study followed the principles outlined in the Declaration of Helsinki for Human Studies.

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RESEARCH ARTICLE

COMPARISON OF EFFECTIVENESS ASCORBIC ACID AND MAGNESIUM ON RENAL ISCHEMIA-REPERFUSION MODEL IN RATS

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ABSTRACT

Purpose: This study aims to determine the effectiveness of ascorbic acid and magnesium administration separately or combined before ischemia in the rat renal ischemia and reperfusion damage of the model.
Material and Methods: Thirty-five Wistar rats were randomized into five groups of seven animals each. Group 1 only underwent laparotomy, followed for 285min until sacrification. Group 2 45min ischemia; 240min reperfusions, no drugs were applied. Group 3 250mg/kg ascorbic acid was applied 60min before ischemia-reperfusion (IR), Group 4 200mg/kg magnesium sulfate was applied 60min before IR, Group5 both drugs were applied at the same doses 60min before IR. Malondialdehyde (MDA) and glutathione (GSH) levels were determined in the renal tissues received, serum blood urea nitrogen (BUN) and creatinine levels were measured in blood samples. Hematoxylin and eosin (H&E) and periodic acid schiff (PAS) paintings for histological examinations. Terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling (TUNEL) immunohistochemical staining for apoptotic cell examinations.
Results: Oxidative stress parameter MDA value was found significantly lower in Group3 (p:0.034) and Group5 (p<0.001) compared to Group2. Endogen antioxidant parameter GSH value of Group 4 (p<0.001) and Group 5 (p<0,001) were significantly higher than the other groups. TUNEL counts of the Group5 was significantly lower than the counts Group2 (p:0,005).
Conclusion: It was concluded that before ischemia, ascorbic acid and magnesium sulfate combination reduce the kidney damage in the rat renal IR model.

Keywords: ascorbic acid, ıschemia-reperfusion ınjury, magnesium, rat

INTRODUCTION

Ischemia is a reduction or cessation in blood flow to a tissue or organ, while reperfusion is defined as the renewal of blood flow to the tissue or organ after ischemia. Reperfusion may cause more damage to tissue than ischemic injury (1). This situation is called

reperfusion injury. One of the organs primarily exposed to IR injury is the kidneys and acute kidney injury is defined as a critical clinical situation related to high morbidity and mortality. Kidney IR injury may occur in a variety of clinical conditions like kidney transplantation, partial nephrectomy, cardiopulmonary bypass, sepsis, urologic interventions, and hydronephrosis (2). Among the reasons for the kidneys being sensitive to IR injury are the complicated microvascular network structure and high energy requirements (3).

In the formation of IR injury, local and systemic responses play roles characterized by the production of reactive oxygen radicals, complement activation, leukocyte-endothelial cell adhesion, transendothelial leukocyte migration, platelet-leukocyte aggregation, increased microvascular permeability, and endothelium-linked increase permeability (4). The production of increased reactive oxygen radicals causes dysfunction of the antioxidant system. This dysfunction causes tubular cell injury and apoptosis. The immune response begins as a result of the release of inflammatory cytokines. The activated complement system forms another injury path (5).

Ischemic preconditioning, defined as the adaptation of tissue through protective mechanisms against stress, was identified as the best-known mechanism to protect against IR injury (6). Among methods used for ischemic preconditioning, the most commonly chosen are distant ischemic preconditioning and pharmacologic conditioning (7).

Many studies have researched the protective efficacy of different agents applied for pharmacologic conditioning for renal IR injury. There are studies showing agents like ascorbic acid (8-14), magnesium sulfate (2,15-18), vitamin E (9), hydrocortisone (9), L-arginine (10), atorvastatin (19), tadalafil (20), monoaminoxidase inhibitors (21), dexmedetomidine (22) and mannitol (23) reduce IR injury.

High-dose ascorbic acid inhibits nicotinamide adenine dinucleotide phosphate (NADP)-oxidase and inducible nitric oxide synthase (NOS) activation to reduce and repair microcirculation disorders linked to oxidative injury; thus, preclinical studies have shown that it may reduce the effects of IR injury (9,11). Varieties of mechanisms have been proposed for how ascorbic acid intervenes in the oxidative-derived interaction between inflammatory and endothelial cells. P-selectin may affect aqueous-phase oxygen radicals, while ascorbic acid was proposed to neutralize these radicals and reduce the upregulation of this critical adhesion molecule (10). At the same time, water-soluble antioxidants cut aqueous-phase oxygen radicals before affecting lipoprotein lipids and thus, were stated to prevent the initiation of lipid peroxidation (10).

Magnesium is an electrolyte found in large amounts in both intracellular and extracellular areas. At the same time, it is a co-factor included in more than 300 enzyme systems regulating biochemical reactions in the body including protein synthesis, muscle and nerve conduction, neuromuscular signal conduction, blood sugar control, and blood pressure regulation. It is an ion channel regulator contributing to the preservation of cellular ion balance. It plays an important role in the active transport of calcium and potassium ions along cell membranes (16).

The intracellular calcium increase occurring with stimulation of L-type calcium channels increases the inflammatory response; and was identified to cause disruption of mitochondrial functions, necrosis, or apoptosis in the cell (2). The L-type calcium channel blockage formed by the L-type calcium channel blocker of magnesium was stated to be protective against IR injury (2). Magnesium is also a well-known NMDA receptor antagonist (15). It directly inhibits lipid peroxidation and was shown to reduce endothelial and neuronal reperfusion injury (18).

To date, there are many studies investigating the effectiveness of ascorbic acid and magnesium on IR damage in separate applications. Our study aimed to investigate the effectiveness of the combined application of ascorbic acid and magnesium on IR damage.

MATERIAL AND METHODS

Ethical Considerations

This study investigating the efficacy of ascorbic acid and magnesium in a renal IR model in rats was completed in Dokuz Eylul University Multidisciplinary Experimental Animal Laboratory with ethical approval from Dokuz Eylul University Animal Experiments Local Ethics Committee (Date: 31.10.2018, Decision No: 47/2018). The study included 35 adult male Wistar rats with weights from 250-300 gr. Subjects obtained from Dokuz Eylul University Faculty of Medicine (DEUFM) Experimental Animals Laboratory were fed with standard rat feed and water ad libidum. Rats were housed in standard laboratory conditions (12-hour day-12-hour night lighting; lights on at 07:00 am, 22-24 °C room temperature, 50-60% humidity).

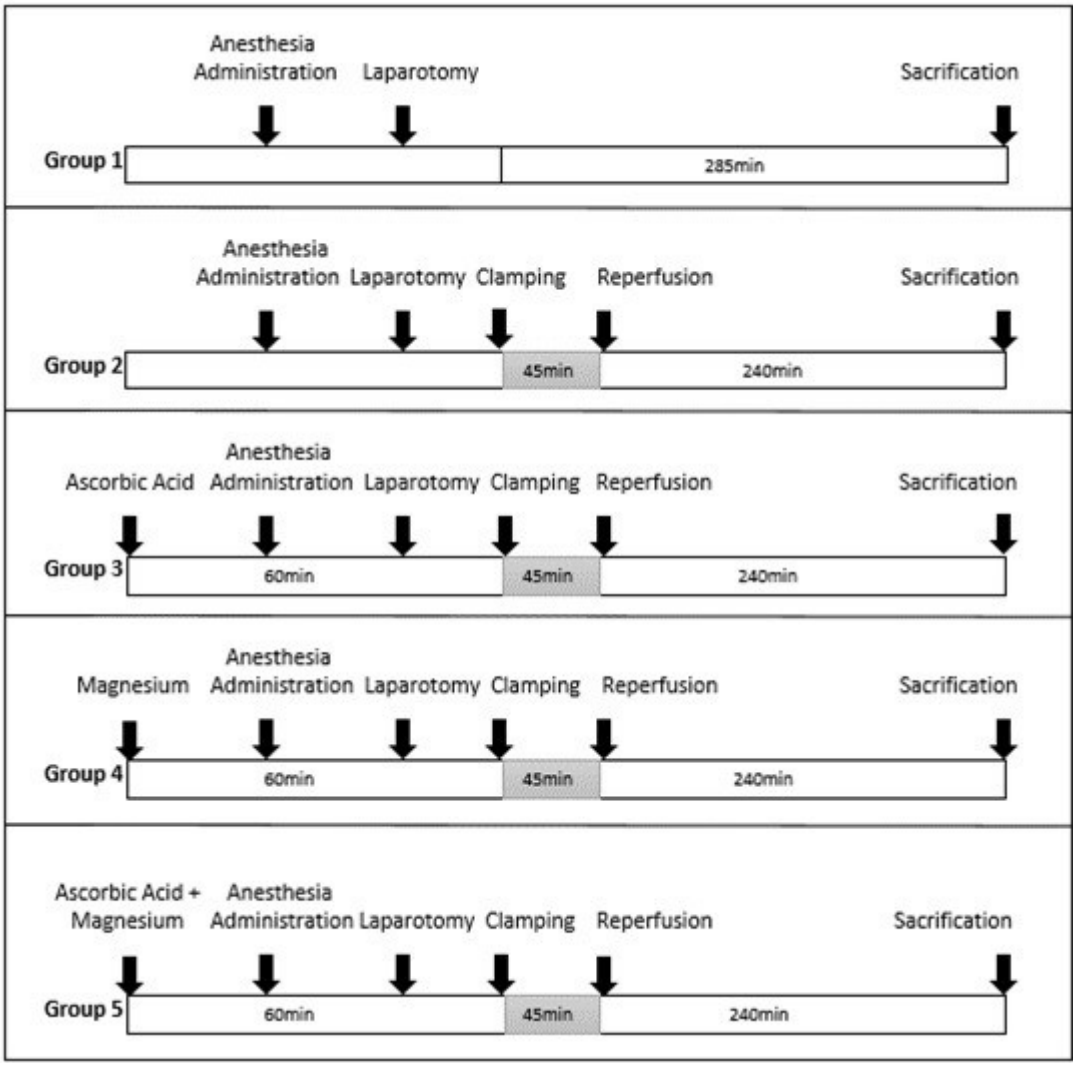

Figure 1. The schematic appearance of the experimental model

During experimental procedures, the care of laboratory animals abided by international guidelines.

Anesthesia

Subjects had anesthetized with 50 mg/kg ketamine (Ketalarflc., Pfizer Pharma GMBH, Germany) and 7 mg/kg xylazine hydrochloride (Alfazyne 2%, Alfasan International, Holland) i.p. When necessary, ketamine (25 mg/kg) was administered to keep anesthesia depth fixed by examining reflex responses (pedal reflex, palpebral and corneal reflexes when given painful stimulation to the foot with tweezers).

Experiment Groups and Protocol

The duration of ischemia in the kidneys is usually limited to 30 to 60 minutes. It has been suggested that renal ischemia lasting more than 60 minutes causes acute tubular necrosis and renal failure. If the duration of renal ischemia is shorter than 30 minutes, rapid proliferation of tubular epithelial cells can repair damaged renal tubules and may be accompanied by improvement in renal function. The effects of renal IR injury begin at the earliest at 4 hours and reach their peak at 24 hours. In this study, an ischemia time of 45 minutes and a reperfusion time of 4 hours were selected based on information obtained from the literature.

Sham Group (Sham, Group 1, n=7): Right and left renal pedicles opened after laparotomy. Rats were left under anesthesia for 285 min without any other intervention.

Ischemia-Reperfusion Group (IR, Group 2, n=7): Right and left renal pedicles opened after laparotomy, both pedicles placed in atraumatic clamp for 45 min ischemia then clamps opened for 240 min reperfusion.

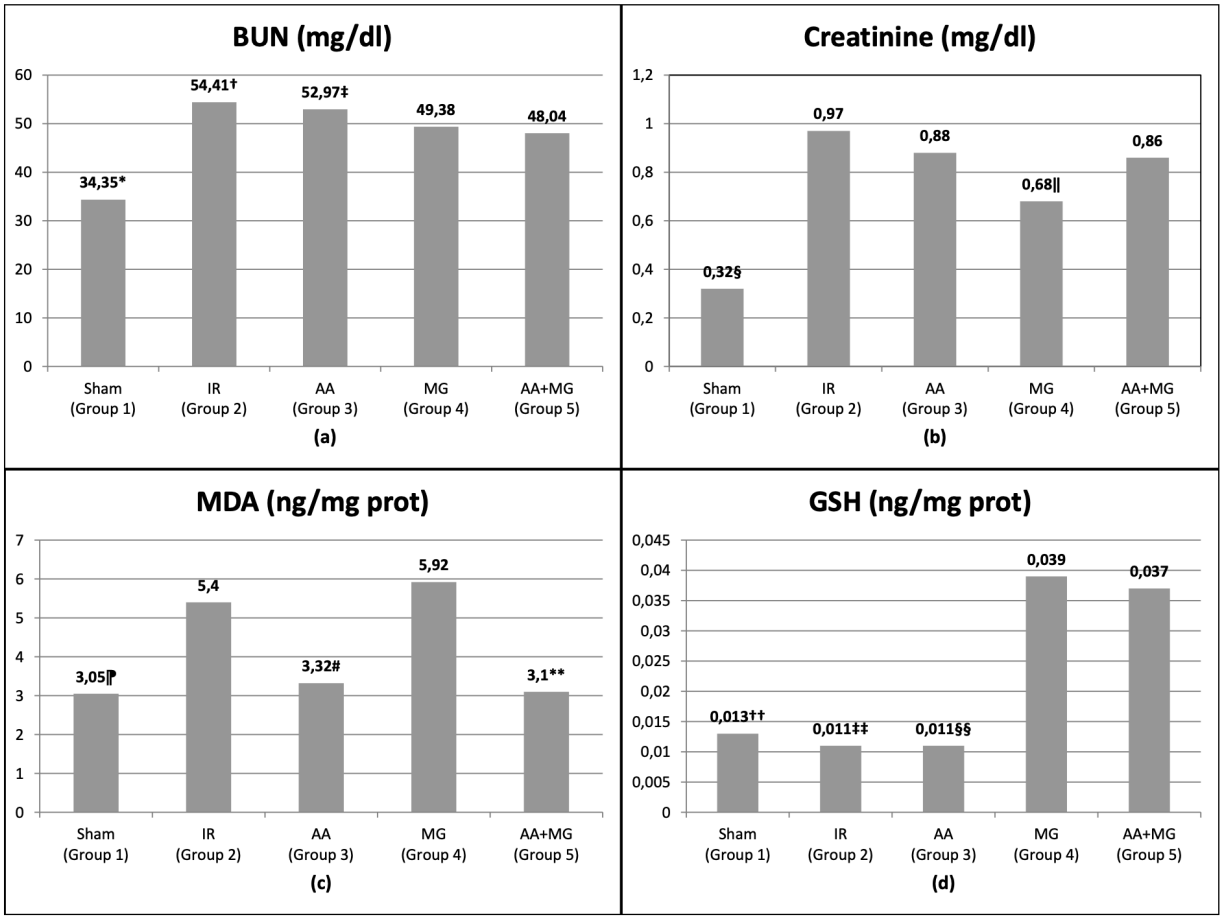

Figure 2. Comparisons between groups used the Kruskal-Wallis analysis, while in-group comparisons used the Mann-Whitney U test. (a) *significant difference compared to Group 2 (p: 0.009), Group3 (p: 0.006) and Group4 (p: 0.025); †significant difference compared to Group 5 (p: 0.021); ‡ significant difference compared to Group5 (p: 0.035). (b) § significant difference compared to Group 2, Group 3, Group 4 and Group 5(p: 0.002); ‖ significant difference compared to Group 2 (0.002) and Group 3 (0.035). (c) ⁋ significant difference compared to Group2 (p: 0.008) and Group 4 (p: 0.001); # significant difference compared to Group 2 (p: 0.034) and Group4 (p: 0.003); ** significant difference compared to Group 2(p: 0.014) and Group 4 (p: 0.001). (d) †† significant difference compared to Group 4 (p<0.001) and Group 5(p<0.001); ‡‡ significant difference compared to Group 4 (p<0.001) and Group 5(p<0.001); §§ significant difference compared to Group 4 (p<0.001) and Group 5(p<0.001).

Ascorbic Acid Group (AA, Group 3, n=7): Before laparotomy, 250 mg/kg ascorbic acid diluted with 0.9% NaCl to total volume 1 mL was administered through i.p route. Right and left renal pedicles opened after laparotomy (1 hour after medication administration), both pedicles placed in atraumatic clamp for 45 min ischemia then clamps opened for 240 min reperfusion.

Magnesium Group (MG, Group 4, n=7): Before laparotomy, 200 mg/kg magnesium sulfate diluted with 0.9% NaCl to total volume 1 mL was administered through i.p route. Right and left renal pedicles opened after laparotomy (1 hour after medication administration), both pedicles placed in atraumatic clamp for 45 min ischemia then clamps opened for 240 min reperfusion.

Ascorbic Acid + Magnesium Group (AA+MG, Group 5, n=7): Before laparotomy, 250 mg/kg ascorbic acid and 200 mg/kg magnesium sulfate diluted with 0.9% NaCl to total volume 1 mL was administered through i.p. route. Right and left renal pedicles opened after laparotomy (1 hour after medication administration), both pedicles placed in atraumatic clamp for 45 min ischemia then clamps opened for 240 min reperfusion.

Ascorbic acid dose (8-14) and magnesium dose (2,15-18) in this study were determined based on previous studies in the literature.

Table 1. Scoringmethod to be used in the histomorphological evaluation

Score	Indication
0	No change
1	Slight brush edge loss and no signs of necrosis
2	Medium brush edge loss and no signs of necrosis
3	Severe brush edge loss and no signs of necrosis

The detailed schematic appearance of the experimental model applied to the groups is presented in Figure 1.

Experimental Study Model

All rats were pinned to the operation table in the supine position after anesthesia and an abdominal midline incision was used. The right and left kidneys were opened and pedicles dissected. Rats were heated with a heating lamp on the operation table during the study to protect against hypothermia and body temperature was held to 37-37.5 ºC by measuring with a rectal probe. To prevent dehydration, a subcutaneous physiologic serum solution was administered with a 3 mL/kg dose per hour. During the waiting period, the abdomen was closed with damp sterile buffers and surgical forceps. Left and right renal total ischemia were induced by compressing the renal pedicles with an atraumatic clamp. Sufficient occlusion was observed with the formation of renal paleness. After the ischemia duration ended, clamps were free and reperfusion was allowed.

Rats in all groups had anesthesia administered and after laparotomy, right and left nephrectomy was performed for histopathologic investigation, all blood was taken for biochemical tests with a cardiac puncture. At the same time, the study was ended with this method, and rats were sacrificed by exsanguination.

Biochemical Assessment

To evaluate oxidative status, oxidative stress parameter MDA and an endogen antioxidant GSH were used. The right and left kidney samples were collected and stored at -80 °C. Homogenization was performed with tissue lyzer (For 0.1 g tissue to 1000 µl PBS). After centrifugation, the supernatants were collected for the assay. The protein concentrations of the samples were defined with BCA kit (Thermo Fisher Scientific, Inc.). MDA and GSH values were determined by using Elabscience MDA and GSH ELISA kits (Elabscience Biotech, China) according to the kit protocols. The results were given as MDA (ng/mg prot) and GSH (ng/mg prot). Assessment of these biochemical parameter kits was completed in DEUFM Medical Biochemistry Department. Serum blood urea nitrogen (BUN) (mg/dL) and creatinine (mg/dL) values in blood samples were determined in DEUFM Hospital Central Laboratory.

Histomorphologic Assessment of Renal Tissue

After the sacrifice of subjects, kidneys were sliced horizontally from the renal pelvis and placed in 10% formol solution for tissue fixation. After 48-hours of fixation, tissues had routine tissue monitoring procedures completed and were submerged in paraffin blocks. Tissues submerged in paraffin blocks had sections of 5 µm thickness taken and histologic immunohistologic staining was performed. For histologic investigations H&E and PAS staining were used, while TUNEL immunohistochemical staining was performed for apoptotic cell investigations.

H&E staining was used to assess general tissue morphology. PAS staining was used to investigate glomerular, tubular, interstitial, and vascular lesions. Five different standard randomized areas were chosen from the cortical region and investigated at x20 magnification with a light microscope. A total of 15 images were taken, with three images for each standard area. The scoring to be used in the assessment is specified in Table 1.

Preparates with TUNEL immunohistochemical staining were investigated. For the quantitative assessment of apoptotic cells, each section had 10 random areas with x20 magnification with nearly 200 cells counted. All histomorphologic analyses were performed by two histologists blind to the experimental groups.

Exclusion

It was planned that rats requiring resuscitation would be excluded from the study.

Table 2. Histomorphological Evaluation Score (values presented as mean ± SD)

	Group 1 Sham (n=7)	Group 2 IR (n=7)	Group 3 AA (n=7)	Group 4 MG (n=7)	Group 5 AA+MG (n=7)	p
Historical Damage Score	**0.20 ± 0.069***	**2.20 ± 0.106†**	**1.14 ± 0.117**	**1.05 ± 0.112**	**1.14 ± 0.124**	**<0.001**

* Significant difference compared to Group 2 (p<0.001), Group 3 (p<0.001), Group 4 (p<0.001) and Group 5 (p<0.001);† significant difference compared to Group 3 (p<0.001), Group 4 (p<0.001) and Group5 (p<0.001).

Statistical Assessment

The statistical assessment used the Statistical Package of Social Sciences 15 (SPSS 15.0, Chicago, IL, USA) program. Comparisons between groups used the Kruskal-Wallis analysis, while in-group comparisons used the Mann-Whitney U test. Values are presented as mean± standard deviation (mean ± SD). Values of p<0.05 were accepted as statistically significant.

RESULTS

This study completed in Dokuz Eylül University Multidisciplinary Experimental Animals Laboratory included a total of 35 male Wistar rats with a mean weight of 281.65 g (±15.77). There was no rat requiring resuscitation, and all rats completed the study.

Biochemical Results

The biochemical assessments for rats in all groups are presented in Figure 2.

Comparison of BUN in Group 1, Group 2, Group 3, Group 4, and Group 5 found the differences were statistically significant (p:0.003). The BUN values in Group 1 were determined to be significantly lower than Group 2 (p:0.009), Group 3 (p:0.006), and Group 4 (p:0.025). This result is interpreted as showing that IR injury was induced; hence, experimental conditions were provided. The BUN value in the Group 5 was found to be significantly low compared to both Group 2 (p:0.021) and Group 3 (p:0.035), leading to consideration that ascorbic acid and magnesium together were successful in lowering BUN values.

When assessed in terms of creatinine values, the differences between the Group 1, Group 2, Group 3, Group 4, and Group 5 were statistically significant (p<0.001). The creatinine value in Group 1 was determined to be significantly low compared to the other four experimental groups (p:0.002). This result may be interpreted as showing that IR injury was induced and experimental conditions provided, similar to the results obtained for BUN. In Group 4, creatinine values were lower compared to both Group 2 (p:0.002) and Group 3 (p:0.035). This may be interpreted as showing that magnesium may reduce the elevation in creatinine, at least at the dose and duration used in the experiment.

In terms of MDA values, the differences between the groups were found to be statistically significant when Group 1, Group 2, Group 3, Group 4, and Group 5 were compared (p<0.001). The MDA values in Group 3 were statistically significantly lower compared to Group 2 (p:0.034) and Group 4 (p:0.003). Similarly, the MDA values in Group 5 were statistically significantly low compared to Group 2 (p:0.014) and Group 4 (p<0.001). Both groups administered ascorbic acid had a fall in MDA values compared to the sham group leading to the consideration that ascorbic acid along is sufficient to lower MDA levels.

In terms of glutathione values, when Group 1, Group 2, Group 3, Group 4, and Group 5 are compared, there were statistically significant differences found between the groups (p<0.001). The significant differences identified between Group 4 and Group 5 compared with the other groups (p<0.001) are interpreted as showing that magnesium alone is beneficial to elevate GSH levels.,

Histomorphologic Finding

Histologic investigations analyzed tissue morphology, glomerular, tubular, interstitial, and vascular lesions with H&E and PAS staining. These assessments were performed and interpreted by two different experts blind to the groups. Both expert opinions were compatible with rats in all groups. Administration of ascorbic acid and magnesium separately and together was identified to have a preventive effect on IR injury in all samples.

The scores obtained as a result of the histomorphological evaluation of renal tissue are presented in Table 2.

Table 3. TUNEL count results (values presented as mean ± SD)

	Group 1 Sham (n=7)	Group 2 IR (n=7)	Group 3 AA (n=7)	Group 4 MG (n=7)	Group 5 AA+MG (n=7)	p
Apoptotic Cell Count	**29.28 ± 4.49***	**96.85 ± 12.4†**	**65.14 ± 29.72**	**57.28 ± 11.04**	**63.28 ± 20.53**	**<0.001**

*significant difference compared to Group 2 (p: 0.002), Group 3 (p: 0.04), Group 4 (p: 0.002) and Group 5 (p: 0.007); † significant difference compared to Group 3 (p: 0.002), Group 4 (p: 0.002) and Group 5 (p: 0.005).

When renal sections belonging to the Group 1 are evaluated, the fibrous capsule was intact outside the cortex, and the renal corpuscles in the cortex had normal structure. The proximal tubules, distal tubules, and collector tubules were observed to have normal structure. The characteristic structure and features of epithelial cells were preserved with normal structure observed for glomeruli.

When renal sections from Group 2 are investigated, the widening of cortical veins was observed. In the cortical region, there was more mononuclear cell infiltration in the peritubular area, brush-like edge loss in proximal tubule cells, tubular atrophy, and tubular dilatation. Especially in the corticomedullar junction, peritubular erythrocyte extravasation was observed.

When sections from Group 3 are investigated, mononuclear cell infiltration in the cortical peritubular area, degeneration observed in tubule cells, and erythrocyte extravasation in the cortex was clearly reduced compared to Group 2. Additionally, the brush-like edge loss observed in tubules, tubular atrophy, and tubular dilatation was observed at lower rates in the ascorbic acid.

When sections belonging to Group 4 are investigated, a clear reduction was observed in mononuclear cell infiltration in the cortical peritubular area, degeneration observed in tubule cells, and erythrocyte extravasation in the cortex compared to the IR group. Additionally, the brush-like edge loss observed in tubules, tubular atrophy, and tubular dilatation was observed at lower rates in the magnesium group.

When sections from Group 5 are investigated, the variations forming as a result of separate administrations were not encountered in the preparate structure with different features. The administration together was not observed to be superior to administration separately.

PAS staining observed villus loss in the Group 2 group. Especially in medullar cells, vacuolization and PAS+ intense areas were observed. Though there were clear improvements in Group 3, Group 4 and Group 5 villi did not recover, and the presence of PAS+ accumulation in the medulla was preserved. Occasional reductions were observed in vacuolization in medullar cells.

According to the TUNEL immunohistochemical staining results obtained from renal tissue investigation, the mean apoptotic cell counts are presented in Table 3.

When mean apoptotic cell counts according to TUNEL staining are compared, there were statistically significant differences between Group 1, Group 2, Group 3, Group 4, and Group 5 ($p<0.001$). The mean cell counts in Group 1 were determined to be significantly low compared to Group 2 (p:0.002), Group 3 (p:0.04), Group 4 (p:0.002), and Group 5 (p:0.007). This result is interpreted to show that IR injury was induced and experimental conditions created.

The mean apoptotic cell counts in Group 3 (p:0.02), Group 4 (p:0.002), and Group 5 (p:0.005) groups were identified to be significantly low compared to Group 2. This leads to the consideration that the ascorbic acid and magnesium administration reduced apoptotic cell counts by similar degrees.

DISCUSSION

In this experimental study evaluating the efficacy of ascorbic acid and magnesium administered separately and together before ischemia in a rat renal IR model, both agents were identified to positively change biochemical parameters, histomorphologic and immunohistologic outcomes.

Renal IR injury may be induced experimentally with two different methods. One of these is unilateral nephrectomy and clamping of the contralateral renal artery or renal pedicle and the other is bilateral clamping of the renal pedicle or renal arteries. The most well-defined method identified uses temporary closure of only the renal artery or the renal artery and vein together. Results obtained from studies suggest

that clamping of the renal artery and veins together is the ideal method to induce ischemia injury (24,25). In this study, bilateral renal ischemia was induced with atraumatic microvascular clamps. Observation of paleness in the kidney when perfusion is stopped and disappearance of arterial pulse confirm ischemia, similarly when blood perfusion is begun again after opening the clamps at the end of the ischemia duration, physical examination determined arterial pulse in the renal pedicles and change in a pale color. The ischemia-reperfusion duration is stated to be as important as the renal ischemia method. Reference scans identified that when different durations are used to induce IR injury, variations may occur according to organ and the duration. The critical ischemia duration is linked to the organ; with more than 5 min ischemia of the brain causing notable degrees of neuron death and infarctus, while this duration is reported as 15-20 minutes for the liver and kidneys(11). The ischemia duration in kidneys is generally limited to 30 to 60 minutes. A renal ischemia duration of more than sixty minutes is proposed to cause acute tubular necrosis and renal failure. If the renal ischemia duration is shorter than 30 minutes, the rapid proliferation of tubular epithelial cells may repair injured renal tubules and may be accompanied by improvement in renal functions (26-28). Different data were obtained about the duration necessary to cause injury to the kidney with ischemia (29-36). In this study, a 45-min ischemia duration was applied in light of the information obtained from the literature.

Williams et al. researched renal IR injury effects with reperfusion lasting 0, 0.5, 1, 2, 4, 6, 9, and 24 hours and 1 week after 45 minutes of ischemia in blood and tissue samples (28). According to these researchers, the effects of renal IR injury begin in the 4th hour at the earliest and peak in the 24th hour. Cochrane et al. stated that renal injury after reperfusion was observed in the 4th hour at the earliest (37). In accordance with this data, a 4-hour reperfusion duration was chosen in the experimental model in our study.

The histomorphologic, immunohistologic, and biochemical data obtained in our study displayed a significant difference in the IR group compared to the sham group showing the chosen ischemia and reperfusion durations were sufficient to induce injury and confirm the IR model was correctly applied.

In the literature, there are studies of protective pharmacologic agents used separately and together for rat renal IR injury. However, the agents researched to prevent renal IR injury and the studies have no definite standards. Partial positive effects on IR injury were researched for vitamin E(9), hydrocortisone(9), L-arginine(10), atorvastatin(19), tadalafil(20), monoaminoxidase inhibitors(21), dexmedetomidine(22), and mannitol(23); but there was no study encountered administering ascorbic acid and magnesium together to prevent renal IR injury.

Determination of the excessive production of reactive oxygen species (ROS) and the role of antioxidant enzymes in the pathogenesis of mitochondrial injury induced as a result of IR brought antioxidant and free radical scavenger treatments to the agenda. With the aim of preventing or treating renal IR injury, one of the methods applied to ascorbic acid was shown to have positive effects on IR injury in many previous studies. Seo et al. in studies assessing a hepatic ischemia-reperfusion injury in rats administered 30 mg/kg, 100 mg/kg, 300 mg/kg, and 1000 mg/kg doses to four different groups 5 minutes before ischemia (14). While antioxidant efficacy was shown at low doses, the administration of high doses showed prooxidant effects. In this study, the ascorbic acid dose was chosen as 250 mg/kg.

Mohamed et al. performed a study researching the protective role of vitamin C and L-arginine in a rat renal IR model (10). In this study with 30 min ischemia and 30 min reperfusion duration planned, 3 groups were created with one administered 500 mg/kg i.p. vitamin C 24 hours before ischemia, one group administered 400 mg/kg i.p. L-arginine for 3 days before ischemia and the final group administered both together. The groups administered vitamin C and both medications had BUN, creatinine, and MDA measurements which showed a protective effect against renal IR injury. Ji et al. studied the same model to identify the acceptable durations as renal ischemia markers (38). With this aim, they determined serum BUN and creatinine values in blood samples taken at 0, 6, 12, 24, 48, and 72 hours and concluded the increase in the 24th hour was significant. Shokeir et al. in a study examining BUN and creatinine values for renal injury in the 2nd, 24th, and 48th hours, identified that the BUN and creatinine reached the highest values atthe 24th hour and had fallen at the 48th hour (6). In our study, a significant fall was not identified in BUN and creatinine values in the groups administered ascorbic acid which was concluded to be due to this study not waiting for the 24-hour duration as in these studies.

The results for BUN and creatinine in Figure 2 demonstrate statistically significant changes across groups. However, to assess the clinical importance of these findings, it is critical to compare these levels to baseline healthy renal function and consider their implications in the context of IR injury.

BUN and creatinine are key markers for renal function, with elevated levels often indicating impaired kidney function due to reduced filtration capacity. In this study, BUN and creatinine levels were significantly elevated in the IR group compared to the sham group, confirming the establishment of renal ischemia-reperfusion injury. Importantly, the reductions observed in BUN and creatinine levels in the groups treated with magnesium sulfate and ascorbic acid—especially when used in combination—indicate a potential protective effect against IR injury.

Clinically, even modest reductions in BUN and creatinine levels can be meaningful as they suggest a lower degree of renal injury and better preservation of renal function. Previous studies have shown that BUN and creatinine levels are highly sensitive indicators of renal damage and recovery post-IR injury. For instance, Ji et al. observed significant elevations in these markers at 24 hours post-ischemia, correlating with peak renal damage (38). Similarly, Shokeir et al. noted that BUN and creatinine peaked at 24 hours and began to decline by 48 hours, aligning with partial recovery of renal function (6). Although the reperfusion time in this study was limited to 4 hours, the significant reductions in BUN and creatinine in treated groups indicate early signs of renal protection.

The reduction in creatinine levels in the magnesium group suggests that magnesium may play a role in mitigating renal IR injury by stabilizing mitochondrial function and reducing calcium overload, mechanisms that are known to contribute to acute renal failure during IR injury (16,39). Furthermore, the combination of ascorbic acid and magnesium appeared to enhance the protective effects, possibly through synergistic antioxidant and anti-inflammatory actions. This combination may optimize the mitigation of oxidative stress and apoptosis, key contributors to IR-induced renal damage.

These findings align with those of Akan et al., who demonstrated that magnesium sulfate significantly reduced BUN and creatinine levels in a diabetic rat renal IR model (2). Additionally, the observed reductions are consistent with the hypothesis that magnesium can act as a cofactor for enzymatic processes, including glutathione synthesis, contributing to antioxidant defense mechanisms (45). In conclusion, the changes in BUN and creatinine observed in this study are both statistically and biologically significant, providing evidence of the potential renal protective effects of ascorbic acid and magnesium, either alone or in combination, in the early stages of renal IR injury.

Ergin et al. in studies researching the effect of ascorbic acid in an IR injury model in rats administered 100 mg/kg bolus ascorbic acid 15 minutes before ischemia and then 50 mg/kg/hr dose as an infusion in the 2-hour reperfusion period (8). They showed ascorbic acid had a reducing effect on IR injury in tissue MDA levels. Similarly, in our study, an increase in MDA levels was identified in Group 2 in MDA results from renal tissue samples and this data is interpreted as showing IR injury increased lipid peroxidation. The administered ascorbic acid lowered MDA levels, while magnesium administration was not identified to have any effect. In the group with ascorbic acid and magnesium administered together, the reduction in MDA levels was similar to Group 3 confirming that the MDA reduction was linked to ascorbic acid. A study by Korkmaz et al. with the same ischemia duration as this study identified a significant fall in GSH values in the IR group after 3 hours of reperfusion (13). Contrary to the similar ischemia duration, Zhu et al. applied 48 hours of reperfusion and stated the GSH values fell in the IR group (12). Contrary to different reperfusion durations, contrary to these studies where GSH values were identified to fall, in the IR group in our study a fall in GSH values was not observed. A possible reason for this is that the oxidative stress induced in the model was within the compensatory capacity of GSH. Azari et al. created four groups in a study researching the effects of hydrocortisone, vitamin C, and vitamin E against renal IR damage (9). They administered 50 mg/kg vitamin C intravenous, 20 mg/kg vitamin E intramuscular, 50 mg/kg hydrocortisone intravenous and the final group had all three agents administered immediately before reperfusion. The histologic assessment used a score formulated according to histomorphologic data based on tubular degeneration, necrosis, and degree of inflammation in tissue, and the group with combination treatment was shown to have IR injury significantly reduced in renal tissue. In our study, sections stained with H&E and PAS were assessed

by two expert histologists. After histomorphologic investigation, ascorbic acid and magnesium were shown to have a preventive effect against IR injury.

Previous studies have demonstrated that magnesium impacts ion channels (inhibits calcium ions) and inhibit NMDA glutamate receptors, thereby preventing sustained stimulation of these receptors. Also magnesium inhibits enzyme activity, regulates substance metabolism, and regulates excitability of cholinergic neurons by acting on hormone receptors. It has similar effects to adrenocortical hormones, and presents obvious anti inflammatory and immunoregulatory effects (39,40). MDA and GSH levels are critical biomarkers for assessing oxidative stress and the antioxidant defense system. In this study, the observed increase in MDA levels in the IR group confirms the occurrence of lipid peroxidation, a hallmark of oxidative stress in renal ischemia-reperfusion injury. Conversely, the administration of ascorbic acid and magnesium sulfate, both separately and in combination, resulted in significant reductions in MDA levels and increases in GSH levels, highlighting their potential as protective agents.

The relationship between increased MDA and GSH levels is pivotal in understanding the oxidative stress mechanisms at play. MDA is a byproduct of lipid peroxidation, and its reduction indicates decreased oxidative damage to cellular membranes. On the other hand, GSH is a critical endogenous antioxidant, and its elevated levels in the groups treated with magnesium alone or in combination with ascorbic acid suggest enhanced antioxidant defense. Magnesium may contribute to GSH synthesis by acting as a cofactor for glutathione synthetase, as reported in the literature (45). This finding aligns with previous studies demonstrating magnesium's role in improving oxidative stress parameters via direct and indirect mechanisms (16,39).

The combined administration of ascorbic acid and magnesium led to the most pronounced reductions in MDA and increases in GSH, suggesting potential additive or synergistic effects. While ascorbic acid primarily acts as a direct scavenger of ROS, neutralizing free radicals and preventing lipid peroxidation, magnesium contributes by stabilizing cellular ion balance and enhancing enzymatic antioxidant defenses. The dual mechanisms may provide complementary protection against oxidative stress, resulting in a more effective mitigation of renal IR injury. These findings are consistent with previous studies indicating that combining antioxidants and ion channel modulators can produce synergistic protective effects in oxidative stress models (2,39).

Future research should focus on delineating the precise molecular pathways underlying this synergistic interaction, including the modulation of signaling pathways like nuclear factor erythroid 2-related factor 2 (Nrf2), which regulates the expression of antioxidant enzymes. Nonetheless, the current data strongly support the hypothesis that the combination of ascorbic acid and magnesium sulfate offers enhanced protection against renal IR injury through complementary antioxidant mechanisms.

The 200 mg/kg magnesium dose chosen in this study was selected from doses recommended in the literature (2,8,39). A study by Akan et al. researching the protective effects of magnesium sulfate in a diabetic rat renal IR injury model administered 20 mg/kg i.p. magnesium sulfate 5 minutes before ischemia and used BUN, creatinine values, and histomorphologic scoring for identification of bilateral renal IR injury (2). The data from this study, where magnesium was identified to induce significant variations in all three parameters, were assessed as showing reduced acute renal injury. In our study, magnesium was identified to cause a significant fall in creatinine values compared to Group 2. Results from H&E, PAS, and TUNEL immune staining indicated a significant reduction was caused in IR injury. The TUNEL test ensuring in situ recognition of DNA cleavage for identification of apoptotic cells in renal tissue in rats is a marker accepted by the majority of histologists (42). Yoon Kyung et al. proposed TUNEL staining showed apoptosis to assess mean apoptotic cell counts in an induced renal IR model (43). However, Yang et al. compared by calculating the apoptotic index instead of mean counts when assessing apoptotic cells in a renal IR model (44). In this study, the assessment was performed taking the mean counts proposed by Yoon Kyung et al. and a clear increase in TUNEL positive cells was observed in Group 2 compared to Group 1. Administration of ascorbic acid and magnesium had a significant reduction in apoptotic cell counts compared to Group 2. This significant reduction identified in apoptosis indicates that the important pathway in IR injury of apoptosis may be prevented by administering magnesium and/or ascorbic acid. Pundir et al. in studies researching the effects of NMDA receptor agonists and antagonists on acute renal injury in rats with bilateral renal IR injury model induced and

administered 600 mg/kg i.p. magnesium sulfate 1 hour before ischemia and for the previous 4 days (total 5 days). The researchers identified significant reductions in creatinine and GSH values in the study and showed magnesium reduced acute renal injury. In our study, creatinine values in Group 4 were significantly reduced compared to Group 2, and kidneys were protected from IR injury. In our study, increases in GSH levels were identified in Group 4 and Group 5, with no change in GSH in the group administered ascorbic acid. The increase in GSH in Group 4 and Group 5 leads to the consideration that GSH increased linked to magnesium and this may be linked to magnesium being a cofactor for GSH synthase, a key enzyme for GSH synthesis (45).

CONCLUSION

In this study, we evaluated the effects of 250 mg/kg ascorbic acid and 200 mg/kg magnesium sulfate on the IR model induced in rats by tissue MDA and GSH, serum BUN and creatinine measurements, and histomorphologic and immunohistologic investigation results.

Administration of 250 mg/kg ascorbic acid and 200 mg/kg magnesium separately and together before ischemia in a rat renal IR model was identified to reduce IR injury.

The administration of ascorbic acid and magnesium sulfate before ischemia to prevent IR injury is considered a simple, noninvasive, and reliable method.

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RESEARCH ARTICLE

HEALTHY LIFESTYLE BEHAVIORS OF UNIVERSITY STUDENTS: THE ROLE OF SENSE OF COHERENCE AND FAMILY HEALTH CLIMATE

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ABSTRACT

Purpose: This study aims to examine the predictive role of individual sense of coherence, family sense of coherence and family health climate variables on university students' healthy lifestyle behaviors.

Material and Methods: The sample of the study consisted of 371 university students aged 18-25. Sociodemographic Information Form, Healthy Lifestyle Behaviors Scale, Sense of Coherence Scale, Family Sense of Coherence Scale, Family Health Climate Scale were applied to the participants in order to collect the research data. Correlation analysis, independent two-sample t-test, one-way ANOVA test and multiple linear regression analysis were used to analyze the data.

Results: According to the results of correlation analysis, a positive relationship was found between healthy lifestyle behaviors and individual sense of coherence, family sense of coherence and family health climate ($p<.05$). As a result of the multiple linear hierarchical regression analysis, after controlling for the sex variable, individual sense of coherence and family health climate variables significantly predicted healthy lifestyle behaviors ($p<.05$), while family sense of coherence had no significant predictive role on healthy lifestyle behaviors ($p>.05$).

Conclusion: The findings show that individual sense of coherence, family sense of coherence and family health climate variables are essential on university students' healthy lifestyle behaviors. The sense of coherence provides significant protection in adopting health behaviors that will determine future health and well-being. Similarly, increasing healthy living practices within the family is of great importance for young people to adopt healthy lifestyle behaviors.

Keywords: healthy lifestyle behaviors, ındividual sense of coherence, family sense of coherence, family health climate

INTRODUCTION

The World Health Organization (WHO) characterizes health as "a condition of holistic well-being encompassing physical, mental, and social aspects, rather than just the absence of disease or infirmity" (1). For overall well-being, there is a need for everyone in the society to take responsibility for protecting, developing and managing their own health (2). Healthy lifestyle behaviors, described as selection and implementation of behaviors to improve

health in daily activities, have a critical place in the development and maintenance of health (3). Health responsibility, exercise, nutrition, self-actualization, interpersonal support, stress management are accepted as healthy lifestyle behaviors (4).

The foundation for adopting healthy lifestyle behaviors is laid during one's youth (5). Failure to develop healthy lifestyle behaviors among young people has the potential to lead to inadequacies in nutrition, exercise, and stress management, affecting both individual and societal well-being. The health status of young people is essential not only for their future physical and psychological well-being, but also for societal development (6).

The 18-25 age period, referred as young adulthood, is characterized as a period in which major changes occur in lifestyles with the transition to university, parental control decreases, individual autonomy increases, and the person begins to take responsibility for health (7). Considering the potential of the university period to be a productive period in terms of gaining healthy lifestyle behaviors (2), it is crucial to determine the behaviors for the protection and improvement of students' health. Based on all these, the current study aims to focus on the healthy lifestyle behaviors of university students.

The sense of individual coherence refers to the level of perceiving the world and life as understandable, manageable and meaningful (8). This perception enables the person to manage tension by allowing them to identify, evaluate and mobilize their internal and external resources. Research shows that a high sense of coherence is associated with positive health outcomes (9-10). Sense of coherence is a vital resource for both preventive health behaviors against risky behaviors and preventive health behaviors for health promotion and maintenance (11-12). Therefore, it is thought that individual sense of coherence should be taken into consideration when evaluating the health behaviors of university students. However, healthy life behaviors are shaped not only individually but also within a social context, for example, by family, school or peer groups. In particular, the family environment is known to be critical for health development and has a direct influence on health behaviors (13). Family coherence is defined as the cognitive map that enables the family to cope with stress throughout its life (14). Galette et al. (2019) propose that the sense of coherence is an orientation that strengthens individuals' resilience to the stressors encountered in daily life (9). . Individuals exhibiting healthy behaviors in terms of nutrition, physical activity, sleep, smoking and alcohol use have a high family sense coherence (15-16). Hence, aside from an individual's sense of coherence, the impact of family sense of coherence on health behaviors appears to be significant.

The family environment is the first place where health protection and development behaviors are learned. The impact of family members' interactions, attitudes, values, and changes within the family on the individual is undeniable (15). A positive health climate within the family, as well as a sense of family coherence, affects the individual's health behaviors (17). The interdependent dynamics among family members influence the physical activity and eating behaviors of individuals, collectively referred to as the "family health climate" (18). Support among family members, family meals, joint physical activities, communication on health issues, and availability of healthy foods at home are indicators of a positive family health climate (19). The physical activity level of family members is associated with the physical activity levels of university students (12). Parents' attitudes towards nutrition affect the eating behaviors that children will develop in their future lives (20). Therefore, family health climate shapes the daily health habits of family members inside and outside the family (19).

Considering all these factors, it can be said that individual sense of coherence, family sense of coherence and family health climate are factors that begin to develop in childhood in a family environment and shape the individual’s health behaviors (8, 16-17). One significant aspect influenced by these variables is healthy lifestyle behaviors. Studies in the literature reveal the relationship between individual sense of coherence and healthy lifestyle behaviors, and family sense of coherence. This research aims to investigate whether family sense of coherence, individual sense of coherence and family health climate variables together significantly predict healthy lifestyle behaviors.

MATERIAL AND METHODS

Ethical Considerations

Ethics committee approval of the study was obtained from FMV Işık University Ethics Committee with the decision dated 30.03.2023 and numbered 2023/04. All individuals participating in the study provided consent. Additionally, written permission from the scale owners was obtained for the use of the scales.

Table 1. Demographic Variables

Baseline Characteristics	***N:371***	**%**
Sex		
Women	275	74
Men	96	26
Grade level		
Prep class	25	7
1st grade	89	24
2nd grade	85	23
3rd grade	90	24
4th grade	74	20
5th grade	3	1
Graduate	5	1
The people they live together		
Dormitory	81	22
Friend	20	5
Alone	30	8
Family (mother/father/sibling)	233	63
Romantic partner	4	1
Spouse/child	3	1
Socio-economic level		
Low	52	14
Middle	216	58
High	103	28
Smoking		
Yes	222	60
No	149	40
Psychiatric diagnosis status		
Yes	45	12
No	326	88
Psychiatric medication use		
Yes	18	5
No	353	95

Study Design and Sample

The study was conducted between June-July 2023 with a cross-sectional and correlational design. G Power software was used to determine the sample size of the study. Based on “multiple linear regression modeling”, when the effect size is 0.15 (medium) and alpha level is 0.05, at least 89 participants are required to reach 0.95 power (21).The population of the study consisted of university students studying in 22 different cities with smaller populations (ranging from 0.2% to 0.8%), mostly in Istanbul (57%) and larger cities such as Ankara (24%), Trabzon (5%), Izmir (2%), Samsun (1%), Eskişehir (1%). Within the scope of the study, convenience sampling method was preferred and a total of 433 participants from different regions of Turkey, undergraduate and graduate students from different faculties and departments were reached. In order to increase generalizability; students from various departments such as psychology, engineering, business administration, communication, management information systems, advertising, chemistry, physics, dentistry, nursing, cookery, architecture, literature,

Table 2. Comparison of healthy lifestyle behaviors according to sociodemographic variables

		Healthy Lifestyle Behaviors			*t/F*	*p*
		N:371	***Mean***	***SD***		
	Women	275	137.35	21.17		
Sex	***Men***	96	143.33	24.33	-2.290	.023
Smoking	***Yes***	222	140.36	20.83		
	No	249	136.71	23.90	1.516	.131
The people they live	***Family***	233	139.78	22.93		
together	***Other***	138	137.41	20.75	.995	.131
Socio-economic level	***Low***	52	136.44	3.05		
	Middle	216	137.87	1.44		
	High	103	142.30	2.34	1.774	.171

political science, nutrition and dietetics participated in the study.

The inclusion criteria were being between the ages of 18-25 (young adulthood) and being a university student. A “leave this question blank” question was added at certain intervals to test whether the respondents answered the questionnaire carefully. The exclusion criterion is answering attention questions that should be left blank. After the eliminations, the study was conducted with 371 participants.

Data Collection

The data collection process was carried out online via Google Forms based on the principle of volunteerism. The administration of the scales took approximately 10 minutes.

Sociodemographic Information Form

The researchers gathered demographic information of the participants, including sex (22), age (7), faculty and department (23), through a prepared form.

Healthy Lifestyle Behaviour Scale II (HLBS II)

This scale was developed by Walker et al. (1987) and revised in 1996 (4). The Turkish adaptation of the scale was conducted by Bahar et al. (2008) (24). The scale aims to measure the health behaviors developed by the individual related to healthy lifestyle. The scale consists of 52 items in total and the rating is on a 4-point Likert scale. The lowest score that can be obtained from this scale is 52 and the highest score is 208. In this study, Cronbach's Alpha reliability coefficient of the HLBS II was determined as .92.

The Revised Sense of Coherence Scale (SOC-R)

The SOC-R was developed by Antonovsky (1993) and revised by Bachem and Maercker (2018) (25-26). The adaptation study of the scale into Turkish was conducted by Tekin and Kırlıoğlu (2019) (27). The scale aims to measure assess one's capacity to view life events as interconnected and to maintain a balance between positive and negative evaluations of life experiences. The SOC-R consists of 13 questions and the rating is on a 5-point Likert scale. The lowest score that can be obtained from this scale is 13 and the highest score is 65. In this study, Cronbach's

Table 3. Correlation between healthy lifestyle behaviors and other variables

Variables	**1**	**2**	**3**	**4**
1- HLBS[1]	1			
2-SOC-R[2]	.442**	1		
3-FSOC-S[3]	.436**	.265**	1	
4-FHC-PA[4]	.535**	.266**	.514**	1
5-FHC-NU[5]	.517**	.275**	.581**	.595**

*p<.01; **p<.001. *Note: HLBS1: Healthy Life Style Behaviour Scale; SOC-R 2: Sense of Coherence Scale-Revised, FSOC-S3: Family Sense of Coherence Scale, FHC-PA4: Family Health Climate-Physical Activity, FHC-NU 5: Family Health Climate-Nutrition*

Table 4. Stepwise multiple linear hierarchical regression analysis results on the predictive effects of independent variables on healthy lifestyle behaviors

Variables	B	S.E.	*B*	t	R	R^2	ΔR^2
Constant	137.353	1.328	-	103.406**	-	-	-
Sex	5.981	2.6111	.118	2.290*	-	-	-
Constant	46.743	7.180		6.510**			-
Sex	.864	2.039	.017	.424	.118[a]	.014	.011
FHC-PA[1]	.511	.083	.305	6.129**	.539[b]	.291	.378
SOC-R[2]	1.004	.147	.288	6.823**	.619[c]	.383	.287
FHC-NU[3]	.399	.078	.255	5.111**	.652[d]	.425	.418

*p<.05; **p<.01. FHC-PA[1]: Family Health Climate-Physical Activity, SOC-R[2]: Sense of Coherence Scale Revised, FHC-NU[3]: Family Health Climate-Nutrition. [a] Predictors: (Constant), Sex. [b] Predictors: (Constant), Sex, FHC-PA. [c] Predictors: (Constant), Sex, FHC-PA, SOC-R. [d] Predictors: (Constant), Sex, FHC-PA, SOC-R, FHC-NU

Alpha reliability coefficient of the SOC-R was determined as .72.

Family Sense of Coherence Scale- Short Form (FSOC-S)

The original FSOC-S was developed by Antonovsky and Sourani (1998) and its short form was created by Sagy (1998) (28-29). The adaptation of the scale into Turkish, validity and reliability study was conducted by Çeçen (2007) (30). The scale measures the sense of coherence that individuals perceive towards their families. The scale consists of 12 questions the rating is on a 7-point Likert scale. The lowest score that can be obtained from this scale is 12 and the highest score is 84. In this study, Cronbach Alpha reliability coefficient of the FSOC-S was determined as .86.

Family Health Climate Scale (FHC-scale)

The original FHC-scale was developed by Niermann et al. (2014) (18). The adaptation study of the scale into Turkish was conducted by Güney et al. (2021) (31). The scale aims to assess the effect of family environment on physical activity and nutrition behaviors. FHC-scale consists of two integrated scales, the Physical Activity Scale (FHC-PA) and the Nutrition Scale (FHC-NU). The scales are 5-point Likert type. The FHC-PA consists of 14 items whereas the FHC-NU consists of 17 items. The lowest and highest scores that can be obtained from the scales are 0-42 and 0-51 for FHC-PA and FHC-NU, respectively. In this study, Cronbach's Alpha reliability coefficient was determined as .94 for FHC-PA and .93 for FHC-NU.

Statistical Analysis

The collected data were evaluated with the SPSS program (version 20.0, IBM Corporation). All scale scores were normally distributed within the ±1 range and demographic data were based on descriptive statistics (percentage and mean). Statistical analysis was conducted using correlation analysis, independent two-sample t-test, one-way ANOVA testFollowing the correlation analysis, multiple linear hierarchical regression analysis was conducted to examine the effect of sense of coherence, family sense of coherence and family health climate on healthy lifestyle behaviors.

RESULTS

The participants' age range was 18-25 and the mean age was 21.22 (SD=.43). 275 (74.1%) of the participants were female and 96 (25.9%) were male. The majority of the participants have a middle socio-economic level (58.2%) and the majority of them live with their families (62.8%) Other demographic characteristics of the participants are given in Table 1.

Independent two-sample t-test and one-way ANOVA test were executed to determine which demographic characteristics differentiate healthy lifestyle behaviors according to the groups. According to the results of the independent two-sample t-test, sex makes a significant difference on healthy lifestyle behaviors (t(269)=-2.290, p<.05). The level of healthy lifestyle behaviors of men (Mean=143.33, SD=24.33) is higher than the level of healthy lifestyle behaviors of women (Mean=143.33, SD=24.33). However, socio-

economic level (F(2.368)=1.1774, p>.05), the people they live together (t(.369)=.995, p>.05) and smoking (t(287.360)=1.516, p>.05) had no significant effect on healthy lifestyle behaviors. (Table 2)

The findings of Pearson Correlation Analysis conducted to test the direction and strength of the relationship between healthy lifestyle behaviors and individual sense of coherence, family sense of coherence and family health climate are presented in Table 3.

Multiple linear hierarchical regression analysis was conducted to examine the effect of individual sense of coherence, family sense of coherence and family health climate on healthy lifestyle behaviors. In this analysis, sex was transformed into a dummy variable and included in the model as a control variable. Based on the analysis results, individual sense of coherence and family health climate had a significant effect on healthy lifestyle behaviors. However, no significant effect of family sense of coherence was observed in the model (F(4.366)=68,722, p<.001). All variables explained 42.3% of healthy lifestyle behaviors (Table 4).

DISCUSSION

This study examined the effect of individual sense of coherence, family sense of coherence and family health climate on healthy lifestyle behaviors of university students, and it was found that individual sense of coherence and family health climate predicted healthy lifestyle behaviors when all variables were together.

In this study, differences between sexes were evaluated and men were found to perform better in healthy lifestyle behaviors. The literature reveals that, in studies conducted on university students, females generally achieve higher scores than males in overall healthy lifestyle behaviors (7, 22). However, males perform better in healthy lifestyle behaviors such as physical activity, stress management and sleep (22, 32).. Kargun et al. (2013) found that male students achieved higher scores in the sub-dimensions of spiritual development, exercise and stress management, while females scored higher in the sub-dimensions of health responsibility, interpersonal relationships and nutrition (33). The analysis shows that sex differences emerge in the subdimensions of healthy lifestyle behaviors.

Considering the relationship among the variables, it was observed that as the individual's sense of coherence increased, healthy lifestyle behaviors also increased. Sense of coherence is related with university students' pro-health behavior tendencies such as actively using their free time, coping better with stress, and paying attention to sleep patterns (23). Several studies have found that a sense of individual coherence is associated with physical activity, interpersonal relationships, stress, and healthy eating in young people (34-35). The results obtained in this study align with existing literature. As a person's sense of coherence improves, they tend to adopt healthier habits. Sense of coherence has a particularly important impact on the health-related behaviors of university students. These findings suggest that interventions to improve the health behaviors of university students may benefit from focusing on strengthening their sense of coherence.

In this study, it was found that as the family health climate increased, healthy lifestyle behaviors also increased. The family health climate functions as a framework for an individual's daily health behavior and forms the basis of health-related regulation (18). Studies indicate that family health climate increases the tendency to exhibit pro-health behaviors in both physical activity and nutrition dimensions (12, 20). Furthermore, engaging in physical activities, sharing family meals, and collectively making food choices within the family environment significantly influence the health behaviors of individuals in their future lives (16). From that point of view, it can be asserted that parents who integrate healthy lifestyle behaviors into their daily routines will effectively serve as role models for their children. This could enhance the probability of individuals adopting healthy lifestyle behaviors.

In this study, it was observed that as the sense of family coherence increased, healthy lifestyle behaviors increased as well as individual sense of coherence. Previous studies also show that family coherence is associated with positive health behaviors (15-16, 36). A study conducted with adolescents reported that parental control and family coherence were positively associated with children eating breakfast and consuming healthy foods, but negatively associated with buying their own food and consuming unhealthy foods (37). Accordingly, it can be said that family coherence increases the tendency to embrace health-conscious lifestyle habits.

Healthy lifestyle behaviors are shaped not by a single factor but by the interaction of individual and family dynamics (17). Within the scope of this study, after controlling for sex, it was found that individual sense

of coherence and family health climate significantly predicted healthy lifestyle behaviors, while family sense of coherence did not have significant predictive power. Moksnes et al. (2012) found that the sense of individual coherence predicted adolescents' emotional health by controlling the sex variable in line with our research findings (38). In a study, it is indicated that the sense of individual coherence affects smoking habits and eating behaviors in young people (11). These findings also reveal that the sense of personal coherence enables individuals to exhibit health-promoting behaviors by mobilizing their internal and external resources (10).

In the current study, the predictive power of family health climate on healthy life behaviors was revealed. The literature supports our current finding and suggests that support for physical activity in the family environment predicts physical activity (39), physically active parents enable children to do more sports (40), and family health climate predicts physical activity and nutritious dietary practices (17). The family health climate triggers the intrinsic motivation which facilitates the maintenance of healthy lifestyle behaviors (17). Also, parental support and parental role modeling improve healthy life behaviors by providing self-efficacy (39). All these findings indicate that exposure to a positive family health climate at an early age increases the predisposition to adopt healthy life behaviors in adulthood.

Another of the current research findings is that the sense of family coherence does not have a predictive effect on health behaviors. On the other hand, it is known that family sense of coherence is closely related to family functioning and family resilience (14). Family functioning plays a pivotal role in influencing behaviors related to weight and overall health (41). Similarly, family resilience contributes to young people's participation in physical activity (42). A longitudinal study shows that family cohesion, parent-child communication and parental involvement predict the level of physical activity of their children for both sexes one year later (36). Besides that, the family environment in which conflict is perceived as high and harmony and warmth are perceived as low contributes to adolescents' low body image perception and dietary problems, and high family harmony and communication are protective factors against smoking (15). In this study, the family sense of coherence did not predict healthy lifestyle behavior. This suggests the presence of other variables influencing family sense of coherence. For example, Wahlqvist et al. (2020) found that family climate was strong, and sense of family coherence was weak in families with deaf parents (43). Although all variables showed a positive relationship with each other in the current study, for example, the presence of a sick family member may have weakened the predictive effect of family sense of coherence. Therefore, the predictive effect of these variables on healthy lifestyle behaviors can be re-evaluated by controlling the health status of family members in future studies.

The fact that sense of family coherence lost its predictive effect when combined with individual coherence and family health climate may also be due to the relationship among the variables themselves. Family environment and relationships are the basis for the development of individual cohesion (44). It is suggested that individuals who experience a supportive family environment and encounter less tension in their relationships with parents during childhood tend to develop a heightened sense of coherence (45). Some research indicates that family coherence can function as a foundation for fostering a healthy family climate (46).

The mediating role of family cohesion and cohesion in the relationship between family meals eaten together in childhood and healthy life behaviors exhibited in later ages is pointed out (46). Similarly, family cohesion has a partial mediating role in the relationship between family meals eaten together and sugar intake (48). According to the literature, the sense of coherence may play a mediating role in the relationship between healthy lifestyle behaviors and family sense of coherence. Moreover, family sense of coherence might serve as a mediating factor in the relationship between healthy lifestyles and the family health climate.

Limitations and Recommendations

Along with the important results obtained, the study has some limitations. More clear results can be achieved by improving the balance between the number of women and men participating in this research. In future studies, it is recommended that demographic information that may affect family dynamics, such as the health status of family members, be considered as control variables. Finally, addressing these variables with longitudinal studies and including mediation hypotheses can make significant contributions to the literature.

CONCLUSION

The findings suggest that the sense of coherence and family health climate are factors that predict healthy lifestyle behaviors among young people. In conclusion, understanding the health behavior patterns of young people, who will constitute the adult population of the future, is a considerable guide for public health studies. Especially adolescence and young adulthood is a critical period for the cultivation of a sense of coherence and behaviors related to health. The sense of coherence developed in this process has a protective and developmental role in coping with stress factors in life and adopting certain health behaviors that will affect future health and well-being. Therefore, various intervention programs can be designed for the development of individual sense of coherence, especially in universities with a young population. In addition, family activities that support the health climate in the family can be included at an early age to help young people develop healthy lifestyle behaviors. Increasing healthy living practices within the family seems to be critical for young people to develop healthy lifestyle behaviors.

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RESEARCH ARTICLE

DO DIFFERENT VISUAL CONDITIONS AFFECT TACTILE ACUITY AT HAND IN HEALTHY YOUNG ADULTS?

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ABSTRACT

Purpose: Subjects should not see the area tested during the two-point discrimination (TPD) test. In literature, various methods are used to hide the test area. However, there is yet to be a consensus on which method is the best. This study investigated the tactile acuity of different methods to obscure the line of vision in TPD testing in healthy young adults.
Material and Methods: We evaluated the TPD thresholds of the dominant hand's three regions in 30 healthy young adults under four different visual conditions (two eyes open and two eyes closed). The statistical analysis involved the use of the Friedman test and the Wilcoxon signed-rank test.
Results: In all areas tested, it was found that the distance between two stimuli was smaller with eyes open. 46.70% of the participants reported being more comfortable and could distinguish two points more quickly when their eyes were open.
Conclusion: Although the threshold values of TPD are within clinically appropriate ranges in all four visual conditions, we suggest performing TPD testing while the patients' eyes are open. Clinicians may obtain more accurate results during TPD tests if subjects are tested with their eyes open rather than closed.

Keywords: somatosensory function, tactile acuity, touch perception, two-point discrimination, young adults

INTRODUCTION

The somatosensory system is a multimodal system that defines, identifies, and distinguishes sensory patterns to guide different types of stimuli, such as joint position (proprioception), thermal, noxious, and tactile stimuli (1,2). The sense of touch, or tactile sensation, is responsible for processing information from our surroundings. Tactile information is crucial for daily activities, differentiating and protecting the body from the environment. It is also essential for physical and social interaction (3).

Tactile acuity is the ability to accurately detect the location and quality of touch (4). Tactile acuity can be impaired in a wide range of clinical conditions such as multiple sclerosis (5,6), cerebral palsy (7), chronic musculoskeletal pain (8,9,10), migraine (11), and carpal tunnel syndrome, etc. (12), so tactile acuity

evaluation is essential for clinical examination and follow-up of these conditions. The Two Point Discrimination (TPD) test is a clinical measure of tactile acuity. TPD test has traditionally been used to measure cutaneous innervation density and evaluate the central somatosensorial functions (13). The TPD test is a simple and cost-effective method for measuring the smallest distance at which an individual can consciously identify two pressure points applied simultaneously to nearby areas of the skin (14,15). As the distance required to distinguish between the two stimuli decreases, the precision of tactile sensation improves. So, shorter distances indicate a higher level of tactile acuity (16).

During testing of TPD, subjects should not see the tested area. In the literature, different methods were used to hide the tested area. In some studies, the eyes were closed with a blindfold (17,18), or the participants were either instructed to keep their eyes closed (19-24) or asked to look away from the evaluated area (19,25). In other studies, a folder was preferred for obscuring the line of vision (26-28). According to Bell-Krotoski (2011), using a folder to obstruct the line of vision is recommended instead of a blindfold to cover the eyes (29). Different methods have been used to blind individuals or patients during tests, but it is still unclear which method works best for consistent assessments. For this reason, this study was planned to investigate the tactile acuity of different methods that obscure the line of vision in TPD testing.

MATERIALS AND METHODS

Participants

A total of 30 healthy young adults aged between 18 and 35 years were included in the study. All participants were informed about the study's aim and methods and signed an informed consent form. The study excluded participants who reported any of the following criteria: 1) current acute or chronic pain symptoms (especially in the evaluated areas or widespread) within the last two years due to traumatic injuries, overuse, rheumatoid arthritis, complex regional pain syndrome, or systemic musculoskeletal pain disorders such as fibromyalgia; 2) previous surgeries in the evaluated regions; 3) any skin conditions such as skin allergy or skin burn; 4) amputation; 5) neurological conditions such as peripheral nerve injuries or neuropathies; 6) any neurological signs such as numbness or tingling; 7) diabetes mellitus; 8) inability to follow instructions due to attention or cognitive disorders; 9) pregnancy.

Procedure

Prior to commencing the study, the Clinical Research Ethics Committee of the Kutahya Health Sciences University approved the ethical considerations (Date: 08.08.2018; Decision No: 2018/10-7). The data were collected between March 2019 and July 2019. Prior to conducting the TPD test, we recorded the participant's age, gender, and dominant hand. The same physical therapist carried out all of the evaluations. The testing environment was maintained at 25℃ with a relative humidity of 30%.

The TPD test was performed in four different visual conditions in order. The first two visual conditions were performed on the first day, and the last two were performed on the second day of evaluation to prevent mental fatigue and reduce the possibility of accommodating the test stimuli. At least five minutes of rest periods were given between each visual condition tested. The visual conditions were as follows.

I. Visual condition: Eyes closed with a blindfold
II. Visual condition: Eyes closed, in which the participants were asked to keep their eyes closed.
III. Visual condition: Eyes open, but a folder obscured the vision
IV. Visual condition: Eyes open, but the participants were asked to look away from the evaluated area

Test instruments and areas

The TPD test was conducted using a commercially available mechanical caliper (Aesthesiometer, Baseline® Two-Point Discriminator, 12-1481, New York, USA). The test was performed on three regions in the dominant hand of each participant: the palmar surface of the distal phalanx of the long finger (for the median nerve), the palmar surface of the distal phalanx of the little finger (for the ulnar nerve), and the area over the first dorsal interosseal muscle (for the radial nerve). For the distal phalanges of the long and little fingers, the caliper was positioned perpendicular to the skin so that the stimulus was applied perpendicular to the digit's axis. The caliper was parallel to the skin and the peripheral nerve trunk, which innervates the area over the first interosseal muscle (14,15).

Determination of two-point discrimination threshold

In the TPD test, two non-harmful, light touch stimuli are applied to the skin at equal pressures as a separate stimulus with two tips of the mechanical caliper. During the TPD assessment, the participants were comfortably seated in a relaxed position. They were asked to indicate if they felt one or two points of pressure by either verbally expressing it or holding up one or two fingers. In case of uncertainty, they were instructed to report a single point. The participants were also asked to indicate if they perceived two points due to a temporal delay between each point of the caliper. If the report was not accepted, the stimulus was reapplied. A TPD demonstration was performed before the actual administration while the participants' eyes were open.

The distance between the tips is measured by increasing or decreasing until the participants perceive two points or only one point, respectively. The study involved conducting three sets of ascending and descending assessments. The results of six measurements were averaged and used for statistical analysis. During the descending assessments, the test began at the maximum distance that the subjects could easily perceive as two separate points of the caliper. The distance between the two points of the caliper was then gradually decreased by one mm increments until the subjects could only perceive one point. Conversely, the ascending assessments began testing from the distance where the subjects perceived a single point and gradually increased the distance by one mm increments until the subjects could perceive two points separately.

During the testing process, the evaluator applied single stimuli randomly between one to three times to reduce the possibility of participants guessing the pattern. Attention was given to applying the stimuli simultaneously and with equal pressure. After completing all visual conditions, the participants were asked to state which visual condition they were more comfortable with and distinguish two points of the caliper more easily and clearly (22).

Statistical analysis

All data were analyzed using SPSS® Statistics 15 (Chicago, IL, USA). Due to the non-normal distribution of variables (Shapiro-Wilk test), all data were presented as medians and inter-quartile ranges (IQR). The study used the Friedman test to compare the threshold values of various visual conditions. A p-value of less than 0.05 was considered significant for the Friedman test. The Wilcoxon signed-rank test was employed to discern variations between every visual condition. For multiple pair-wise comparisons, the alpha value was adjusted and set at 0.00625 with the Bonferroni correction method, and the confidence interval was set at 95%.

RESULTS

A total of 30 healthy young adults (22 female, 8 male) with a median age of 22 (IQR=21-27) years were included in the study. The right hand was dominant for most of the participants (90%, n=27). TPD threshold values for each visual condition and test region were reported in Table 1. The comparison of each visual condition with each other was also reported in Table 1. The distance required to perceive two distinct stimuli was smaller for all test regions in III and IV visual conditions when compared to I and II. A statistically significant difference was found between TPD threshold values of I-III visual conditions and I-IV visual conditions in the distal phalanx of the long and the little fingers, respectively. Moreover, a significant difference was found between the threshold values of the first dorsal interosseal muscle in I-III, I-IV, II-III, and II-IV visual conditions (Table 1).

46.70% of the participants reported being more comfortable and distinguished two points easily when their eyes were open (III and IV visual condition). 23.30 % reported difficulty in distinguishing two points when their eyes were closed (I and II visual conditions). 16.70% of the participants could not decide which condition was more comfortable.

DISCUSSION

The TPD test is a widely used neurosensory test to assess mechanoperception in a clinical setting (30). However, it has been criticized for the unexplained variability within and between subjects and studies (31,32). This criticism stems from the need for a standard procedure for measuring TPD (33), making it difficult to compare findings across studies or clinics and create normative data (34). A standardized TPD protocol should be developed to reduce variability and minimize clinician judgment. As in all other sensory tests, some method of occluding the patients' vision should be used during the evaluation of TPD. Preventing visual input during testing reduces compensation of a sensory deficit and improves test accuracy (35). However, there is no standardization

Table 1. Comparison of the TPD threshold values of four visual conditions used in clinical sensory testings.

Test region	Different visual conditions	Mean ± SD	Median (IQR)	Friedman test p-value	Wilcoxon signed rank test	I.-II. visual condition	I.-III. visual condition	I.-IV. visual condition	II.-III. visual condition	II.-IV. visual condition	III.-IV. visual condition
The palmar surface of distal phalanx of long finger	I.visual condition	2.50 ± 0.45	2.50 (2.16-2.83)	**0.041***	**Z**	-2.049[b]	-2.915[b]	-2.560[b]	-1.094[b]	-1.225[b]	-0.041[b]
	II.visual condition	2.30 ± 0.46	2.41 (1.83-2.66)								
	III.visual condition	2.23 ± 0.40	2.24 (2.00-2.50)		***p-value***	0.040	**0.004****	0.010	0.274	0.221	0.967
	IV.visual condition	2.23 ± 0.44	2.00 (1.83-2.70)								
The palmar surface of distal phalanx of little finger	I.visual condition	2.61 ± 0.52	2.66 (2.16-3.04)	0.096	**Z**	-1.853[b]	-2.375[b]	-2.778[b]	-0.582[b]	-0.757[b]	-0.350[b]
	II.visual condition	2.44 ± 0.48	2.50 (2.12-2.83)								
	III.visual condition	2.37 ± 0.50	2.33 (1.83-2.70)		***p-value***	0.064	0.018	**0.005****	0.560	0.449	0.726
	IV.visual condition	2.39 ± 0.55	2.33 (1.83-2.83)								
Over 1st dorsal interosseous muscle	I.visual condition	20.60 ± 2.38	20.91 (19.53-22.04)	**0.001***	**Z**	-0.238[b]	-3.305[b]	-2.892[b]	-2.836[b]	-2.734[b]	-0.768[b]
	II.visual condition	20.53 ± 2.31	21.16 (19.07-22.04)								
	III.visual condition	19.80 ± 2.40	19.58 (18.77-21.08)		***p-value***	0.812	**0.001****	**0.004****	**0.005****	**0.006****	0.442
	IV.visual condition	19.65 ± 2.51	19.50 (18.66-21.16)								

*IQR= inter-quartile range, SD: standard deviation, *p <0.05, ** p < 0.00625 (Bonferroni correction)*

of the occlusion method in TPD testing. This was achieved in different ways by different authors. Traditionally, blindfolds or closed eyes were commonly used to block a person's vision. However, in cases of central nervous system dysfunction, such methods may cause anxiety or disorientation if used for an extended period. In such situations, using a small screen or folder as a visual barrier is recommended to limit the amount of visual input (35). In our study, the vision was obscured by four different methods. In the first two methods, the eyes were closed (I and II visual condition), and in the other two methods (III and IV visual condition), the eyes were open during testing. From these four methods, nearly half of the participants felt more comfortable and distinguished two points more easily in the test procedures when their eyes were open (III and IV visual condition). These patients claimed they were distracted, their concentration decreased, and they fatigued quickly while their eyes closed. Also, the distance required to perceive two points as distinct stimuli was smaller in these two test procedures. Smith (2016) recommends sensory evaluations with eyes open, in line with current findings.

The learning effect is a challenge encountered in sensory evaluation. In the study of Meiner et al. (1996), TPD assessments were carried out within 14 days by the same examiner at the same location and time of day, following a standardized procedure. The results showed that thresholds decreased in all conditions across the three testing sites during the second session. This decrease, or in other words,

improvement, may result from the change in perception thresholds on the second day of the assessment and may support the existence of a learning effect between the first and second sessions (37). Several studies have also indicated that the learning effect can influence TPD measurements, although these effects are often not clinically significant. Research involving healthy volunteers and patients with multiple sclerosis (MS) has shown slightly enhanced perception in repeated test sessions, suggesting the presence of a learning effect (38,39). In contrast, a study in children with cerebral palsy (CP) indicated that static and moving TPD tests may be less sensitive to learning effect (40). In our study, we tested four different visual conditions on two separate days. The first two visual conditions—where participants either wore a blindfold or kept their eyes closed—were assessed on the first day. The other two conditions—where participants' eyes were open but their vision was obscured by a folder or they were instructed to look away from the evaluated area—were tested on the second day. The improvement in tactile acuity during the last two visual conditions (eyes open) may be attributed to a learning effect present on the second day of evaluation. This represents a limitation of our study. Further research is necessary to determine whether the TPD threshold varies when the order of the visual conditions changes.

CONCLUSION

In our study, we tried to find the most sensitive method for occluding the participants' vision in the TPD test. Although the threshold values of the TPD test were in clinically appropriate ranges in all visual conditions, we suggest performing the TPD test while the participants' eyes are open.

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RESEARCH ARTICLE

DISASTER RESPONSE SELF-EFFICACY OF MEDICAL STUDENTS AND RELATED FACTORS: A CROSS-SECTIONAL STUDY

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ABSTRACT

Purpose: The purpose of this study was to assess the self-efficacy of 4th-6th grade medical students in disaster response and to investigate the related factors.
Material and Methods: This is a cross-sectional study. The sample of the study consisted of 209 4th-6th grade medical students studying at a medical faculty in the 2022-2023 academic year. Data were collected by applying a questionnaire to 4th-6th grade medical students between April-June 2023. The questionnaire included sociodemographic characteristics, Disaster Response Self-Efficacy Scale (DRSES).
Results: : A total of 209 medical students participated in this study. The mean age of the participants was 23.4±1.3 years, and 56.4% were women. Of the participants, 84.5% had no previous training in disaster preparedness and 92.8% required training in disaster preparedness. The DRSES scores were higher among senior medical students (p=0.001), those who had previously received disaster response training (p=0.005), those who had previously participated in a disaster drill (p=0.017), those who felt prepared for possible disasters (p<0.001), those who had information about the disaster risks of the region where they lived (p=0.003), and those who knew the disaster assembly areas of the hospital (p=0.023). The DRSES score did not differ according to the sex of the participants.
Conclusion: The The majority of participants needed disaster preparedness training. Self-efficacy in disaster response was higher among those who had previously received disaster response training and among final-year medical students.

Keywords: disaster medicine, medical students, self-efficacy

INTRODUCTION

Disasters are events that disrupt the order of a society, causing economic and social losses that either halt or interrupt people's daily lives and activities and deeply affect societies (1). Disasters continue to occur around the world, causing negative social and individual impacts at national and international levels (2, 3). According to the World Disasters Report, 97.6 million people were affected by disasters in 2019, and 24,396 people lost their lives. Of the people affected by disasters, 97% were affected by climate and weather-related disasters. According to the report, the most common disasters in 2019, in order of frequency, were floods, storms, epidemics, and earthquakes (4).

Human activities are negatively affecting natural climate variations and are rapidly causing global climate change. The Mediterranean region, including Turkey, is expected to be more affected by climate change. The expected effects of global climate

change include an increase in the number and severity of disasters caused by natural hazards such as floods, fires, storms, and earthquakes; erratic precipitation; extreme heat waves; and increased frequency of epidemics. Although climate change is slow, its consequences are devastating (5).

Turkey is located on the active Anatolian plate, where major earthquakes have occurred throughout history. In the last hundred years, 269 earthquakes have occurred in Turkey, 20 of which had a magnitude greater than 7.0 on the Richter scale. The largest earthquakes in terms of loss of life and severe damage were the 2023 Kahramanmaraş, 1939 Erzincan and 1999 Gölcük centered Marmara earthquakes. Due to its location, Turkey is one of the most earthquake-prone countries in the world. On February 6, 2023, two earthquakes occurred in Turkey with epicenters in Pazarcık and Elbistan districts of Kahramanmaraş with magnitudes of 7.7 and 7.6 on the Richter scale. On February 20, 2023, an earthquake with a magnitude of 6.4 on the Richter scale occurred in Turkey with the epicenter in Yayladagi, Hatay. A total of 11 provinces were devastated by these earthquakes. In terms of severity and area affected, these earthquakes are unprecedented in recent history. In fact, more than 48,000 people lost their lives and hundreds of thousands were injured as a result of these earthquakes (6).

During disasters, the provision of health services may be disrupted and health facilities and personnel may be unable to meet the increased demand (7). In such cases, the level of disaster-related knowledge of healthcare workers becomes critical and once again highlights the importance of disaster medicine training. From the past to the present, medical students have been directly involved in patient care during large-scale events that deeply affect society, such as disasters. Medical students have participated in care and intervention during earthquakes, floods, the Spanish flu of 1918, the September 11 massacre, and many other disasters (8-12). During the 2005 H5N1 pandemic, the Royal Belgian Academy of Medicine (13) suggested that medical students could play an important role in pandemic planning, but the study showed that they were not ready (14). Although volunteers are expected to respond to disasters, we find that there is little or no training in disaster response or disaster medicine in current medical curricula worldwide (15-17). In disaster management, doctors are an important part of the workforce. Interventions by doctors can be life-saving (17). Physicians acquire their basic professional competencies during their medical education. According to the National Core Curriculum for Pre-Graduate Medical Education, providing health care services in emergencies is one of the basic medical practices, and a general practitioner is expected to perform these practices in an emergency in accordance with the guidelines (18).

Although there are many studies in the literature evaluating the level of knowledge and awareness of healthcare professionals about disasters, the number of studies measuring medical students' self-efficacy toward disasters is quite limited. This study is one of the few studies that measured medical students' disaster self-efficacy and examined the factors that influence it.

The aim of this study was to evaluate the self-efficacy of 4th-6th grade medical students in disaster response and related factors in a medical school in Turkey.

Study questions are listed below:

- What is the disaster self-efficacy of 4th-6th grade medical students?
- What are the variables that affect the disaster self-efficacy of 4th-6th grade medical students?
- What is the self-efficacy of students trained in disaster medicine?

MATERIAL AND METHODS

Study Design and participants

This is a cross-sectional study. The study was conducted in May-June 2023 at Ondokuz Mayis University Faculty of Medicine. Due to the earthquake, the preclinical period students switched to online education, so the population of the study consisted of 825 4th, 5th, and 6th year students of Ondokuz Mayıs University Faculty of Medicine. The inclusion criteria for this study were 4th, 5th and 6th year medical students and volunteering to participate in the study. There was no age limit for participation in the study. The sample size was calculated as 263 with 95% confidence interval (α=0.05), 5% margin of error and 50% unknown prevalence. Taking into account the possibility of missing data, it was planned to reach a total of 289 people. Of these 825 medical students, 291 who volunteered to participate in the study comprised the sample of the study. A method of probability sampling was not used in this study.

Table 1. Characteristics of the participants

Variable	Category	n(%)
Sex	Female	164(56.4)
	Male	127(43.6)
Education year	4	132(45.4)
	5	78(26.8)
	6	81(27.8)
Previous disaster experience	Yes	31(10.7)
	No	260(89.3)
Previous family disaster experience	Yes	86(29.6)
	No	205(70.4)
Previous disaster preparedness training status	Yes	45(15.5)
	No	246(84.5)
Previous participation in a disaster drill	Yes	172(59.1)
	No	119(40.9)
Need for disaster preparedness training	Yes	270(92.8)
	No	21(7.20)
Feeling prepared for possible disasters	Yes	31(10.7)
	No	260(89.3)
Concern about possible disasters in the region where they live	Yes	249(85.6)
	No	42(14.4)
Knowledge of disaster risks in the region where they live	Yes	211(72.5)
	No	80(27.5)
Knowledge of the hospital's disaster assembly areas	Yes	43(14.8)
	No	248(85.2)

Ethical Considerations

This study was conducted according to the tenets of the Declaration of Helsinki. Ethics committee approval was obtained from the Clinical Research Ethics Committee of Ondokuz Mayıs University before the start of the study (Date:10.05.2023, Decision No: OMÜKAEK 2023/137). In addition, verbal permission was obtained from the participants.

Data Collection Tools

The mean score obtained from the DRSES were the dependent variable of the study whereas the participants' characteristics were the independent variables of the study. A questionnaire developed from a literature review was used. A pilot study was carried out with 15 participants. Afterwards, adjustments were made regarding the clarity and fluency of the questionnaire and the final version of the questionnaire was created. The questionnaire consisted of two parts. In the first part, the socio-demographic characteristics of the participants were asked. The Disaster Response Self-Efficacy Scale (DRSES) was used in the second part. The purpose of the study was explained to the participants at the beginning of the questionnaire. The questionnaire was administered face to face.

The sociodemographic information form asked about age, gender, class, previous disaster experience, presence of someone in the family who has experienced a disaster, previous disaster preparedness training, previous participation in a disaster drill, presence of disaster preparedness training needs, feeling prepared for possible disasters, concern about possible disasters in the region where they live, knowledge of disaster risks in the region where they live, and knowledge of disaster assembly areas of the hospital where they work.

The original version of the Disaster Response Self-Efficacy Scale (DRSES) was developed by Li et al. (19) The Turkish adaptation, validity and reliability study of the Disaster Response Self-Efficacy Scale (DRSES) was conducted by Toraman et al. (20) The scale is a 5-point Likert-type scale consisting of 19 items. Each item is scored from 1 to 5 (1 = No confidence at all, 2 = Basically no confidence, 3 = Little confidence, 4 = Basically confident, 5 = Complete confidence). Higher scores indicate higher self-efficacy in disaster response. In this study, the

Table 2. Students' responses to the DRSES

Variable	No confidence at all	Basically no confidence	Little confidence	Basically confident	Complete confidence
	n(%)	n(%)	n(%)	n(%)	n(%)
1. Debridement, hemostasis, bandaging and immobilization	36(12.4)	56(19.2)	125(43.0)	59(20.3)	15(5.20)
2. Lifting/Transportation	25(8.60)	48(16.5)	90(30.9)	99(34.0)	29(10.0)
3. Transferring	23(7.90)	57(19.6)	99(34.0)	86(29.6)	26(8.90)
4. Techniques for emergency rescue	35(12.0)	67(23.0)	110(37.8)	66(22.7)	13(4.50)
5. Intensive care of critical patients	91(31.3)	104(35.7)	79(27.1)	11(3.80)	6(2.10)
6. Communicable disease prevention and control in the disaster area	40(13.7)	86(29.6)	106(36.4)	48(16.5)	11(3.80)
7. Initial psychological assessment of disaster victims	39(13.4)	64(22.0)	111(38.1)	67(23.0)	10(3.40)
8. Recognize common post-disaster psychiatric and psychological problems such as post-traumatic stress disorder, depression, and anxiety.	24(8.20)	50(17.2)	97(33.3)	99(34.0)	21(7.20)
9. Provide basic psychological treatment to disaster victims	38(13.1)	70(24.1)	120(41.2)	51(17.5)	12(4.10)
10. Referral of disaster victims in need of psychological and psychiatric treatment in the disaster area	20(6.90)	44(15.1)	88(30.2)	118(40.5)	21(7.20)
11. Identifying the damage caused by the disaster	42(14.4)	72(24.7)	95(32.6)	72(24.7)	10(3.40)
12. Accurately and quickly identify injuries	14(4.80)	54(18.6)	113(38.8)	99(34.0)	11(3.80)
13. Assess post-disaster outbreaks such as infectious diseases or acute poisonings	25(8.60)	69(23.7)	118(40.5)	74(25.4)	5(1.70)
14. Identify vulnerable populations such as the chronically ill or disabled	13(4.50)	48(16.5)	94(32.3)	113(38.8)	23(7.90)
15. Triage techniques	20(6.90)	65(22.3)	106(36.4)	81(27.8)	19(6.50)
16. Regulate own psychological state and adapt quickly to the work environment	17(5.80)	23(7.90)	78(26.8)	115(39.5)	58(19.9)
17. Good communication and cooperation with other team members	13(4.50)	9(3.10)	49(16.8)	143(49.1)	77(26.5)
18. Communicate effectively with victims and their families and establish a good patient-doctor relationship	14(4.80)	11(3.80)	60(20.6)	143(49.1)	63(21.6)
19. Adhere in a humane, fully empathetic and loving manner to professional ethical principles.	12(4.10)	11(3.80)	41(14.1)	141(48.5)	86(29.6)

Cronbach's alpha value of the DRSES scale was 0.91.

Statistical analysis

The data were analyzed using IBM SPSS Statistics, version 21.0. Categorical data were expressed as numbers and percentages. Continuous variables were expressed as mean±standard deviation. The chi-squared test was used to compare categorical data. Data distribution was evaluated by tests and graphs. Independent samples t-test and ANOVA were used to compare continuous variables with

Table 3. The relationship between participant characteristics and DRSES score

Variable	Category	DRSES score	
		Mean±SD	p*
Sex	Female	59.49±11.83	0.591
	Male	58.72±12.74	
Education year	4	56.77±11.61[a]	**0.001**
	5	59.13±12.45[ab]	
	6	63.06±12.11[b]	
Previous disaster experience	Yes	62.68±13.66	0.090
	No	58.73±12.00	
Previous family disaster experience	Yes	60.33±12.84	0.290
	No	58.66±11.95	
Previous disaster preparedness training status	Yes	63.89±12.60	**0.005**
	No	58.29±11.97	
Previous participation in a disaster drill	Yes	60.58±11.94	**0.017**
	No	57.10±12.38	
Need for disaster preparedness training	Yes	58.92±12.22	0.245
	No	62.14±12.16	
Feeling prepared for possible disasters	Yes	66.87±12.43	**<0.001**
	No	58.23±11.89	
Concern about possible disasters in the region where they live	Yes	59.23±12.44	0.791
	No	58.69±10.93	
Knowledge of disaster risks in the region where they live	Yes	60.46±11.93	**0.003**
	No	55.70±12.37	
Knowledge of the hospital's disaster assembly areas	Yes	63.07±11.22	**0.023**
	No	58.48±12.28	

a-b: No significant difference between similar letters in categories in the same column.
* Independent samples t-test for two groups and ANOVA for groups of three or more.

normal distribution. p<0.05 was considered statistically significant.

RESULTS

A total of 291 medical students were participants in this study. The mean age of the participants was 23.4±1.3 years. Of the participants, 56.4% were female and 45.4% were 4th year students. Of the participants, 89.3% had never experienced a disaster, 84.5% had never received disaster preparedness training, 40.9% had never participated in a disaster drill, 92.8% needed disaster preparedness training, and 89.3% did not feel prepared for possible disasters (Table 1).

The least confident items on the disaster response self-efficacy scale were "providing intensive care to critically ill patients" with 67.0%, "preventing and controlling infectious diseases in the disaster area" with 43.3%, and "assessing the damage caused by the disaster" with 39.1%. The most confident items on the disaster response self-efficacy scale were "adhere to professional ethical principles in a humane, fully empathetic and loving manner" at 78.1%, "establish good communication and cooperation with other team members" at 75.6%, and "communicate effectively with victims and their families and establish a good patient-doctor relationship" at 70.7% (Table 2).

The mean DRSES score of the participants was 59.15±12.21. When the DRSES scores of the students were compared, the DRSES scores of the senior students were higher (p=0.001). The DRSES scores of students who had previously received any disaster preparedness training were higher (p=0.005). The DRSES scores of students who had previously participated in a disaster drill were higher (p=0.017). The DRSES scores of participants who felt prepared for possible disasters were higher (p<0.001). The DRSES scores of the participants who had information about the disaster risks of the region where they lived were higher (p=0.003). The DRSES scores of the participants who knew the disaster assembly areas of the hospital where they worked were higher (p=0.023). The DRSES scores of the

participants did not differ according to gender, previous disaster experience, previous disaster experience in the family, need for disaster preparedness training, and concern about possible disasters in the region where they lived (Table 3).

DISCUSSION

As the global climate crisis worsens by the day, the occurrence of disasters caused by natural hazards is inevitable (5). Since the place and time of disasters caused by natural hazards cannot be predicted in advance, physicians in the disaster area should take the lead in providing medical services until official help arrives. In mass casualty disasters, help is expected from all organizations not affected by the disaster. Therefore, physicians are expected to participate in disaster health care regardless of their specialty (21). Unfortunately, physicians who are not trained in disaster medicine may not be able to work efficiently.

In this study, 10.7% of the participants reported having experienced a disaster situation, while 42.9% of the participants reported having experienced a disaster situation in the study conducted by Demiray et al. (22) with 6th grade medical students. In the studies conducted by Yiğit et al. (23), Şekerci et al. (24), these rates were 52.8% and 53.4%, respectively. In these studies, which were conducted in different parts of Turkey at different times and in different regions, the difference in disaster experience may be related to the diversity and frequency of disasters occurring in different geographical areas of the country.

In this study, 15.5% of 4th-6th grade medical students reported receiving disaster medicine training. In a study by Demiray et al. (22) of 6th grade medical students, 59.2% of the participants reported receiving disaster training. The reason for the low rate in this study may be the presence of 4th and 5th grade students in the sample group.

In this study, 40.9% of participants reported that they had not participated in a disaster drill. In studies conducted among health professionals, more than half of the participants reported that they had not participated in any disaster drills or courses (25-27). The reason why this rate was lower in this study may be that disaster medicine education in medical school is higher than other health personnel education.

In this study, 89.3% of the participants reported that they did not feel prepared for possible disasters. In the studies of Yiğit et al., Arslan et al., and Demiray et al., 78.8%, 88.2%, and 89.2% of the students, respectively, did not feel prepared for possible disasters (22, 23, 28). The rate of students who did not feel prepared for disasters was found to be consistent with the literature.

In this study, the majority of medical students reported that they had not received any training in disaster preparedness and that they needed training in disaster preparedness. Similar to this study, Ragazzoni et al. found that most students did not attend any academic or non-academic courses on disaster medicine and that they wanted a course on disaster medicine to be added to their curriculum (29).

In this study, the mean DRSES scores of the participants were similar to the study of nursing students by Yıldız et al. (30). Yıldız et al. (30) and Demiray et al. (22) found a significant increase in the mean DRSES score after disaster medicine training . In the study of Demiray et al. (22), the mean DRSES scores of final year medical students before receiving disaster medicine training were similar to this study. There are previous studies indicating that disaster medicine is inadequately covered in medical school curricula (21, 29, 31). Therefore, there is a need for more research on medical student self-efficacy in disaster response.

In this study, final year medical students were found to be more confident in disaster response than the other two years. This may be due to their increased experience in medicine with more patient care in clinical settings in the final year.

This study found that participants who had previously received disaster preparedness training or participated in disaster drills were more confident in responding to disasters. A systematic review by Ashcroft et al. found that implementing disaster education programs for medical students improves medical students' preparedness, knowledge, and skills in disaster situations (32). This study showed that medical students who felt prepared for disasters were more confident in responding to disasters. We believe that student-centered, interactive practical and theoretical disaster medicine training will significantly contribute to students' self-efficacy. Thus, medical students who feel prepared for disasters can play a greater role in disaster health care.

Limitations

The most important limitation of this study is that it was conducted only with 4th, 5th and 6th year

students of a medical faculty because the preclinical period students switched to online education due to the earthquake that occurred during the study period. Therefore, it may not be representative of all medical students in Turkey. Another limitation of this study is that one of the probability sampling methods was not the choice.

CONCLUSION

Most of the students who participated in this study did not receive disaster preparedness training during their medical school programs and did not feel prepared for disasters. However, the participants needed training in disaster response and wanted to increase their knowledge in this area. Students would welcome more applicable courses on disaster medicine in the standard medical curriculum in Turkey. Further studies are needed to investigate how prepared medical students, the future physicians, are to respond to disasters.

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RESEARCH ARTICLE

THE RELATIONSHIP BETWEEN ANTICIPATORY NAUSEA AND VOMITING WITH SOCIAL SUPPORT AND ANXIETY IN CANCER PATIENTS UNDERGOING CHEMOTHERAPY

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ABSTRACT

Purpose: This study aims to investigate the relationship between anticipatory nausea and vomiting in cancer patients undergoing chemotherapy with social support and anxiety.
Material and Methods: This descriptive-correlational study involved 134 cancer patients receiving chemotherapy at a university hospital and experiencing anticipatory nausea. Anxiety levels were measured using the Beck Anxiety Inventory (BAI), while the severity of nausea was assessed using the Visual Analog Scale (VAS-nausea), and social support levels were evaluated using the Cancer Patient Social Support Scale (CPSSS). Descriptive statistics were employed to analyze demographic characteristics. Independent sample t-tests, Kruskal-Wallis, one-way analysis of variance (ANOVA), Mann-Whitney U test, Cronbach's alpha coefficient, and Pearson correlation analysis were used for data analysis.
Results: Results showed that 62.7% experienced moderate to severe nausea post-chemotherapy, with 57.5% having received prior chemotherapy. 61.2% reported minimal anxiety. Patients under moderately emetogenic treatments exhibited higher anticipatory nausea scores. Anxiety inversely correlated with social support, but no significant link was found between anticipatory nausea severity and anxiety/social support levels.
Conclusion: The findings indicate that there is no relationship between anticipatory nausea and vomiting, social support, and anxiety among cancer patients undergoing chemotherapy.

Keywords: anticipatory nausea and vomiting, anxiety, cancer, chemotherapy, nursing, social support

INTRODUCTION

Cancer, characterized by the uncontrolled growth and proliferation of deformed cells due to various etiological factors, represents tumor formation. Chemotherapy stands as a fundamental component in almost every cancer treatment plan (1), though it induces several side effects. One such side effect is nausea and vomiting. It is a debilitating side effect of cancer treatment and affects 40% of patients (2). When left untreated, it affects 60-80% of cancer patients. Early discontinuation of treatment causes dehydration, electrolyte imbalances, increased cost of care, and ultimately a decrease in treatment success (3). In a survey conducted with 212 European oncology nurses, only 19% reported that the majority of their patients had their nausea and

vomiting optimally controlled (4). Nausea and vomiting due to untreated chemotherapy can affect 60% to 80% of cancer patients (3,4). In the study conducted by Yu Sun and colleagues, it was determined that nausea and vomiting occurred at rates of 30.1% in highly emetic chemotherapy regimens, and 21.5% in moderately emetic ones (5).

Anticipatory nausea and vomiting are defined as episodes preceding medication administration (6,7). Investigations suggest that anticipatory nausea and vomiting may arise in approximately 20-30% of patients based on their chemotherapy cycles (8). Being a conditioned response, it emerges subsequent to adverse vomiting experiences during prior chemotherapy sessions (7). A study conducted across eight European countries by Molassiotis et al. (8) suggests a prevalence of approximately 8-14% for anticipatory nausea and vomiting, which escalates in subsequent chemotherapy cycles. It is widely believed that 25% of patients develop this response by the fourth treatment cycle (9).

Anticipatory nausea and vomiting encompass psychologically associated variations of chemotherapy- induced nausea and vomiting. Psychological mechanisms and demographic factors contribute to the onset, frequency, severity, and duration of anticipatory nausea and vomiting. Three distinct yet interrelated contributing factors exist: classical conditioning leading to anticipated gastric distress, demographic, clinical, and treatment-related factors capable of predicting the risk of anticipated gastric distress, and anxiety or negative expectations triggering and exacerbating anticipatory gastric distress (10).

Feeling anxious immediately before a chemotherapy appointment has been associated with an increased tendency toward anticipatory nausea and vomiting, as it reflects a general predisposition to feel anxious in various situations. These anticipatory symptoms, commencing one or more days before a patient's clinical appointment, significantly escalate the burden experienced during chemotherapy, intensify concerns, and prolong discomfort (11).The dual-process model in a study by Montgomery and Bovbjerg (2003) asserts a strong contribution of concurrent emotional distress to this experience. Social learning theory supports the notion that expectations of involuntary consequences should be based on prior experiences (12). These researchers tested the impact of emotional distress and prior experience on patients' expectations of side effects within a sample of 80 breast cancer patients receiving outpatient adjuvant chemotherapy. Bivariate analyses suggested an early contribution of emotional distress to the treatment process (13).

Social support, aiding individuals in navigating their illness-related processes, constitutes assistance obtained from their surroundings when faced with stressful circumstances (14). Notably, support from immediate family members influences cancer patients' coping strategies (15). A study revealed that family support mediates patients' anxiety levels and the severity of post-treatment nausea, and it is associated with the severity of anticipatory nausea directly (16). Changes in family relationships, including adaptation, expression, and conflict, were examined for their impact on patients' physical adherence to chemotherapy. An increase in family conflict was found to correlate with prolonged post-treatment nausea and a higher degree of anticipatory nausea in younger adult patients and women (17).

Several factors may influence the development of depression and anxiety among individuals with cancer, yet these remain poorly understood and require further investigation. Among individual risk factors that may elevate the risk of depression and anxiety, similar to the general population, include demographic factors like age and gender, along with social and economic factors such as unemployment, lower educational qualifications, and a lack of social support (18). Consequently, the deficiency in social support can be construed to correlate with anxiety. Studies have also supported the notion that anxiety forms a basis for anticipatory nausea and vomiting.

Social support significantly influences cancer patients' coping strategies (19), while family support mediates anxiety levels and post-treatment nausea severity (3). Changes in family relationships impact patients' physical adherence to chemotherapy (7). Various factors can affect the development of depression and anxiety among cancer patients (7), with social support deficiency correlating with anxiety (8).

In Turkey, no study has been encountered examining the relationship between anticipatory vomiting, social support, and anxiety in cancer patients undergoing treatment. Other country are limited to a very small number of studies, and there is no existing research conducted using the scales we could utilize. Therefore, our study contributes new data to the literature.

Table 1. Patient Characteristic (n:134)

Variables (n:134)	$\overline{X}$	**SD**
Age	57.2	13.9
Characteristic	**n**	**%**
Gender		
Woman	79	59.0
Male	55	41.0
Marital Status		
Married	99	73.9
Single	35	26.1
Level of education		
Primary education and below	49	36.6
Secondary education	25	18.7
High school	36	26.9
Undergraduate and above	24	17.9
Working Status		
Working	20	14.9
Not working	114	85.1
Moderate or severe nausea and vomiting after the last chemotherapy course		
Yes	84	62.7
No	50	37.3
Emetogenic risk status of current chemotherapy treatment		
Low	54	40.3
Middle	64	47.8
High	16	11.9
Having a chronic illness		
Yes	66	49.3
No	68	50.7
Previous chemotherapy treatment		
Yes	70	52.2
No	64	47.8
Nausea and vomiting during previous chemotherapy sessions		
Yes	77	57.5
No	57	42.5
Drinking alcohol		
Yes	23	17.2
No	111	82.8

Table 2. Anxiety and Social Support Levels of Patients (n:134)

Scales and Sub-Dimensions	Mean±SS	Min- Max	α
Beck Anxiety Scale	7.50±7.67	00.0-40.0	.88
Cancer Patient Social Support Scale	144.29±20.58	48.0-174.0	.85
CPSSS Sub-Dimensions			
Trust Support	57.44±9.57	13.0-65.0	.94
Emotional Support	49.89±8.42	16.0-60.0	.83
Information Support	36.94±6.73	18.0-50.0	.62

α: Cronbach Alfa

MATERIAL AND METHODS

Ethical Dimension of the Study

This study was approved by the Non-Interventional Research Ethics Committee of Dokuz Eylul University (Date: 17.11.2021, Decision Number: 2021/33-07). Permission was obtained from the Dokuz Eylül University Oncology Service and Day Treatment Center for the collection of data. Permissions from researchers who conducted the Turkish validity and reliability studies of the scales were obtained for their use. Additionally, prior to their participation, individuals volunteering for the research were verbally informed about the purpose of the study and provided written consent through an "Informed Consent Form" before the administration of surveys and scales.

Type of Study

This study was conducted in a descriptive correlational design. Social support, anxiety levels, and anticipatory nausea and vomiting were measured among cancer patients receiving chemotherapy at a university hospital, and their correlations were assessed.

Research Questions

- What is the level of anticipatory nausea and vomiting in cancer patients undergoing chemotherapy?
- What is the level of social support among cancer patients undergoing chemotherapy?
- What is the level of anxiety among cancer patients undergoing chemotherapy?
- Is there a relationship between the anxiety level and the level of social support among cancer patients undergoing chemotherapy?
- Is there a relationship between anticipatory nausea and vomiting and social support among cancer patients undergoing chemotherapy?
- Is there a relationship between anticipatory nausea and vomiting and anxiety among cancer patients undergoing chemotherapy?

Study Design and Sample

Participant data was collected through face-to-face interviews. The rules of social distancing, mask-wearing, and hygiene were adhered to due to Covid-19. The study population comprised all patients receiving chemotherapy treatment in the Oncology Service and Day Treatment Center between April 2021 and September 2022. The study sample included cancer patients aged 18 and above, undergoing chemotherapy, and having a VAS-nausea score of 5 or higher. The sample size was determined using the G*Power-3.1.9.7. program, conducting a power analysis. The power analysis indicated that to detect a Pearson correlation coefficient of r =.30 with 95% power (alpha = .05, two-tailed), the study needed to reach 134 participants. Consequently, 134 patients meeting the inclusion criteria constituted the study sample.

Data Collection Instruments

Data Collection Tools: The data were collected using various tools including the "Descriptive Information Form," prepared by reviewing relevant literature, encompassing 15 questions related to sociodemographic characteristics and factors contributing to anticipatory nausea (3,10,12,16). Within the demographic information, the emetogenic risk of chemotherapy received by patients was

Table 3. Anxiety Levels of Patients (n:134)

Anxiety Classification	n	%
Minimal anxiety symptoms (0-7 point)	82	61.2
Mild anxiety symptoms (8-15 point)	37	27.6
Moderate anxiety symptoms (16-25 point)	10	7.5
Severe anxiety symptoms (26-63 point)	5	3.7

classified using the Multinational Association of Supportive Care in Cancer (MASCC) guidelines, categorizing each chemotherapy drug into minimal, low, moderate, or high emetogenic potential. The regimen's emetogenicity was categorized into three groups (low/minimal, moderate, high) based on the chemotherapy drug with the highest emetogenic potential (10,12).

Beck Anxiety Scale (BAS): The Turkish validity and reliability study of the scale, which was originally developed by Doctor Aaron T. Beck and his colleagues in 1988, was conducted by Ulusoy et al. This 21-item scale was created to measure the severity of anxiety and evaluate the frequency of anxiety symptoms. The reliability of the scale is α = .93. Participants answer the questions as “not at all (not at all = 0)”, “mildly (mild = 1), “moderately (moderate = 2)” and “serious (serious = 3)” (20). The score varies between 0 and 63, with 0-7 points indicating minimal anxiety symptoms, 8-15 points indicating mild anxiety symptoms, 16-25 points indicating moderate anxiety symptoms, and 26-63 points indicating severe anxiety symptoms. In this study, Cronbach's alpha coefficient was found to be .93.

Cancer Patient Social Support Scale (CPSSS): The validity, reliability and factor structure study of the Cancer Patient Social Support Scale is a 5-point Likert type scale developed by Eylen in 2002. It is a 35-item 5-point Likert scale measuring the perceived level of social support from family members among cancer patients. The scale consists of three subscales: "trust support," "emotional support," and "information support." The Cronbach's alpha values for the subscales are 0.88 for trust support, 0.88 for emotional support, and 0.87 for information support. The score of perceived social support is calculated by reversing the scores obtained from negative items and adding them to the scores obtained from positive items. A higher score on the scale indicates a higher perceived level of social support from the cancer patient's family (21).

Visual Analog Scale (VAS): This scale was used to subjectively evaluate the severity of nausea experienced by patients before or during chemotherapy. The scale consists of a horizontal line measuring 100 mm/10 cm in length. The left end of the line represents 0, indicating "No Nausea," while the right end represents 10, indicating "Severe Nausea." The distance of the patient's mark from the left end was measured using a ruler. This measured distance in millimeters was considered as the "score" and recorded. A measurement of 5 mm or less indicates "no nausea," whereas values above 5 mm suggest that the individual "experienced nausea" (8,22,23).

Data Analysis

The obtained data in the study were analyzed using the "SPSS (Statistical Package for Social Sciences) for Windows 25.0" software (24). Descriptive statistical methods (number, percentage, mean, standard deviation) were employed for data evaluation. Normal distribution was checked through normality tests and examining kurtosis and skewness values. To assess the consistency of expressions within CPSSS and BAS and to determine if they measure the same construct, reliability analysis was conducted, and Cronbach's Alpha values were examined. For the comparison of mean scores of CPSSS, BAS, and VAS-nausea according to patients' descriptive characteristics, the Kruskal-Wallis test was used for age, cancer type, education status, emetogenic risk of current chemotherapy, and number of chemotherapy cycles. Independent sample t-tests were utilized for the analysis of gender, marital status, experiencing moderate or severe nausea-vomiting after the last chemotherapy cycle,

Table 4. Comparison of Patients' Descriptive Characteristics and Visual Analogue Scale Total Score Averages

Patient Characteristic	n	Visual Analogue Scale Total Score $\bar{X}$±SS	Test	P value
Gender			t test	.27
Woman	79	6.10±1.43	.65	
Male	55	6.38±1.49		
Marital Status			t test	.54
Married	99	6.26±1.47	.03	
Single	35	6.09±1.44		
Level of education			H test	
Primary education and below	49	6.24±1.54	2.47	
Secondary education	25	5.80±1.11		.48
High school	36	6.28±1.48		
Undergraduate and above	24	6.50±1.56		
Working Status				
Working	20	6.85±2.00	Z testi	.16
Not working	114	6.11±1.32	-1.40	
Moderate or severe nausea and vomiting after the last chemotherapy course			t test	.21
Yes	84	6.33±1.55	5.17	
No	50	6.02±1.28		
Emetogenic risk status of current chemotherapy treatment			H test	**.01***
Low	54	5.91±1.33	8.44	
Middle	64	6.59±1.55		
High	16	5.75±1.18		
Having a chronic illness			t test	.88
Yes	66	6.20±1.47	.05	
No	68	6.24±1.40		
Previous chemotherapy treatment			t testi	.71
Yes	70	6.17±1.43	.63	
No	64	6.27±1.50		
Nausea and vomiting during previous chemotherapy sessions			T test	.16
Yes	77	6.06±1.27	1.20	
No	57	6.42±1.49		
Drinking alcohol			U test	.09
Yes	23	6.74±1.73	-1.65	
No	111	6.11±1.38		

*p<.05 t: Independent Samples t-test, H test: Kruskal Wallis test, U test: Mann Whitney U testi

additional medical conditions, additional medication use, history of prior chemotherapy, and experiencing nausea during past chemotherapy. Mann-Whitney U test was applied for the analysis of employment status and alcohol use. The relationship between CPSSS, BAS, VAS-nausea scales, and their subscales was examined using "Pearson correlation analysis".

RESULTS

It was determined that the mean age of 57,2±13,9 (years). It was determined that 59.0% of them were woman and 73.9% of them were married. It was determined that 88.1% of the patients received chemotherapy treatment with medium or low emetogenic risk. 57.5% of patients reported experiencing nausea and vomiting during previous chemotherapy treatment, and 62.7% reported experiencing moderate or severe nausea and vomiting after their last course. Data regarding the descriptive characteristics of the patients are given in Table 1.

When examining the descriptive statistics related to the scales utilized, it was observed that the average total score for the Beck Anxiety Scale (BAS) was 28.50±7.67. Cancer Patient Social Support Scale

Table 5. Relationship between Patients' Visual Analog Scale, Beck Anxiety Scale, Cancer Patient Social Support Scale and Sub-Dimensions Scores (n:134)

	1	*2*	*3*	*4*	*5*	*6*
1)Visual Analogue Scale	1					
2)Cancer Patient Social Support Scale	-.06	1				
3)Trust Support Sub-Dimension	-.07	.86**	1			
4)Emotional Support Sub-Dimension	-.04	.94**	.80**	1		
5)Information Support Sub-Dimension	-.05	.63**	.22**	.50**	1	
6) Beck Anxiety Scale	.11	-.22**	-.17*	-.22*	-.16	1

*p<.05 **p<.01

(CPSSS) displayed an average total score of 144.29±20.58, and the Visual Analog Scale for nausea (VAS-nausea) showed an average total score of 6.22±1.46.

Upon further inspection of the CPSSS subdimensions, the mean score for the confidence support dimension was identified as 57.44±9.57, the emotional support dimension yielded a mean score of 49.89±8.42, and the information support dimension exhibited a mean score of 36.94±6.73 (Table 2).

Upon comparison of the descriptive characteristics of patients and the average scores obtained from the visual analog scale used to measure the severity of nausea and vomiting, factors such as gender, marital status, level of education, working status, emetogenic risk status of current chemotherapy treatment, having a chronic illness, number of chemotherapy courses, previous chemotherapy treatment, nausea and vomiting during previous chemotherapy sessions, drinking alcohol no statistically significant relationship was observed (Table 4).

However, upon assessing the emetogenic risk associated with patients' ongoing chemotherapy treatments in correlation with the mean visual analog scale scores, a statistically significant association emerged (p<.05). Notably, patients undergoing chemotherapy with a moderate emetogenic risk demonstrated higher visual analog scale scores, suggesting an elevated severity in reported nausea and vomiting symptoms.

A statistically significant and positive relationship was found between the patients' level of social support and its subdimensions: confidence support (p<.01: r=.86), emotional support (p<.01: r=.94), and information support (p<.01: r=.63). As the scores on the Cancer Patient Social Support Scale increased, the total scores for information support, emotional support, and confidence support also increased. Furthermore, a statistically significant and negative relationship was observed between the total scores of the Cancer Patient Social Support Scale and the Beck Anxiety Inventory scores (p<.01: r= -.22). As the total scores on the Cancer Patient Social Support Scale decreased, the Beck Anxiety Inventory scores increased. Moreover, a statistically significant and positive relationship was identified between the subdimensions of the Cancer Patient Social Support Scale, specifically confidence support (p<.01: r= .80), and the emotional support dimension. As the score for confidence support increased, the scores for emotional support also increased.Similarly, a statistically significant and positive relationship was observed between the subdimensions of the Cancer Patient Information Support Scale, particularly confidence support (p<.01: r= .22), and the emotional support dimension. As the score for confidence support increased, the scores for emotional support also increased.Additionally, a statistically significant and positive relationship was found between the subdimensions of the Cancer Patient Information Support Scale, specifically information support (p<.01: r= .50), and the emotional support dimension. As the score for information support increased, the scores for emotional support also increased. Finally, when comparing the scores of the subdimension of confidence support in the Cancer Patient Social Support Scale with the Beck Anxiety Inventory (p<.05: r=-.17), a statistically significant and negative relationship was established. This indicated that as confidence support scores increased, anxiety scores decreased. Likewise, a statistically significant and negative relationship was determined when

comparing the scores of the subdimension of emotional support in the Cancer Patient Social Support Scale with the Beck Anxiety Inventory ($p<.05$: $r=-.22$). As emotional support scores increased, anxiety scores decreased (Table 5).

DISCUSSION

The mean age of the patients was 57.26±13.95, with 59.0% being female and 73.9% being married. Consistent with several studies, the high prevalence of female gender and married status among patients experiencing anticipatory nausea and vomiting aligns with findings from this investigation (8,25,26,27,28).

It was identified that 88.1% of the patients received chemotherapy treatments categorized as having moderate or low emetogenic risk. This finding resonates with Hunter et al.'s research, which yielded similar results (26). When comparing the emetogenic risk of the current chemotherapy treatment with VAS-nausea scores, a statistically significant relationship ($p<.05$) was established. Patients undergoing treatments with moderate emetogenic risk exhibited higher levels of nausea. However, no significant correlation was found with other sociodemographic factors. Additionally, 57.5% of patients reported experiencing nausea and vomiting during previous chemotherapy treatments, while 62.7% reported moderate or severe symptoms after their last cycle. Similar studies have supported that experiencing nausea and vomiting during previous chemotherapy cycles constitutes the strongest risk factor for anticipatory nausea and vomiting (8,29). Consequently, while patients' negative experiences were influential in the development of anticipatory nausea and vomiting, these factors were not correlated with the severity of nausea.

Nurses play a critical role in managing symptoms such as nausea and vomiting throughout patients' treatment processes. Data indicating that a majority of patients are female and married suggests the necessity for a more tailored approach in managing symptoms within this group. Additionally, nurses should consider evaluating parameters such as patients' previous experiences of moderate or severe nausea, aiming to personalize treatment plans accordingly. Therefore, the correlation between the emetogenic risk levels of patients' forthcoming chemotherapy and the severity of nausea emphasizes the necessity for nurses to personalize their treatment plans. Nurses should evaluate patients' emetic risks before treatment and determine suitable therapeutic strategies.

The mean score for the Beck Anxiety Inventory (BAI) was 7.50±7.67, with 61.2% experiencing minimal anxiety. Similar findings have been observed in various studies involving cancer patients undergoing chemotherapy (30,31,32).

Patients' chemotherapy treatments were evaluated from the 1st to the 57th cycle. As the number of cycles increased, the number of patients experiencing anticipatory nausea and vomiting decreased. In a study conducted by Kurt (2021), it was concluded that as the number of chemotherapy cycles increased, the likelihood of experiencing anticipatory nausea and vomiting also increased (33). Patients undergoing a higher number of cycles seemed to experience more symptoms related to chemotherapy. Effectively managing common symptoms like nausea and vomiting can prevent the onset of anticipatory symptoms. While past mismanagement of nausea and vomiting might lead to anticipatory symptoms, finding the correct management strategy could subsequently alleviate future expectations.

A statistical analysis revealed a significant negative correlation ($p<.01$: $r= -.22$) between patients' social support and anxiety levels. However, no direct correlation was observed between the severity of nausea and vomiting and either social support or anxiety. This finding holds significant implications for nursing care. Nurses should understand that there is no direct relationship between the severity of nausea and vomiting and either anxiety or social support while managing patients' symptoms. Therefore, a different focus might be required for effective symptom management. Nurses should continue to monitor patients' anxiety levels while focusing on managing symptoms and provide appropriate support and counseling when needed.

The absence of a direct link between anxiety or social support levels and nausea and vomiting underlines the necessity for nurses to approach treatment strategies and patient support from a different perspective. Nurses can concentrate more on symptom management and effective treatment options to cope with patients' symptoms. This approach might require a focus on effectively controlling symptoms to enhance patients' quality of life.

The limitation of this research lies in the evaluation of data obtained from a single institution. Therefore, parameters should also be assessed in larger sample

sizes and sample groups that encompass institutional diversity. Despite the constraints regarding the sample size and institutional scope, this study contributes to the literature on a relatively underexplored topic in the field.

Limitations

Findings related to anticipatory nausea and vomiting in cancer patients undergoing chemotherapy were limited to the oncology ward and daytime treatment center.

CONCLUSION

In this study, factors contributing to anticipatory nausea and vomiting in cancer patients undergoing chemotherapy were identified. Previous chemotherapy treatment, negative vomiting experiences during prior chemotherapy sessions, a lower number of chemotherapy cycles, and experiencing moderate to severe nausea and vomiting after the last chemotherapy session were determined as potential influential factors. However, it was observed that the severity of anticipatory nausea and vomiting did not exhibit a significant relationship with anxiety levels and social support alongside these factors.

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RESEARCH ARTICLE

CONTRIBUTIONS TO RARE PHENOTYPES IN KLINEFELTER SYNDROME

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ABSTRACT

Purpose: Klinefelter Syndrome (KS; 47, XXY) and Y chromosome microdeletions are the most common genetic causes of male infertility. Our goal was to assess these factors contributing to male infertility in our region.
Material and Methods: In this current study, 58 patients diagnosed with azoospermia/oligozoospermia were invited to the polyclinic and 2 ml peripheral blood samples were collected. Genotyping by employing PCR based-fragment analysis was conducted after isolating genomic DNA from the peripheral blood samples of patients who consented to participate in our study. Patients' FSH, LH, and testosterone levels, as well as their physical examinations, were carefully evaluated.
Results: We found that high follicle stimulating hormone (FSH) value can be used as a predictive factor in azoospermia. We successfully revealed the potential of KS (3.2%) but no Y chromosome microdeletions are responsible for primary male infertility. A patient with KS was identified, exhibiting not only short stature but also a lack of breast enlargement.
Conclusion: Non-genetic factors such as varicocele (28%) and smoking (28%) may have greater potential to explain primary infertility in our region. Physicians should be aware that unexpected features such as short stature may accompany KS in adult patients who have not received growth hormone treatment.

Keywords: Klinefelter Syndrome, short stature, Y-chromosome microdeletion, delayed diagnosis, smoking.

INTRODUCTION

According to the definition of the World Health Organization (WHO), infertility; is the absence of pregnancy as a result of sexual intercourse 2-3 times a week regularly and unprotected during a year (1). About 15% of couples of reproductive age are affected by infertility (2). Male factors account for half of infertility cases, and recently, the rise in infertility rates has gained attention, particularly due to declining sperm quality. Azoospermia, or the absence of sperm in semen analysis, is observed in 11.2% of infertile men (3). The most common genetic cause of infertility is Klinefelter syndrome (47, XXY), which is a sex chromosome anomaly, and this syndrome is also the most common (1/650 newborn male) sex chromosome anomaly (4). In this syndrome, spermatogenesis and androgen hormone production are interrupted (5). High LH (luteinizing hormone) production affects estradiol production and causes gynecomastia, and insufficient Leydig cell number causes low testosterone levels (6). Klinefelter syndrome (KS) is observed in 3% of all infertile men, whereas KS is seen in 14% of non-obstructive azoospermic cases (7–9).

According to the data obtained from the studies carried out between 1984-2003; chromosomal anomaly is seen in 13.1% of azoospermic cases (67% KS), while a chromosomal anomaly is observed in 4.3% of oligozoospermic cases (12% KS) (10). Y-chromosome microdeletions are one of the most common genetic cause of male infertility (11). Y-chromosome hosts the AZF (AZoospermia Factor) region, which has important roles in spermatogenesis, and there are 4 different AZF

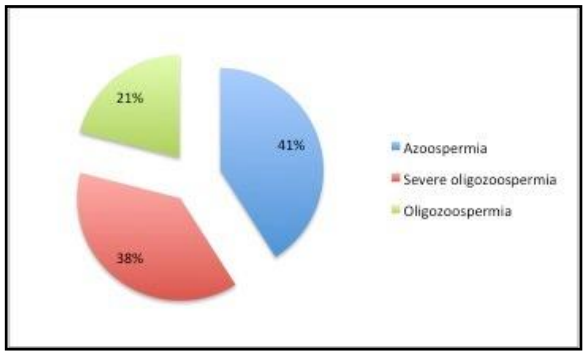

Figure 1. Classification of patients by sperm count

regions (AZFa, AZFb, AZFc and AZFd) (12). Three genes (*DDX3Y*, *USP9Y*, *UTY*) and 1 testis-specific transcription unit (*TTTY15*) located in the AZFa locus are present in a region of 1150 (kb), and single or combined deletions of these first 3 genes occur in Sertoli cell only (SCO) cases (9-55%) have been reported (13–15). The deleted AZFb locus, which is also associated with infertility, has 6 protein-coding genes and 9 TTTYs in a 6.2 Mb region, where the genes are *EIFA1Y*, *RPS4Y*, *SMCY*, *HSFY*, *PRY*, and *RBMY* genes (16). Mass deletions of AZFc are seen in 5-15% of infertile men and are responsible for oligozoospermia (17–20). In recent studies, the AZFd region is located between the AZFb and AZFc regions as a separate gene region, and patients with AZFd deletion may have mild oligozoospermia or normal sperm counting. However, it has been shown that they have a dysmorphic sperm structure (12).

We assessed the potential of the most common genetic and non-genetic causes of oligozoospermia and azoospermia in our region for the first time.

MATERIAL AND METHODS

Peripheral blood samples in K2-EDTA tubes by filling in informed consent form 58 patients who came to the Urology Polyclinic of Yozgat Bozok University, Medical Faculty Hospital due to primary infertility and had azoospermia/oligozoospermia in their semen analysis. The ethical approval of the current study was granted by the Clinical Research Ethics Committee of Faculty of Yozgat Bozok University (Date: 25.09.2019, Decision No: 2017-KAEK-189_2019.09.25_20).

This study was conducted in line with the principles of the "Helsinki Declaration". DNA isolation was performed from peripheral blood samples from 58 patients using commercial kits (PureLink™ Genomic DNA Mini Kit-Invitrogen). Polymerase chain reaction of the regions of interest was performed on a thermal cycler (SimpliAmp-Applied Biosystems Thermo Fisher Scientific) in accordance with the kit manual (GT AZFScreen Plus). FSH, LH and testosterone hormone levels were measured by Cobas series equipment (Roche Diagnostics, GmbH, Mannheim, Germany).

PCR conditions of initial denaturation (95°C- 20 mins), 30 cycles of (95°C- 1 min; 63°C- 90 sec; 70°C- 2 mins), final elongation (70°C- 20 mins), storage (4°C- ∞) was employed in thermal cycler. Samples were loaded into ABI Prism 3130 XL Genetic Analyzer and analyzed based on the pics of the fragments.

The spermiogram values of the patients; were divided into 3 groups and evaluated as azoospermia (zero sperm count), severe oligozoospermia (<5 million sperm/mL), and oligozoospermia (5-20 million sperm/mL). Figures were created and analyzed by choosing One-way-ANOVA (analysis of variance) and/or Kruskal-Wallis methods in the GraphPad Prism Version 8 software.

RESULTS

24 out of 58 patients who accepted to participate in the study had azoospermia, 22 had severe oligozoospermia, and the remaining 12 patients had oligozoospermia phenotype (Figure 1). The hormone levels we used as a reference in our project are given in table 1. The majority of the patients (n=47) were born in Yozgat and its surrounding districts (81.03%) (Supplemental Table 1).

When the patients were examined in 3 groups according to their sperm count, we found a significant difference between all groups (Figure 2). Accordingly, the mean sperm count of the patients with oligozoospermia was 13.4 ± 4.1 million/mL, which was higher than the mean of the patients with severe oligozoospermia, which 2.2 ±1.3 million/mL. The difference between the azoospermia group (zero sperm) and both severe oligozoospermia and oligozoospermia groups was also statistically significant ($p < .05$).

Table 1. Male reference hormone values

Hormon	Minumum	Maximum	Unit
FSH	0.95	11.95	mIU/mL
LH	0.57	12.07	mIU/mL
Total testosteron	1.4	9.2	ng/mL
Estradiol(E2)	11	44	pg/mL
Prolactin	3.46	19.40	ng/mL

FSH, LH, testosterone values were examined according to the groups, we found a significant difference between azoospermia and FSH levels (Figure 3). The mean FSH (15.0 ± 9.4 mIU/mL) in patients with azoospermia was higher than both the mean FSH in the patient group with oligozoospermia (5.5 ± 4.5 mIU/mL) and the mean FSH in the patient group with severe oligozoospermia (8.5 ± 7.2 mIU/mL). The mean FSH level of the patient group with severe oligozoospermia the was higher than the mean FSH level of the patient group with oligozoospermia, but it was not statistically significant (p> .05). Differences between mean LH or testosterone levels were also not statistically significant (p> .05). The mean FSH level was 10.5 ± 8.5 mIU/mL, the mean LH level was 6.2 ± 3.2 mIU/mL, and the mean testosterone level was 5.4 ± 2.9 ng/mL (Figure 4).

It was found that 5 of the married couples were consanguineous (8.8%). When evaluating male infertile patients by occupation, it was noted that 5 patients (8.8%) were security guards and 4 patients (7.0%) were construction workers. Additionally, a smoking history was observed in 16 patients (28.1%), with 12 of them (75%) consuming more than one pack of cigarettes per day.

Fifteen patients (26.3%) had a history of varicocele with a grade lower than three. In 6 patients out of 15 with varicocele had a history of varicocele operation. In addition to the 15 patients mentioned earlier, another patient (patient #22) experienced physical trauma to his testicles in his youth. Fifteen patients had a history of undergoing TESE/mTESE (microsurgical testicular sperm extraction). Among these 15, sperm retrieval was unsuccessful in 5 patients, all of whom were diagnosed with azoospermia.

A non-smoker patient without a history of varicocele had a history of pituitary adenoma (prolactinoma) and was followed for a long time using psychiatric drugs (patient #13). Despite being azoospermic, blood tests showed relatively normal follicle-stimulating hormone (FSH) at 2.6 mIU/mL and luteinizing hormone (LH) at 0.9 mIU/mL, while testosterone was low at 3 ng/mL. Long-term use of high-dose colchicine due to familial mediterranean fever (FMF) was observed in a patient with oligozoospermia (patient #58). A patient with azoospermia working in the construction industry (patient #30), who stated that he smoked 1 pack of cigarettes a day, and he had received chemotherapy 10 years ago for lung cancer. A patient with

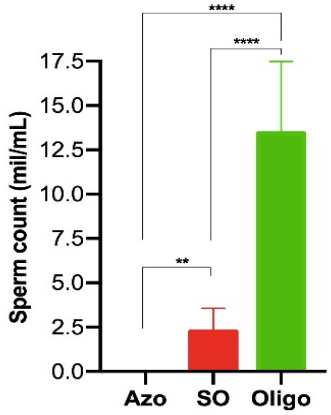

Figure 2. Demonstration of the difference between the sperm counts of the patients (** p = .0094, ****, p < .0001, Kruskal-Wallis)

azoospermia (patient #9) had a history of left orchiectomy in 2014 for sertoli cell only syndrome (SCO) and testicular cancer.

TESE history was followed up in 15 patients (26.3%). One of these patients (patient #48) had a healthy baby with the ROSI (round sperm injection) method. Apart from this patient, it was learnt that a patient with a sperm count of 100 thousand/mL (patient #57) fathered a healthy girl later on in a normal way. Excluding these two cases, we learned about two additional patients who achieved successful pregnancies with ART (assisted reproductive techniques) despite having azoospermia and severe oligozoospermia, respectively. Notably, these outcomes were achieved without the use of TESE. Additionally, it was observed that a patient with oligozoospermia (patient #35) successfully fathered a daughter naturally, without the need for ART.

KS (47, XXY) was detected in 2 patients (patient #27 and #49). Patient 27 did not present the phenotypic features of KS such as enlarged breasts and lack of facial and body hair. The patient was not tall (1.70 meter) either. Additionally, no microdeletions were found in the AZF regions of the Y chromosome in any of the patients, and none exhibited XYY syndrome.

Patient #45 underwent a male infertility panel that evaluated five genes: *CFTR*, *CATSPER1*, *LHCGR*, *AR*, and *FSHR*. This analysis revealed a heterozygous pathogenic mutation (p.G178R) in the *CFTR* gene. Additionally, conventional cytogenetic testing confirmed a normal chromosomal karyotype (46, XY) for this patient.

When providing counseling upon receiving the genetic results, it was realized that the azospermic patient 21 did not fully meet the criteria for primary

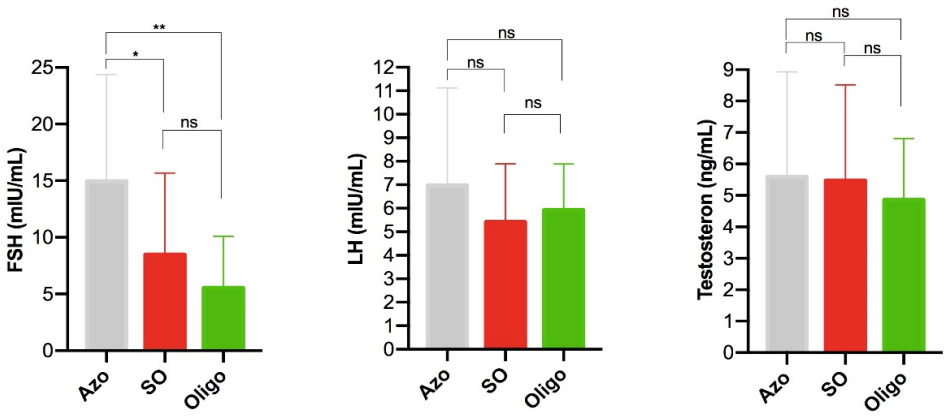

Figure 3. FSH, LH and testosterone hormone levels according to the sperm count profile (FSH group * p = .0195, ** p = .0036, ns: not significant = not significant, p = .5491, Ordinary one-way ANOVA, expressed as ns: p < .05 for LH and testosterone groups)

infertility (duration is 10 months but not a year). Therefore, the patient was not included in the results section. So, we decided to give patient number as 57 while calculation the ratios.

DISCUSSION

Sperm count was below 5 million in 79% of primary infertile men in this current study. When the patients are grouped in terms of their hormone profiles, we can say that high FSH value can be used as a predictive factor in azoospermia (Figure 4). The same is not the case with the other two hormones (LH and testosterone).

In line with the tests we conducted within the scope of the project, we determined genetically factors that could explain infertility in 2 patients (3.5%). These patients were also azoospermic. In the literature, the incidence of KS in azoospermic cases is up to 12.6% and its frequency has been reported as up to 3% in infertile men (21–23). Considering our 23 cases of azoospermia, our rate is slightly low (8.7%), and it is almost the same when considering our 57 infertile cases. In a retrospective study, in which only cases with azoospermia and severe oligozoospermia were evaluated, KS was found to be 8% in total, 3.2% in men with azoospermia, and 11.5% in men with severe oligozoospermia (24).

One distinctive feature of our study is the existence of unexpected combination of relatively short stature in one patient with KS (BMI-body mass index; 24.2). Short stature which was explained by partial growth hormone deficiency in some previous pediatric KS cases (25–27) , was observed in the adult patient in our case (170 cm). Enlarged breast structure and lack of hair growth, which are specific findings of KS, were not present in our case either. The hormone profile of this patient was high FSH (21.8 mIU/mL), and borderline testosterone 152.4 ng/dL while there is normal LH (8.7 mIU/mL) level. One very rare adult case report was also found in literature that acromegaly can accompany with Klinefelter syndrome as a result of high growth hormone concentration (15.1 ng/mL, reference range is 0.01-3.6 ng/mL) due to the existence of pituitary adenoma (28). The height of this adult patient (age 27) was found to be 2 cm taller than our patient but was considered as short compared to the patients with KS. We couldn't find any documentation in the patients' medical records regarding growth hormone testing or its results. The unusual presentation of KS in this patient may have led to a delayed or missed diagnosis by their physicians. This could be due to the presence of unexpected and uncommon features associated with the syndrome.

AZF region deletion was not found in any of the infertile male patients included in our study. Our findings suggest that Y-chromosome microdeletions are not a major contributing factor to male infertility in our patient population. Deletions in the AZF regions of the Y chromosome are the second most frequent genetic cause of male infertility, following KS, with a reported prevalence of up to 10% (29). Among Y-chromosome microdeletions, the most common condition is AZFc deletions. In a study originating from Turkiye and finding the lowest rate of AZFc deletions (in only 1 azoospermic patient), this rate was found to be 1.3%, while AZFa and AZFb region deletions were not observed (30). The prevalence of Y-chromosome microdeletions can vary across populations. An Iranian study, for instance, found a higher frequency of AZFb deletions (66.7%)

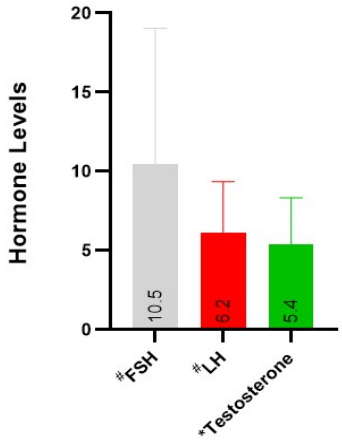

Figure 4. FSH, LH and testosterone hormone levels in primary infertile male patients (#:mIU/mL for FSH and LH levels, * ng/mL for testosterone levels)

compared to AZFc deletions (41.7%), with an overall microdeletion rate of 12% (31).

While our study did not identify the most common genetic contributors to infertility in this patient group, it does not rule out a genetic cause altogether. Further investigation into chromosomal abnormalities, such as Robertsonian and reciprocal translocations, is necessary. Techniques such as conventional cytogenetics or array CGH (comparative genomic hybridization) can be employed for this purpose. Emerging research suggests a significant role for de novo mutations in male infertility. Studies have identified at least 94 genes potentially contributing to this condition (32).

CONCLUSION

We can conclude that etiological factors like varicocele (28%), smoking (28%), working in intensive jobs (15.8%), long-term drug use (1.8%) such as cholchisine, chemotherapy history (1.8%) have more potentials to explain male infertility in our primary infertile patient group than the genetic factors such as XXY syndrome and Y chromosome microdeletions. Since ratio of smokers is significantly higher in Türkiye than western countries, conducting studies with a larger number of patients divided into two distinct groups, smokers and non-smokers, would yield healthier results. To prevent missed/overlooked diagnoses of KS in patients presenting with atypical symptoms, physicians should consider including common causes of primary male infertility in their differential diagnoses.

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Supplemental Table 1. The hormone levels and some clinical outputs of 58 patients in current study

Patient No	Infertility (month)	Relativity status	Sperm million/mL	LH mIU/mL	FSH mIU/mL	T. ng/mL	Smoking status	Var*/operation torsion-trauma	TESE status
1	36	No	20	3.7	18.5	2.1	1 pack	operated	No
2	18	Yes	0	6.5	15	5.8	No	No	No
3	18	No	3	2.8	5.8	3.1	No	No	No
4	36	No	0	6.8	21.1	4.3	No	No	No
5	48	No	3	3.4	3	2.3	No	No	No
6	36	Yes	2.5	7.1	25.6	5.3	No	No	No
7	72	No	0	4.8	7.6	8.7	No	No	No
8	18	No	2	5.9	8.5	5.3	No	No	No
9	12	No	0	14.3	38.4	3.1	No	left orchiectomy	Yes
10	12	No	2	4.4	18.9	7	No	No	No
11	24	Yes	0.5	5.2	7.3	5.7	No	Var.	No
12	24	No	0	5.8	5.3	2.4	No	No	Yes
13	36	No	0	0.9	2.6	3	No	No	Yes
14	12	No	0.5	7	6	7.7	No	No	No
15	12	No	2.5	4	3.9	3.1	No	operated	No
16	84	No	4	4.3	2.7	6	No	No	No
17	12	Yes	0	1.8	3.5	5.8	No	Var.	Yes
18	48	No	0	2.6	5.3	3.8	No	operated	No
19	24	No	4	4.7	5	5.8	No	No	No
20	12	No	0.1	2.6	3.6	4.2	No	No	No
21	NA	NA	0	69.1	136	1.6	No	torsion	No
22	18	No	0	11.5	19.3	3.3	3-4 butt	trauma	No
23	30	No	12	8.2	5.8	4.9	No	No	No
24	18	No	2	4.5	3.5	3.3	No	No	No
25	48	No	1	5.4	24.1	6.9	1 pack	No	No
26	72	Yes	3	3	4.5	3.5	1 pack	Var.	No
27	276	No	0	12	20	166	4 packs	operated	Yes
28	12	No	12	5.4	1.9	2.2	No	No	No
29	42	No	0	4	22.2	3.7	No	No	Yes
30	60	No	0	6.3	10.5	4.6	1 pack	No	No
31	18	No	14	6.6	5.2	5.6	3-4 butt	No	No
32	18	No	18	6.8	5	7	No	Var.	No
33	12	No	3	11.9	21.7	4.8	No	No	No
34	12	No	0.1	9.1	4.3	4	No	No	No
35	12	No	14	5.1	5.3	3.9	1 butt	No	No
36	12	No	0	7.9	15.7	7.4	No	No	No
37	18	No	4	5	5.6	5.2	No	operated	No
38	12	No	15	7.6	2.3	8	No	No	No
39	12	No	3	8.8	3.4	3.7	No	No	No
40	24	No	6	4.5	2.8	5.5	No	operated	No

Supplemental Table 1 Continue.

Patient No	Infertility (month)	Relativity status	Sperm million/mL	LH mIU/mL	FSH mIU/mL	T. ng/mL	Smoking status	Var*/operation torsion-trauma
41	24	No	14	5.7	3.9	6.2	No	No
42	36	No	4	2.7	7.3	2.9	No	Var.
43	156	No	7	9.9	3.7	5.4	No	No
44	96	No	0	6.6	19.9	2.3	No	Var.
45	72	No	0	8.6	12.5	2.8	No	No
46	96	No	12	3.9	2.7	5.5	30 butt	Var.
47	20	No	17	3.7	9.3	2	5-6 butt	Var./right orchiectomy
48	180	No	0	1.5	2.8	14.7	1 pack	No
49	12	No	0	18.4	12.1	9.4	No	No
50	180	No	0	8	12	5.5	1 pack	No
51	18	No	2	6.7	6.6	5.2	1 pack	Var.
52	60	No	0	6.4	12.5	12.4	No	No
53	40	No	0	6.3	30	6	1 pack	No
54	72	No	0	7.1	5.3	2.8	1 pack	No
55	150	No	0	8.8	25.5	8.7	1 pack	No
56	120	No	0	3.6	24.8	6.2	1 pack	No
57	24	No	0.1	8.2	11.8	8.3	No	No
58	24	No	2.8	2.5	3.2	17	No	No

Abbreviations: T.:Testosteron,*Var. represents the varicocele status is present but patient has not been operated. Rows in green show patients with KS.

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RESEARCH ARTICLE

ARE VESTIBULAR FUNCTION OR VISUOSPATIAL PERCEPTION AFFECTED IN INDIVIDUALS WITH IDIOPATHIC SCOLIOSIS?

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ABSTRACT

Purpose: It was aimed to investigate the relationship between characteristics of the curve and balance, vestibular dysfunction, visuospatial perception, navigation performance, and quality of life in idiopathic scoliosis.
Material and Methods: Thirty-three participants aged 10-25 were included. The Cobb angle of the participants was recorded. The degree of rotation of the curve with the mobile application called ScolioDetector; balance parameters with the duration of unipedal stance test (eyes open-closed, right-left foot, hard-soft ground); vestibular dysfunction with the Utenberger test; visual-spatial perception with the Corsi Block Tapping test; navigation performance with the triangle completion task; and quality of life was assessed with the Scoliosis Research Society-22. In the comparison made according to type and direction of scoliosis curve, analysis was performed with the independent sample t-test or Mann-Whitney U test; Pearson correlation or Spearman correlation test was used in the relationship between Cobb and rotation angle and other parameters.
Results: In the comparison made according to the curve type, only the right eyes closed unipedal stance test duration ($p=0.022$) and Unterberger test rotation angle were found to be significantly different ($p=0.045$). According to the direction of the curve, except for the right foot unipedal stance test (eyes open) on soft ground ($p=0.009$) and Unterberger test displacement distance ($p<0.05$) and the degree of rotation with eyes open ($p=0.007$), no significant difference was found ($p>0.05$). A significant correlation was shown only between the rotation angle and the right foot eyes closed single leg stance test on soft ground. No significant correlation was found between Unterberger and Visual-Spatial Memory tests.
Conclusion: The characteristics of the curve (C or S; right or left scoliosis) affect balance and vestibular dysfunction. The rotation angle is only related to balance; it was observed that the curve features were not associated with visuospatial perception and navigation performance.

Keywords: Balance, idiopathic scoliosis, pain, vestibular dysfunction, visuospatial perception

INTRODUCTION

Idiopathic scoliosis is the most prevalent form of scoliosis, accounting for approximately 80% of structural scoliosis cases, with its underlying cause remaining largely unknown (1, 2). Anatomical abnormalities in the vestibular system, medulla oblongata, pons, and midbrain have been reported in individuals with idiopathic scoliosis (3). In MRI studies conducted between healthy individuals and individuals with idiopathic scoliosis, have revealed morphological differences in the vestibular system, including regional brain volume, corpus callosum, white matter, internal capsule, and especially semicircular canal alignment (3-5). Visual, vestibular,

proprioceptive, and postural control abnormalities involving the cerebral hemispheres, brainstem, and corpus callosum have been demonstrated in idiopathic scoliosis patients (4-6). In idiopathic scoliosis, there is a disorder in the postural reflex mechanism originating from the proprioceptive organs, the vestibule of the inner ear, and the visual system during the vertebral growth period. It is also known that balance problems occurring in the balance function center in the brainstem are associated with the displacement of the vertebrae. Therefore, it is thought that brainstem dysfunction may cause idiopathic scoliosis (7). Since the vestibulospinal pathway affects the hypothalamus and cerebellum, the vestibular system has been suggested to be a possible cause of the morphological, hormonal, and neurosensory abnormalities observed in individuals with idiopathic scoliosis (8). In the etiology and definition of idiopathic scoliosis and the three-dimensional deformity of the spine, many factors such as postural asymmetry, dysfunction in the proprioceptive, vestibular, and vestibulospinal systems, and postural stability defects are noted (9). An asymmetry in vestibular function can create unbalanced stimulation of the spinal musculature and thus contribute to the development of scoliosis. One study showed that individuals with idiopathic scoliosis exhibited greater vestibular asymmetry than healthy participants (10). Research on adolescent idiopathic scoliosis has reported significant differences in balance and vestibular function compared to control groups (11,12). Apart from the typical symptoms associated with vestibular dysfunction, various cognitive processes, such as visuospatial perception ability, memory, attention, and executive function, provide insight into this system. Visuospatial perception is a term used to describe how the mind understands and organizes two- and three-dimensional space. It involves a variety of skills, including spatial memory, mental imagery, rotation, distance and depth perception, navigation, and visuospatial structure (13). Spatial memory, orientation, and mental rotation have been examined about vestibular dysfunction (13). A person's perception is the result of vestibular, visual, and somatosensory sensory integration. Studies have shown that animals with vestibular lesions have impaired visuospatial perception abilities (14). It has been proposed that alterations or absence of vestibular input may result in disruptions to an individual's mental representation of three-dimensional space. Furthermore, patients with vestibular deficits have demonstrated impaired spatial navigation abilities, particularly in environments devoid of visual cues (15).

It is thought that vestibular dysfunction may be effective in the etiology of patients with idiopathic scoliosis. Additionally, vestibular dysfunction has been associated with visuospatial perception performance. In the literature, to our knowledge, there is no study evaluating visuospatial perception performance in patients with scoliosis. Based on the information in the literature, in this study was aimed at investigating whether there is any impairment in vestibular function and visuospatial perception performance in individuals with idiopathic scoliosis and revealing the dependent/independent relationship between the two parameters.

MATERIAL AND METHODS

Study Design

This study was designed as a cross-sectional, single-center study. Study data was collected between August 2021 and June 2022. The research was previously reviewed and approved by the Inonu University Clinical Research Ethics Committee (Date: 30.06.2021, Decision No: 2021/144). This study was conducted in accordance with the principles of the Declaration of Helsinki. Informed consent was obtained from the parents of the participants under 18, and the participants over 18 read and signed the informed consent.

Participants

Participants were selected through a non-probability random sampling method from patients with idiopathic scoliosis who presented to the orthopedics outpatient clinic at Turgut Ozal Medical Center. Although it was initially planned to include a healthy control group in the study, the ongoing pandemic conditions prevented the recruitment of a sufficient number of participants, and thus the control group could not be included. Scoliosis-specific analysis was performed. Individuals who had no additional joint deformities, no cognitive impairment, and were diagnosed with idiopathic scoliosis by an orthopedist between the ages of 10-25 were included in the study. Individuals with other types of scoliosis, those with severe hearing and visual impairment other than idiopathic scoliosis, those with any neurological, orthopedic, metabolic, or rheumatological disorders, those with benign paroxysmal positional vertigo,

Table 1. Demographic characteristics of individuals with idiopathic scoliosis

	Total (n=33)
Age, year, mean± SD	15.27±2.28
Gender, F/M	25/8
BMI, mean± SD	18.95±3.31
Dominant hand, R/L	32/1

F: Female, M: Male, BMI: Body mass index, R: Right, L: Left, SD: Standard deviation

Meniere's primary pathologies of the ear, and those with a history of serious infection (ear, internal organs), and those who had undergone a scoliosis-specific exercise program within the last year were excluded from the study. Participants who did not comply with the evaluations, who left the evaluations unfinished, and who did not sign the informed consent form were excluded from the study.

Sample Size

In the power analysis performed before the study, it was calculated that at least 14 participants should be included, assuming that idiopathic scoliosis is seen in 1% of the general population (16). The sample size calculation was performed using OpenEpi version 3 (http://www.openepi.com).

Measurements

Demographic variables such as age, gender, weight, height, as well as dominant hand information of all participants were recorded.

Balance performance was evaluated with a unipedal stance test. Individuals' right and left foot single leg stance times were recorded in seconds with a stopwatch on soft/hard ground and with eyes open/closed (17).

Vestibular dysfunction was evaluated with the Unterberger test (Fukuda stepping test). In this test, participants counted in place with 45 degrees of hip flexion for 50 steps, and after 50 steps, the rotation angle between the initial position and the final position of the right foot was measured with a goniometer, and the displacement distance was measured with the help of a tape measure The test was applied in two different positions, with eyes open and closed (18,19).

Spatial memory for visuospatial perception was evaluated with the mobile application called Visuospatial Memory Test, which was inspired by the Corsi Block Tapping test. In the application, pink squares appear on the screen, and the squares that individuals need to mark are indicated. Participants were asked to make the resulting markings in the same way. As soon as he confused the order of the markings, the test ended, and the highest span scores were recorded (20). Navigation performance was evaluated with the triangle completion task. In the navigation performance, two triangles (one equilateral triangle and one right triangle, at angles of 30°, 60°, and 90°, respectively) with lengths between 150 and 300 cm were marked on the ground. Participants were asked to place their feet on the pre-marked area at the common starting point of the two triangles and to complete the two triangles with their eyes open, first the larger triangle and then the smaller triangle. The test with eyes open was a trial phase. During the main assessment, participants were asked to complete triangle tasks with their eyes closed. The distance between the initial and final positions of the reference right big toe was recorded in centimeters (21).

The degree of rotation was evaluated with the help of a mobile application called ScolioDetector. They were asked to bend forward with their hands. During this process, care was taken to ensure that their bodies were parallel. The highest point of the hump on the spine was determined, and the smartphone was placed perpendicular to the spine. The degree of rotation was recorded (22).

Quality of life was evaluated with the Scoliosis Research Society-22 questionnaire. The survey consists of a total of 36 items and 8 sub-parameters: body pain, physical function, emotional well-being, limitation due to emotional problems, limitation due to physical problems, social function, energy/fatigue, and general health perception. Low scores on the questionnaire were associated with poor quality of life (23,24).

The Cobb angle, type and direction of the curve, and localization of scoliosis were evaluated through routine radiography taken by the orthopedist. In Cobb angle measurement, tangent lines were drawn from the upper-end plate of the upper vertebra participating in the curvature and from the lower-end plate of the lowest vertebra participating in the curvature. The angle formed where these two lines intersected was recorded as the Cobb angle (25).

Statistical Analysis

Normality evaluation of the participants' data was made with the Shapiro-Wilk Test. Among descriptive statistics, mean and standard deviation were used. In

Table 2. Comparison of balance, vestibular dysfunction, visuospatial perception, navigation performance, and quality of life according to the type of curve

	C Scoliosis (n=17)	**S Scoliosis (n=16)**	***p***
Cobb Angle (°)	22 (10/53)	29 (10/51)	0.211[a]
Rotation Angle (°)	2 (1/33)	7 (2/20)	0.102[b]
Unipedal Stance Test, s			
Rigid Surface, EO, R	60 (16.59/60)	60 (4.73/60)	0.759[b]
Soft Surface, EO, R	56.61 (5.14/60)	22.32 (5.62/60)	0.115[b]
Rigid Surface, EO, L	60 (0.19/60)	60 (24.07/60)	0.450[b]
Soft Surface, EO, L	42.12 (4.46/60)	19.13 (4.95/60)	0.410[b]
Rigid Surface, EC, R	20.46 (3/60)	7.61 (2.22/46.20)	**0.022[b]**
Soft Surface, EC, R	6.11 (1.64/22.35)	3.63 (1.65/60)	0.171[b]
Rigid Surface, EC, L	11.42 (2.37/48.70)	9.78 (4.29/47)	0.829[b]
Soft Surface, EC, L	6.76 (1.85/60)	4.86 (1.77/19.55)	0.150[b]
Unterberger test			
Distance of Displacement, EO, m	14.5 (1/87)	135 (1/57.50)	0.564[b]
Distance of Displacement, EC, m	45 (6/115)	48.75 (13.50/99)	0.745[a]
Angle of rotation, EO, (°)	13 (2/72)	24 (8/73)	**0.045[b]**
Angle of rotation, EC, (°)	20 (3/83)	16.5 (3/57)	0.601[b]
Visuospatial Memory Test			
Highest Span	5 (4/9)	6 (3/6)	0.734[b]
Reaction time	1020.70 (460.40/1438.50)	928.45 (489/1913.50)	0.729[a]
Navigation Performance			
30-60-90 Triangle, m	45 (6/157)	54.50 (7/147)	0.871[b]
60-60-60 Triangle, m	26 (1,5/101)	30.50 (2/170)	0.957[b]
SRS-22			
Function	4.6 (2.60/5)	4.60 (2.20/5)	0.645[b]
Pain	4.2 (1.80/5)	3.90 (2.80/5)	0.704[b]
Body image	3.20 (1.60/5)	3.20 (2.20/4.60)	0.640[a]
Mental health	3.2 (1.80/5)	3.70 (1.60/4.60)	0.772[b]

EO: Eyes open, EC: Eyes close, R: Right, L: Left, s: second, m: Meter, SRS-22: Scoliosis Research Society- 22. [a]Independent Sample T test, [b] Mann Whitney U test

the comparison made according to the type of scoliosis curve and the direction of the deficit, analysis was performed with the independent sample t-test or Mann-Whitney U test, depending on the normality of the data. In the correlation analysis, the Pearson correlation test, or Spearman correlation test, was used according to the normal distribution in the relationship between Cobb and rotation angle and other parameters. Correlation coefficient (r); It was interpreted as 0.00–0.20 poor, 0.21–0.40 fair, 0.41–0.60 good, 0.61–0.80 very good, 0.81–1.0 excellent $p<0.05$ was considered statistically significant. The data were analyzed with Statistical Package for the Social Sciences (SPSS) version 25.0.

RESULTS

During the study period, 38 patients were evaluated for eligibility. A total of 2 patients (short limbs, genetic disorder) were not included because they did not meet the eligibility criteria. 36 patients participated in the evaluation. Three participants could not be included in the analyses due to missing data, and the analysis had been carried out with a total of 33 participants. Demographic data and dominant hand information of the individuals are given in Table 1.

In the analysis performed according to the type of curve, there was no difference in Cobb and rotation angles between the groups ($p>0.05$). In the comparison of the unipedal stance test between the groups, it was found that the right foot eyes closed test performance in the group with only the S scoliosis curve was significantly lower than in the C scoliosis group ($p = 0.022$). There was no difference in other unipedal stance test performances between the groups ($p>0.05$). In the Unterberger test, the eyes-open rotation angle in the S scoliosis group was significantly higher than the C scoliosis group ($p = 0.045$). There was no significant difference between

Table 3. Comparison of balance, vestibular dysfunction, visiospatial perception, navigation performance and quality of life according to the direction of the primary curve

	Right (n=22)	**Left (n=11)**	***p***
Cobb Angle (°)	24 (10/51)	31 (10/53)	0.467[b]
Rotation Angle (°)	4.50 (1/33)	8 (2/20)	0.286[b]
Unipedal Stance Test, s			
Rigid Surface, EO, R	60 (4.73/60)	60 (12.20/60)	0.319[b]
Soft Surface, EO, R	24.99 (5.14/60)	60 (7.96/60)	**0.009[b]**
Rigid Surface, EO, L	60 (0.19/60)	60 (28.84/60)	0.279[b]
Soft Surface, EO, L	26.89 (4.46/60)	33.86 (12.20/60)	0.691[b]
Rigid Surface, EC, R	9.79 (3/34.65)	14.10 (2.22/60)	0.422[b]
Soft Surface, EC, R	4.14 (1.65/22.35)	5.09 (1.64/60)	0.661[b]
Rigid Surface, EC, L	11.72 (2.37/47)	10.20 (5.23/48.70)	0.789[b]
Soft Surface, EC, L	6.73 (1.77/60)	4.27 (1.85/19.55)	0.169[b]
Unterberger test			
Distance of Displacement, EO, m	12.75 (1/52.50)	24 (1/87)	**0.049[b]**
Distance of Displacement, EC, m	40 (6/99)	61.50 (33/115)	**0.008[a]**
Angle of rotation, EO, (°)	23.35 (5/73)	10 (2/40)	**0.007[b]**
Angle of rotation, EC, (°)	25.50 (3/83)	14 (5/48)	0.089[b]
Visuospatial Memory Test			
Highest Span	5 (4/9)	6 (3/7)	0.389[b]
Reaction time	1037 (489/1438.50)	848.90 (460.40/1913.50)	0.693[a]
Navigation Performance			
30-60-90 Triangle, m	49.50 (8/157)	50 (6/147)	0.660[b]
60-60-60 Triangle, m	30.50 (1.50/170)	24 (2/92)	0.593[b]
SRS-22			
Function	4.50 (2.20/5)	4.60 (3.20/5)	0.845[b]
Pain	4 (1.80/5)	4.20 (3/4.80)	0.618[b]
Body image	3.20 (1.60/5)	3 (2.20/4.40)	0.952[a]
Mental health	3.40 (1.60/5)	3.80 (2.20/4.20)	0.388[b]

EO: Eyes open, EC: Eyes close, R: Right, L: Left, s: second, m: Meter, SRS-22: Scoliosis Research Society- 22. [a]Independent Sample T test, [b] Mann Whitney U test

the groups in Visuospatial Memory Test scores, navigation performance, and SRS-22 survey sub-scores (p>0.05) (Table 2).

Cobb and rotation angles were similar in the groups according to the direction of the curve opening (p>0.05). Unipedal stance test performance had no difference between the groups in all subparameters (p>0.05), except for the duration of the right extremity standing on one foot with eyes open on soft ground (p=0.009). The Unterberger test showed that the amount of displacement with eyes open and closed was significantly lower in individuals with a right-facing curve than in the group with a left-facing aperture curve (p=0.049, p=0.008, respectively). While there was a significant difference between the groups in the eyes-open rotation angle (p=0.007), there was no difference between the groups in the eyes-closed rotation angle (p>0.05). There was no significant difference between the groups in Visuospatial Memory Test scores, navigation performance and SRS-22 survey sub-scores (p>0.05) (Table 3).

In participants with scoliosis, a negative correlation was shown between the Cobb angle and the duration of unipedal stance test on the right and left soft ground with eyes closed, respectively at a moderate (p = -0.313) and good level (r = -0.414). There was no significant correlation between other unipedal stance test performances (p>0.05). There was a fair negative correlation between the Cobb angle and the eyes-closed rotation angle of the Unterberger test only (p=-0.300). There was no significant correlation between Cobb angle and navigation performance and SRS-22 (function, body image, mental health) (p>0.05). There

Table 4. Relationship of Cobb and rotation angle with other parameters

		Cobb Angle	Rotation Angle
Unipedal Stance Test			
Rigid Surface, EO, R	**r**	-0.078	0.061
	p	0.665[b]	0.737
Soft Surface, EO, R	**r**	-0.118	0.115
	p	0.513[b]	0.525
Rigid Surface, EO, L	**r**	0.085	0.188
	p	0.638[b]	0.294
Soft Surface, EO, L	**r**	-0.132	0.114
	p	0.464[b]	0.526
Rigid Surface, EC, R	**r**	-0.214	-0.164
	p	0.231[b]	0.361
Soft Surface, EC, R	**r**	-0.313	**-0.433***
	p	0.076[b]	**0.012**
Rigid Surface, EC, L	**r**	-0.038	-0.015
	p	0.834[b]	0.934
Soft Surface, EC, L	**r**	-0.414	-0.274
	p	0.017[b]	0.123
Unterberger test			
Distance of Displacement, EO, m	**r**	0.052	0.113
	p	0.775[b]	0.530
Distance of Displacement, EC, m	**r**	-0.202	-0.023
	p	0.259[a]	0.898
Angle of rotation, EO, (°)	**r**	0.195	0.208
	p	0.277[b]	0.245
Angle of rotation, EC, (°)	**r**	-0.300	-0.254
	p	0.090[b]	0.154
Visuospatial Memory Test			
Highest Span	**r**	-0.237	-0.087
	p	0.183[b]	0.629
Reaction time	**r**	0.130	0.123
	p	0.470[a]	0.495
Navigation Performance			
30-60-90 Triangle, m	**r**	-0.089	0.030
	p	0.622[b]	0.869
60-60-60 Triangle, m	**r**	0.040	0.008
	p	0.827[b]	0.963
SRS-22			
Function	**r**	-0.291	-0.215
	p	0.100[b]	0.230
Pain	**r**	-0.383	-0.231
	p	0.028[b]	0.195
Body image	**r**	-0.115	-0.135
	p	0.525[a]	0.452
Mental health	**r**	-0.185	-0.190
	p	0.304[b]	0.289

EO: Eyes open, EC: Eyes close, R: Right, L: Left, m: Meter, SRS-22: Scoliosis Research Society- 22. [a]Pearson correlation test, [b]Spearman correlation test

was a moderate negative correlation with the SRS-22 pain sub-parameter (p = -0.383) (Table 4).

There was no significant difference between all subparameters of the unipedal stance test (p>0.05), except for the rotation angle and the duration of standing on a single leg with eyes closed on the right soft ground (p=-0.433). There was no significant correlation between rotation angle and Unterberger test, visuospatial memory test, navigation performance and SRS-22 (p>0.05) (Table 5).

Table 5. The relationship between vestibular dysfunction and visuospatial perception disorder in individuals with scoliosis

			Visuospatial Memory Test[a]	
			Highest Span	Reaction time
Unterberger test[a]	Distance of Displacement, EO, m	r	0.041	-0.018
		p	0.823	0.920
	Distance of Displacement, EC, m	r	0.006	0.061
		p	0.975	0.735
	Angle of rotation, EO, (°)	r	-0.043	0.110
		p	0.810	0.543
	Angle of rotation, EC, (°)	r	-0.145	0.020
		p	0.421	0.912

EO: Eyes open, EC: Eyes close, m: Meter. [a]Spearman correlation test

Additionally, there was no significant correlation between Unterberger test subparameters and Visuospatial Memory Test scores (p>0.05).

DISCUSSION

In the analysis according to the type of curve; although there is a similarity in cobb and rotation angles, in the test performance in the eyes closed right standing (unipedal stance test) and eyes open rotation angle (unterberger test); it is seen that patients with S scoliosis are more affected than patients with C scoliosis. The direction of curvature is in the right group; It is observed that the duration of standing on soft ground with eyes open (unipedal stance test) with the right extremity has lower scores than the left extremity. In addition, it was observed that the amount of displacement with eyes open and closed (Unterberger test) had lower scores in the group with right-sided curvature than in patients with left-sided curvature. Negative correlation (moderate and good) was observed between the cobb angle and unipedal stance test, unterberger test rotation angle, SRS-22 pain sub-parameter with eyes closed. In addition, a negative correlation was observed between the rotation angle and the duration of standing on soft ground with the right extremity.

Three-dimensional deformity of the spine in patients with adolescent idiopathic scoliosis leads to negative changes in sensory and motor processes (26). Negative effects on sensory and motor processes cause asymmetries in muscle strength around the spine, leading to changes in the center of body mass. This leads to an increase in balance and postural oscillations (27). In studies in which patients with AIS and healthy individuals were examined; Gauchard et al. reported significant differences in both static and dynamic balance parameters (28), Simoneau et al. reported that patients with AIS were weaker in standing balance (29). Force transfers in the form of kinetic chains will cause major changes in balance while performing static and dynamic activities, along with changes in spinal stability and neutral spinal alignment (30). Our study findings are consistent with the literature. In the light of the information in the literature, we think that spinal stability and neural spine alignment might be adversely affected in patients with S scoliosis compared to patients with C scoliosis. With this situation, we hypothesize that the effects that may occur in the muscle and soft tissue structures around the spine will be greater. We hypothesize that these effects may exacerbate adverse events in the vestibular, somatosensory and visual systems, resulting in worse balance and vestibular function in patients with S scoliosis than in patients with C scoliosis.

In a study examining the relationship between foot stability and postural changes in patients with AIS, a decrease in foot stability was observed in patients with AIS. It was reported that decreased foot stability negatively affected balance (31). In a study in which the relationship between standing balance parameters and body somatotype was investigated in girls with AIS, it was reported that increased oscillations was observed in children with endormorphic structure compared to the healthy group, while children with ectomorphic structure tended to lean backwards compared to the healthy group. As a result, it was reported that postural responses and balance were related with body somatotype (32). It has been reported that pelvic abnormal growth in girls with right thoracic scoliosis negatively alters the relationship between body and trunk center of mass (33). As far as we reviewed the literature, there are studies reporting that hand preferences in patients with scoliosis are related with the strength of the direction of asymmetry and trunk asymmetry, curve pattern and convexity of scoliosis (34, 35). In young adults with AIS, it has been reported that static balance is mostly related to sagittal balance, 67% of patients with a leftward

orientation of curvature place weight on the back, whereas this rate is 89% in patients with a rightward orientation of curvature. In addition, it has been reported that regardless of whether the direction of curvature is left or right, there is a tendency to place more weight on the right (36). Considering the patient profile in our study, right dominance (32 right, 1 left) and right predominance in the direction of the curve aperture (22 right, 11 left in the direction of the curve aperture) were prominent. Balance of standing on the limbs and vestibular involvement are more common on the right side. We hypothesize that patients expending more energy on the dominant side, putting more weight on the right side and loading most of the weight on the back may negatively affect the balance, and that the body somatotype, right dominance and the direction of the curve of the patients included in the study may be due to different compensation mechanisms of right dominance in order to achieve balance.

Negative correlation (moderate and good) was observed between the cobb angle and unipedal stance test, unterberger test rotation angle, SRS-22 pain sub-parameter with eyes closed. In addition, a negative correlation was observed between the rotation angle and the duration of standing on soft ground with the right extremity. When the studies in the literature are examined, it is reported that patients with AIS have increased body oscillations when the eyes are closed and have difficulties in maintaining balance (37,38). It has been reported in the literature that Cobb angle is associated with trunk morphology, asymmetric bone growth, ground reaction force and neuromuscular control (41-43). The findings of our study are consistent with the studies in the literature. In cases where vision is eliminated, increased Cobb angle in patients is associated with poor balance and vestibular function. Based on the literature, the increase in the curve in individuals with scoliosis explains the impairments in balance and vestibular systems due to impaired neuromuscular control.

The limitations of our study are that our patients with scoliosis did not show homogeneous distribution according to curve type and direction of the curve, we did not have a control group, and the physical activity levels of the patients were not evaluated when balance and vestibular functions were considered. In addition, the strengths of this research were that we made detailed analyses according to the direction of the curve. Additionally, using a mobile application to evaluate visuospatial memory performance is important for the objectivity and applicability of our results.

CONCLUSION

This study is, to our knowledge, the first to investigate the relationship between curve features, unipedal stance test performance, vestibular dysfunction, navigation performance, visuospatial memory, and quality of life in individuals with idiopathic scoliosis. As a result, it was shown that there was no difference in visuospatial memory, navigation performance, and quality of life in individuals with idiopathic scoliosis, depending on the type of curve and the direction of the opening of the curve. In particular, it was found that the direction of the curve caused a difference in terms of vestibular dysfunction. There were no significant correlations between Cobb and rotation angle and unipedal stance test, vestibular dysfunction, visuospatial memory, navigation performance, and quality of life. However, no relationship was shown between vestibular dysfunction and visuospatial memory performance in individuals with scoliosis. Further studies are needed to investigate the relationship between these parameters in order to understand the etiology of scoliosis.

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RESEARCH ARTICLE

EXAMINATION OF THE RELATIONSHIP BETWEEN BODY MASS INDEX AND THE SKIN-EPIDURAL SPACE DISTANCE MEASURED BY ULTRASOUND IN THE LUMBAR REGION

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ABSTRACT

Purpose: The aim of the study; to investigate whether there is a relationship between body mass index (BMI), abdominal subcutaneous adipose tissue, waist circumference, and the skin-epidural space distance measured by USG.

Material and Methods: The research was carried out in the block room in the preoperating room of Dokuz Eylul University Practice and Research Hospital and with 42 volunteer operating room workers aged between 18-59 years. Height, weight and waist circumference measurements were made with standard measuring instruments, and other measurements were completed by ultrasonography (USG). For the examination of the skin-to-epidural space distance, a convex probe was used with the transverse median approach in ultrasonography. Abdominal subcutaneous fat thickness measurements were made with a linear probe in the supine position. The statistical correlations of all measurements were examined.

Results: The BMI of the volunteers included in the study was found to be 18-29.9 kg/m2, waist circumference 70-115 cm, and abdominal subcutaneous fat thickness 0.55-4.69 cm. It has been observed that the epidural space distance varies between 3.35-5.47 cm at the L2-L3 level and 3.56-6.09 cm at the L3-L4 level in the sitting position. There was a moderate correlation between BMI and skin-epidural space distance, while a high correlation was found with waist circumference and subcutaneouse abdominal fat distance. It has been observed that people with very similar BMI may have different skin-epidural thicknesses.

Conclusion: It is observed that the skin-to-epidural space distance does not always show high or very high levels of correlation with anthropometric measurements in individuals. Therefore, if epidural anesthesia is required for individuals with higher weight, the assumption that the skin-to-epidural space distance will also be significantly greater should be avoided.

Keywords: Epidural fat, epidural, neuroaxial anesthesia, waist circumference, ultrasound, epidural ultrasound, abdominal subcutaneous fat

INTRODUCTION

According to the World Health Organization (WHO), obesity is a disease defined in ICD-10, which is the accumulation of fat to a degree that can harm health. The BMI unit is kg/m2 and if it is below 18.5 it is classified as underweight; 18.5-24.9 is considered

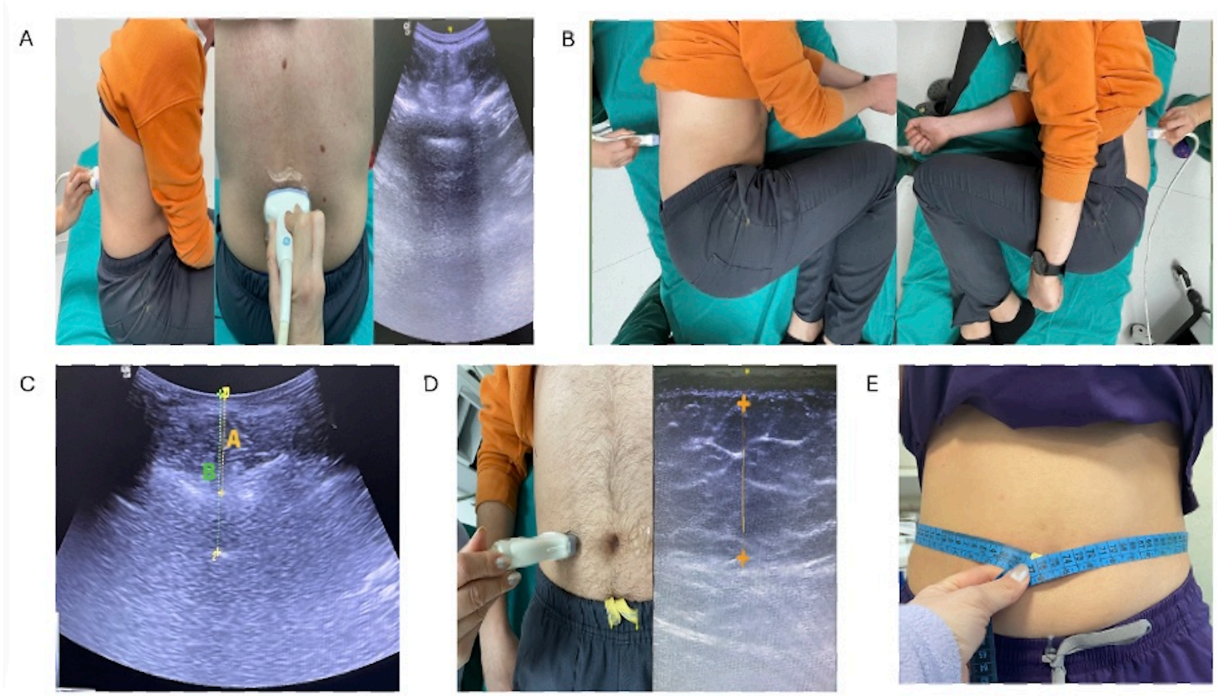

Figure 1. (A) The measurement of skin-epidural space distance in the lumbar region using ultrasound-guided transverse median approach. (B) The measurement of skin-epidural space distance in the right lateral and left lateral decubitus positions. (C) Measurement of skin-epidural space distance A: Skin-Dural Composite Distance (SDCD) B: Skin-Vertebral Body Dura Anterior Complex Distance, Distance B minus A: Intrathecal space. (D) Measurement of subcutaneous abdominal fat thickness. (E) Measurement of waist circumference with a standard measuring tape.

normal weight; 25-29.9 is classified as overweight, and over 30 is classified as obesity (1).

According to BMI, patients classified as obese may exhibit variability in subcutaneous fat tissue at the lumbar level, influenced by age, gender, abdominal fat thickness, and sometimes even race (2). It's observed that in some cases, a patient's adiposity is centralized around the abdomen or hip area, not affecting the lumbar region, while at times, the back region may display fatty tissue accumulation. This situation might differ in pregnant patients. In pregnancy, owing to hormonal fluctuations affecting the entire body, there's an increase in edema affecting the connective tissue in the perivertebral area. These alterations could pose challenges during the application of neuroaxial anesthesia due to this edema (3).

In a study conducted by R.W. Watts et al.(4) (1993), a total of 248 patients, 69% of whom were in the lateral lying position during pregnancy and 31% were in a sitting position while not pregnant, underwent epidural insertion at the L3-L4 level to determine depth. A moderate correlation between BMI and skin-to-epidural space distance was observed in patients with a BMI below 25 kg/m2, both obstetric and non-obstetric, whereas in patients with a BMI >25 kg/m2, a weak correlation was found.

Awasthi et al.'s study (5) (2021) examined 100 female patients aged 40-65, ASA I-II, with a BMI range of 18.5-30 kg/m2. In the group evaluated using ultrasound in the para-sagittal oblique plane, epidural depth demonstrated a weak correlation with BMI ($r^2 = 0.367$, $P = 0.01$).

Based on both the aforementioned studies and similar studies, as well as our own clinical experience and observations, BMI should not always bias neuraxial blocks applied to the lumbar region (spinal or epidural). The aim of our study is to reveal the relationship between BMI, waist circumference, abdominal fat tissue thickness and skin-epidural space distance.

MATERIAL AND METHODS

This methodological trial was registered at clinicaltrials.gov (NCT06316622) on March 16, 2024. After obtaining ethical approval from Non-Interventional Research Ethics Committee of Dokuz Eylul University (Date: 08.03.2021, Decision No: 2021/08-42), study commenced. The first patient enrollment is dated October 1, 2021, and the last patient enrollment is dated January 15, 2022. It is a methodological study conducted at the Block Room of LOGIQ-E, GE Medical Systems® USG at Dokuz Eylul University Training and Research Hospital,

Table 1. Anesthesiologist and radiologist measurement values

	ANESTHESIOLOGIST			RADIOLOGIST		
	L3-4 skin-epidural distance (cm)	L4-5 skin-epidural distance (cm)	Subcutaneous abdominal fat (cm)	L3-4 skin-epidural distance (cm)	L4-5 skin-epidural distance (cm)	Subcutaneous abdominal fat (cm)
1	3.71	3.82	0.87	3.79	3.79	0.88
2	4.25	4.53	2.49	4.64	4.50	2.35
3	4.92	5.15	3.53	4.27	4.98	3.51
4	4.29	4.56	3.23	4.77	5.24	3.18
5	4.56	5.00	1.84	4.71	5.00	2.02
6	4.80	5.75	4.47	4.77	5.29	4.43
7	3.49	3.85	2.20	3.38	4.20	1.98
8	5.73	6.12	3.37	5.56	5.74	3.36
9	4.39	4.55	2.38	4.25	4.56	2.29
10	4.61	4.55	1.70	4.51	4.75	1.85
11	4.72	4.50	1.33	4.23	4.42	1.40
12	5.42	5.72	4.69	5.19	6.06	5.17
13	4.41	4.64	1.83	3.77	4.60	1.60
14	4.36	4.69	1.37	3.77	4.34	1.93
15	4.57	4.52	0.55	4.57	4.55	0.69
16	4.29	4.31	2.70	4.04	4.33	2.79
17	4.45	4.50	2.59	4.62	4.57	2.85
18	4.59	4.36	2.11	4.53	4.59	2.11
19	4.41	4.84	3.55	4.16	4.75	3.86
20	4.34	4.41	1.73	4.23	4.64	1.63
21	3.65	3.81	0.52	3.74	3.79	0.78
22	3.79	4.37	0.89	3.99	3.74	1.01
23	5.26	5.34	3.21	5.21	5.32	3.17
24	3.77	3.77	0.96	3.57	3.74	1.01
25	4.29	4.22	0.84	4.02	4.07	0.81
26	3.89	4.12	3.24	3.82	3.87	3.11
27	4.98	5.00	2.53	4.67	4.94	2.72
28	3.74	4.09	1.12	3.79	4.17	1.08
29	3.83	4.04	0.78	4.09	4.16	0.84
30	3.51	3.83	2.26	3.54	3.79	2.09
31	4.36	4.62	2.79	4.50	4.71	2.71
32	3.35	3.79	3.28	3.42	3.86	3.47
33	4.11	4.34	1.57	4.23	4.33	1.74
34	4.22	4.69	2.82	4.28	4.36	2.97
35	4.20	4.70	1.79	4.30	4.68	2.15
36	4.62	4.91	3.03	4.47	4.81	3.24
37	5.10	5.68	3.56	5.10	5.20	3.78
38	4.32	4.34	1.77	4.30	4.02	2.04
39	3.49	4.04	1.32	3.40	3.93	1.23
40	3.48	3.68	1.97	3.33	3.81	2.14
41	3.33	3.95	2.28	3.33	3.89	2.35
42	4.92	5.07	1.81	4.80	5.10	1.65

Izmir. This research is in accordance with the CONSORT statement.

The study sample consists of volunteers working at Dokuz Eylul University Hospital who do not meet the exclusion criteria. Since the initiation of the study, individuals aged between 18 and 59 were asked whether they agreed to undergo lumbosacral spine ultrasonography (USG) and participate in our study. Those who consented were included in the study based on their arrival order at the operating room. Three volunteers were excluded from the study as proper USG imaging couldn't be obtained, and measurements couldn't be taken. After reaching the required number of 42 eligible volunteers, the study was concluded.

Inclusion Criteria: 1) Age between 18 and 59 years old. 2) ASA I-II classification. 3) Body Mass Index (BMI): 18.5-29.9 kg/m2.

Table 2. Paired samples test

Paired Samples Test									
		Paired Differences					t	df	p
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	Radiolog-Anesthesiolog Abdominal Subcutaneous Fat	,06071	,22333	,03446	-,00888	,13031	1,762	41	,086
Pair 2	Radiolog-Anesthesiolog L3-4 Skin-Epidural Distance	-,06810	,24128	,03723	-,14328	,00709	-1,829	41	,075
Pair 3	Radiolog-Anesthesiolog L4-5 Skin-Epidural Distance	-,03762	,23822	,03676	-,11185	,03662	-1,023	41	,312

Exclusion Criteria: 1) Individuals under 18 or over 59 years old. 2) Those with a history of vertebral surgery. 3) Patients diagnosed with spinal deformities (excluding scoliosis ≤10 degrees, kyphosis <30 or >80 degrees). 4) Individuals with rheumatologic diseases affecting skeletal structure such as ankylosing spondylitis, rheumatoid arthritis. 5) Presence of wounds or infections in the lumbar region. 6) Volunteers who underwent epidural or spinal interventions in the lumbar region within the last month. 7) Individuals with bone implants affecting posture, such as hip or knee prostheses. 8) Pregnant women. 9. Individuals with conditions like Cushing's syndrome, hypothyroidism, acromegaly causing lumbar edema. 10) Volunteers using corticosteroids. 11) Obese individuals (BMI: >29.9kg/m2). Consent was obtained from the volunteers included in the study, and their weight and height measurements were taken using calibrated standard Mechanical Scale and Mesilife JSA-180® Height-Measuring Scale and recorded. The study was conducted on volunteers working in the operating room.

Measurements of the skin-epidural space distance were performed using the LOGIQ-E, GE Medical Systems® convex ultrasound probe by an anesthesiologist with over five years of experience and a radiologist with over five years of experience. Each patient was positioned in the left lateral, right lateral, and then sitting positions, approached with a transverse-median approach by an anesthesiologist followed by a radiologist, targeting the intended space.

The sitting position involved the patient's legs hanging off the edge of the bed, with feet placed on an immobile stool. The patient's back leaned towards the investigator on the other side of the bed, with the backs of the knees against the edge of the bed as much as possible. Patients were asked to symmetrically 'hunch' over their shoulders on the hips to allow flexion of the lumbar spine. As shown in Figure 1A, patients usually positioned their arms with elbows bent, forearms, and hands resting lightly on their thighs. The lateral position during epidural and spinal anesthesia in the operating room involved the patient's back towards the edge of the table, close to the clinician, with the bed edge parallel and vertical, and the hips stacked on top of each other. Thighs were pulled in maximum flexion, and the patient was asked to 'push out' or 'roll' their lower back. Patients instructed to 'curve' typically arch their upper backs, causing rotation of the spine as the lower shoulder remains fixed on the mattress. As shown in Figure 1B, dependent shoulder may need to be pulled forward by an assistant. During measurements, data recording forms captured the palpable spinal level points: superior to the anterior superior iliac spine (ASIS) (estimated L3-L4) and inferior to the ASIS (estimated L4-L5). Skin-dural composite (SDC) and skin-vertebral body, anterior complex (posterior longitudinal ligament) distance measurements were separately recorded by both experts. Since the primary objective was to measure the skin-to-epidural distance, only the SDC measurements were used for statistical analysis. As shown in Figure 1A, determination of intervals was ensured by counting palpable points from the sacrum parasagittally towards the cephalad direction in difficult-to-palpate areas.

Additionally, the measurement of subcutaneous abdominal fat thickness for all volunteers was conducted using the linear probe of the ultrasound machine (LOGIQ-E, GE Medical Systems, China) to ensure consistency. As shown in Figure 1D, measurements were taken 2 cm away from the umbilicus at two points (right lateral, left lateral) and averaged then recorded. Individuals were instructed to hold their breath after expiration and release

Table 3. Measurement and demographic data

		Age	Weight (kg)	Height (cm)	BMI	Waist Circumference (cm)	WC/W	Subcutaneous Fat (cm)	L3-4 Skin-Epidural (cm)	L4-5 Skin-Epidural (cm)
Man N:20 (%47.6)	Mean	31.0	81.83	175.45	26.56	96.50	0.55	2.64	4.69	4.86
	Median	28.0	81.50	176.00	26.85	95.50	0.55	2.56	4.68	4.85
	Std. Deviation	8.07	8.85	6.763	2.12	8.16	0.04	0.96	0.48	0.51
	Minimum	22.0	68.00	166	22.53	79	0.47	1.33	3.66	3.79
	Maximum	58.0	100.00	185	29.75	115	0.62	4.69	5.74	5.65
		Age	Weight (kg)	Height (cm)	BMI	Waist Circumference (cm)	WC/W	Subcutaneous Fat (cm)	L3-4 Skin-Epidural (cm)	L4-5 Skin-Epidural (cm)
Woman N:22 (%52.4)	Mean	33.7	60.94	162.68	23.13	82.36	0.50	1.81	4.02	4.26
	Median	33.0	62.00	163.00	22.80	81.50	0.49	1.81	3.91	4.35
	Std. Deviation	8.93	7.41	6.04	2.91	9.54	0.06	0.95	0.40	0.46
	Minimum	21.0	49.00	155	18.00	70	0.42	0.52	3.35	3.10
	Maximum	58.0	71.00	174	29.91	100	0.67	3.53	4.77	4.99
		Age	Weight (kg)	Height (cm)	BMI	Waist Circumference (cm)	WC/W	Subcutaneous Fat (cm)	L3-4 Skin-Epidural (cm)	L4-5 Skin-Epidural (cm)
Total N:42 (%100)	Mean	32.45	70.89	168.76	24.76	89.10	0.52	2.21	4.34	4.55
	Median	30.00	69.50	168.50	24.92	91.00	0.53	2.15	4.39	4.47
	Std. Deviation	8.548	13.266	9.031	3.069	11.33	0.05	1.03	0.55	0.56
	Minimum	21.00	49.00	155	18.00	70	0.42	.52	3.35	3.10
	Maximum	58.00	100.00	185	29.91	115	0.67	4.69	5.74	5.65

Values are shown as mean ± SD (median)

abdominal muscles during measurement. Waist circumference was measured in all volunteers using a standard measuring tape at the level of the umbilicus. As shown in Figure 1E, during measurement with the tape, patients were asked to stand upright, hold their breath after expiration, and relax abdominal muscles. During data collection in the preoperative assessment room, while the anesthesiologist conducted measurements, there was no radiologist present, and vice versa when the radiologist performed the measurements. Because, since objectivity was desired during these precise measurements, it was also aimed to objectively demonstrate that the radiologist, who is more experienced in ultrasonography, and the anesthesiologist provided consistent measurements.

Statistical Analysis

SPSS 24.0 statistical software package was utilized for data analysis. Data were summarized using percentage distribution, mean, standard deviation, median, minimum and maximum values. For categorical variables, dependent group analysis employed Chi-square test and Kappa agreement test. In the case of measurement variables, dependent group analysis utilized either t-test or Wilcoxon test based on the normality distribution of the data. Correlation analysis was conducted using either Pearson correlation test or Spearman correlation test. A significance level of $p<0.05$ was considered. Volunteers aged 18-59, working in the operating room of Dokuz Eylul University Hospital and willing to undergo lumbar vertebral ultrasonography, were

Table 4. Pearson correlation analysis

		1	2	3	4	5	6	7	8	9	10
1	Weight										
2	Height	.753**									
		0									
3	BMI	.822**	0.253								
		0	0.105								
4	Waist Circumference	.900**	.507**	.887**							
		0	0.001	0							
5	Waist/Height	.651**	0.074	.898**	.894**						
		0	0.643	0	0						
6	Abd Subc Fat (Radio)	.672**	0.302	.724**	.762**	.719**					
		0	0.052	0	0	0					
7	Abd Subc Fat (Anesth)	.642**	0.248	.729**	.753**	.733**	.985**				
		0	0.113	0	0	0	0				
8	L3-4 skin-epidural (Radio)	.754**	.647**	.548**	.670**	.437**	.606**	.574**			
		0	0	0	0	0.004	0	0			
9	L3-4 skin-epidural (Anesth)	.742**	.684**	.508**	.630**	.376*	.572**	.602**	.892**		
		0	0	0	0	0.029	0	0	0		
10	L4-5 skin-epidural (Radio)	.716**	.566**	.544**	.594**	.373*	.603**	.590**	.855**	.851**	
		0	0	0	0	0.015	0	0	0	0	
11	L4-5 skin-epidural (Anesth)	.692**	.574**	.526**	.602**	.370**	.639**	.628**	.784**	.850**	.862**
		0	0	0	0	0.01	0	0	0	0	0

* Correlation is significant at the 0,05 level (2-tailed). ** Correlation is significant at the 0,01 level (2-tailed). Abd Subc Fat: Abdominal Subcutan Fat, Anesth: Measurements performed by the anesthesiologist, Radio: Measurements performed by the radiologist

included in the sample of this study. Using G Power 3.1.9.7 free version software, a minimum of 42 individuals were determined for inclusion in the research, considering a medium effect size at 0.05 alpha level and 95% power. Three individuals were excluded from the study due to inadequate imaging and measurement possibilities via ultrasonography. As the required number of volunteers was attained, the study was concluded upon reaching 42 participants.

RESULTS

A total of 42 volunteers, comprising 23 females and 19 males, within the age range of 18-59, were enrolled in the study conducted in the central operating room of Dokuz Eylul University Hospital. No volunteers were excluded from the study, and data from all 42 individuals were utilized in the analysis. The measurement values of the anesthesiologist and radiologist are shown in Table 1.

Our study revealed no significant difference between measurements performed by the radiologist and the anesthesiologist (p >.05). This paired samples test analysis are shown Table 2. Measurement and demographic data are shown in Table 3. The normality of the data (n=42) was assessed using the Kolmogorov-Smirnov test, and it was determined that the data were normally distributed. For data conforming to normal distribution, Pearson correlation analysis was applied (2-tailed, p<.01, r=.00-.20: No or very low correlation; r=.20-.40: Low correlation; r=.40-.60: Moderate correlation; r=.60-.80: High correlation; r=.80-1.00: Very high correlation). The results of the statistical analysis are shown in Table 4 and Table 5.

The interpretation of the table below is based on the anesthesiologist's measurements.

There was identified a significantly positive high-level correlation between weight and parameters indicating skin-epidural space distance. As weight increases, these parameters show a significant increase. The correlation in the L3-L4 level (r: 0.742) is stronger than the correlation in the L4-L5 level (0.692). There was identified a significantly positive high-level correlation between height and parameters indicating skin-epidural space distance at the L3-L4 level (r: 0.684), and a moderately high-level correlation at the L4-L5 level (r: 0.574).

There was identified a significantly positive moderately high-level correlation between body mass index (BMI) and parameters indicating skin-epidural space distance at both L3-L4 and L4-L5 levels (r: 0.508, r: 0.526).

There was identified a significantly high-level positive correlation between waist circumference and parameters indicating skin-epidural space distance at both L3-L4 and L4-L5 levels (r: 0.630, r: 0.602).

There was identified a significantly positive low-level correlation between the waist-to-height ratio and parameters indicating skin-epidural space distance at both L3-L4 and L4-L5 levels (r: 0.376, 0.370).

There was identified a significantly high-level positive correlation between subcutaneous abdominal fat tissue thickness and parameters indicating skin-

Table 5. Pearson correlation analysis (simplyfied)

Skin-Epidural Distance	Weight	Height	BMI	Waist Circumference	Waist/Height	Abdominal Subcutan Fat
L3-L4	0,742	0,684	0,508	0,630	0,376	0,602
L4-L5	0,692	0,574	0,526	0,602	0,370	0,628

epidural space distance at both L3-L4 and L4-L5 levels (r: 0.602, r: 0.628).

In summary, when examining the correlations with skin-epidural distance, it can be observed that waist circumference and subcutaneous abdominal fat thickness have a higher correlation than BMI. As shown in figure 2A and 2B, from the data, when examining the correlation distribution graphs of skin-epidural space distance with BMI and subcutaneous abdominal fat thickness, it can be seen that patients with similar fat thickness or similar BMI exhibit variability in skin-epidural space distance. Furthermore, although the results indicate that abdominal subcutaneous fat thickness and waist circumference are better indicators than body mass index (BMI), none of the parameters showed a very high degree of correlation (r:0.8-1.0). In other words, it cannot be definitively stated that the skin-to-epidural space distance is also increased based solely on body anthropometric measurements.

DISCUSSION

The escalating body weight, fat percentage, and the distribution of this fat tissue in body composition not only impact health problems such as cardiac risks, diabetes mellitus, sleep apnea but also affect intubation difficulties, general anesthesia complications, and regional anesthesia challenges from an anesthetic perspective. The notion that foreseeing these difficulties and risks solely through BMI may not yield entirely accurate results is being supported by recent studies (6).

In a study conducted by Erika and colleagues (7) (2020) on 246 males and 357 females, body composition was assessed using DXA, and visceral fat mass was measured using Corescan. It was observed that individuals with similar BMI had significantly different body compositions in terms of internal organ fat percentage and lean body masses. The main premise of this study is that BMI does not reflect body fat distribution, and an increase in BMI does not always correspond to an increase in the skin-to-epidural space distance (8).

In addition, there is a biased approach to regional interventions in overweight patients due to increased skin-epidural fat deposition and in pregnant individuals due to increased edema. In fact, contrary to the assumption that the skin-epidural fat deposition increases uniformly in every overweight or pregnant patient, clinical observations and our experiences indicate that it does not increase to the same extent in all cases, despite the encountered difficulties (3).

The reason for using ultrasound (USG) is its practical and easy applicability, as well as the ability to achieve a large sample size quickly without radiation risk and without causing stress to the patients (9).

A study conducted by Sprung and colleagues (10) involving 595 patients recorded spinal/epidural anesthesia procedures. A low-degree correlation was found between the success of the interventions and BMI. It was concluded that BMI may only pose challenges in identifying anatomical landmarks. Therefore, focusing on measurements such as abdominal fat thickness and waist circumference may lead to more accurate results. In a study conducted by Mauad and colleagues (11) in 2017, the correlation between CT and USG in abdominal fat measurement was examined. The probe was placed 1 cm above the umbilical level at the midclavicular line, and the deep fat tissue was measured using a convex probe, while the subcutaneous fat tissue was measured using a linear probe with ultrasound. A higher correlation was found between body fat measurements obtained by CT and ultrasound. In this study, the reason for measuring the skin-epidural space distance only in the transverse-median (TM) plane is that in clinical practice, anesthetists usually perform epidural needle insertion in the TM plane. After imaging, the needle entry to reach the epidural space at the same skin-epidural distance should mimic the angle of the probe in this imaging (12). In a study conducted by Gnaho and colleagues (2012) on 31 patients, measurements of skin-dura with transverse median, L3-L4 level USG imaging performed by an anesthetic specialist correlated with the actual needle depth of another

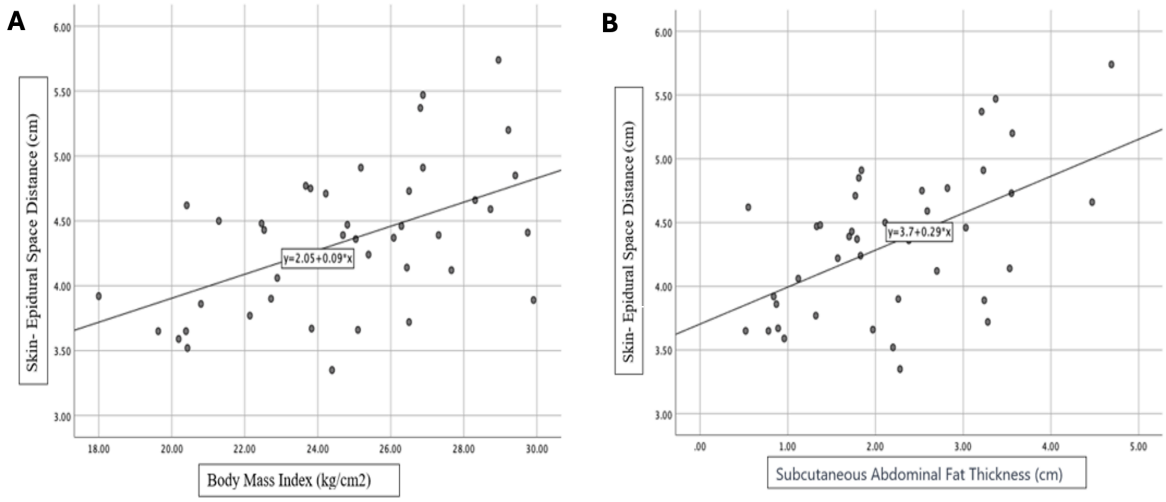

Figure 2. (A) Correlation distribution graphic of skin- epidural space distance with BMI. (B) Correlation distribution graphic of skin-epidural space distance with subcutaneous abdominal fat thickness

anesthetist who was unaware of the measurements (13).

In a study conducted by Eley and colleagues (14) on pregnant women beyond 37 weeks, published in 2019, the relationship between increased BMI and abdominal subcutaneous fat thickness with the skin-epidural space distance was investigated. There was a strong correlation between abdominal subcutaneous fat thickness and BMI, a moderate correlation between subcutaneous fat thickness and skin-epidural space distance, and a moderate correlation between BMI and skin-epidural space distance. BMI does not necessarily increase the difficulty of placement but is a more valuable indicator than subcutaneous fat measurement.

While similar studies have been predominantly conducted on pregnant women (15) and obese patients, at the initiation of this study, there were very few examples in the normal population, mostly involving magnetic resonance imaging (16). A very similar study was conducted by Harshvardhan and colleagues in 2021. This study included 100 individuals aged 40-65 with ASA I/II, a BMI between 18.5 and 30 kg/m2, who were scheduled for surgery with lumbar epidural block. In contrast to this study, only female patients were selected, and measurements were taken in only the sitting position, with parasagittal oblique and transverse median approaches. Consistency was assessed by performing the procedure with an epidural needle (5). Due to the limited number of volunteers, groupings could not be made according to age, occupational groups, ethnicities, and gender differences. Measurements could only be taken from the L3-L4 and L4-L5 levels. However, since there are clinical situations where applications need to be made from higher and lower levels, the levels of measurements can be expanded. Verification measurements can be performed not only in the transverse median but also in the parasagittal oblique plane to minimize skin-fat compression. While the measurement of visceral abdominal fat is of great importance in determining body fat, our study only measured subcutaneous fat. Furthermore, by evaluating the entire body composition and determining the fat percentage, more advanced examination tools such as MRI, DXA, CT can be used.

To support the findings, it is recommended to increase the number of prospective randomized studies in individuals with different anthropometric measurements and to evaluate these individuals separately based on factors such as occupational groups or gender, which may affect the fat-muscle composition in the lumbar region.

CONCLUSION

In conclusion, this study demonstrates that excess weight does not always significantly affect the skin-to-epidural space distance. While the results indicate that abdominal subcutaneous fat thickness and waist circumference are better indicators than body mass index (BMI), none of the parameters showed a very high degree of correlation. Therefore, each patient should be evaluated individually, and the assumption that patients with higher weight will have a greater skin-to-epidural space distance should be avoided. Ultrasound-guided pre-assessment can facilitate interventions and help mitigate the risks of

anesthesia, particularly in situations where general anesthesia may pose disadvantages (e.g., difficult intubation, respiratory comorbidities, cardiac issues).

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RESEARCH ARTICLE

THE RELATIONSHIP BETWEEN BODY MASS INDEX AND GAIT CHARACTERISTICS AND GAIT PERFORMANCE OF CHILDREN WITH DUCHENNE MUSCULAR DYSTROPHY

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ABSTRACT

Purpose: Obesity is a common problem in Duchenne Muscular Dystrophy (DMD) and adversely affects disease progression. This study aimed to investigate the relation between Body Mass Index (BMI) and gait characteristics and gait performance in patients with DMD.
Material and Methods: Weight and height were measured to calculate BMI. BMI z-scores were used to categorise weight. Height, weight, and BMI were standardised to sex- and age-corrected z-scores based on the Turkish population. The gait characteristics were evaluated with the Gait Assessment Scale for Duchenne Muscular Dystrophy (DMD-GAS) and Quality and Independence of Gait Classification Scale for Duchenne Muscular Dystrophy (QIGS-DMD). The gait performance was assessed by the 6-minute walk test and the 10-meter walk/run test. The correlation between body composition and gait parameters was analysed.
Results: Eighty-six children with DMD were included in the study. Among the participants, 40.7% had a BMI above the normal range. There were moderate correlations between DMD-GAS and height, weight, and BMI and moderate correlations between QIGS-DMD and height, weight, and BMI ($p<0.01$).
Conclusion: The current findings revealed that an increase in BMI affects the characteristics, quality, and independence of gait in children with DMD, highlighting the need for regular monitoring and timely intervention.

Keywords: Duchenne Muscular Dystrophy, obesity, body mass index, gait, gait analysis

INTRODUCTION

Duchenne muscular dystrophy (DMD) is a devastating childhood disease with an X-linked recessive inheritance (1). Proximal muscle weakness, joint contractures, gait abnormalities and postural disorders are common characteristics of the disease in ambulatory period which limit functional abilities (1, 2). Progressive muscle weakness and joint contractures result in distinctive gait which is characterized by increased lumbar lordosis and base of support, decreased stride length and gait speed, and toe walking as a compensatory mechanism (3-5). Gait abnormalities including gait performance and characteristics, like other associated symptoms of the disease, become more severe as the disease progresses (6, 7). Children with this condition usually lose the ability to walk in their teens and become wheelchair-dependent (2).

Table 1. Descriptive characteristics of the study population (n=86)

	Mean (SD)	Min – Max
Age (month)	107.90 (26.53)	60 – 175
Height (cm)	126.25 (13.12)	103 – 161
Weight (kg)	30.77 (10.58)	17 – 68
BMI (kg/m^2)	18.80 (3.13)	13.08 – 28.80
Height Z-score	-0.85 (1.41)	-3.99 – 2.71
Weight Z-score	0.17 (1.06)	-2.23 – 2.68
BMI Z-score	0.72 (0.98)	-1.98 – 2.63
Height percentile	30.32 (31.51)	0 – 99.66
Weight percentile	54.57 (30.02)	1 – 100
BMI percentile	70.72 (27.03)	2 – 100
	N	**%**
BMI Percentile		
• ⩽5th	1	1.2
• >5th and <85th	50	58.1
• ⩾85th and <95th	19	22.1
• ⩾95th	16	18.6
Steroid usage		
• Yes	77	89.5
• No	9	10.5

BMI: body mass index; SD: Standard deviation

In addition to physical impairments, nutritional disorders are also among the frequently observed symptoms in children with DMD. Willig et al found that the highest prevalence of obesity (54%) occurs around the age of 13; and undernutrition occurs after the age of 14, with a similar prevalence of underweight at the age of 18 (8). The results of a study conducted in France by Martigne et al. that 73% of children were classified as obese by the age of 13, while 34% were underweight and 47% were obese by the age of 18 in a DMD population (9). A study conducted by Davidson et al. in an Australian DMD population reported that children with DMD aged 3-12 years had a higher body mass index (BMI) than typically developing children, and that steroid users had a higher BMI than non-steroid users (10). Additionally, the literature reported that steroid treatment, a common medical intervention for DMD (11), had a detrimental impact on BMI (12). Once BMI increases, it negatively affects sleep quality, physical and lung functions, and increases metabolic risk factors in children with DMD (13-16).

As previously stated, obesity is prevalent among these children, and emerging evidence indicates that obesity has a negative impact on the progression of disease. However, to the best of our knowledge, no studies have examined the impact of obesity on gait in DMD. Investigating the effect of differences in BMI on gait in children with DMD may be a step towards filling the gap in this area. Thus, it was planned to investigate the relationships between BMI and gait characteristics and performance in a Turkish DMD cohort.

MATERIAL AND METHODS

Participants

This study was conducted at the Department of Physiotherapy and Rehabilitation between November 2022 and November 2023. The study included 86 children with DMD diagnosed through genetic testing or muscle biopsy. The inclusion criteria were as follows: a diagnosis of DMD, an age of at least five years, and the ability to walk at least 10 meters independently (17, 18). Individuals with a history of lower extremity surgery or trauma within the previous six months, as well as those who were unable to comply with the physiotherapists' instructions, were excluded from the study. The children and their parents signed the informed consent form. Ethical approval was obtained from the Non-Interventional Clinical Research Ethics Committee of the Hacettepe University (Date: 01.11.2022, Decision no: 2022/18-23). The study adheres to the mandates of the Declaration of Helsinki.

Assessments

All assessments performed in the study were performed by physiotherapists with at least 6 years of

Table 2. The gait characteristics and gait performance test results of the children (n=86)

Variables	Mean (SD)	Min – Max
DMD-GAS (0-20)	11.98 (4.93)	1 – 20
6MWT (m)	384.52 (93.89)	111 – 568
10MWT (sec)	8.47 (2.06)	5.55 – 16.46
	N	**%**
QIGS-DMD		
√ Level-1	8	9.3
√ Level-2	44	51.2
√ Level-3	34	39.5

DMD-GAS: Gait Assessment Scale for Duchenne Muscular Dystrophy; 6MWT: 6-minute walk test; 10MWT: 10-meter walk/run test; QIGS-DMD: Quality and Independence of Gait Classification Scale for Duchenne Muscular Dystrophy; SD: standard deviation

experience in DMD management and assessments. All tests were also performed in a quiet room to minimize potential distractions for the children during their routine follow-up visits.

Body Mass Index

The children's height was measured using a portable stadiometer and weight was measured using a bascule. Children's height, weight and BMI were standardized as sex- and age-adjusted z-scores based on the Turkish population (19). Using the criteria defined by the World Health Organisation (WHO), BMI Z-scores by age are graded as follows (20): Normal = > -1 to < +1 SD; overweight= > +1SD; Obesity= > +2SD; Thinness= < -2SD; Severe Thinness= < -3SD. BMI percentiles were also expressed as follows: ⩽5th percentile= underweight; >5th and <85th percentile= normal weight; ⩾85th and <95th percentile= overweight; ⩾95th percentile= obese (9).

Gait Assessments

The gait characteristics were evaluated by the Gait Assessment Scale for Duchenne Muscular Dystrophy (DMD-GAS) and the Quality and Independence of Gait Classification Scale for Duchenne Muscular Dystrophy (QIGS-DMD). These scales were developed specifically for DMD and found valid and reliable [(ICC>0.90), and (K>0.80), respectively] (18, 21). The 10 item DMD-GAS evaluates the compensatory movements of body parts such as the foot, knee, hip, and trunk during walking and the time-distance characteristics of gait, such as speed, stride length, and base of support in children with DMD. Scores ranged between 0 and 20 which lower scores indicated impaired gait characteristics (18). The QIGS-DMD classifies the independence and quality of gait in 5 levels. Level 5 indicates that child has lost the ability to walk, while level 1 indicates that child is able to walk independently without compensation (21).

The gait performance was evaluated by using the 6-minute walk test (6MWT) and the 10-meter walk/run test, which were valid and reliable measures in DMD. The 6MWT is considered as the primary endpoint in clinical trials with DMD populations. The distance taken during the 6MWT were recorded in meters (22, 23). The 10MWT is another outcome measure used in studies with DMD populations. The time taken during walking a 10 meter walkway was recorded in seconds (22).

Data Analysis

IBM SPSS Statistics software version 26.0 was used for data analysis. Descriptive characteristics were defined by mean and standard deviation (mean±SD) for quantitative data and number/percentages for qualitative data. After verifying the absence of a standard statistical normal distribution using the Kolmogorov-Smirnov test, Spearman's correlation coefficient was used to assess the relation between body composition and gait data of the children. The strength of the correlations (r) was graded as: 0.05 to 0.30 weak; 0.30 to 0.70 moderate; and 0.70 to 1.00 strong (24, 25). Chi-square test was used to compare gait quality and independence by BMI percentile. Statistical significance was set at a $p<0.05$.

G* Power (Version 3.1.9 Universität Düsseldorf, Düsseldorf, Germany) was used for post-hoc power analysis. Taking into account the relationship between BMI and DMD-GAS in the current study, the

Table 3. The correlations between the gait characteristics and gait performance of the children and body composition (Spearmen's *r*)

Gait Assessments	Height	Weight	BMI	Height Z-score	Weight Z-score	BMI Z-score	BMI percentile
DMD-GAS	**-0.36****	**-0.44****	**-0.36****	0.07	-0.06	-0.10	-0.10
QIGS-DMD	**0.44****	**0.48****	**0.35****	-0.07	0.06	0.07	0.08
6MWT	-0.05	-0.12	-0.15	0.10	0.04	-0.02	-0.02
10MWT	-0.01	0.07	0.15	-0.16	-0.06	0.06	0.06

*** p<0.01*

effect size of the current study was 0.36 and the post-hoc power was 91.8%.

RESULTS

The results related to the descriptive characteristics of children were shown in Table 1. According to BMI percentile, a total of 40.7% of the cohort had a BMI above the normal range in which 22.1% were overweight and 18.6% were obese.

The results related to the gait characteristics and gait performance of the children were given in Table 2.

There were moderate correlations between DMD-GAS and height, weight, and BMI (r=-0.36, r=-0.44, r=-0.36, respectively) and moderate correlations between QIGS-DMD and height, weight, and BMI (r:0.43, r:0.48, r:0.35, respectively) (p<0.01). The findings related to the correlations of the test parameters were shown in Table 3.

Statistical differences were found in the comparison of gait quality and independence by BMI percentile (p<0.05) (Figure 1).

DISCUSSION

Prenatal, genetic, developmental and psychosocial risk factors are important determinants of susceptibility to obesity. Reduced energy expenditure due to limited physical activity and mobility and steroid use leads to increased risk of obesity in individuals with DMD (26). Excessive weight gain worsens many functional and daily life factors in children with DMD. Previous studies have shown that higher BMI and fat mass contribute to clinical milestones, metabolic risk factors, and respiratory function in children with DMD (13, 14, 16, 27, 28). In this study which was aimed to investigate the relations between excessive weight gain and gait characteristics showed that a significant proportion of the cohort were overweight or obese, and the increased weight and higher BMI might contribute to the deterioration of gait characteristics including gait performance and quality.

Considering the BMI analysis of our study, it can be interpreted that the obesity rate of the cohort is approximately similar to the rate in different countries which highlights the vulnerability of DMD population to obesity (8-10, 29). The Z-scores of the studied population showed that this cohort included both thin and obese children. It has been reported in the literature that younger children with DMD are at risk of obesity, whereas older children with DMD are at risk of underweight due to muscle damage, nutritional problems and dysphagia (10, 14). With these results, it can be stated that the body composition characteristics of the population in this study which included a wide age range by including children up to 14.5 years of age, are compatible with the literature (8-10, 29).

As indicated in several studies, obesity or overweight is a potential risk for gait characteristics in children with various musculoskeletal disorders or typical development (30-33). Hills et al. showed that the cadence, cycle duration, velocity, and stance duration of gait of obese children were worser than children with normal weight (30). Another study showed the negative effect of obesity on the stance duration of gait pattern in children with Down Syndrome (DS) (31). Similarly, Elshemy et al. reported that children with DS had a lower cadence and a shorter stride length than healthy children and non-obese children with DS (32). Obesity has also been reported as a contributor to the disability of children with Charcot-Marie-Tooth (33) and excessive weight was shown to be a potential risk for normal gait characteristics in children with cerebral palsy (34). Similar to the results of the studies above, the current study provided evidence that gait characteristics, gait quality and independency worsened as children's height, weight and BMI increased in DMD. Weight gain was related to the shorter stride length, increased trunk movement, arm swing, base of support, and lumbar lordosis, decreased gait speed, or adverse joint kinematics according to DMD-GAS. On the other

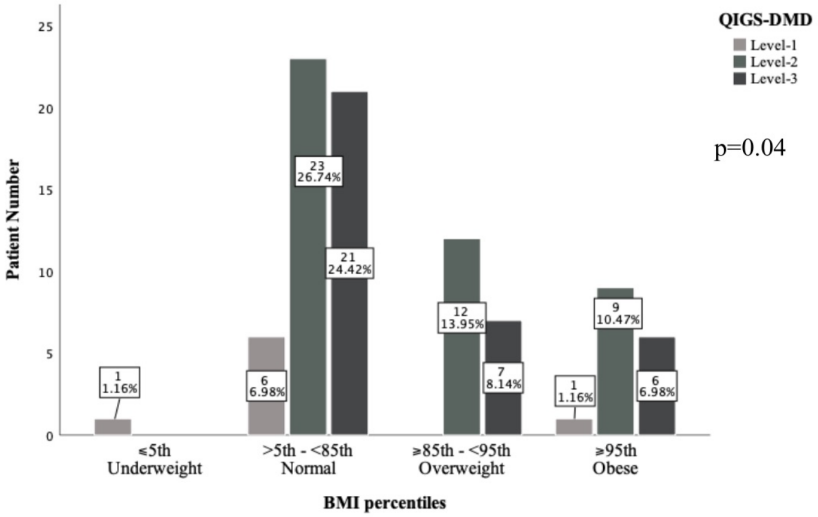

Figure 1. Distribution of QIGS-DMD levels according to the BMI percentile

hand, obesity is known to impair balance and constitutes a risk factor for falls (35). Thus, the authors hypothesized that children with DMD may have developed a strategy to maintain their balance by altering their gait characteristics. It can also be interpreted that overweight or obese children with DMD might use their muscles in a way that caused more compensatory movements and affecting gait characteristics more to maintain upright stability.

In addition to the common effects of obesity in DMD, there have also been reports of adverse effects on motor function (14, 36). A previous study showed that body composition and anthropometry were associated with the functional level measured by Gross Motor Function Classification System (GMFCS) in children with DMD (36). Billich et al. found that obesity increased the risk of fracture in younger DMD children, and that obesity at 8 years was associated with 10-m walk/run test results, but did not affect other physical functions [supine-to-stand and climbing four stairs, North Star Ambulatory Assessment (NSAA), 6MWT] and forced vital capacity. They also found that obesity in other age groups was not associated with 10-m walk/run scores (14). Similarly, in this study, there was no association between obesity and children's performance tests (6MWT and 10-m walk/run). There are 2 hypotheses considered as a reason for this. First, the mean age of our cohort indicated the early-mid stage of the disease, and although BMI was known to be higher in children in this period (37), they might have more preserved muscle mass (38). This means that younger DMD children with more muscle mass may be able to maintain gait performance despite an increase in body composition by modifying their gait characteristics. Second, our cohort included children predominantly on steroids. Although it has been known that obesity is more common in steroid-treated children, it is also known that steroids protect muscle mass and functional ability (10, 39). Approximately 90% of the children in this study were on steroids, which might explain why the gait performance of the children was preserved despite the increased BMI.

This study had several limitations. First, the gait parameters could not have been assessed by an objective method. Second, since the BMI data alone might not be sufficient to further interpretation of the effects of obesity, the lack of muscle-fat ratio measurement can be considered as another limitation. Studies that eliminate these limitations may provide a better understanding of the effect of excessive weight gain on gait characteristics in this population. There is also a need for studies that include more patients, a homogeneous population of patients of similar age, and longitudinal studies.

CONCLUSION

It is concluded that weight control during the disease process is essential since there may be deterioration in gait function related to the change of body composition, especially by the excessive weight gain in children with DMD. As a recommendation, regular monitoring of the weight in each visit might lead to timely interventions to maintain gait ability of children with DMD.

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Author contribution: Conceptualization: Güllü Aydın-Yağcıoğlu and Öznur Tunca. Methodology: Güllü Aydın-Yağcıoğlu, İpek Alemdaroğlu-Gürbüz and Öznur Tunca. Data curation: Güllü Aydın-Yağcıoğlu and Numan Bulut. Investigation: Güllü Aydın-Yağcıoğlu, Numan Bulut, İpek Alemdaroğlu-Gürbüz, and Öznur Tunca. Formal analysis: Güllü Aydın-Yağcıoğlu and Numan Bulut. Supervision: İpek Alemdaroğlu-Gürbüz and Öznur Tunca. Writing—original draft: Güllü Aydın-Yağcıoğlu, Numan Bulut, İpek Alemdaroğlu-Gürbüz and Öznur Tunca. Writing—review and editing: Güllü Aydın-Yağcıoğlu, Numan Bulut, İpek Alemdaroğlu-Gürbüz and Öznur Tunca.

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RESEARCH ARTICLE

SHORT-TERM EFFECT OF DIFFERENT PHYSICAL THERAPY MODALITIES ON NECK MOBILITY IN HEALTHY YOUNG ADULTS

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ABSTRACT

Purpose: To investigate the short-term effects of hot pack, intermittent traction and low-level laser combined with stretching exercises and stretching exercises alone on neck mobility in individuals with no past pain history but flexibility loss.
Material and Methods: Sixty (60) participants were randomly divided into 4 groups. All participants performed cervical stretching exercises. Cervical intermittent traction wad added to Group I, low level laser therapy was added to group II, hot pack was added to group III. Range of motion (ROM) of the neck, occiput-wall distance (OWD) and, coronoid process- tragus distance (CPTD) was assessed at the beginning and end of the study.
Results: The results of this study showed that all physical therapy modalities were effective in increasing lateral flexion ROM and OWD score ($p<.05$). Hot packs combined with stretching exercises increased the CPTD score ($p=.001$) in addition to lateral flexion ROM and OWD score. However, plus interventions were not superior ($p>.05$) to stretching exercises alone in increasing neck mobility.
Conclusion: In conclusion, both stretching exercises alone and combined with hot pack, intermittent traction, and low-level laser increased neck mobility in healthy young adults. Even if not combined with any physical therapy modality, stretching exercises alone may increase neck mobility.

Keywords: Hot pack, intermittent traction, laser therapy, neck mobility, stretching exercise

INTRODUCTION

The cervical region is the most flexible part of the spine. Vertebral bones, muscles, nerves, blood vessels, lymphatics and other connective tissues constitude this region (1). The seven cervical vertebrae, together with cartilages, ligaments and muscles, generate an advanced and flexible structure that allows for a variety of head/neck movements (2). Many muscles originating from the cervical region create the flexibility of the cervical spine, and this flexibility provides a range of motion in many anatomical planes (3). Considering that decreased muscle flexibility and limited range of motion increases the risk of injury (4), maintaining muscle flexibility is even more important.

Neck pain is a highly prevalent among middle aged population (5) and it affects two-thirds of the adult population (6). Neck pain usually resolve within days or weeks, but considering that the pain can become chronic in 5-7% of cases, it is important to understand the factors that predispose to the development of future neck pain (7,8). Predisposing factors may categorised as as physical, psychosocial, and individual (9). Among the physical factors, muscle

strength and neck mobility are the main ones (10,11). When the literature is examined, a relationship between decreased neck muscle strength and neck pain is detected (10), and it has also been proven that neck pain decreases after muscle strengthening exercises (12). Similarly, decreased neck mobility has been observed in individuals with neck pain compared to those without neck pain (13). It remains unclear whether neck pain causes decreased neck mobility or decreased neck mobility causes neck pain. Therefore, increasing the neck mobility of individuals with decreased neck mobility but without neck pain may be a preventive public health option to prevent future neck pain. We examined different physical therapy modalities to improve neck mobility in healthy subjects with decreased neck mobility.

Physical therapists use a wide variety of interventions as traction, therapeutic exercises, mobilizations, thermotherapy and electrophysical agents to improve neck mobility (14). Thermotherapy used to improve, blood flow, pain, muscle fatigue and muscle flexibility (15). Cervical traction stretches the spine at a specific angle using external force (16). Laser therapy has been used to improve pain and skin resistance by means of increasing blood perfusion, collagen proliferation, peripheral nerve stimulation, anti-inflammatory, and direct analgesic effects (17). On the other hand, stretching exercises are commonly used to obtain muscle relaxation and pain relief, in addition to other applications (18). According to Behm et al (19), static stretching exercise is an effective method to increase ROM and reduce the risk of activity-related injuries in physically active individuals. Also, intermittent traction plus traditional physical therapy program was effective in improving pain, disability and ROM in patients with neck pain (20). According to Kocabal and Gündüz (21) low level laser therapy increased neck ROM by reducing tension in the trapezius muscle. It has been observed that physical therapy modalities mentioned above increase neck ROM. However, it is unclear whether these modalities increase neck mobility by reducing pain and enabling tissue healing or by increasing muscle flexibility. In order to determine whether these modalities increase muscle flexibility, they should be applied to individuals with no past pain history but flexibility loss and the results should be evaluated. If these modalities increase neck mobility, they will make a significant contribution to the literature on protective and preventive strategies to prevent future neck pain. For this reason, current study investigated the short-term effects of hot pack, traction and laser applications combined with stretching exercises and stretching exercises alone on neck mobility in individuals with no past pain history but flexibility loss.

MATERIAL AND METHODS

Study Design

The randomized controlled clinical study was conducted at Sarayköy Vocational School in Turkey. This study was approved by Non-Interventional Clinical Research Ethics Committee of Pamukkale University (Date:26.06.2018; Decision Number:13), and while conducting the study, the principles of the Declaration of Helsinki were complied and written informed consent was obtained from the participants. The inclusion criteria of the current study were as follows: under 30 years of age, being inactive, having right or left upper trapezius muscle shortness, not having neurological, musculoskeletal, and metabolic diseases and did not undergo surgery at the cervical region of spine. The exclusion criteria of the current study were as follows: malignancy, pregnancy and not actively involved in any sport or not participated in a regular exercise program. Inactivity was defined as exercised less than 30 minutes of moderate physical activity for five times a week were accepted as inactive (22).

Flexibility loss was tested by evaluating the upper trapezius muscle length. Participants with flexibility loss were included in the study. Participants were asked to sit on a stool without back support and stand upright with their arms at their sides, facing forward. The assessor stood behind and asked the participant to laterally flex the neck, ckecked the tested shoulder and then asked to perform contralateral rotation of the head. Afterwards, the assessor then asked the participants to pronate and flex their elbows to 90°. He grasped the forearm below the elbow and elevated the upper extremity. If visually observable excessive head motion occurred, the test result recorded as “short”. Test–retest reliability of this test was implemented by Manske et al (23).

One hundred and two participants assessed. Eighteen participants were excluded from the study because of not meeting the inclusion criteria (n=12) and not willing to participate to the study (n=6). A randomization list was prepared before the study for random group assignment of participants. Finally, eighty-four participants were randomly divided into 4 groups (21 participants in each group) (Figure 1).

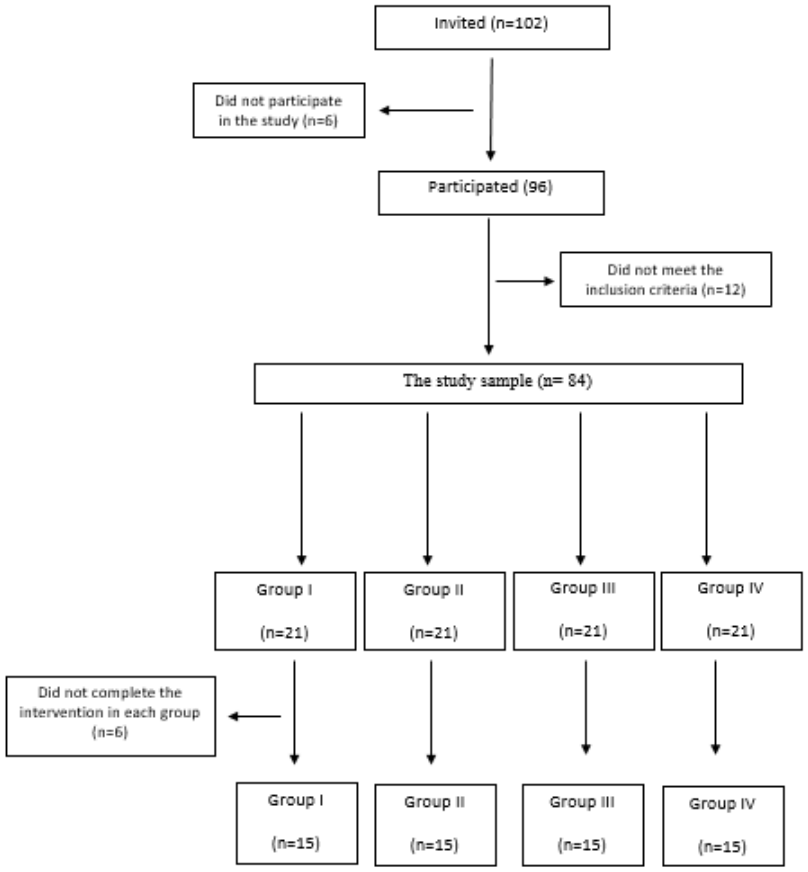

Figure 1. Flow chart of the study design

All participants performed cervical stretching exercises. The supervised upper trapezius, sternocleidomastoid (SCM) and scalene muscle stretching applied for 30 seconds and repeated three times for each muscle. The volunteers rested for 15 seconds between each stretch. In addition to stretching exercises, different physiotherapy modalities were applied to Groups I, II and III. All interventions applied 3 times a week for 6 weeks.

Group I

Participants assigned to group I received cervical intermittent traction (60- second tractions, 60-second inter-traction rest, period for 15 min) in addition to stretching exercises applied 3 times a week for 6 weeks (24). It was applied in a supine position with the neck in 25-30° flexion. The weight was gradually increased from low to the target weight. The target weight was 10% of the patient's weight (25,26).

Group II

Participants assigned to group II received low level laser therapy in addition to stretching exercises applied 3 times a week for 6 weeks. Galyum Arsenic laser device was used transforaminal to each of 6 points at an intensity of 2 J/cm² for 120 seconds. The laser treatment probe was kept in constant contact with the skin (27,28). The wavelength was 904 nm, and the total dose was 12 J.

Group III

Participants assigned to group III received hot pack for 20 minutes in addition to stretching exercises applied 3 times a week for 6 weeks. Hydrocollator hot packs sized 25 to 50-cm dimensions were applied to the cervical region at 45°C in a seated position.

Group IV

Participants assigned to group IV received cervical stretching exercises alone. Supervised self-streching exercises applied 3 times a week for 6 weeks. The upper trapezius, SCM and scalene muscles stretching applied for 30 seconds. Each streching exercises repeated three times for each muscle with a 15 second rest between each strech. Demographic data of the participants (age, Body Mass Index (BMI) and gender) were recorded. All measurements were

Table 1. Demographics data of the groups

Variables	Group I		Group II		Group III		Group IV		p
	Mean±SD (Median)		Mean±SD (Median)		Mean±SD (Median)		Mean±SD (Median)		
Age (Year)	19.40±0.89		19.33±1.44		19.33±0.81		19.53±0.99		0.816
BMI (kg/cm^2)	21.04±2.79		21.49±3.95		23.24±4.00		21.70±4.38		0.256
	Female n (%)	Male n (%)	Female n (%)	Male n (%)	Female n (%)	Male n (%)	Female n (%)	Male n (%)	
Gender	8(53.3)	7(46.7)	11(73.3)	4(26.7)	11(73.3)	4(26.7)	13(86.79	2(13.3)	0.249

BMI: Body Mass Index; SD: Standart Deviation

done at the beginning and end of the study. Cervical flexion, lateral flexion and rotation range of motion (ROM), occiput-wall distance (OWD) and, coronoid process- tragus distance (CPTD) was assessed.

Cervical ROM assessed with the CROM device. It has been determined that this device is a valid and reliable for assessing neck ROM (29). The device was fixed to the participants according to standard rules. Participants were seated on a chair with their back supported, feet in contact with the ground, and shoulders relaxed. Cervical flexion, lateral flexion and rotation movements were demonstrated before the measurements. The participants were asked to perform the movements that mentioned above up to the maximum possible range. The compensatory movements were prevented either by manual contact or verbal instructions. cervical movements were performed in the order specified above. Movements was performed for three times with a 5 s rest between each movement. The values on the unit at neutral position and after the completion of maximum range in every direction were recorded. The difference between the recordings was calculated. The measurements for each direction repeated 3 times, and the average value recorded.

OWD

Participants were asked to lean against the wall with their heels, coccyx and back touching the wall. With a neutral head position, the distance between their occiput and the wall was measured with a ruler. The 3 measurement was performed, and the average distance was recorded (30).

CPTD

The participants asked to stand upright, and laterally flex the cervical spine. The distance between the coronoid process and tragus was measured with a tape measure. And then the participants asked to laterally flex the cervical spine and the distance was measured again. The relative distance was recorded in cm (31).

Statistical Analysis

The sample size was estimated based on the primary endpoints, which were defined as the effect of the ultrasound therapy on neck mobility. The overall effect size of the reference study was large (13) with an index for (f=3.07) (32). Therefore, we included a 4-group comparison with a large effect size (f = 0.5). Accordingly, at least 56 participants (at least 14 for each group) will be included in the study to achieve 80% power with 95% CI.

Within-group change scores were analyzed with Paired samples t-test (for parametric test assumptions) or Wilcoxon signed-rank test (for non-parametric test assumptions- Δ CPTD score of groups I, Δ right-left lateral flexion and CPTD scores of group II, Δ OWD score of group IV). İntergroup difference was analyzed with One-way ANOVA (for parametric test assumptions) or independent samples Kruskal– Walli's test (for non-parametric test assumptions- Δ right and left lateral flexion scores-). Statistical significance was set at $p < 0.05$.

RESULTS

A total of 84 participants were enrolled for the current study. Six participants from each group could not complete the study due to the curfew during the Covid-19 pandemic. Finally, the study completed with sixty participants (15 participants for each group). Of the 60 participants (age =18-25), 43 were females, and 17 were males. The mean age, BMI of the participants were 19.40±1.06, 21.86 ± 3.80, respectively. Descriptive data of the participants according to groups were shown in Table 1.

Cervical Flexion ROM measurements were significantly increased ($p<.001$ for Group I, $p<.05$ for

Group III and p<.01 for Group IV) after interventions compared to pre-intervention values in all groups except Group II (p>.05). Right and left lateral flexion ROM measurements were significantly increased (p<.01 for Group I, p<.05 for Group II, p<.05 for Group III and p<.001 for Group IV) after interventions compared to pre-intervention values in all groups. Right rotation ROM measurements were significantly increased after interventions compared to pre-intervention values in Group II (p=0.025) and Group IV (p=0.04). Left rotation ROM measurements were significantly increased (p<.017 for Group I, p<.01 for Group II, p<.05 for Group IV) after interventions compared to pre-intervention values in all groups except Group III (p>.05). There was no statistical difference between groups in terms of cervical ROM (Table 2).

CPTD scores at right and left directions were significantly increased after interventions compared to pre-intervention values in Group III (p<.001). OWD scores were significantly increased (p<.01 for Group I, p<.001 for Group II, p<.05 for Group III and p=0.01 for Group IV) after interventions compared to pre-intervention values in all groups. There was no statistical difference between groups in terms of CPTD and OWD scores (Table 3).

DISCUSSION

The aim of this study was to compare short-term effects of different physical therapy modalities on neck mobility in individuals with no past pain history but flexibility loss. The results of this study showed that all physical therapy modalities were effective in increasing lateral flexion ROM and OWD score. Hot packs combined with stretching exercises increased the CPTD score in addition to lateral flexion ROM and OWD score. However, plus interventions were not superior to stretching exercises alone in increasing neck mobility.

Spinal mobility plays an important role in performing many daily, occupational and recreational activities. The decrease in spinal mobility increases the load on the spine and causes tissue degeneration. This may result in pain and similar clinical symptoms (32). Therefore, spinal mobility assessment is widely used in clinical musculoskeletal system evaluation. It has been proven in the literature that there is a positive correlation between joint stiffness, age, BMI and trapezius muscle elasticity (33). In this sense, we added a stretching exercise to increase the flexibility of the upper trapezius muscle to our treatment program, which aims to increase neck mobility. We found that hot pack, traction and laser combined with stretching exercises increased neck mobility.

Literature shows that mechanical traction is often used with different exercise interventions (34,35,36). According to fritz et al, cervical traction plus exercise resulted in less disability and pain, especially in long-term follow-up in specific subgroups of patients with neck pain (36). Infrared, massage, stretching, and strengthening exercises plus cervical traction improved disability and pain compared to other interventions in young adults with mechanical neck pain (37). In line with the literature, we combined intermittent traction with stretching exercises. Current study results concluded that intermittent traction plus stretching exercises improved cervical flexion, lateral flexion, left rotation and OWD scores. However, it was not superior to other physical therapy modalities.

According to Momenzadeh et al (38), low-level laser therapy decreased neck pain compared to sham application. Low-level laser therapy was effective in pain and ROM in patients with myofascial pain syndrome of the upper trapezius muscle (39). Therefore, we combined low-level laser therapy with stretching exercises to enhance more relive in upper trapezius muscle and current study results showed improvement in flexion, lateral flexion and rotation ROM and CPTD scores. Thermotherapy is used in musculoskeletal pain as a complementary intervention to increase muscle flexibility (40). Fujita et al (41) suggested that hot pack plus stretching exercise increased ROM, but hot pack was not superior to stretching exercise in increasing hamstring flexibility. Additionally, thermotherapy before 30 sec of stretching exercise increased the extensibility of the muscle in children with hypertonia (42). Current study results showed that hot packs plus stretching exercise improved cervical flexion, cervical lateral flexion, OWD and CPTD scores, but was not superior to stretching exercise alone in increasing neck mobility. We supervised peri cervical stretching exercises for 6 weeks and elicited similar improvements with the plus interventions. Anderson et al (43) showed that six-week of peri cervical muscle stretching and strengthening exercises increased muscle endurance and cervical motion in young adults. According to current study results, 6 weeks of peri cervical stretching exercise alone improved cervical ROM. We did not assess muscle strength or endurance in this study. Therefore, we cannot compare our study findings with the relevant study,

Table 2. Comparison of pre- and post-intervention ROM values of groups

Variables	Group I	Group II	Group III	Group IV	p
	Mean±SD (95% CI)	**Mean±SD (95% CI)**	**Mean±SD (95% CI)**	**Mean±SD (95% CI)**	
ROM- Flexion					
Baseline	55.13 ±10.54	56.67±9.82	50.93±11.94	53.40±10.07	0.497
6W	65.93±9.56	60.13±6.78	57.73±7.66	61.40±7.65	
6W-Baseline change	10.80±9.91	3.46±8.65	6.80±9.96	8.00±10.19	0.232
p	**0.001**	0.143	**0.019**	**0.009**	
ROM-Right Lateral Flexion					
Baseline	50.07±11.57	46.73±9.15	46.00±9.59	45.80±8.43	0.632
6W	59.07±6.71	51.93±10.00	53.33±5.83	53.60±9.92	
6W-Baseline change	9.00±9.90	5.20±5.12	7.33±7.23	7.80±6.43	0.392
p	**0.007**	**0.004**	**0.005**	**0.001**	
ROM-Left Lateral Flexion					
Baseline	53.67±11.06	46.33±9.30	46.27±7.77	45.80±6.16	0.091
6W	58.00±8.71	53.33±10.66	54.27±7.24	54.00±8.48	
6W-Baseline change	4.33±11.15	7.00±6.55	8.00±9.28	8.20±8.16	0.852
p	**0.039**	**0.002**	**0.011**	**0.004**	
ROM-Right Rotation					
Baseline	69.67±9.64	65.67±10.90	65.47±11.89	65.47±10.94	0.655
6W	73.67±10.34	73.47±10.21	71.73±9.13	76.07±10.36	
6W-Baseline change	4.00±12.58	7.80±11.98	6.26±12.98	10.60±12.11	0.529
p	0.239	**0.025**	0.083	**0.004**	
ROM-Left Rotation					
Baseline	64.07±14.71	67.20±8.89	67.33±11.50	64.93±18.42	0.929
6W	74.53±11.52	73.73±10.79	67.20±13.49	75.60±13.65	
6W-Baseline change	10.46±14.92	8.53±12.07	-0.13±16.68	10.66±17.72	0.194
p	**0.017**	**0.016**	0.976	**0.035**	

SD: Standart Deviation: CI: Coincidence İnterval; W: Week; ROM: Range of Motion

Table 3. Comparison of pre- and post-intervention flexibility values of groups

Variables	Group I	Group II	Group III	Group IV	p
	Mean±SD (95% CI)	**Mean±SD (95% CI)**	**Mean±SD (95% CI)**	**Mean±SD (95% CI)**	
CPTD-right					
Baseline	9.64±2.83	8.97±1.82	9.68±1.71	9.43±1.57	0.818
6W	8.32±3.12	8.00±2.41	7.94±1.49	8.51±2.15	
6W-Baseline change	-0.88**±**2.14	-0.70**±**1.76	-1.74±1.04	0.34±4.61	0.792
p	0.137	0.146	**0.001**	0.169	
CPTD-left					
Baseline	9.99±2.09	9.01±1.48	9.75±1.29	9.16±1.83	0.461
6W	8.72±2.44	8.12±2.36	7.97±1.44	8.71±1.82	
6W-Baseline change	-0.84**±**2.20	-0.65±1.25	-1.78±1.08	0.76±4.22	0.361
p	0.139	0.062	**0.001**	0.093	
OWD					
Baseline	2.71±1.08	2.73±0.74	2.71±0.65	2.32±0.47	0.399
6W	1.78±0.70	1.66±0.46	2.09±0.90	1.75±0.59	
6W-Baseline change	-0.92±0.92	-1.07±0.77	-0.62±0.77	-0.56±0.63	0.241
p	**0.005**	**0.001**	**0.011**	**0.008**	

SD: Standart Deviation: CI: Coincidence İnterval; W: Week; cm: centimeter; CPTD; Coronoid Process-Tragus Distance; OWD: Occiput-Wall Distance

but we can comment that exercise therapy can improve pain and muscle endurance even if there is no physiotherapy modality applied additionally. Ibrahim et al (44) suggested that supervised exercise was superior to unsupervised neck exercises in patients with chronic neck pain. We judge from the current study results that supervised peri cervical stretching exercise can improve neck mobility even if not adding any physical therapy modalities.

Limitations

The sample size of the current study was restricted to young adults aged between 18-25. Although standard random sampling techniques were used to assign volunteers to groups, this study was designed to evaluate young undergraduate students in a specific geographic location. In this study, participants were not assigned to groups based on gender, so the groups were not homogeneous in terms of gender. During the study, volunteers with muscle tightness in the upper trapezius were included, but it was not assessed after the interventions. We think that this study should be repeated in a larger population and by considering other factors that may affect neck mobility.

CONCLUSION

In conclusion, both stretching exercises alone and combined with hot packs, intermittent traction, and low-level laser increased neck mobility in healthy individuals. Increasing neck mobility, which is among the predisposing factors for future neck pain, is important for preventive public health. If neck pain becomes chronic, it can lead to degenerative spinal disorders, and gradual loss of muscle strength and forward head/neck posture are important symptoms of chronic neck pain (45,46,47). In order to prevent these symptoms, it is important to eliminate the predisposing factors that may cause pain before it occurs. Therefore, it is important to maintain neck mobility at a young age before chronic neck pain occurs. The findings of this study showed that stretching exercises can increase neck mobility regardless of which physical therapy modality they are used with, or even alone. The short-term results of this study will shed light on preventive physiotherapy interventions. In order to prevent degenerative spinal disorders, physiotherapists working in the field of preventive health should add neck stretching exercises to their exercise routines.

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RESEARCH ARTICLE

INVESTIGATION OF THE EFFECT OF PREOPERATIVE FASTING PERIOD ON COMFORT ACCORDING TO KOLCABA'S COMFORT THEORY

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ABSTRACT

Purpose: This study was aimed at investigating the effect of preoperative fasting periods on the preoperative comfort levels of patients according to Kolcaba's Comfort Theory.
Material and Methods: This descriptive and correlational study included 267 patients. The study data were collected using the General Comfort Questionnaire. In the data analysis, the independent sample t test, one-way analysis of variance, Pearson correlation analysis and linear regression analysis were used.
Results: While the increase in the duration of preoperative fluid fasting caused a decrease in their mean scores for the relief and transcendence sub-dimensions of the sense dimension, and the physical, environmental and sociocultural comfort sub-dimensions of the context dimension; the increase in the duration of preoperative solid fasting caused a decrease in their mean scores for the relief and transcendence sub-dimensions of the sense dimension, and the physical, environmental and sociocultural comfort sub-dimensions of the context dimension and the overall questionnaire ($p<0.05$).
Conclusion: The increase in the patients' durations of both preoperative fluid and solid fasting decreased their mean scores for the most of sub-dimensions; the increase in the patients' durations of preoperative solid fasting also decreased their mean score for the overall questionnaire.

Keywords: Preoperative care, preoperative period, patient comfort, perioperative nursing

INTRODUCTION

Fasting of patients scheduled for elective surgery has been accepted as one of the cornerstones of safe preoperative care (1-4). Due to fear of aspiration, the negative effects of preoperative fasting are ignored, which leads to excessive fluid and solid food restriction (2,5,6). Theoretically, preoperative fasting may reduce the risk of aspiration, but the evidence does not support prolonged fasting (7). According to guidelines, clear fluids up to 2 hours and a light meal up to 6 hours before elective procedures requiring general anesthesia, regional anesthesia or procedural sedation (8-11). Despite the evidence, current recommendations for prolonged fasting are a significant problem (5,11,12). Medical and nursing staff concerned with the safety and comfort of patients strive to establish safe and comfortable preoperative fasting levels, which prevents patients from starving unnecessarily (7,13).

The nurse confirms that the patient is fasting before going to the operating room by following the steps stated in the Surgical Safety Checklist (4). The nurse plays an important role in establishing a safe fasting level before surgery. Although the length of preoperative fasting period is determined by the physician, the nurse has essential responsibilities

such as informing patients about fasting before surgery and monitoring them for compliance and adverse effects (13,14). A longer preoperative fasting period causes a decrease in preoperative comfort (2,7,15). The best preoperative fasting regimen minimizes not only the risk of aspiration or regurgitation but also possible adverse effects on comfort (7). Several systematic reviews have determined that the comfort level increases when the preoperative fasting period is shortened (12,16).

Comfort is a desired outcome of nursing care during perianesthesia. It is also an umbrella term for surgery- or procedure-related discomfort experienced by patients (17). Comfort interventions are implemented at each period of perianesthesia. One of the aims of the pre-anesthesia period is to assess risk factors affecting anesthesia one of which is the restriction of fluid and solid food intake. Nurses should advocate implementing recommendations stated in current guidelines for preoperative fluid and solid food management (18). Guidelines innate features of each nursing conceptual model or theory, guide nursing practices and research (19). In a research context, theory brings results into meaningful form and summarizes existing knowledge in coherent systems (20). Since Kolcaba's Comfort Theory includes a measurable, holistic, and nurse-sensitive result, it guides nursing practices and research (21). Comfort theory formed the conceptual framework of the study. Patient comfort, defined as the immediate state of being strengthened by having needs met in four contexts of human experience (physical, psychospiritual, environmental, and sociocultural). This theory describes traditional nursing practice as humanistic, needs-related, and holistic (22). This theory guides nurses to detect comfort needs of patients that are not being addressed and to develop interventions to meet those needs (23).

The study's main aim is to investigate the effect of preoperative fasting periods on the preoperative comfort levels of patients according to Kolcaba's Comfort Theory. Among the other aims are to investigate whether there are significant differences in preoperative comfort levels according to the sociodemographic and clinical characteristics of the patients, whether sociodemographic and clinical characteristics of the patients affect their preoperative comfort levels, and what the relationship between the durations of preoperative fluid and solid fasting in the patients and their preoperative comfort levels is like.

MATERIAL AND METHODS

Study Design/Setting

The study is a descriptive and correlational study.

The study was conducted in the cardiovascular surgery, general surgery, neurosurgery, ophthalmology, orthopedics and traumatology, otolaryngology, otolaryngology, and urology clinics of a state hospital. Patients are admitted to the clinic one or a few days before the surgery. After the surgery, they stay in the hospital for at least one day, depending on their general condition and the extent of their surgery. During the pre-surgery preparation, patients are informed by the physician or, upon the physician's request, by nurses that they should not drink or eat anything after midnight. The preoperative fasting requirement does not vary by clinic, procedure or anesthesia.

The study was conducted between April 2023 and November 2023.

Sample

The population of the study comprised patients who were hospitalized in the clinics of the aforementioned hospital between the dates of the study and who met the sampling criteria. No type of procedure was selected. All patients under general anesthesia and regional anesthesia were included. Inclusion criteria were volunteering to participate in the study, being ≥18 years old, being conscious and cooperative, fasting for at least 2 hours for fluid foods and 6 hours for solid foods, being to go into surgery very soon, and undergoing elective surgery. Patients undergoing day surgery were excluded from the sample.

The minimum sample size was calculated 242 people using the "G. Power-3.1.9.2" program (effect size=0.179, alpha value=0.05, power=80%). However, considering the possibility of withdrawals or losses during the study, it was decided to include 10% more people. Thus, the study was completed with 267 people.

Data Collection Tools

Patient information form: The form prepared by the researchers based on the relevant literature includes 17 items questioning patients' sociodemographic and clinical characteristics such as body mass index (BMI), presence of a chronic disease, having undergone surgery previously, diagnosis, clinic where the respondent is hospitalized, American Society of Anesthesiology (ASA) score, time of

surgery, duration of preoperative fluid and solid fasting (24-26).

General comfort questionnaire (GCQ): The GCQ developed by Kolcaba in 1992 contains 48 items (27). Of the items, 24 are positively keyed, and 24 are negatively keyed. According to the taxonomic structure of comfort, there are three senses (relief, ease and transcendence) and four contexts (physical comfort, psychospiritual comfort, environmental comfort and sociocultural comfort) (28). Responses given to the items are rated on a 4-point Likert type scale. Negatively keyed items are reverse coded. A higher score means a greater degree of met comfort needs. The Cronbach's alpha coefficient for the context sub-dimensions ranged between 0.66 and 0.80. It increased to 0.90 for 35 items (27). The questionnaire was adapted into Turkish by Kuğuoğlu and Karabacak in 2008 and its psychometric properties were tested. The Cronbach's alpha coefficient ranged between 0.55 and 0.70 for the sense sub-dimensions and 0.85 for the overall questionnaire (29). In the study, it ranged between 0.630 and 0.716 for the sense sub-dimensions, between 0.610 and 0.789 for the context sub-dimensions and 0.885 for the overall questionnaire.

Data Collection

Data were collected by the researcher in surgical clinics through face-to-face interviews, just before the patients went to the operating room. During the data collection process, the patients were not insisted on filling out the data collection tools. Whether their emotional states were suitable for filling out the data collection tools was taken into consideration. Data were collected during working hours.

Data Analysis

Data analyzed using the Statistical Package for Social Science (SPSS) 22.0 were presented as arithmetic mean, standard deviation, number and percentage. Whether the data were normally distributed was checked with kurtosis and skewness values, and it was determined that the data were normally distributed. The independent samples t-test and one-way analysis of variance were used in the analysis of the data. Pearson correlation analysis was used to determine the relationship between continuous data whereas the linear regression analysis was used to determine how much of the dependent variable was explained by the independent variables. The statistical significance level in the study was accepted as $\alpha=0.05$.

Ethical Issues: Before the study was conducted, ethical approval was obtained from Non-Interventional Clinical Research Ethics Committee of Izmir Katip Celebi University (Decision Date: 18.05.2023, Decision No: 0216). The Informed Consent Form was obtained from the patients participating in the study.

RESULTS

The distribution of the sociodemographic and clinical characteristics of the participants was presented in Table 1.

The participants' mean durations of preoperative fluid fasting were 12.12±2.43 (minimum=7, maximum=19) hours. The participants' mean durations of preoperative solid fasting were 14.57±2.74 (minimum=8, maximum=21) hours. The mean scores the participants obtained from the sense sub-dimensions of the GCQ were as follows: 2.64±0.36 for the relief, 2.67±0.37 for the ease and 2.59±0.46 for the transcendence. The mean scores they obtained from the context sub-dimensions of the GCQ were as follows: 2.33±0.42 for the physical comfort, 2.62±0.50 for the psychospiritual comfort, 2.93±0.37 for the environmental comfort, and 2.63±0.42 for the sociocultural comfort. The mean score they obtained from the overall questionnaire was 2.63±0.36.

There were statistically significant differences between the participants' mean scores for the overall questionnaire and its sense and context sub-dimensions except for psychospiritual and environmental sub-dimensions in terms of variables such as age, employment status, presence of a chronic disease, and having undergone surgery previously. There were statistically significant differences between the participants' mean scores for the overall questionnaire and all the sense and context sub-dimensions in terms of the variables such as sex, educational status, marital status, the clinic where the respondent is hospitalized, and ASA score ($p<0.05$). There was no statistically significant difference in any mean score according to the participants' BMI ($p>0.05$).

Three models were created to investigate the effects of the participants' sociodemographic and clinical characteristics on their mean scores for the overall

Table 1. Distribution of the sociodemographic and clinical characteristics of the participants

Characteristics		N	%
Age (years) (X̄±SD=52.43±18.84)	≤44	94	35.2
	45-54	40	15.0
	55-64	49	18.4
	≥65	84	31.5
Sex	Women	127	47.6
	Men	140	52.4
Educational status	Illiterate	28	10.5
	Elementary school and junior high school	146	54.7
	Senior high school	63	23.6
	University	30	11.2
Employment status	Employed	88	33.0
	Not employed	179	67.0
Marital status	Married	188	70.4
	Single	79	29.6
BMI (X̄±SD=26.98±4.19)	Normal (18.50-24.99)	87	32.6
	Overweight (25.00-29.99)	114	42.7
	Obese (≥30.00)	66	24.7
Presence of a chronic disease	Yes	115	43.1
	No	152	56.9
Having undergone surgery previously	Yes	169	63.3
	No	98	36.7
Clinic where the respondent is hospitalized	General surgery	91	34.1
	Otolaryngology	45	16.9
	Orthopedics and traumatology	93	34.8
	Urology	26	9.7
	Others	12	4.5
ASA score	ASA I	49	18.4
	ASA II	195	73.0
	ASA III	23	8.6

N=267, BMI=Body Mass Index, ASA=American Society of Anesthesiology

Table 2. Effects of the participants' sociodemographic and clinical characteristics on their mean scores for the overall GCQ

	β	SE	t	p	VIF	F	p	Adj.R^2	DW
Constant	2.744	0.088	31.063	0.000		12.682	**0.000**	0.235	1.927
Sex reference category=Women									
Sex=Men	0.102	0.039	2.575	**0.011**	1.044				
Educational status reference category=Illiterate									
Educational status=Elementary school and junior high school	0.052	0.070	0.751	0.453	3.646				
Educational status=Senior high school	0.185	0.077	2.402	**0.017**	4.094				
Educational status=University	0.282	0.085	3.337	**0.001**	2.893				
Marital status reference category=Married									
Marital status=Single	-0.225	0.047	-4.830	**0.000**	1.219				
ASA score reference category=ASA I									
ASA score=ASA II	-0.229	0.053	-4.313	**0.000**	1.731				
ASA score=ASA III	-0.407	0.083	-4.935	**0.000**	1.561				

Dependent variable=mean score for the overall GCQ, ASA=American Society of Anesthesiology, GCQ=General Comfort Questionnaire

Table 3. Relationship between the participants' durations of preoperative fluid and solid fasting, and their mean scores for the overall GCQ and its sub-dimensions

Sub-dimensions		Duration of preoperative fluid fasting	Duration of preoperative solid fasting
Relief	r	-0.127*	-0.158**
	p	**0.038**	**0.010**
Ease	r	-0.060	-0.049
	p	0.327	0.421
Transcendence	r	-0.132*	-0.135*
	p	**0.031**	**0.027**
Physical comfort	r	-0.180**	-0.198**
	p	**0.003**	**0.001**
Psychospiritual comfort	r	0.041	0.010
	p	0.500	0.869
Environmental comfort	r	-0.140*	-0.171**
	p	**0.022**	**0.005**
Sociocultural comfort	r	-0.175**	-0.106
	p	**0.004**	0.084
Overall GCQ	r	-0.120	-0.129*
	p	0.050	**0.036**

**p<0.05, **p<0.01, GCQ=General Comfort Questionnaire*

value of the model and were insignificant were removed from the model. The model was re-evaluated. The p values of the variables were checked, and it was decided whether they would remain in the model or not. While evaluating the model, first statistical significance and then theoretical significance were considered (Table 2).

The relationship between the participants' durations of preoperative fluid and solid fasting, and their mean scores for the GCQ were presented in Table 3.

The effects of the participants' durations of preoperative fluid fasting on their mean scores for the GCQ were analyzed. The effects of the duration of preoperative fluid fasting on the mean scores obtained from the relief and transcendence sub-dimensions of the sense dimension, and physical comfort, environmental comfort, and sociocultural comfort sub-dimensions of the context dimension were statistically significant for all the models created ($p<0.05$) (Table 4).

The effects of the duration of preoperative solid fasting on the mean scores obtained from the overall questionnaire were statistically significant for the model created ($F=4.459$, $p=0.036$). A 1-unit increase in the duration of preoperative solid fasting caused a decrease of 0.017 units in the mean score obtained from the overall questionnaire ($\beta=-0.017$). In the model, the duration of preoperative solid fasting explained 1.3% of the change in the mean score obtained from the overall questionnaire (Adj. $R^2=0.013$) (Table 5).

DISCUSSION

In good research, a link is established between existing knowledge and the new study by reviewing previous research conducted on the topic and by identifying an appropriate theory (20). Theory provides concrete structures for interpreting situations and events (19,30), and it is sometimes used as an organizing framework (20). Kolcaba's Comfort Theory provided a structure for the interpretation of the concepts of preoperative fasting and comfort and explained the relationship between these concepts and thus contributed to the formation of the conceptual framework of the study. The study's mean durations of preoperative fluid and solid fasting were 12.12 and 14.57 hours, respectively. In several international studies, these durations ranged between 5.80 and 15.30 hours and between 12.10 and 16.10 hours, respectively (31-39). In studies conducted in our country, these durations ranged between 10.57 and 13.54 hours and between 11.40 and 14.80 hours, respectively (24,26,40-45). The guidelines endorse a 2-hour preoperative fasting period for clear fluids and a 6 hours period for solid foods (8-11). The results of the study, which were consistent with the results of all other studies, demonstrated that the durations of preoperative fluid and solid fasting were longer than the durations recommended in the guidelines. These results suggest that evidence-based knowledge has not yet been transferred to practice and that traditional preoperative fasting practices continue.

Table 4. Effects of the participants' durations of preoperative fluid fasting on their mean scores for the overall GCQ and its sub-dimensions

	β	SE	t	p	F	p	Adj.R^2	DW
Relief								
Constant	2.867	0.112	25.641	**0.000**	4.336	**0.038**	0.012	1.702
Duration of preoperative fluid fasting	-0.019	0.009	-2.082	**0.038**				
Ease								
Constant	2.782	0.116	23.912	0.000	0.966	0.327	0.000	1.633
Duration of preoperative fluid fasting	-0.009	0.009	-0.983	0.327				
Transcendence								
Constant	2.895	0.144	25.641	0.000	4.683	**0.031**	0.014	1.577
Duration of preoperative fluid fasting	-0.025	0.012	-2.082	**0.031**				
Physical comfort								
Constant	2.713	0.130	20.858	0.000	8.849	**0.003**	0.012	1.51
Duration of preoperative fluid fasting	-0.031	0.011	-2.975	**0.003**				
Psychospiritual comfort								
Constant	2.517	0.155	16.288	0.000	0.457	0.500	0.000	1.796
Duration of preoperative fluid fasting	0.008	0.012	0.676	0.500				
Environmental comfort								
Constant	3.190	0.116	27.580	0.000	5.319	**0.022**	0.016	1.680
Duration of preoperative fluid fasting	-0.022	0.009	-2.306	**0.022**				
Sociocultural comfort								
Constant	2.993	0.131	27.881	0.000	5.319	**0.022**	0.016	1.680
Duration of preoperative fluid fasting	-0.031	0.011	-2.891	**0.004**				
Overall GCQ								
Constant	2.847	0.112	25.512	0.000	3.867	0.050	0.011	1.568
Duration of preoperative fluid fasting	-0.018	0.009	-1.966	0.050				

GCQ=General Comfort Questionnaire

Empirical indicators also include the processes used for data collection and analysis, and can be used to calculate quantitative scores (19). The GCQ used to measure the comfort level in the study is an empirical indicator representing the comfort theory. According to comfort theory adapted to outcome research, the level of patient comfort should be assessed with a questionnaire developed based on the taxonomic structure (22). When Kolcaba developed the GCQ, she took this taxonomic structure as a guide (27). The GCQ was also used in the study to assess the participants' comfort levels.

According to the results of the study, the participants' preoperative comfort level was moderate to high in the relief, ease, and transcendence sub-dimensions of the sense dimension. It was low to moderate in the physical comfort sub-dimension of the context dimension and moderate to high in the psychospiritual comfort, environmental comfort and sociocultural comfort sub-dimensions of the context dimension. It was moderate to high for the overall

Table 5. effects of the participants' durations of preoperative solid fasting on their mean scores for the overall GCQ and its sub-dimensions

	β	SE	t	p	F	p	Adj. R^2	DW
Relief								
Constant	2.943	0.119	24.796	0.000	6.815	**0.010**	0.021	1.698
Duration of preoperative solid fasting	-0.021	0.008	-2.611	**0.010**				
Ease								
Constant	2.768	0.124	22.296	0.000	0.648	0.421	0.000	1.637
Duration of preoperative solid fasting	-0.007	0.008	-0.805	0.421				
Transcendence								
Constant	2.925	0.153	19.113	0.000	4.950	**0.027**	0.015	1.571
Duration of preoperative solid fasting	-0.023	0.010	-2.225	**0.027**				
Physical comfort								
Constant	2.780	0.138	20.108	0.000	10.770	**0.001**	0.035	1.50
Duration of preoperative solid fasting	-0.031	0.009	-3.282	**0.003**				
Psychospiritual comfort								
Constant	2.593	0.165	15.719	0.000	0.027	0.869	0.000	1.798
Duration of preoperative solid fasting	0.002	0.011	0.165	0.869				
Environmental comfort								
Constant	3.270	0.123	26.642	0.000	8.018	**0.005**	0.026	1.680
Duration of preoperative solid fasting	-0.023	0.008	-2.832	**0.005**				
Sociocultural comfort								
Constant	2.993	0.131	22.881	0.000	8.359	**0.004**	0.027	1.634
Duration of preoperative solid fasting	-0.031	0.011	-2.891	**0.004**				
Overall GCQ								
Constant	2.879	0.119	24.214	0.000	4.459	**0.036**	0.013	1.566
Duration of preoperative solid fasting	-0.017	0.008	-2.112	**0.036**				

GCQ=General Comfort Questionnaire

questionnaire. In a study, the comfort level was moderate in the physical comfort sub-dimension of the context dimension, and above moderate in all the other sub-dimensions of the context dimension, in all the sub-dimensions of the sense dimension, and in the overall questionnaire (24). It was low to moderate in all the sub-dimensions of the sense dimension in another study (46). In another study, comfort was moderate (26).

In the study, sex, educational status, marital status and ASA score explained 23.5% of the change in the mean score of the overall questionnaire. In a study, it was found that women had higher levels of fatigue and nausea than men, and those with university and higher education had higher levels of dizziness than those with primary and high school education. On the other hand, it was determined that there was no significant difference in thirst, hunger, dry mouth, nausea, headache, dizziness, fatigue and irritability levels according to age and BMI (26).

The "I am hungry." item of the GCQ is in the cell where the relief sub-dimension of the sense dimension and physical comfort sub-dimension of the context dimension emerge (28). In surgical patients, pain and nausea are located in the same cell in the taxonomic structure of comfort needs (17). Relief is the patient's experience of having his or her specific needs met (47), and indicates his or her urgent

comfort need that has just been met (27). In perianesthesia nursing, relief refers to a situation in which a specific discomfort is eliminated. Two of the common discomforts associated with this in the perianesthesia procedure are pain and nausea (17). The physical dimension is related to bodily sensations (47). Physical discomforts such as pain are included in this dimension (48). In terms of perianesthesia nursing, it covers the deficiencies in physiological mechanisms that are disrupted due to surgical procedures. Observation and treatment of physical needs such as pain and nausea are easier (18). Thus, according to the theory, preoperative fasting can be considered as pain and nausea.

Longer preoperative fasting reduces the patients' comfort. A long fasting period before surgery causes the patient to experience discomfort due to reasons such as thirst, hunger and anxiety (2,7,15). In the study, the increase in the duration of both preoperative fluid and solid fasting of the patients caused a decrease in the mean scores obtained from the relief and transcendence sub-dimensions of the sense dimension, and in the mean scores obtained from the physical comfort, environmental comfort and sociocultural comfort sub-dimensions of the context dimension. The increase in the duration of preoperative solid fasting also led to a decrease in the mean score obtained from the overall questionnaire. The review of systematic reviews in which the relationship between the duration of preoperative fasting and preoperative comfort was investigated revealed that the comfort level significantly increased in patients whose fasting duration was short (12,16). In a study, comfort levels were high in patients whose durations of preoperative fluid and solid fasting were short (25). According to another study, no relationship was determined between the duration of preoperative fluid fasting and the mean scores obtained from the overall questionnaire and its sub-dimensions, but there was a positive relationship between the duration of preoperative solid fasting and the ease sub-dimension of the sense dimension, and between the physical comfort and sociocultural comfort sub-dimensions of the context dimension (24). In another study, it was determined that duration of preoperative fluid or solid fasting did not lead to any difference in comfort levels (26).

According to comfort theory, a stimulating situation creates alpha press. Alpha press activates obstructing forces that reveal health care needs. Needs arise from a stimulating situation and cause negative tension, which decreases comfort levels (21,49). Within the framework of comfort theory, it can be concluded that in the study, preoperative fasting created negative tension and reduced comfort levels. Health care including physical needs refer to needs which arise in stressful health care situations (22). In the study, the participants' leading healthcare need was the reduction of preoperative fasting.

Comfort theory guides nurses in determining patients' comfort needs and initiating interventions. According to the comfort theory, there are three types of comfort interventions, one of which is technical interventions (23). Preoperative fasting practice is a technical intervention. All the needs of people, including physical ones, determine the nursing tasks to be performed (50). Conscious use of the theory strengthens improvement programs and facilitates assessment of their effectiveness (51). The study's results may guide future studies in which nurses plan comfort interventions to meet patients' comfort needs, such as arising from preoperative fasting.

The current practice according to enhanced recovery after surgery (ERAS) protocols is to allow the intake of fluid foods up to 2 hours before surgery and solid foods up to 6 hours before surgery. This practice has been shown to reduce metabolic stress and improve well-being during the perioperative period (52). Nurses play a role in identifying patient needs and initiating interventions to address them in case of discomfort that occurs when these protocols are not followed and the preoperative fasting period is prolonged.

Limitations

The study has some methodological limitations. Because it was conducted in a single hospital, it is impossible to make a general comment on the subject. The results of the study are applicable only to the participants hospitalized in the surgical clinics of the aforementioned hospital between the dates during which the study was conducted. Data on the durations of preoperative fluid and solid fasting and the comfort levels of the participants are based on their statements.

CONCLUSION

According to the results of this study, durations of preoperative fluid and solid fasting were longer in the participants of the study than were those recommended in the guidelines. The comfort was low

to moderate in the physical comfort sub-dimension of the context dimension, and medium to high in the overall questionnaire, all sub-dimensions of the sense dimension and the sub-dimensions of the context dimension other the physical comfort sub-dimension. The increase in the durations of both preoperative fluid and solid fasting of the patients caused a decrease in the mean scores obtained from the relief and transcendence sub-dimensions of the sense dimension and in the scores obtained from the physical comfort, environmental comfort, and sociocultural comfort sub-dimensions of the context dimension. The increase in the duration of preoperative solid fasting also led to a decrease in the mean score obtained from the overall questionnaire.

In line with these results, it is recommended to take precautions against situations that will delay the surgery time and prolong the preoperative fasting period, adopt a multidisciplinary approach to preoperative fasting, and updating preoperative fasting practices in institutions in line with evidence-based practices. In order to raise the awareness of nurses about the negative effects of prolonged preoperative fasting, they can be trained on this issue. It is also recommended that nurses should determine the patients' preoperative comfort needs, plan interventions to increase the patients' preoperative comfort levels and evaluate patient comfort as a whole with all its dimensions. Nurses should be aware that patient comfort will decrease especially when traditional fasting periods are applied before surgery, and should diagnose the patient needs that arise in this period, plan interventions for them and evaluate the patients' comfort level in line with Kolcaba's Comfort Theory. Researchers are advised to prepare studies in which the preoperative comfort levels of patients who undergo evidence-based preoperative fasting periods are compared with those of patients who undergo traditional preoperative fasting periods and to test the comfort theory in their studies.

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RESEARCH ARTICLE

THE RELATIONSHIP BETWEEN VIOLENCE TENDENCY AND DATING VIOLENCE ATTITUDE IN STUDENTS OF HEALTH SCIENCES

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ABSTRACT

Purpose: The aim of this study was to determine the levels of violence tendency and dating violence attitudes of university students and the risk factors affecting violence tendency and dating violence attitudes.

Material and Methods: The study is a cross-sectional. University students between the ages of 18-25 who were studying at the faculty of health sciences and had at least one dating experience were included in the study and 365 students participated in the study. Data were collected face-to-face using the “Student Information Form”, “Violence Tendency Scale” and “Dating Violence Attitude Scale”. Violence tendency and dating violence attitudes were dependent variables of the study. Multivariate linear regression analysis was performed to predict sociocultural factors with tendency to violence and dating violence attitudes.

Results: University students studying at the faculty of health sciences have low violence tendencies and positive attitudes towards dating violence. Exposure to domestic violence (B = 1.393, 95% CI [0.111, 2.674], p = .013) and exposure to partner violence (B = 2.970, 95% CI [0.773, 5.167], p = .008) significantly increased violence tendency, while other variables showed no significant effects (R^2 = .145, Adj. R^2 = .129). Negative attitudes toward dating violence were significantly associated with being from a broken family (B = -0.166, 95% CI [-0.312, -0.020], p = .026), higher father’s education level (B = 0.013, 95% CI [0.003, 0.024], p = .013), and exposure to partner violence (B = -0.279, 95% CI [-0.414, -0.143], p < .001) (R^2 = .190, Adj. R^2 = .174).

Conclusion: Family dynamics and exposure to violence can influence people's tendency to violence and their attitudes towards dating violence. In addition, women are more exposed to domestic violence and dating violence. This situation reveals gender inequality even among young individuals with high education levels. It is important to regularly screen young people for early detection of violent tendencies and to establish referral systems to ensure that victims of violence receive support.

Keywords: violence tendency, dating violence, dating violence attitude, health sciences students.

INTRODUCTION

The World Health Organization defines violence as 'the use of physical force or threats against itself, another person, a particular community or group, which may result in injury, physical harm, developmental disorders, deprivation or death' (1). Individuals' feelings, thoughts and behaviors towards violence indicate a tendency towards violence (2). The tendency towards violence among young people is increasing and it is important to determine the levels of violence and dating violence among young people for healthy dating experiences. Dating

violence is defined as physical, emotional, psychological or sexual abuse that occurs in an unmarried dating relationship, including stalking, and includes all forms of violence that occur from the beginning of the relationship until cohabitation (3).

Dating violence is generally classified into three main categories: physical, psychological and sexual violence. Physical violence includes all violent behaviors that are consciously carried out to harm the partner by using physical force (3,4). Sexual violence, on the other hand, is forcing the partner to participate in sexual acts without their consent, and sexual contact in cases where the partner does not consent or cannot refuse. Sexual violence is also recognized as making unwanted sexual remarks; attempts to rape; spreading sexually explicit rumors about a partner (5).

Psychological violence is the use of verbal and non-verbal behaviors to gain control over a person, which may lead to psychological intimidation and damage to self-esteem. Psychological violence also includes name-calling, insulting, using inappropriate words, discouraging, threatening and shouting with the intention of demoralizing the partner (6). Studies show that psychological violence leads to eating disorders, causes self-neglect and isolates the individual (7,8). Dating violence is a psychosocial problem that is common among young people, affects their lives and requires effective prevention strategies. It is an important issue to identify the factors affecting young people's attitudes towards dating violence and to raise awareness of young people (9). Dating violence has been found to cause physical, mental, behavioral and financial health problems and exacerbate existing health problems, which negatively affects academic performance (10). In addition, dating violence is associated with increased depression, anxiety and stress disorders (11).

In studies conducted on university students' attitudes towards dating violence, it has been observed that female students' awareness of violence is higher than male students, and there is a significant relationship between being exposed to violence in the past, witnessing violence, being a child of indifferent parents, being abused, and violence against one's partner (12-14). It is known that people studying in health sciences are potential health service providers and will provide services to people who are exposed to/perpetrate dating violence in the future. It is necessary to determine the violence tendency and dating violence attitudes of students. In this context, understanding the violence tendency levels and dating violence attitudes of students studying in health sciences and the factors affecting them will be effective in preventing violence. The aim of this study was to determine the levels of violence tendency and dating violence attitudes of university students and the risk factors affecting violence tendency and dating violence attitudes.

MATERIALS AND METHODS

The study is a cross-sectional. Purposeful sampling method was used while calculating the sample. The sample was determined according to the Purposeful sampling method, which is one of the non-probability sampling methods. The population of the study consisted of all students (N= 2221) studying at Istanbul University Cerrahpaşa Faculty of Health Sciences between 2022-2023 academic years. The Raosoft Sample Size program was used to calculate the sample size of the population. Accordingly, the sample size was determined to be at least 328 with a 5% margin of error and 95% confidence interval. 365 university students with at least one dating experience were included in the study. A total of 1050 female and 614 male participants were included in the study. Based on previous research, it was determined that 26% of female participants and 15% of male participants experienced sexual or physical violence before the age of 18 (15). In this study, there were 273 female and 92 male participants. The obtained data were evaluated by taking into account the rates of violence experience in determining the sample size.

The dependent and independent variables are as below:

Dependent variables: Violence Tendency Scale score, Dating Violence Attitude Scale score

Indpendent variables: Gender, age, current dating status, current dating duration, mother's education, father's education, family structure, exposure to domestic violence, and exposure to partner violence.

Measures

"Student Information Form", "Violence Tendency Scale" and "Dating Violence Attitude Scale" were used as data collection tools. The data were obtained face-to-face with the forms distributed to the students on the university campus.

Student Information Form: It consists of 19 questions created by the researchers in line with the literature (16,17). The form includes information on gender, age, current dating status, current dating duration, mother's education, father's education, family structure, exposure to domestic violence, and exposure to partner violence.

Violence Tendency Scale: This scale was developed by Haskan and Yıldırım (18) to measure the violence tendency levels of individuals. The scale consists of 20 items and is 3-point Likert type. It consists of four sub-dimensions: "feeling of violence", "violence through information technologies", "thoughts of harming others". A score of 20-60 is obtained from the Violence Tendency Scale and the higher the score, the higher the tendency towards violence. The reliability coefficient was reported as 0.83 by the researcher (18). According to the findings of this study, the reliability coefficient was found to be 0.86.

Dating Violence Attitude Scale: It was developed by Terzioğlu et al. (19) to determine university students' attitudes towards dating violence. The scale consists of 28 items and is 5-point Likert type. It consists of 5 sub-dimensions: "general violence", "physical violence", "emotional violence", "economic violence" and "sexual violence". As the score obtained from the scale approaches 5, it is interpreted that the individual has an attitude that does not support dating violence. The reliability coefficient was reported as 0.91 by the researcher (19). According to the findings of this study, the reliability coefficient was found to be 0.83.

Data Analysis

The data obtained in this study were analyzed using SPSS (Statistical Package for Social Sciences) for Windows 27.0 program. Number, percentage, median, standard deviation were used as descriptive statistical methods in the evaluation of the data. Pearson correlation analysis was used to determine the relationship between the scale scores. Multivariate linear regression model was performed to predict the relationship between violence tendency and dating violence attitudes and sociodemographic data. Reference values in the regression analysis were determined as follows: "male" for gender, "nuclear family" for family structure, "none" for exposure to domestic violence, and "none" for exposure to partner violence. All tests were performed two-way and p value <0.05 was considered statistically significant.

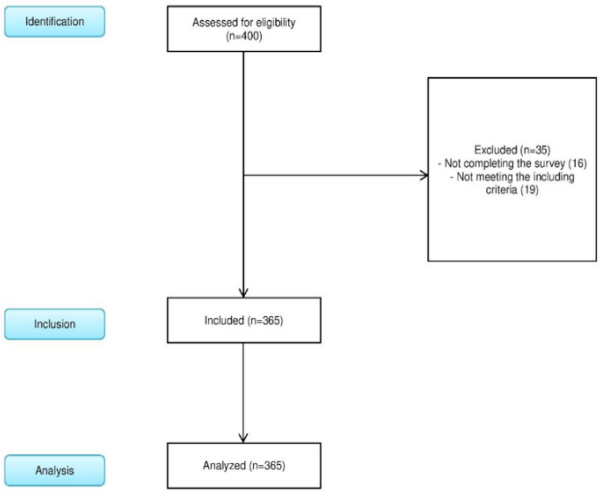

Figure 1. Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) flow diagram

Table 1. Correlation between violence tendency and dating violence attitude

	Min	Max	Median
Violence Tendency Scale	21	58	29
Dating Violence Attitude Scale	3.04	5.00	4.57
	r_s:-0.286 p <.001*		

Min: Minimum, Max: Maximum, SD: Standard deviation, r: Spearman's rho correlation. p < 0.05

Ethics

Before the research was conducted, permission was obtained from Istanbul University – Cerrahpasa Social and Human Sciences Research Ethics Committee (Date: 06.12.2022, Decision Number: 2022/427) and institutional permission was obtained from Istanbul University – Cerrahpasa Faculty of Health Sciences Dean's Office. Permission to use the scales was obtained. The study process was explained to the university students who volunteered to participate in the study and their consent was obtained. Helsinki Declaration rules were followed in the study.

RESULTS

A total of 365 university students were included in this study. The median age of the participants was 21 years. The rate of current dating was 39.5% (n = 144)

Table 2. Multivariate linear regression model for the association between sociodemographic data with violence tendency

Violence Tendency	B	95 % CI for B		SE B	β	p value	R^2	Adj. R^2
		Lower Bound	Upper Bound					
Model							.145	.129
Constant	29.206	27.456	30.955	.890		<.001*		
Extended family	-.953	-2.652	.746	.864	-.058	.271		
Broken family	-.184	-2.555	2.187	1.206	-.008	.879		
Mother's education	.095	-.077	.267	.088	.070	.279		
Father's education	-.129	-.301	.043	.087	-.094	.141		
Exposure to domestic violence	1.393	.111	2.674	.652	.113	.013*		
Exposure to partner violence	2.970	.773	5.167	1.117	.142	.008*		

B: Unstandardized beta, 95% CI: 95% confidence interval, SE: Standard error, β: Beta, R2: R-squared, coefficient of determination Adj. R2: Adjusted R-squared, p < 0.05

Table 3. Multivariate linear regression model for the association between sociodemographic data with dating violence attitude

Dating Violence Attitude	B	95 % CI for B
		Lower Bound
Model		
Constant	4,445	4,337
Extended family	-.023	-.128
Broken family	-.166	-.312
Mother's education	-.001	-.012
Father's education	.013	.003
Exposure to domestic violence	-.022	-.101
Exposure to partner violence	-.279	-.414

B: Unstandardized beta, 95% CI: 95% confidence interval, SE: Standard error, β: Beta, R2: R-squared, coefficient of determination Adj. R2: Adjusted R-squared, p < 0.05

and the median duration of dating was 7.5 months. The majority of the participants were female (n = 273, 74.8%). Median mother's education was 8 years and the median father's education was 12 years. 77.8% (n = 284) of the participants lived in nuclear families. Among the participants, 34.5% (n = 126) reported having experienced domestic violence at least once and 8.5% (n = 31) reported having experienced dating violence at least once. 79.4% (n = 100) of those who had experienced domestic violence and 71% (n = 22) of those who had experienced dating violence were women.

There was a statistically significant, weakly negative linear relationship between violent tendency and dating violence attitudes (r_s = -0.286, p < 0.001) (Table 1).

Multivariate linear regression analysis was performed to predict gender, family structure, mother's and father's education levels, exposure to domestic violence, exposure to partner violence and tendency to violence. As a result of the analysis, the model was found to be statistically significant F(6,358) = 2.781, p = 0.012. Among the variables included in the model, exposure to domestic violence and exposure to partner violence were found to be statistically significant predictors of tendency to violence (p < 0.05). Accordingly, being exposed to domestic violence and being exposed to partner violence increase the tendency to violence (Table 2).

Multiple linear regression analysis was performed to predict gender, family structure, mother's and father's education levels, exposure to domestic violence, exposure to partner violence and dating violence attitudes. As a result of the analysis, the model was found to be statistically significant F(6,358) = 5.882, p < 0.001). Among the variables included in the model, family structure, father's education level and exposure to partner violence were found to be statistically significant predictors of dating violence attitudes (p < 0.05). Accordingly, having a high level of father's education increased the attitude towards dating violence, while having a broken family

structure and being exposed to partner violence decreased the attitude towards dating violence (Table 3).

DISCUSSION

A total of 365 university students participated in the study. The participants scored an average of 29.34 ± 5.86 points on the Violence Tendency Scale and 4.52 ± 0.37 points on the Dating Violence Attitude Scale. Considering the scores obtained, it is seen that the university students participating in the study have a low tendency to violence and do not support dating violence. Young people, the demographic group in which the research was conducted, are particularly susceptible to problems such as violence and dating violence due to their critical developmental stages (20). It is suggested that dating violence is linked to other health risks in young people's relationships, especially risky sexual behaviors. The combination of risks associated with dating violence and sexual risk behaviors is particularly serious for young women (21).

Exposure to violence is thought to cause serious negative consequences, including homicides, and to be a predictor of intimate partner violence in adulthood (22). Dating violence is known to be very common among young people. According to the CDC report, one out of every 12 people states that they have been exposed to dating violence (3). Similarly, considering the results of this study, the rate of exposure to dating violence at least once is 8.5%. In the literature, it is seen that the victims of violence are often women and the perpetrators of violence are men. It is also known that women are frequently affected by exposure to violence (22,23). In our study, 79% of those who were exposed to domestic violence at least once and 71% of those who were exposed to partner violence were women. The study result is similar to the literature.

It is reported that the problem that frequently emerges in studies is due to gender inequality and that this situation is related to social roles. The fact that men are the perpetrators of violence points to patriarchy, which is understood as a hierarchical power system (24). Therefore, while perpetrators of violence legalize violence, those who are exposed to violence consider it normal to be exposed to physical abuse in dating relationships (9). In a systematic review of 16 studies, it was reported that men were frequently the perpetrators of dating violence. It is seen that traditional gender roles and concepts of male superiority are the underlying causes of dating violence perpetrated by men against women. It is stated that dating violence is associated with negative childhood experiences, lack of interpersonal communication and conflict resolution skills, and alcohol use (25). In a study conducted in adolescents, it was reported that there were differences between genders in exposure to dating violence; males were significantly more likely to be victims of physical abuse and females were significantly more likely to be victims of sexual abuse (26). In another study, 61.9% of health sciences students experienced abusive behavior at least once in their relationships. Especially, men were found to show sexist attitudes that legitimize gender-based violence (27).

Factors such as exposure to childhood abuse, witnessing interparental violence, and the presence of beliefs that justify and accept violence seem to be effective in exposure to or perpetration of dating violence (28,29). A study shows that childhood trauma is associated with exposure to/perpetration of dating violence (30).

At the same time, exposure to intimate partner violence in childhood significantly increases the tendency towards dating violence in adulthood (31). In our study, similar to the literature, it was found that those who were exposed to domestic or intimate partner violence at least once had an increased tendency towards violence. In a study examining the exposure of women of reproductive age to dating violence, women's age, education level and family structure were found to be important determinants of exposure to dating violence. Accordingly, it is stated that exposure to dating violence is less in women with higher welfare and education levels and living in nuclear families (32).

Even though it was not possible to compare the exposure to dating violence according to educational level in our study group, which consisted entirely of university students, the fact that these students had higher attitudes towards dating violence may support the literature. Accordingly, the attitude towards dating violence is positively affected by higher education level. In addition, in our study, while father's education was not effective in violence tendency, it was found to affect the attitude towards dating violence. Accordingly, as the father's education level increased, the attitude towards dating violence was positively affected. In addition, attitudes towards dating violence were positively affected in young people with nuclear families.

Limitations

This study was conducted with university students studying at the faculty of health sciences of a university and the results of the study cannot be generalized. The fact that one of the probability sampling methods was not used prevents the generalizability of the results to the population. Since it was a cross-sectional study, it was not possible to determine the causal direction of the relationships found. In addition, the technique used to distribute the questionnaire to the students made it impossible to determine the non-response rate. In addition, due to the low number of male participants, it was not possible to conduct further analyses by gender. The study does not reflect the results of people diagnosed with violent tendencies and perpetrators of violence. In the study, health problems that may be seen in people exposed to dating violence were not questioned. Finally, regression analyses showed that the variance explained was relatively low. This finding may be a limitation of the study.

CONCLUSION

Dating violence is an important and common problem among young people. According to the results of the study, university students studying at the faculty of health sciences have low violence tendencies and positive attitudes towards dating violence. However, the rate of those exposed to domestic or dating violence is high. In addition, more than two thirds of the population exposed to violence are women. The results of the study reveal gender inequality even in young individuals with a high level of education. It may be recommended to implement prevention programs to educate young people about healthy interpersonal relationships. Health professionals have an important role to play in this. Regular screening of young people for early detection of violent tendencies and the establishment of referral systems to ensure that victims of violence receive the necessary support can be recommended.

In addition, health sciences students will be the ones who provide care/treatment to patients in their professional life. It is also a matter of curiosity whether the violence perceptions of these individuals will affect their professional experiences in the coming years. Future studies can be planned by considering these variables. Follow-up studies to be conducted for this purpose will facilitate the realization of the measures planned to be taken as a result of finding a place in the literature.

This study has the strength of being a research that includes a representative sample of health sciences students of both genders in the young age group. Therefore, it is a pioneering study that examines the relationship between health sciences students and violence before they become members of health professions in the future. In addition, the assessment of students' violence tendency and dating violence attitudes with a scale is one of the strengths of the study.

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RESEARCH ARTICLE

BIRTH-RELATED PSYCHOLOGICAL TRAUMA PERCEPTION SCALE DEVELOPMENT AND PSYCHOMETRIC PROPERTIES: A METHODOLOGICAL STUDY

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ABSTRACT

Purpose: The purpose of this study was to develop a valid and reliable measurement tool for assessing mothers' psychological trauma perception regarding birth**.**

Materials and methods: This is a methodologically type study. This study was conducted with 430 mother who had normal delivery. Personel information form and Birth-related psychological trauma perception scale draft form were used to data curation.

Results: The statistics show that the sample is sufficient for factor analysis (Kaiser-Meyer Olkin measure = 0.858) and that the correlation between the questions is appropriate (Bartlett's test of sphericity, $\chi 2$ = 9211.281, p = 0.001). The total explained variance of the scale is 28.309%. It was determined that the scale consists of 39 items and a single sub-dimension. The internal consistency coefficient of the total scale was calculated as 0.92. The scale's invariance over time shows that its reliability is high (r = 0.83, p< 0.001).

Conclusion: Results showed that the developed scale is a valid and reliable tool for measuring the perception of traumatic birth. This scale can be used to determine whether women are susceptible to psychological trauma from the first postpartum month to a year later.

Key words: Birth, perception, psychological, scale, trauma.

INTRODUCTION

Traumatic childbirth is defined as an event occurring during the labor and birth process that involves actual or threatened serious injury or death to the mother's or infant's physical or emotional integrity (1-6). Women who experienced traumatic childbirth depict the moment of birth as a moment of helplessness, loss of control, intense fear, and horror (7). Besides, these women may also exhibit symptoms of posttraumatic stress such as a strong recall of childbirth, dreams about the event and recurrent memories (1,2,7,8). Studies have revealed that 3-4% of the women in the postnatal period and 15-19% of the women who experienced high-risk, complicated, or preterm deliveries exhibited post-traumatic stress disorder symptoms (9,10).

In the literature, there are measurement tools that evaluate the childbirth experience from different aspects and measure the attitudes of women towards pregnancy and childbirth (9,11-16). Some of these were developed in Turkish, while Turkish validity and reliability studies were performed for some of them, on the other hand (13-19).

There are scales developed regarding birth trauma in the literature. One of these scales is a scale

developed in Turkish that can be applied to both women with and without birth experience. The primary goal here is to measure women's perceptions of birth-related trauma, which they acquire through environmental and cultural factors, rather than their subjective experiences. Additionally, the items of this scale are evaluated with a visual analog scale (0-10 points). With this type of evaluation, women are expected to express their feelings of trauma, which is a psychological perception, with quantitative data. This may negatively affect the reliability of the application (16). Another scale is the City Birth Trauma scale, published by Ayers et al. (2018). This scale is mostly aimed at evaluating the symptoms and diagnostic criteria of postpartum post-traumatic stress disorder. For this reason, it was thought to be inadequate in measuring women's perception of birth-related trauma. These limitations in scales made us think about the need for a new scale. After a comprehensive literature review, including DSM-V (Diagnostic and Statistical Manual of Mental Disorders) criteria, it is understood that the situation whose perception is to be measured must be experienced by individuals (20). The perception of traumatic birth can only occur in women who have experienced birth. The scale developed in this study is only for the sample of women who have given birth. The aim of this study is to develop the birth-related psychological trauma perception scale and determine its psychometric properties.

MATERIALS AND METHODS

Design and setting

This methodological study was carried out in the Gynecology and Pediatrics Hospital affiliated with a City Hospital and Family Health Centers in Turkey between May 2019 and September 2019.

Participants and sample

The sample size of this study consists of 430 women who meeting the inclusion criteria. When calculating the sample size in scale development studies, it is generally recommended to select individuals 5 to 10 times the number of items in the draft scale, taking into account the number of items in the draft scale (21). Women aged 18-40 who gave birth at term and vaginally, were at least the 4th week of the postpartum period, whose baby and themselves were healthy, and who had given birth without any problems before (for multiparous women) were included in the study. Mothers with low cognitive and communication levels and diagnosed psychiatric diseases were not included in the study. Additionally, women who experienced serious birth trauma were not included in the sample.

Data collection

Data were collected in a separate interview room, taking care of privacy, and the interview duration was approximately 10-15 minutes. Personel information form and Birth-related psychological trauma perception scale draft form were used to data collection.

Personel information form: This form consisted of a total of 11 questions regarding the sociodemographic (age, education, marital status, etc) and obstetric characteristics (mode of delivery, interventions at birth, etc.) of women.

Birth-related psychological trauma perception scale (BRPTPS): The scale was developed by the researchers to determine the perception of psychological trauma related to birth. Before preparing the scale draft, factors associated with traumatic birth were explored in a comprehensive literature review (22-24). After to develop scale a 46-item pool was created in line with the literature information and the knowledge and experience of the researchers (4,10,16,19,25-30). To assess the content validity of the BRPTPS draft form, 12 experts were consulted, the 12 experts whose opinions were obtained were working in the field of obstetrics and women’s health nursing. The study calculated the Content Validity Index (CVI) values for the scale items using Lawshe's content validity testing technique. Experts was sent information on the purpose of the BRPTPS and instructions about how to evaluate content validity. According to expert evaluations, the CVI values of the scale items ranged between 0.33 and 1.00. Since the number of experts was 12, items with a CVI value of 0.56 and above were included in the scale (31). By removing four items from the draft form, CVI was calculated as 0.83 for the 42-item scale. To test face validity, a pilot study was conducted with 30 mothers (15 primiparous and 15 multiparous) who had similarities with the characteristics of the sample group to check whether the expressions of the scale items were clear. No changes were made to the scale items after the pilot study.

As a result of all validity and reliability analyses, BRPTPS, which aims to measure women's

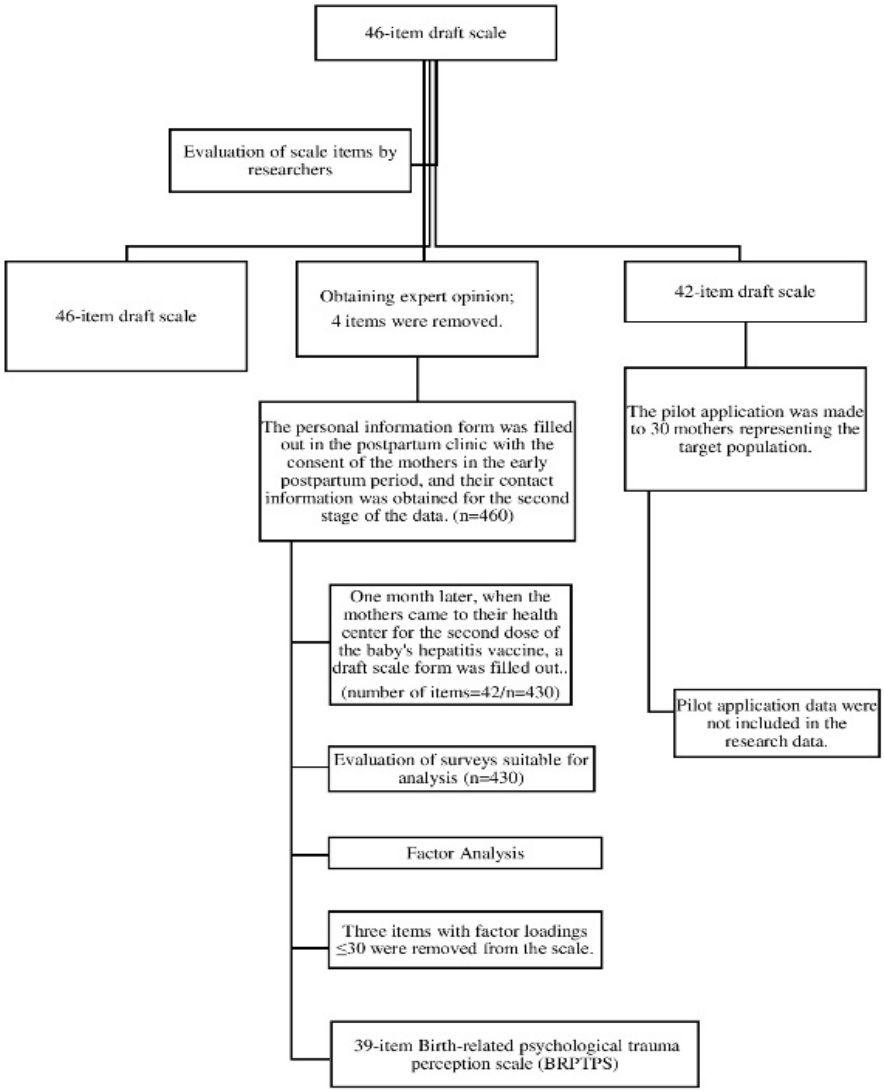

Figure1. Flow diagram for development and validation of *BRPTPS*

Table 1. Sociodemographic and obstetric characteristics of the mothers

Characteristics		**n**	**%**
Age (year)	18-24	127	29.5
	25-29	122	28.4
	30-34	121	28.1
	35 and above	60	14.0
Educational status	Primary school	36	8.4
	Middle school	62	14.4
	High school	215	50.0
	University and above	117	27.2
Duration of marriage (year)	1	50	11.7
	2-4	150	34.9
	5-9	121	28.1
	10 and above	109	25.3
Number of birth	Primiparous	196	45.6
	Multiparous	234	54.4

perception of psychological trauma regarding birth, consists of 39 items and a single dimension. BRPTPS can be used from the first month to one year postpartum. The scale is a five-point Likert model. For the negative items in the rating scale, 1 was taken as "strongly disagree", 2 as "disagree", 3 as "undecided", 4 as "agree", and 5 as "strongly agree". Positive items are answered in the opposite way. There are 11 items (4, 5, 11, 13, 15, 17, 18, 29, 30, 31, 36) that need to be reversed in calculating the total scale score. Total scale scores range between 39-195. An increase in the score obtained from the scale indicates that the woman's perception of trauma is high.

Data Anlaysis

Data analysis was performed by using SPSS version (22; SPSS Inc., Chicago, IL) and Lisrel 8.0 software (Scientific Software International, Inc., Lincolnwood, IL) Number, percentages, mean, and standard deviations were utilized for the statistical analysis. The analysis and techniques used to conduct validity and reliability analyses are presented below.

Validity analysis

To assess the construct validity of the scale, an exploratory and confirmatory factor analysis was conducted. Prior to factor analysis, Kaiser-Meyer-Olkin (KMO) and Bartlett tests were used to assess the sample size of the scale and its suitability for factor analysis. The lowest value of the KMO index is given as 0.50 and is used to determine the adequacy of the sample size (24,32,33). The significance of the Bartlett test indicates the suitability of the data set for factor analysis. Principal components analysis were used in exploratory factor analysis (EFA) and examined using the varimax rotation method. After EFA, confirmatory factor analysis was performed for structural equation modeling.

Reliability analysis

For reliability analysis, item-total score analysis (Pearson correlation test), and Cronbach alpha, the Split-Half method, Spearman-Brown Correlation Coefficient, and Guttman Split Half Correlation test were used. The 27% subgroup and upper group discrimination analysis was also used for reliability analysis. To determine the invariance of the scale over time, a test-retest analysis was performed. The duration suggested by the test-retest method generally varies between two and six weeks. Therefore, the scale was re-administered to 30 mothers 15 days after the first application. To determine the correlation between two test-retest reliability measures, Pearson's product-moment correlation coefficient was used. The calculated correlation coefficient must be at least 0.70 (34,35).

Ethical Approval: The ethics committee approval was obtained from the Ethics Committee of Ataturk University, Faculty of Health Sciences before conducting the study (Date: 22/11/2018, Decision No: 05/01). Permission was obtained from the institution where the research was conducted. Additionally, verbal consent was obtained from each of the participants. This study was carried out by the ethical standards of the Declaration of Helsinki.

RESULTS

Characteristics of the participants

It was determined that 29.5% of the mothers participating in the research were between the ages of 18-24, and 50% of them had high school education. Additionally, 34.9% of the mothers have been married for 2-4 years and 54.4% are multiparous (Table 1). In the literature, it has been suggested that the floor and ceiling effect should be below 15%. The values obtained from the group in which the research was conducted (0.4%-5.6%) meet this criterion.

Findings of validity

Before the principal components analysis, the KMO and Bartlett's tests were carried. In the analysis conducted, the KMO value was found to be 0.85. Similarly, as a result of Bartlett's test, it was found that $\chi 2 = 9927.444$, $P = 0.000$. A KMO value of 0.850 indicates that the scale is suitable for principal component analysis and the sample size is sufficient. Likewise, Bartlett test results show that the data are related to each other and are suitable for factor analysis.

Exploratory factor analysis (EFA)

The factor structure of the scale was revealed by using the principal components analysis and varimax rotation method. The factor loads of items ranged from 0.305 to 0.789, and the factor load of each item except for three items was higher than 0.30. 28.30% of the total variance was explained. Therefore, these three items with factor loads below 0.30 were removed from the scale and were again submitted to an examination in one-factor structure (Table 2).

Table 2. Factor Analysis Findings of Birth-Related Psychological Trauma Perception Scale (Single Factor Structure, 39 items)

	Items	Factor Loadings		Items	Factor Loadings
1	I could not control my pain at birth.	0.318	**21**	I was not allowed to have someone I wanted at birth.	0.394
2	Midwives (health professionals) did not support me enough at birth.	0.566	**22**	I was very uncomfortable that I was not allowed to eat or drink during labor.	0.338
3	For me, birth is a painful event.	0.418	**23**	Health professionals did not listen to me at birth.	0.753
4	The behavior of midwives (health professionals) at birth made me feel comfortable.*	0.746	**24**	I was not informed about the progress of the birth at birth.	0.758
5	It was comforting to me that the midwives (health professionals) provided information about what I had to do during the birth.*	0.789	**25**	The behavior of other pregnant women in the delivery room frightened me.	0.388
6	I felt bad during the vaginal examinations in the delivery room.	0.305	**26**	The physical conditions of the delivery room were not good.	0.439
7	Midwives (health professionals) asked my permission before doing a vaginal examination.	0.462	**27**	Too much medical intervention (artificial pain, episiotomy) at birth bothered me.	0.387
8	I felt like I was going to die while I was push on at birth.	0.555	**28**	Hearing my baby's voice at birth relieved me.*	0.406
9	I was afraid of harming my baby while I was push on at birth.	0.405	**29**	As a mother, I feel happy.*	0.537
10	I was afraid that something would happen to my baby during the birth.	0.359	**30**	As a mother, I feel successful and strong.*	0.578
11	I was extremely positive at birth.*	0.586	**31**	I feel happy when I think of my birth.*	0.382
12	I felt very lonely at birth.	0.603	**32**	I don't want to remember my birth.	0.459
13	I felt so strong during the birth pains.*	0.550	**33**	I have bad dreams about my birth.	0.463
14	I felt scared during the birth.	0.547	**34**	I feel disconnected from my baby.	0.599
15	I felt safe at birth.*	0.601	**35**	I'm trying to avoid things that remind me of my birth.	0.553
16	I was extremely panicked at birth.	0.552	**36**	I wanted to breastfeed my baby after birth.*	0.303
17	I did not lose physical control at birth.*	0.619	**37**	I didn't want to see my baby after the birth.	0.494
18	I was afraid that I would die at birth.	0.595	**38**	I feel incapable of taking care of my baby.	0.721
19	I was afraid of getting a cesarean.	0.385	**39**	I don't want to talk about my birth.	0.586
20	I felt helpless at birth.	0.608			
	Described variance (%)				**28.309**

*Inverted items

Table 3. Determined Adjustment Index Values, Normal and Acceptable Values of Birth-Related Psychological Trauma Perception Scale

Index	Normal value	Acceptable value	Determined value
x^2/SD	≤2	≤5	8.72
GFI	≥0.95	≥0.90	0.90
AGFI	≥0.95	≥0.90	0.91
CFI	≥0.95	≥0.90	0.90
RMSEA	≤0.05	≤0.08	0.079
SRMR	≤0.05	≤0.08	0.08

Confirmatory factor analysis (CFA)

Confirmatory factor analysis was conducted to verify the harmony between the explanatory factors and that the factor structure was preserved. Many indices were used to examine the model fit of the BRPTPS. Among them, the values were found as follows: x2/SD=8.72, goodness of fit index (GFI) 0.90, djusted goodness of fit index (AGFI) .91, comparative fit index (CFI) 0.90, root mean square error of approximation (RMSEA) 0.079, and standardized root mean square residual (SRMR) 0.08 (36,37). It was determined that all relevant fit index values were within the range of reference values (Table 3).

Findings of reliability analysis

Item analysis was performed on the 39 items and the results are presented in Table 2. The item total score correlation values were between 0.25 and 0.71. Cronbach's alpha level which reflects internal consistency was calculated as 0.92. In addition, there was no item in which the Cronbach alpha coefficient increased if it was removed from the scale (Table 4). Therefore, no item was removed from the scale. We also applied the Split-Half method, Spearman-Brown Correlation Coefficient, and Guttman Split Half Correlation for reliability analysis. The Split-Half value for the first half of the scale (20 items) was calculated as 0.79, and the Split-Half value for the second half (19 items) was calculated as 0.88. As a result of the analysis, Spearman-Brown Correlation Coefficient (0.82) and Guttman Partition Coefficient (0.81) were calculated. Split-Half value > 0.70, Spearman-Brown Correlation Coefficient > 0.70 and Guttman Split Halves Correlation Coefficient > 0.70 indicate that the scale has high reliability [38]. Another method in reliability analysis is to compare the lower 27% and upper 27% groups according to total scores. The lower and upper 27% slices of the total scale scores were calculated, and the significance of the difference between them was tested with the t-test. According to the total scale score, the significance of between the 27% slices was found as p< 0.001.

In the test-retest reliability analysis, it was determined that there was a positively high (r = 0.83) correlation between the first and second application scores and the correlation between two measurements was statistically significant (P < 0.001).

DISCUSSION

The study developed a birth trauma perception scale. The developed scale is a measurement tool that helps measure mothers' experiences, feelings and thoughts in the postpartum period and allows mothers to evaluate how they perceive the experience of childbirth, which has a very important place in their lives. The scale items were designed so that mothers could easily express their feelings and thoughts. This scale is believed to facilitate the identification of individuals vulnerable to birth trauma. The present study examined content and construct validity to examine the validity of the scale. The scale was administered to 12 experts in the item list to determine its content validity. According to DeVellis (2012) in the study of adaptation and development of the scale, the number of specialists consulted varies from three to 20. To confirm the content validity with numerical values, the Lawshe technique was used and the CVI of the scale was determined to be 0, 83 set. Karakoc & Donmez (2014) stated that the CVI score should be 0.80 or higher in content importance, assessed using the Lawshe technique. The scale is therefore satisfactory in terms of content validity. In order to evaluate the characteristics measured with the prepared scale and to interpret the results of the people on whom the scale was used, the construct validity of the scale is assessed. The factor analysis method is often used to measure construct validity by collecting related variables in a given set. Explanatory factor analysis is used to determine the number of subscales in a particular case. The use of this large-scale analysis method is tied to a specific sample size (39). To understand the adequacy of the sample size, KMO analysis was performed. To examine the significance of the relationship between variances, Bartlett analysis was performed. A KMO test result above 0.50 indicates that the sample size is sufficient for validity analysis (34,39).

Table 4. Item-Total Correlations and Cronbach α Coefficients of the Birth-Related Psychological Trauma Perception Scale

Items	Arithmetic mean	Standard deviation	Item total correlation	Item deleted α	Items	Arithmetic mean	Standard deviation	Item total correlation	Item deleted α
Item 1	3.48	1.32	0.303	0.927	**Item 21**	2.96	1.56	0.362	0.927
Item 2	2.36	1.44	0.510	0.925	**Item 22**	2.51	1.49	0.322	0.927
Item 3	4.12	1.14	0.397	0.926	**Item 23**	2.36	1.49	0.709	0.923
Item4*	2.18	1.32	0.696	0.923	**Item 24**	2.19	1.45	0.717	0.923
Item 5*	2.13	1.39	0.737	0.923	**Item 25**	3.42	1.58	0.360	0.927
Item 6	3.98	1.23	0.296	0.927	**Item 26**	2.30	1.51	0.402	0.926
Item 7	3.04	1.59	0.411	0.926	**Item 27**	3.57	1.50	0.364	0.927
Item 8	3.75	1.31	0.529	0.925	**Item 28***	1.67	1.15	0.345	0.927
Item 9	3.67	1.35	0.384	0.927	**Item 29***	1.77	1.27	0.491	0.925
Item 10	3.96	1.26	0.347	0.927	**Item 30***	1.73	1.25	0.530	0.925
Item 11*	3.31	1.32	0.548	0.925	**Item 31***	3.22	1.52	0.361	0.927
Item 12	2.67	1.38	0.573	0.925	**Item 32**	2.95	1.45	0.430	0.926
Item 13*	2.75	1.44	0.491	0.925	**Item 33**	1.83	1.20	0.423	0.926
Item 14	3.59	1.42	0.512	0.925	**Item 34**	1.67	1.07	0.564	0.925
Item 15*	3.23	1.35	0.561	0.925	**Item 35**	2.38	1.50	0.510	0.925
Item 16	3.51	1.45	0.519	0.925	**Item 36***	2.05	1.31	0.251	0.928
Item 17*	3.05	1.51	0.582	0.924	**Item 37**	2.06	1.32	0.450	0.926
Item 18	3.25	1.39	0.568	0.925	**Item 38**	2.09	1.41	0.678	0.924
Item 19	3.30	1.50	0.363	0.927	**Item 39**	2.86	1.46	0.548	0.925
Item 20	3.18	1.37	0.582	0.925					
*Inverted items									**0.927**

As a result of the explanatory factor analysis, the KMO value of 0.85 indicates that the sample is suitable for factor analysis, while the Bartlett test, which is at a highly significant level (p=0000), shows that the matrix of item correlation is suitable Suitable for factor analysis. A different number of subscales were tested in the studies, with the items being combined into a single subscale according to the theoretical structure. After examining the one-factor structure, it was found that the factor loading

coefficients of all items except items 17, 33, and 42 were greater than 0.30. The single-factor structure of the scale shows that trauma cannot be examined under a stereotyped structure, that it is multifactorial, and that birth trauma is a reflection of each woman's own feelings, thoughts, experiences and subjective responses. In addition, the fact that the items determined in the scale affect each other may explain the single-factor structure of the scale. In the confirmatory factor analysis performed to test whether the single-factor structure obtained was confirmed or not, the fit indices of the model were examined and it was seen that the fit index values were sufficient for the fit of the model.

Confirmatory factor analysis is not only an extension of explanatory factor analysis, but also evaluates the underlying structure of the data. Confirmatory factor analysis tests the factors identified by explanatory factor analysis. In confirmatory factor analysis, the degree of conformity of the model with the theory is decided and the evaluation is carried out based on various fit index results rather than on the result of a single value (40). In this study, according toresults of confirmatory factor analysis; The chi-square value was = 8.72; IGF = 0.90; AGFI=0.91; FCI=0.90; RMSEA=0.079; and SRMR=0.08. Regarding the scale fit indices, the fit of the observed data to the model was considered good. Cronbach's alpha is one of the most commonly used parameters for assessing the internal consistency reliability of scales. Cronbach's alpha reliability coefficient is often used to determine the internal consistency of Likert scales. Sencan (2005) suggests that Nunnelly's alpha value should be greater than 0.70 (40). On the other hand, George and Mallery argued that (2003) an alpha value > 0.90 indicates “perfect” reliability of the scale (41). In our study, the Cronbach alpha value was 0.92, while the scale based on the internal consistency coefficient was very reliable. The alpha value of the Cronbach scale indicates that this scale can be used to determine birth trauma and is a scale that can measure the trauma of any woman who gives birth vaginally. In order to test the distinctiveness of the items of the scale, the 27% with the highest score from each of the items of the scale and the 27% with the lowest score were compared with the t test analysis and it was determined that the results were statistically significant and all items had discrimination. Based on this, we can say that trauma is affected by the subjective responses of individuals, as mentioned in DSM-IV. The distinctiveness of the items in the scale makes the scale more usable in the field of birth trauma. On the other hand, this situation may also form the basis for individualized care given by midwives to two different women who experienced traumatic birth in the clinic. Because every woman's perception of birth and trauma is different and may be based on different reasons. The performance of a prepared scale in a test-retest reliability analysis is evaluated to produce consistent results across applications and to demonstrate invariance over time. If you apply the same scale to the same people at different times, the responses should be similar. This is the sine qua non for a reliable measuring instrument (39,42,43). In this study, it was found that the test-retest comparison results were statistically significant and the relationship between the first and second measurements was high and positive (r = 0.831, p = 0.000). Test-retest analysis indicates that the result would be valid if the scale items measured birth trauma at a time designated for use of the scale. The process of perception, interpretation and acceptance/rejection of the birth experience as traumatic continues in the postpartum period. It is stated that after birth, women care about sharing their birth experiences with health professionals and perceive this as support. However, the majority of women do not ask the health personnel about their feelings about their birth, and when they express their feelings, they do not respond well enough. They stated that they were not understood and ignored. It has been found that providing women with the opportunity to discuss their birth experiences is very useful in coping with postpartum trauma symptoms. The developed scale allows women to express their birth experiences in the postpartum period. In addition, health professionals can provide individualized care in the postpartum period to women with a high perception of traumatic birth as a result of the scale evaluation. The scale has a distinctive feature in identifying individuals who are special in this regard and need care.

Limitations

The results of the study can be generalized to the mothers who constitute the sample group. The developed scale can be applied from the first postnatal week to one year.

CONCLUSIONS

In the end, this 5-point Likert-type scale was developed to measure women’s childbirth

psychological trauma perception. The scale has one subscale and 39 items. The Cronbach's α internal consistency coefficient of the scale, total item correlation and test-retest analysis were found to have high correlations.

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RESEARCH ARTICLE

DETERMINANTS OF ADHERENCE TO THE MEDITERRANEAN DIET AND DEPRESSIVE SYMPTOMS IN TURKISH YOUNG ADULTS

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ABSTRACT

Purpose: This research aimed to investigate the effects of adherence to the Mediterranean diet (MD) on depression risk in young adults and to understand potential associations.
Material and Methods: An online survey form was sent to university students in Türkiye, and 479 participated in this cross-sectional study. Data was collected based on students' declarations. Adherence to the MD was decided using the Mediterranean Diet Adherence Screener (MEDAS). Beck Depression Inventory (BDI) was applied to measure the presence of manifestations of depression. Multivariate linear regression models were used for the determinants of MEDAS and BDI scores.
Results: 73 males and 406 females with a mean age of 21.6±2.3 years and a mean Body Mass Index (BMI) of 22.0±3.5 kg/m^2 participated in the study. While 61.8% had a moderate adherence to the MD, 54.9% had a mild or moderate BDI level. According to models, regular exercise, presence of NCD(s), and adherence to an adequate/balanced diet were MEDAS score's determinants, and adherence to an adequate/balanced diet and BMI were for BDI score ($p<0.05$). No statistically significant effect of MEDAS and BDI scores on each other was found ($p>0.05$).
Conclusion: The relationship between the MD and depression is complex and encompasses several dimensions. More comprehensive and long-term studies, considering the influence of individual differences and other factors such as genetics, environment, and lifestyle, may help to reveal this effect more clearly.

Keywords: Body mass index, depression, Mediterranean diet, multivariate linear regression

INTRODUCTION

The Mediterranean diet (MD), originating from the Mediterranean region, is characterized by fruits, vegetables, fish, whole grains, and olive oil consumption (1). Current research indicates that following the Mediterranean diet could potentially reduce the risk of various non-communicable diseases (NCDs) such as obesity, diabetes, hypertension, heart disease, and metabolic syndrome, as well as enhance overall well-being (2, 3). Furthermore, recent studies suggest that adherence to the MD may also be an important factor in reducing the risk of depression (4, 5). Depression is a prevalent mental health issue worldwide and can significantly impact individuals' quality of life and functionality (6). Therefore, understanding the effects of the MD on depressive symptoms is important both to improve the mental health of individuals and to promote the overall well-being of society (7, 8).

Scientific evidence shows that following a Mediterranean diet provides a diet rich in omega-3 fatty acids, antioxidants, and other nutrients that support brain health (9, 10). Omega-3 fatty acids are well-known for enhancing brain functions and mental

health (11). In addition, compliance with the Mediterranean diet has also been shown to reduce inflammation. Nutrients in the Mediterranean diet can reduce the inflammatory process in the body, which in turn can decrease the risk of mental health problems, such as depression (12). Chronic inflammation is one of the important factors in depression and other mental health problems development (13). Moreover, it should be noted that adherence to the Mediterranean diet is also associated with social and psychological factors. The Mediterranean diet generally promotes social interactions and encourages the sharing of eating habits and eating together. This could potentially play a positive role in reducing social isolation and loneliness and decreasing the risk of depression (14). The impact of university students' adaptation to the Mediterranean diet on the risk of depression is one of the focal points of recent research. University students are young adults who face a range of stressors, such as academic expectations, social pressures, and lifestyle changes (15). The intense pressures during this period can increase the risk of depression and negatively affect the mental health of young adults (16). Eating habits are also often associated with these stressors, and a healthy diet has been shown to have positive effects on mental health (17). In this context, this research aims to investigate the effects of adherence to the Mediterranean diet on depression risk in university students and to understand potential associations. This research endeavor has the potential to enhance our comprehension of the impacts of the Mediterranean diet on mental well-being, thereby facilitating the development of more efficacious intervention approaches.

MATERIALS AND METHODS

Study Design: The study was planned as self-reported, online, and cross-sectional. The study was conducted with university students studying in Türkiye and the random and snowball sampling methods was used. Participants were reached via social media platforms (Facebook, Instagram, WhatsApp) using snowball methods to guarantee a large-scale distribution and recruitment of participants.

Participants: Sample size calculation was made based on the Multivariate Linear Regression Analysis to be conducted in the study with a 5% margin of error, 95% power level, 0.15 effect size, and 12 possible determinants (age, gender, smoking, alcohol intake, regular exercise, presence of NCD(s), sufficient nutritional knowledge, adherence to an adequate and balanced diet, main meals/day, snacks/day, BMI, and BDI score) of the dependent variable (Mediterranean diet compliance score). The minimum sample size was calculated as 184 on the Free Statistics Calculators website (https://www.danielsoper.com/statcalc/default.aspx). The data collection period was aimed to reach the maximum sample size. The study was conducted with 479 participants aged between 18 and 35, and data were collected through an online survey prepared in Google Forms between November 2023-March 2024. Among the criteria for inclusion in the study; being a volunteer, studying at a university in Türkiye, living in Türkiye, having a device such as a computer, tablet, or phone, having internet access, having enough time to fill out the online survey form. The exclusion criteria of the study were not willing to participate, not living or studying in Türkiye, not being university students, and not having the opportunity to fill out the online survey form. While the universe of the study consisted of all university students studying in Turkey, the sample comprised students who met the study inclusion criteria and were reached during the data collection process of the study. Before participating in the online survey, students were given information related to the study, and their consent was provided by clicking the box in the online survey.

There are reasons for collecting data using the online survey method in this study. Firstly, university students spend long hours a day using the Internet, and paper survey forms might not have interested them. In this study, the online survey form presents time and cost savings (18). In addition, some studies' results indicate that web-based surveys may be more reliable or advantageous than face-to-face surveys and other survey methods (19).

Data collection instruments: An online survey form used in the study was created by researchers after a literature review. The form consists of three sections: i) baseline characteristics (gender, age, living place etc.), ii) health and nutrition status information (presence of diseases, physical activity level, meal consumption), iii) “Mediterranean Diet Adherence Screener (MEDAS)” and “Beck Depression Inventory (BDI)”.

Mediterranean Diet Adherence Screener (MEDAS): MEDAS was created by Martínez-González et al. (20)

and Pehlivanoğlu et al. conducted its validity and reliability study in Turkish. As a result of this study, the Cronbach alpha coefficient of the scale was determined as 0.829, and it was concluded that it is a valid and reliable tool for assessing compliance with MD. It consists of two questions on habits of consuming foods and 12 questions on food consumption frequency, 14 items in total. MEDAS items are scored between 0-1 points, and the cutoffs ≤ 5, 6-9, and ≥ 10 indicate low, moderate, and high adherence, respectively (20, 21).

Beck Depression Inventory (BDI): BDI was developed by Beck et al. (22) to evaluate the existence and severity of depression's affective, cognitive, motivational, psychomotor, and vegetative manifestations. The tool was adapted into Turkish with university students by Hisli (23). The Cronbach alpha coefficient of the valid and reliable tool's Turkish version is 0.80. BDI consists of 21 items, and each item determines a behavioral pattern specific to depression. It contains 21 self-evaluation sentences with four options ranging from least to most (0-3). The score obtained from the scale is evaluated as 0-9 points (minimal), 10-16 points (mild), 17-29 points (moderate), 30-63 points (severe) (22, 23).

Body mass index (BMI) by using participants' reported body weight (kg) and height (cm) information with the formula body weight/height2 (kg/m2) was calculated. The BMI was divided into four groups underweight (<18.5 kg/m2), normal weight (18.5-24.9 kg/m2), overweight (25.0-29.9 kg/m2), and obese (≥30.0 kg/m2) according to the WHO reference classifications (24).

Statistical Analysis: The study's data were processed in the SPSS (Statistical Package for the Social Sciences) package program (IBM SPSS Statistics 23.0. Armonk, NY, USA: IBM Corp; 2013). In the descriptive analyses, categorical data were used as number and frequency (%). The mean, standard deviation (SD) and minimum-maximum (min-max) values were calculated for quantitative data of the participants. To compare two groups and more than two groups, showing normal distribution, were conducted with the Independent Samples T test and One-Way ANOVA test, respectively. The chi-square test (Pearson chi-square) was used to determine significant differences between the frequencies. Multivariate linear regression models were conducted to identify independent predictors of the MD. $p < 0.05$ was taken as the reliability coefficient.

Ethical Approval: The study was carried out with the permission of Mardin Artuklu University Non-Interventional Research Ethics Committee (Date:14.12.2022, Decision no:2022/14-16). The study was carried out following the Declaration of Helsinki principles. Students ticked an informed consent form in the online survey form and could withdraw from participation at any time without providing a reason. Permission was obtained from the authors who performed the validity and reliability study of the scale to administer the MEDAS in this study.

RESULTS

73 males and 406 females, in total 479, young adults from 64 different universities and 23 faculties or vocational schools participated in this study, and general characteristics are presented in Table 1.

Mean age and distributions of living place, habits (smoking, alcohol intake, regular exercise), and presence of NCDs were statistically different in males and females ($p<0.05$).

In terms of self-nutritional assessment, 40.5% of participants, with 38.4% of males and 40.9% of females, have declared having sufficient nutrition knowledge. While the distribution of female students who confidante dietitian on nutrition information was higher than male students. The distribution of female students who confident dietitians on nutrition information was higher than that of male students (80.8% vs 54.3%, $p<0.05$). The distribution of main meals, meal skipping, and snacks were different in genders ($p<0.05$). The mean BMI was 22.0±3.5 kg/m2 and 68.3% of students had a normal BMI with differences between genders ($p<0.05$) (as shown in Table 2).

When examining the distribution of students' adherence levels to the MD, it was determined that 5.0% had high, 61.8% had moderate, and 33.2% had low MD adherence levels. The mean MEDAS score was 6.4±1.9 (in the moderate category), 6.2±2.0 for males vs 6.4±1.9 for females, with no difference between genders. While 33.6% of students had a minimal BDI level, 11.5% of them had a severe BDI level, the distribution in gender groups differed and the mean BDI was 15.3±10.4 (in the mild category), with higher mean BDI score in females (15.7±10.2) than males (13.0±11.4) ($p<0.05$) (Table 3).

Table 1. Characteristics of study participants

Characteristics	**Male (n=73, 15.2%)**		**Female (n=406, 84.8%)**		**Total (n=479)**		**p***
Age (years) mean ± SD (min-max)	22.2±2.6 (18-35)		21.5±2.2 (18-35)		21.6±2.3 (18-35)		**0.028**
	n	**%**	**n**	**%**	**n**	**%**	
Marital status							
Single	73	100.0	395	97.3	468	97.7	0.155
Married	-	-	11	2.7	11	2.3	
Living place							
Dormitory	33	45.2	218	53.7	251	52.4	**0.006**
Home	35	47.9	183	45.1	218	45.5	
Other	5	6.9	5	1.2	10	2.1	
Habits							
Smoking	29	39.7	85	20.9	114	23.8	**0.001**
Alcohol intake	18	24.7	41	10.1	59	12.3	**<0.001**
Regular exercise	36	49.3	106	26.1	142	29.6	**<0.001**
Presence of NCDs	5	6.8	75	18.5	80	16.7	**0.014**

*NCDs: Non-communicable diseases. The bold values indicate statistically significant results (p<0.05). *Chi-Square test and t-test were used.*

Table 2. Self-nutritional assessment and BMI of the participants

Nutritional assessment	**Male (n=73, 15.2%)**		**Female (n=406,84.8%)**		**Total (n=479)**		**p***
	n	**%**	**n**	**%**	**n**	**%**	
Sufficient nutrition knowledge **							
Yes	28	38.4	166	40.9	194	40.5	0.456
No	9	12.3	32	7.9	41	8.6	
Partially	36	49.3	208	51.2	244	50.9	
Confidence in nutrition information							
Dietitians	39	54.3	328	80.8	367	76.6	**<0.001**
Other HP(s)[a]	17	23.3	41	10.1	58	12.1	
Organization(s)[b]	8	11.1	22	5.4	30	6.3	
Other(s)[c]	9	12.3	15	3.7	24	5.0	
Adherence to an adequate balanced diet**							
Yes	16	21.9	64	15.8	80	16.7	0.404
No	28	38.4	159	39.2	187	39.0	
Sometimes	29	39.7	183	45.0	212	44.3	
Main meal(s)/day							
1	2	2.8	11	2.7	13	2.7	**0.002**
2	30	41.7	257	63.5	287	60.2	
≥3	40	55.5	137	33.8	177	37.1	
Main meal skip							
Yes	18	24.7	142	35.0	160	33.4	**0.018**
Sometimes	39	53.4	220	54.2	259	54.1	
No	16	21.9	44	10.8	60	12.5	
Snacks/day							
No	11	15.1	31	7.7	42	8.8	0.091
1	28	38.3	137	33.7	165	34.4	
2	26	35.6	165	40.6	191	39.9	
≥3	8	11.0	73	18.0	81	16.9	
BMI (kg/m^2)[d]							
<18.5 kg/m^2	5	6.8	65	16.0	70	14.6	0.181
18.5-24.9 kg/m^2	53	72.6	274	67.5	327	68.3	
25.0-29.9 kg/m^2	12	16.4	58	14.3	70	14.6	
≥30.0 kg/m^2	3	4.2	9	2.2	12	2.5	
BMI (kg/m^2) mean ± SD (min-max)	23.1±3.2 (16.2-32.3)		21.8±3.5 (15.0-38.8)		22.0±3.5 (15.0-38.8)		**0.004**

*[a]HP(s): health professions (physician, nurse, psychologist=. [b]Health organizations(s) (WHO, Ministry of health). [c]Social environment, media. [d]<18.5 kg/m^2 underweight, 18.5-24.9 kg/m^2 normal weight, 25.0-29.9 kg/m^{2}overweight, ≥30.0 kg/m^2 obese. *Chi-Square test and t-test were used. ** The answers to the questions 'Do you think you have sufficient nutritional knowledge?' and 'Do you think you have adequate and balanced nutrition?' reflect the statements of the participants, respectively.*

Table 4 shows the analysis of determinants of BDI score using multivariate linear regression models. The regression model incorporates variables such as age, gender, smoking, alcohol intake, regular exercise, presence of NCD(s), nutritional parameters (sufficient nutritional knowledge, adherence to an adequate and balanced diet, main meals/day, snacks/day), BMI, and MEDAS score. The variables that were found to be statistically significant ($p<0.05$) in the regression model were regular exercise, presence of NCD(s), and adherence to an adequate and balanced diet.

Table 5 shows the analysis of determinants of MEDAS score using multivariate linear regression models. The regression model incorporates variables such as age, gender, smoking, alcohol intake, regular exercise, presence of NCD(s), nutritional parameters (sufficient nutritional knowledge, adherence to an adequate and balanced diet, main meals/day, snacks/day), BMI, and BDI score. The variables that were found to be statistically significant ($p<0.05$) in the regression model were adherence to an adequate and balanced diet and BMI. A negative effect was found between adherence to the Mediterranean diet and depression, but this effect was not statistically significant in regression analyses ($p>0.05$).

DISCUSSION

University years are an important period in which individuals shape their pre- and post-adult lifestyle habits. Adopting healthy eating patterns, such as the Mediterranean diet, during this period contributes to the formation of sustainable health habits in later ages (25). Particular attention is paid to the Mediterranean diet due to its protective effects against type 2 diabetes, metabolic syndrome, inflammatory diseases, liver diseases, cancer or cardiovascular diseases (26). In addition to these effects on health, the relationship between this eating pattern and depression is also an important issue. The relationship between the Mediterranean diet and depression has been examined by many studies and various findings have been revealed (27-29). This study found a negative effect between compliance with the Mediterranean diet and depression, but this effect was not statistically significant in regression analyses. In a study examining the level of students' commitment to MD and its impact on depression during the COVID-19 epidemic, a negative significant relationship was found between MEDAS scores and depression and anxiety (30). Similarly, among Korean adults, adherence to the Mediterranean diet has been suggested to be inversely associated with depression in both females and males (31). In a systematic review, analyzes of cohort studies revealed that there was no significant relationship between adherence to the Mediterranean diet and the risk of depression (32). Similarly, other studies did not find a relationship between adherence to the Mediterranean diet and depression (33, 34).

Understanding the level of compliance with MD and the factors influencing it is crucial for public health initiatives. A superabundance of studies conducted in the European Mediterranean region assessed the adherence levels to this dietary pattern and identified the associated sociodemographic and lifestyle

Table 3. Mediterranean Diet Adherence Screener and Beck Depression Inventory scores of the participants

Scores	Male (n=73)		Female (n=406)		Total (n=479)		p*
	n	%	n	%	n	%	
Adherence level to the MD							
Low	29	39.7	130	32.0	159	33.2	0.430
Moderate	41	56.2	255	62.8	296	61.8	
High	3	4.1	21	5.2	24	5.0	
MEDAS Score mean ± SD (min-max)	6.2±2.0 (2-11)		6.4±1.9 (1-12)		6.4±1.9 (1-12)		0.405
BDI Level							
Minimal	37	50.7	124	30.5	161	33.6	**0.002**
Mild	17	23.3	120	29.6	137	28.6	
Moderate	9	12.3	117	28.8	126	26.3	
Severe	10	13.7	45	11.1	55	11.5	
BDI Score mean ± SD (min-max)	13.0±11.4 (0-40)		15.7±10.2 (0-52)		15.3±10.4 (0-52)		**0.046**

determinants (35-37). In this study, it was found that the proportion of students with high commitment to MD was low (5.0%), while 61.8% had a medium level of commitment. The mean MEDAS score was 6.4±1.9 (medium category) and there was no difference between genders. Similarly, while a moderate level of adherence was found in many studies examining compliance with the Mediterranean diet (38, 39), this compliance did not differ according to gender was detected (40, 41). In another study, it was determined that the average MEDAS score was 5.6±1.82 and the majority (69.4%) had a low level of adherence to the Mediterranean diet (42). There may be various reasons behind the low Mediterranean diet scores in university students, such as eating habits and preferences, economic factors and accessibility, time

Table 4. Potential factors affecting depression risk via multivariate linear regression models

Factors	Unstandardized Coefficients		Standardized Coefficients	t	P*
	β1 (%95 CI)	Std. Error	Beta		
(Constant)	31.754	8.639	-	3.676	**<0.001**
Age	-0.127	0.209	-0.028	-0.611	0.542
Gender	2.143	1.394	0.073	1.537	0.125
Smoking	-2.199	1.206	-0.090	-1.823	0.069
Alcohol intake	1.896	1.557	0.060	1.218	0.224
Regular exercise	-2.180	1.054	-0.095	-2.069	**0.039**
Presence of NCD(s)	-5.019	1.259	-0.179	-3.987	**<0.001**
Sufficient NK *	0.716	0.521	0.065	1.374	0.170
Adherence to an ABD*	-1.513	0.666	-0.106	-2.270	**0.024**
Main meal(s)/day	-1.372	0.774	-0.082	-1.773	0.077
Snacks/day	-0.129	0.500	-0.012	-0.257	0.797
BMI	0.110	0.137	0.036	0.800	0.424
MEDAS score	-0.148	0.247	-0.027	-0.600	0.549
Adjusted R^2	0.071				

NK: nutrition knowledge, ABD: adequate and balanced diet, BMI: body mass index, CI: Confidence interval, MEDAS: Mediterranean Diet Adherence Screener. Multivariate linear regression models were used. The bold values indicate statistically significant results ($p<0.05$). *The answers to the questions 'Do you think you have sufficient nutritional knowledge?' and 'Do you think you have adequate and balanced nutrition?' reflect the statements of the participants, respectively.

Table 5. Potential factors affecting adherence to the Mediterranean diet via multivariate linear regression models

Factors	Unstandardized Coefficients		Standardized Coefficients	t	p*
	β1 (%95 CI)	Std. Error	Beta		
(Constant)	4.644	1.634	-	2.842	**0.005**
Age	0.028	0.039	0.034	0.713	0.476
Gender	0.252	0.263	0.047	0.958	0.338
Smoking	0.045	0.228	0.010	0.197	0.844
Alcohol intake	-0.493	0.292	-0.085	-1.688	0.092
Regular exercise	0.130	0.199	0.031	0.653	0.514
Presence of NCD(s)	-0.027	0.241	-0.005	-0.111	0.912
Sufficient NK*	-0.033	0.098	-0.016	-0.333	0.739
Adherence to an ABD*	-0.292	0.125	-0.112	-2.331	**0.020**
Main meal(s)/day	0.146	0.146	0.048	1.001	0.318
Snacks/day	0.133	0.094	0.067	1.418	0.157
BMI	0.059	0.026	0.106	2.295	**0.022**
BDI score	-0.005	0.009	-0.029	-0.600	0.549
Adjusted R^2	0.019				

*NK: nutrition knowledge, ABD: adequate and balanced diet, BMI: body mass index, CI: Confidence interval, BDI: Beck Depression Inventory. Multivariate linear regression models were used. The bold values indicate statistically significant results (p<0.05).*The answers to the questions 'Do you think you have sufficient nutritional knowledge?' and 'Do you think you have adequate and balanced nutrition?' reflect the statements of the participants, respectively.*

management and busy schedules, cultural and social factors, and knowledge and awareness levels.

Adherence to an adequate and balanced diet is an important component of a healthy lifestyle, and dietary patterns such as the Mediterranean diet encourage the consumption of foods with high nutritional value (43). In this study, according to the multivariate linear regression model, the factors affecting the MEDAS score were found to be adherence to adequate and balanced nutrition and BMI ($p<0.05$). In one study, it was found that age and knowledge of sustainable nutrition affected compliance with the Mediterranean diet (41). Hashim et al. (2024) found an effect between MD adherence and physical activity and nutritional information from dietitians (44). Vera Ponce et al. reported that the relevant factors were gender, smoking, physical activity level and BMI (45). The findings of our study indicate that high BMI may negatively affect compliance with the Mediterranean diet and therefore MEDAS scores in young people. This dietary pattern generally supports a healthy lifestyle, and as a result, body weight is likely to be under control and a healthy BMI maintained. However, more research is needed to understand this relationship, and it is important to also consider individual factors.

Although no significant effect was found between the Mediterranean diet and depression in this study, determining the prevalence of depression and the effective factors in university students is important to protect students' academic success, social relationships, physical health and general well-being (46). In this study, 26.3% of the students had a moderate BDI level and 11.5% had a severe BDI level, while the average BDI score in females was higher than in males ($p<0.05$). Similarly, in a study conducted on university students, it was found that the depression, anxiety, and stress scores of female participants were higher than male participants (47). In particular, the fact that female students have higher BDI scores than male students indicate the effect of gender on depressive symptoms. This finding suggests that gender differences and mental health status in young females should be considered. According to the results of the multivariate linear regression models of the study, regular exercise, the presence of NCDs, and adherence to adequate and balanced nutrition were found to have significant effects on depression. Although these findings may suggest that both physical activity and dietary habits could be important potential factors in mental health, it should be noted that they are based on student self-reports. The positive effects of regular exercise on depression are widely supported in the literature (48-50). Exercise can improve mood and reduce depressive symptoms by increasing the release of endorphins. The presence of NCDs may negatively affect the quality of life, which may increase the risk of depression. For example, factors such as persistent pain, fatigue, physical limitations, stress and anxiety, social isolation, and treatment compliance problems may contribute to the development of depression. Healthy eating habits can have positive effects on depression; for example, consumption of foods with high nutritional value can have positive effects on brain function and mood.

The study has some strengths and limitations. The study drew attention to the role of the Mediterranean diet, which is accepted worldwide, in improving mental health and alleviating symptoms of depression in young adults. On the other hand, the study was conducted using the snowball sampling method. To generalize the results to all university studies, data was collected from as many universities as possible by using the communication power of technology. The second limitation of the study is that students with psychological problems or diagnosed psychological disorders/diseases were not excluded from the study. It is recommended that this criterion be taken into consideration in future studies on this subject. Finally, participants' nutritional knowledge and adherence to healthy nutrition were obtained based on their statements. It is recommended that valid and reliable measurement tools be used in future studies.

CONCLUSION

In conclusion, it was determined that there was no significant effect between the Mediterranean diet and depression. However, the study identified several factors affecting both the level of adherence to the Mediterranean diet and the prevalence of depression. This may offer perspectives to be considered in the establishing and maintaining healthy eating habits, improving mental health in university students. As the relationship between the Mediterranean diet and depression is complex and multifaceted, more comprehensive, and long-term studies may help to provide a clearer picture of the effects of the Mediterranean diet on health and mental health in young people.

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RESEARCH ARTICLE

HEALTHCARE PROFESSIONALS' KNOWLEDGE LEVEL REGARDING FORENSİC CASES IN THE INTENSIVE CARE AND OPERATING ROOM

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ABSTRACT

Purpose: This study aims to assess the knowledge and practices of healthcare professionals in operating rooms and intensive care units regarding forensic cases.

Material and Methods: A descriptive comparative design study was conducted, involving healthcare professionals from a university hospital in the Eastern Black Sea Region between March 17 and May 1, 2023 (n=116). Data were collected through a questionnaire developed by the researchers, and analysis was performed using descriptive statistics, t-tests, variance analysis, and correlation analysis.

Results: Participants had a mean age of 30.6±7.7 years, with 56% working in operating rooms and 56.9% being nurses. While 53.4% had received training on forensic cases, 82.8% had encountered such cases. Education significantly influenced both knowledge level (p=0.00) and application (p=0.02), with a moderate positive correlation between knowledge and application (r=0.541, p=0.000).
Conclusion: The study highlights a lack of knowledge and practices among healthcare professionals in operating rooms and intensive care units regarding forensic cases.

Keywords: Forensic case, healthcare professionals, intensive care, knowledge, operating room

INTRODUCTION

A forensic case refers to a situation that is considered a criminal or suspicious event that may have legal consequences on individuals or society. Such cases usually require a criminal or legal investigation and require a multi-faceted evaluation of the legal, medical, psychological and social dimensions of the event (1,2) Suspicious deaths, such as firearm injuries, traffic accidents, falling incidents, penetrating sharps injuries, occupational accidents, poisoning, burns, drowning, and suicide attempts, and individuals who are admitted to healthcare facilities for these reasons are accepted as forensic cases. Physicians and nurses are the first group of health professionals who encounter the individual and their family through examination and laboratory samples in forensic cases (3,4). Therefore, all healthcare professionals, especially physicians, and nurses, have a great responsibility to prevent delays or negligence in the processes of identifying, collecting,

storing, transporting, and recording materials that may constitute evidence in forensic cases (5). For this reason, health professionals in all units have an important role in identifying, collecting, and preserving forensic traces, which can include fingerprints, blood, semen, saliva, hair, etc (6). Evidence only have value as evidence in a forensic investigation if they are collected, preserved, stored, and transferred in a suitable (1,6) Thus, important deficiencies and errors can occur in the determination, collection, storage, and transfer processes in forensic cases in the event of a lack or deficiency in training (7).

It is frequently emphasized in the literature that healthcare professionals who encounter forensic cases lack knowledge about the treatment and care of these cases. Since such cases are often intertwined with legal processes and ethical principles, healthcare professionals need to have not only medical knowledge but also forensic and legal knowledge. However, it is reported that many healthcare professionals lack training and experience in forensic cases and that these deficiencies can negatively affect both patient care and compliance with legal processes. In addition, the lack of special training programs for nurses and other healthcare professionals in the management of forensic cases can pose serious risks to both employees and (1,8,9). It is important to identify, collect and properly store evidence for individuals who apply to medical units due to legal cases. Deficiencies in these practices can lead to a large amount of evidence being overlooked, lost or damaged, leading to wrong decisions and unsuccessful cases in court (10). Therefore, the protection of the chain of evidence is very important in healthcare professional practices (11), and all evidence obtained should be kept in sealed envelopes, boxes, or suitable container for the type of sample (12).

Emergency departments typically serve as the initial points of contact for forensic cases and must consistently be ready to provide forensic care to patients. Given that emergency departments are often the first medical facilities consulted in forensic cases, in literatüre extensive research has been conducted in this specific clinical setting (13–16). It is worth noting that the treatment and follow-up of forensic cases after including physical injuries resulting from explosions, burns, severe injuries due to traffic accidents, poisonings, and suicide attempts in fist emergency unit. Treatment and care continues in the operating room and intensive care units (14). There is a high risk that evidentiary material will be ignored while treatment is ongoing. Forensic cases often require rapid intervention and due to their forensic nature, it is vital that treatment and care are immediately implemented while preserving and securing potential evidence. It is critical that such cases are not overlooked, within the scope of the duty, authority and responsibility of healthcare professionals. Forensic cases not only require medical intervention, but must also be handled correctly from a legal and ethical perspective. Healthcare professionals are responsible for both ensuring patient safety and acting in accordance with legal processes by correctly recognizing and documenting forensic cases (1,17). Therefore as healthcare professionals working in operating rooms and intensive care units may come across forensic cases, it becomes imperative for them to possess the necessary knowledge and competence to provide care, including all aspects of medical and technical training related to the collection, preservation, and documentation of forensic traces in forensic cases (5). Detection of the crime, recognition of the offender, protection of the victim and the role of health personnel in protecting the patient's right can be ensured by the correct management of the forensic case (18,19). Maintaining continuity of care in our country, there are no forensic nurses or healthcare professionals whose duties are defined and legally defined. In addition, healthcare professional cannot make the necessary approaches and practices because they do not have sufficient knowledge about forensic medicine. Therefore, the evaluation of forensic cases is mostly performed by nurses who have not received special training (20–24).

According to Article 280 of the Turkish Criminal Code No. 5237 in our country, “A healthcare professional who fails to report the situation to the authorities or shows delay in this regard, despite encountering an indication that a crime has been committed during the performance of his/her duty, will be punished with imprisonment for up to one year (25). For this reason, this study was conducted to determine the knowledge and practice levels of health professionals in the operating room and intensive care units where the treatment of forensic cases continues.

Research Questions:

Do health professionals have sufficient knowledge and practices about forensic cases and types of forensic evidence?

Do health professionals have sufficient knowledge and practices about the preservation and storage of evidence in a forensic case?

Is there a difference in terms of knowledge and attitude between the group that received training on forensic cases and the group that did not?

MATERIALS AND METHODS

Type of Research

The study was designed as a descriptive-comparative design investigation to assess the knowledge and attitudes of healthcare proffesionals working in the operating room and intensive care units regarding forensic cases. The study adhered to the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology guidelines).

Place and Time of the Study

The study was carried out in the operating room and intensive care units of a university hospital located in northern Turkey, within the period from March 17, 2023, to May 1, 2023.

Population and Sample of the Study

The study population included healthcare professionals employed in the operating rooms and intensive care units of the northern Turkish a university hospital (n=116).

Data Collection and Data Collection Tools

The data were collected by a questionnaire that prepared by the researchers the literature rewiev (1). The form consists of two sections and 26 questions. The questionnaire included questions related to the descriptive characteristics of healthcare professionals (age, occupation, gender, marital status, educational background, the voluntary choice of their profession, and prior training on forensic cases) and their knowledge (9 questions) and level of knowledge regarding application (9 questions) regarding forensic cases. Participants were informed that their involvement was entirely voluntary and that the data would only be used for scientific research purposes.The questions regarding are responded as “Yes”, “No” and “I don’t know” and are evaluated those who answered the question correctly were given 2 points, those who did not know were given 1 point, and those who answered incorrectly were given 0 points in knowledge question form, the question correctly were given 3 points, 2 point for participant who answered “sometimes” those who did not know were given 1 point, and those who answered incorrectly were given 0 points in attidues form. Knowledge and apply practice question question form and the lowest and highest scores to be obtained from the information form on knowledge and practices fosensic event were 0-16 and 0-27, respectively. Knowledge level score 0-5 was determined as low level, 6-10 as medium level and 11-16 as high level. The application knowledge level was determined as 0-9 points as low level, 10-19 points as medium level, and 19-27 points as high level. After receiving information about the study’s scope, the questionnaire forms were distributed to those who agreed to participate, including healthcare professionals. Participants were given a specific time to respond to the survey questions and filled out the form face-to-face. Once they completed the questionnaires, they were directed to designated collection points in the operating room and each intensive care unit. The researchers made regular visits to these areas to gather the completed questionnaires. It took participants approximately 3-5 minutes to complete the questionnaire. Cronbach Alpha reliability coefficient of the questions was found 0.621.

Data Analysis

Statistical analysis was conducted using the IBM SPSS 25.0 (IBM Corp., Armonk, NY, USA) software package. In the evaluation of the data, Nonparametric categorical data were compared using X^2 (chi-square) analysis, descriptive statistical methods such as percentage, mean, standard deviation, and Kolmogorov-Smirnov distribution test were used for the normal distribution. For the two-group comparison of quantitative variables, independent group t-test, and for the multiple groups, variance analysis and correlation analysis was used. Statistical significance was accepted at the level of $p<0.05$

Ethical Approval

Ethics committee approval was obtained from the Clinical Research Ethics Committee of Giresun University, Giresun Education and Research Hospital (Date: 13.03.2023, Decision No: 14). Additionally, written permission was obtained from the relevant hospital after explaining the scope of the study. Once volunteers were informed and had filled out the voluntary consent form, the study was initiated. The study was carried out in accordance with the Helsinki Declaration and with the participant's approval.

RESULTS

The study encompassed healthcare workers with a mean age of 30.6 years. Among the participants, 64.7% were female, and 56.0% were working in operating room. Specifically, 56.9% were nurses, and 53.4% had previously received training on forensic cases. The socio-demographic characteristics of the study group are outlined in Table 1.

Table 1. Descriptive Information

Descriptive Information	**n**	**%**
Age (Mean ± Standard Deviation)	116	30.6±7.7
Unit		
Operating Room	65	**56.0**
Intensive Care	51	44.0
Occupation		
Nurse	66	**56.9**
Anesthesia Technician	21	18.1
Specialist Physician	12	10.3
Assistant Doctor	17	14.7
Gender		
Female	75	**64.7**
Male	41	35.3
Receiving Forensic Case Training		
Yes	62	**53.4**
No	54	46.6

Table 2 displays the numerical values of responses concerning the knowledge of healthcare workers about forensic cases. It was observed that 84.8% of nurses, 85.7% of anesthesia technicians, 91.7% of specialist physicians, and 64.7% of assistant physicians in operating rooms and intensive care units had encountered forensic cases during their professional experience. The total mean score of the healthcare professionals' questions related to knowledge regarding forensic cases was 15.62±3.97. Knowledge level score was found as high level.

In Table 3, it was found that 42.2% of the participants answered "don't know" to the premise that damp or wet material should be allowed to dry before packaging. While 54.3% of the participants said yes to the question about visual recording of forensic cases such as photographs and drawings (e.g., bruises, abrasions, or cuts), 56.8% of the participants did not know about the practice of giving these records or documents to patients. The mean total score of application skill knowledge level questions of healthcare professionals was found to be 19.25±7.40. The application knowledge level was found as medi level. The comparison of the mean scores of forensic case practice knowledge level according to the descriptive characteristics of healthcare workers is presented in Table 4. It was determined that the statistical difference between the healthcare professions in the total apply knowledge score regarding forensic cases was not significant ($p>0.05$), but a statistically significant difference was found between the responses to the apply knowledge score regarding previous training on the subject and encountering a forensic case ($p<0.005$).

In Table 4, it was determined that the statistical difference between the healthcare professions in the total knowledge score regarding forensic cases was not significant ($p>0.05$). However, a statistically significant difference was found between the responses to the knowledge score regarding previous training on the subject and encountering a forensic case ($p<0.005$).

A positive, moderately significant relationship was found between the forensic case knowledge mean score and the Applying Knowledge score in Table 5 ($r=0.541$, $p=0.000$).

DISCUSSION

This study, which aims to evaluate the knowledge, competence and education status of nurses, doctors and anesthesia technicians working in operating rooms and intensive care units of a hospital in eastern Turkey, regarding forensic cases, is particularly related to research conducted in a similar field and discussed in the light of literature findings regarding health professionals working in operating rooms and intensive care units. A review of the literature revealed that previous studies on this subject have primarily focused on emergency departments (1,13,19,21,26–28) However, it is important to note that patient transfers from the emergency unit to the operating room or intensive care unit can also occur based on the priority of the forensic case (7,29). During this process, the correct removal and storage of clothing or evidence is crucial for both the criminal and the victim. Because legal proceedings may be initiated as a result of some forensic cases presented to the court (14,30).

Table 2. Questions related to knowledge and ability the apply regarding forensic case

Questions related to knowledge regarding forensic case	Yes- n (%)	No- n (%)	Don't know- n (%)
1. Have you ever encountered forensic cases in the operating room or intensive care unit?			
Nurse **(n=66)**	56 (84.8)	7(10.6)	3(4.5)
Anesthesia Technician **(n=21)**	18(85.7)	2(9.5)	1(4.8)
Specialist Physician **(n=12)**	11(91.7)	-	1(4.8)
Assistant Doctor **(n=17)**	11(64.7)	6(35.3)	-
Total	96 (82.8)	15 (12.9)	5 (4.3)
2. Should a report be prepared when a forensic case is encountered in the operating room or intensive care unit?			
Nurse **(n=66)**	29(43.9)	19(29.8)	18(27.3)
Anesthesia Technician **(n=21)**	8(38.1)	6(28.6)	7(33.2)
Specialist Physician **(n=12)**	6(50)	2(16.7)	4(33.3)
Assistant Doctor **(n=17)**	10(58.8)	2(11.8)	5(29.4)
Total	53(45.6)	29 (25)	34 (29.4)
3. I know who is in charge of preparing reports on forensic cases			
Nurse **(n=66)**	24(36.4)	18(27.2)	24(36.4)
Anesthesia Technician **(n=21)**	5(23.8)	6(28.6)	10(47.6)
Specialist Physician **(n=12)**	5(41.7)	4(33.3)	3(25.0)
Assistant Doctor **(n=17)**	10(58.8)	6(35.1)	1(5.9)
Total	54(46.5)	34(29.4)	38(29.1)
4. I know who is in charge of reporting forensic cases in the operating room or intensive care unit?			
Nurse **(n=66)**	34(51.5)	12(18.2)	20(30.3)
Anesthesia Technician **(n=21)**	11(52.4)	-	10(47.6)
Specialist Physician **(n=12)**	7(58.3)	1(8.3)	4(33.3)
Assistant Doctor **(n=17)**	13(76.5)	2(11.8)	2(11.8)
Total	65(56.0)	15 (13.0)	36 (31.0)
5. I know the unit where forensic cases are reported			
Nurse **(n=66)**	58(87.9)	1(1.5)	7(10.6)
Anesthesia Technician **(n=21)**	18(85.7)	1(4.8)	2(9.5)
Specialist Physician **(n=12)**	10(83.3)	1(8.3)	1(8.3)
Assistant Doctor **(n=17)**	17(100)	-	-
Total	103(88.7)	3(2.7)	10(8.6)
6. I know that there should be a different clinic for the storage of forensic evidence			
Nurse **(n=66)**	13(19.7)	36(40.9)	17(39.4)
Anesthesia Technician **(n=21)**	7(33.3)	1(4.8)	13(61.9)
Specialist Physician **(n=12)**	2(16.7)	3(25.0)	7(58.3)
Assistant Doctor **(n=17)**	4(23.5)	1(5.9)	12(70.6)
Total	26(22.4)	41(35.0)	49(42.2)
7. The institution should have a forensic case archive			
Nurse **(n=66)**	24(34.8)	4(6.1)	39(59.1)
Anesthesia Technician **(n=21)**	8(38.1)	-	13(61.9)
Specialist Physician **(n=12)**	5(41.7)	-	7(58.3)
Assistant Doctor **(n=17)**	5(35.3)	-	11(64.7)
Total	42(36.2)	4(3.4)	70(60.3)
8. An easily accessible evidence collection kit should be available in the operating room or intensive care unit			
Nurse **(n=66)**	17 (25.8)	41 (62.19	8 (12.1)
Anesthesia Technician **(n=21)**	6 (28.6)	14 (66.7)	1 (4.8)
Specialist Physician **(n=12)**	2 (16.7)	8 (66.7)	2 (16.7)
Assistant Doctor **(n=17)**	2 (11.8)	14 (82.4)	1 (5.9)
Total	27(23.2)	77(66.3)	12(10.5)
9. When you encounter a forensic case, do you experience feelings of prejudice, discomfort, anger, or fear toward the patient?			
Nurse **(n=66)**	17 (25.8)	41 (62.1)	8 (12.1)
Anesthesia Technician **(n=21)**	6 (26.8)	14 (66.7)	1 (4.8)
Specialist Physician **(n=12)**	2 (16.7)	8 (66.7)	2 (16.7)
Assistant Doctor **(n=17)**	2 (11.8)	14(82.4)	1 (5.9)
Total	27(23.2)	77(66.3)	12(10.5)
(Mean ± Standard Deviation))(n=116)		15.62±3.97	

Table 3. Ability to Apply Knowledge Forensic Cases

Ability to Apply Knowledge Forensic Cases	Yes- n (%)	No- n (%)	Sometimes-n (%)	Don't know- n(%)
1. The clothes of the forensic case are removed appropriately.				
Nurse **(n=66)**	50 (75.8)	1(1.5)	5(7.6)	10(15.2)
Anesthesia Technician **(n=21)**	13(61.9)	3(15.3)	-	5(23.8)
Specialist Physician **(n=12)**	10(83.3)	-	-	2(16.7)
Assistant Doctor **(n=17)**	12(70.6)	1(5.9)	2(11.8)	2(11.8)
Total	85(73.2)	5(4.3)	7(6.0)	19 (16.5)
2. All materials are labeled and protected because they can be evidence in a forensic investigation.				
Nurse **(n=66)**	52(78.8)	2(3.0)	5(7.6)	7(10.6)
Anesthesia Technician **(n=21)**	14(66.7)	1(4.8)	1(4.8)	5(23.8)
Specialist Physician **(n=12)**	6(50.0)	-	2(16.7)	4(33.3)
Assistant Doctor **(n=17)**	12(70.6)	-	-	5(29.4)
Total	84(72.4)	3(2.7)	8(6.8)	21(18.1)
3. Damp or wet material should be left to dry before packaging				
Nurse **(n=66)**	21(31.8)	15(22.7)	6(9.1)	24(36.4)
Anesthesia Technician **(n=21)**	4(19.0)	6(28.6)	1(4.8)	10(47.6)
Specialist Physician **(n=12)**	1(8.3)	2(16.7)	-	9(75.0)
Assistant Doctor **(n=17)**	2(11.8)	7(41.2)	2(11.8)	6(35.3)
Total	28(24.1)	30(26.0)	9(7.7)	49(42.2)
4. All evidence materials are placed separately (paper packaging or envelopes, etc.).				
Nurse **(n=66)**	46(69.7)	3(4.5)	5(7.6)	12(18.2)
Anesthesia Technician **(n=21)**	11(52.4)	-	1(4.8)	9(42.9)
Specialist Physician **(n=12)**	5(41.7)	2(16.7)	-	5(41.7)
Assistant Doctor **(n=17)**	10(58.8)	1(5.9)	1(5.9)	5(29.4)
Total	72(62.0)	6(5.3)	7(6.0)	39(33.7)
5. Any evidentiary material is properly recorded before it is handed over to the judicial authorities.				
Nurse **(n=66)**	53(80.3)	3(4.5)	3(4.5)	7(10.6)
Anesthesia Technician **(n=21)**	14(66.7)	1(4.8)	-	6(26.6)
Specialist Physician **(n=12)**	7(58.3)	1(8.3)	1(8.3)	3(25.1)
Assistant Doctor **(n=17)**	14(82.4)	-	-	3(17.6)
Total	88(76.0)	5(4.3)	4(3.3)	19(16.4)
6. Materials suitable for sending to the judicial authorities that may be evidence are known.				
Nurse **(n=66)**	41(62.1)	2(3.0)	12(18.2)	11(16.7)
Anesthesia Technician **(n=21)**	10(47.6)	1(4.8)	4(19.0)	6(28.6)
Specialist Physician **(n=12)**	6(50.0)	-	3(25.0)	3(25.0)
Assistant Doctor **(n=17)**	7(41.2)	2(11.8)	4(23.5)	4(23.5)
Total	64(55.2)	5(4.3)	23(19.9)	24(20.6)
7. Visual recording of the forensic case is conducted, such as photographs and drawings (e.g., bruises, abrasions, or cuts).				
Nurse **(n=66)**	38(57.6)	6(9.1)	5(7.6)	17(25.8)
Anesthesia Technician **(n=21)**	8(38.1)	1(4.8)	4(19.0)	8(38.1)
Specialist Physician **(n=12)**	5(41.7)	2(16.7)	2(16.7)	3(25.0)
Assistant Doctor **(n=17)**	12(70.6)	-	-	5(29.4)
Total	63(54.3)	9(7.8)	11(9.5)	33(28.4)
8. Any visual recording in a forensic case requires permission from the patient or his/her relatives.				
Nurse **(n=66)**	27(40.9)	12(18.2)	4(6.1)	23(34.8)
Anesthesia Technician **(n=21)**	8(38.1)	-	1(4.8)	12(57.1)
Specialist Physician **(n=12)**	6(50.0)	-	1(8.3)	5(41.7)
Assistant Doctor **(n=17)**	7(41.2)	5(29.4)	1(5.9)	4(23.5)
Total	48(41.4)	17(14.6)	7(6.0)	44(38.0)
9. Original films that are the result of any imaging method are delivered to the patient (photographs, etc.).				
Nurse **(n=66)**	14(21.2)	10(15.2)	4(6.1)	38(57.6)
Anesthesia Technician **(n=21)**	2(9.5)	3(14.3)	1(4.8)	15(71.4)
Specialist Physician **(n=12)**	3(25.0)	2(16.7)	-	7(58.3)
Assistant Doctor **(n=17)**	4(23.5)	4(23.5)	1(5.9)	8(47.1)
Total	23(19.9)	19(16.3)	6(5.2)	68 (58.6)
(Mean ± Standard Deviation) (n=116)		19.25±7.4		

Table 4. Comparison of the knowledge and level of practice knowledge mean scores of forensic events according to the descriptive characteristics of healthcare professionals (n=116)

Descriptive characteristics	Knowledge (Mean±SD)	Practices Knowledge (Mean±SD)
Occupation		
Nurse	15.62±4.15	18.07±7.03
Anesthesia Technician	16.80±3.74	22.00±7.49
Specialist Physician	15.50±4.92	21.50±7.00
Assistant Doctor	14.29±4.15	15.62±4.15
	F=1.27	F=1.94
	P=0.28	P=0.12
Receiving Forensic Case Training		
Yes	14.40±3.33	17.77±6.46
No	17.03±4.20	20.96±8.08
	t=-3.76	t=-2.32
	P=0.00	**P=0.02**
Have you ever encountered forensic cases in the operating room or intensive care unit?		
Yes	14.89±3.55	17.79±6.34
No	18.53±4.30	26.86±8.06
Don't know	21.00±2.73	24.60±9.02
	F=12.20	F=13.50
	P=0.00	**P=0.00**

Table 5. The Relationship Between Knowledge Status and Ability to Apply Knowledge Mean Score

		Ability to Apply Knowledge
Knowledge	**r**	**0.541**
	p	**0.000**
	n	116

r=pearson corelation, p<0.05

Therefore, forensic cases should not be restricted to the emergency unit alone. Healthcare professionals in the operating room and intensive care units must possess the necessary knowledge and experience to handle such cases. Prior research has highlighted training disparities in fields(14,31,32). In one study, 58% of emergency nurses (33), and in another study, 77% of emergency nurses (34) did not receive training on forensic cases. Similarly, more than half of the physicians and nurses in an operating room (1) study did not receive training. In our study, it was determined that those who received training in forensic medicine course were above average and most healthcare professionals reported receiving training on forensic cases. Differences between the stated situation and the literatureas may have been addressed and incorporated into educational policies over the years. It may also correlated with the importance given to forensic cases by countries and their policies.

Recognizing and knowing about forensic cases also affects the collection, storage, and delivery of forensic evidence. For example, the collection and storage of evidence in forensic cases such as sexual assault, trauma, traffic accident or intoxication are different from each other (35). According to our research findings, it has showed that the knowledge level and practice attitudes of nurses regarding the storage and packaging of forensic evidence were higher than those of other health personnel but not at the expected level (20, 21). According to our research results, it was determined that the level of knowledge

of healthcare workers on the subject was high, but the level of implementation was at a medium level. This finding is based on research findings may be attributed to the infrequent encounter of forensic cases in operating rooms and intensive care units, or to the inadequacy of the training provided, which often lacks visual and practical components. This discrepancy could be related to the content, duration, and structure of the training programs.

It is well-documented in existing literature that individuals lacking adequate knowledge and training may commit errors in the documentation and reporting of forensic cases, which is crucial for the correct legal process (7,18). In our study, significant differences were found among professions regarding practical knowledge, particularly in areas such as "Should a report be prepared when a forensic case is encountered in the operating room or intensive care unit?", "Moist or wet material should be left to dry before packaging", "I know the unit where forensic cases are reported", and "The institution should have a forensic case archive". Therefore, it is believed that receiving appropriate training significantly impacts the handling and reporting of such cases.

In a study, a significant correlation was reported between the level of training and evidence preservation (33,36,37). Similarly in our study, it was found that a positive, moderately significant relationship was found between the forensic case knowledge level.

CONCLUSION

In our study, it was found that there was no significant difference in the theoretical and practical knowledge levels among different occupational groups and also it was concluded that training status positively influenced both theoretical and practical aspects. While healthcare personnel exhibited a high level of theoretical knowledge regarding forensic cases, their practical application was only at a moderate level. This finding highlights the importance of bridging the gap between theoretical knowledge and practical application, suggesting that healthcare personnel can effectively manage forensic cases by translating their acquired knowledge into practice through targeted training and practical experience. Therefore, it is recommended that mandatory practical training, including case scenarios and simulation techniques, be provided to healthcare personnel in operating rooms and intensive care units. Additionally, integrating forensic medicine education into the curriculum of health schools and promoting further research on this topic are encouraged.

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RESEARCH ARTICLE

EFFECT OF TELEREHABILITATION BASED HIGH INTENSITY INTERVAL TRAINING ON BIOCHEMISTRY PARAMETERS AND SYMPTOMS IN PATIENTS WITH FIBROMYALGIA

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ABSTRACT

Purpose: Fibromyalgia syndrome (FMS) is a chronic musculoskeletal disease of unknown etiology accompanied by symptoms such as pain, hyperalgesia, sleep disorders, fatigue and mood disorders. The purpose of this study was to evaluate the effect of telerehabilitation-based high intensity interval training (HIIT) on blood parameters and disease symptoms in FMS.
Material and Methods: 33 fibromyalgia patients were randomly assigned as HIIT (n = 11), moderate-intensity continuous training (MICT) (n=11) and control (CG) (n=11). While the patients in the study groups were given upper extremity exercise with telerehabilitation for 6 weeks, no intervention was applied to the CG. Disease symptoms and blood parameters were evaluated before and after treatment.
Results: In the post-treatment intra-group evaluation, a significant increase was observed in the HIIT group superoxide dismutase (SOD), malondialdehyde (MDA), glutathione peroxidase (GSH-Px), pressure pain threshold, right handgrip values, while a significant decrease was detected in fatigue severity and fibromyalgia impact scores. In the MICT group, a significant decrease was observed in SOD, myeloperoxidase (MPO), fatigue severity values, while a significant increase was detected in pressure pain threshold and left handgrip values. In the inter-group evaluation, significant differences were detected in post-treatment right handgrip, fatigue severity, fibromyalgia impact score, MPO and SOD values ($p<0.05$).
Conclusion: HIIT may be an effective treatment choice for patients.
Keywords: Exercise; Rehabilitation; Oxidative stress

INTRODUCTION

Fibromyalgia syndrome (FMS) is a chronic musculoskeletal disease of unknown etiology accompanied by symptoms such as pain, hyperalgesia, sleep disorders, fatigue and mood disorders. The serotonergic system has been

associated with many symptoms such as depression, sleep problems, anxiety, fatigue, and pain. Tryptophan is the precursor to serotonin in the system (1).

Studies on FMS have reported that plasma tryptophan, serotonin and vitamin D levels (25-OH Vit D) decrease in this patient group. In the treatment of FMS, aerobic exercise reduces the amount of amino acids that compete with tryptophan by allowing the muscles to use branched-chain amino acids (BCCAs) and increases the chances of tryptophan passing the blood-brain barrier. Therefore, it has the potential to increase serotonin in the brain (2). It is stated that physical activity may be a way to achieve higher serum vitamin D levels in the population, and it is suggested that endurance exercises may increase circulating 25-OH Vit D (3).

The type of exercise given to FMS patients is important. Studies have shown that when aerobic exercises are applied regularly for a long time, the level of lipid peroxidation malondialdehyde (MDA), which is an indicator of oxidative stress, decreases and the antioxidant enzyme activity (SOD) increases (4). Studies conducted in recent years focus on high intensity interval training (HIIT). HIIT are shorter in duration compared to moderate intensity continuous training (MICT) and produce the same or more positive effects compared to MICT (5). Compared to lower extremity training, upper extremity HIIT training has received relatively less attention in studies. We thought that this form of exercise could produce beneficial results for the FMS patient group who have problems in upper extremity muscle strength and performance due to pain and physical inactivity and condition (6). During the pandemic period, studies involving video-guided exercise programs accompanied by telerehabilitation in fibromyalgia patients were reported in the literature (7).

The primary aim of this study was to evaluate the effect of telerehabilitation-based upper extremity-HIIT on biochemistry parameters and disease symptoms in FMS patients.

MATERIALS AND METHODS

This study is a prospective, randomized controlled trial with blinded assessors. Ethics committee approval for the study was obtained from the Clinical Research Ethics Committee of Bakırköy Doctor Sadi Konuk Training and Research Hospital (Date: 12.07.2021, Decision No: 2021-14-05). The study was registered on the ClinicalTrial.gov website and the registration number is NCT05502003. The study was conducted with written informed consent from each patient and in accordance with the Declaration of Helsinki.

A total of 33 patients diagnosed with fibromyalgia at the Physical Medicine and Rehabilitation outpatient clinic of Bakırköy Sadi Konuk Research and Training Hospital participated in this study. Patients between the ages of 18-65 years who were followed up in the hospital with the diagnosis of fibromyalgia (American College of Rheumatology 2016) and agreed to participate in the study were included in the study (8). Participants were patients who had regular doctor check-ups and did not participate in any physical therapy or exercise program. All patients were invited to the study by phone call by the responsible physiotherapist. Since they were under routine control in terms of the drugs used, no changes were made. Exclusion criteria were infection, fever, contraindication to physical activity, any known advanced pathology related to the locomotor system, cardiopulmonary problem, presence of autoimmune disease, pregnancy, malignancy, severe psychiatric disorder, neurological disorder and epilepsy. Eligible patients were informed about the study and an informed consent form was filled.

Patients were assigned to HIIT, MICT, and control (CG) groups through a simple randomization technique. The participants were applied one-on-one telerehabilitation (Zoom-video conference) and upper extremity exercise program for 6 weeks/ 3 times a week, accompanied by a physiotherapist (5.9).

The upper extremity aerobic exercise program was performed with the arm ergometer provided to the participants. Pulse oximetry was used to evaluate heart rates during exercise. The exercise intensity was calculated by determining the Maximum Heart Rate (MHR) method. For HIIT, it was started with a warm-up at MHR (50%) for 5 min. The cycles were continued with high intensity at 4 min MHR (80-95%) and active recovery cycles at 3 min MHR (70%) and ended with a cooldown at 5 min MHR (50%) and the program took 35 min in total. Participants in MICT received a total of 55 min of exercise training at 65-70% of Maximum Heart Rate, following the same warm-up and cool-down period as the HIIT group. All evaluations were evaluated by the responsible physiotherapist before and after 6 weeks of treatment. Blood samples for biochemical analysis was performed by a responsible nurse. Biochemical

analyzes were completed by the responsible physician (5).

Biochemical analyzes such as MDA, myeloperoxidase (MPO), SOD, glutathione peroxidase (GSH-Px), 25-OH Vit D, free serum tryptophan, 5 hydroxyindoleacetic acid 5-HIAA were determined in the study. The all blood samples were collected in tubes and the samples were centrifuged for serum removal. The samples were centrifuged at 2000 x g for 10 min and serum samples were stored frozen at −80 °C until study data were completed. Serum concentrations of these parameters were determined with ELISA kits, using a sandwich ELISA technique (Catalogue numbers: E0880Hu for MPO, E1371Hu for MDA, E1912Hu for 5-HIAA, E0918Hu for SOD, E4244hu for tryptophan, E1981Hu for 25 OH Vit D and E0725Hu for GSH-Px). Assays were carried out according to the manufacturer's instructions. The sensitivities of each assay were 0.05 ng/ml for MPO, 0.14 nmol/ml for MDA, 9.29 ng/L for 5-HIAA, 1.52 U/L for SOD, 0.23 ug/ml for tryptophan, 0.23 ng/ml for 25 OH Vit D and 1.12 U/ml for GSH-Px (10). The inter-and intra-assay coefficients of variation were CV<10% and CV<8% and the assay range were 0.1-30 ng/ml for MPO, 0.2 nmol/ml-60 nmol/ml for MDA, 20-4500 ng/L for 5-HIAA, 3-900 U/L for SOD, 0.5-200 ug/ml for tryptophan, 0.5-150 ng/ml for 25-OH Vit D and 2-600 U/ml for GSH-Px.

Algometer (Baseline® Dolorimeter) was used on 18 tender points to evaluate the pressure pain threshold. The evaluation score was obtained by averaging the values obtained at each point. (11). Fatigue Severity Scale, was used for fatigue assessment. A low score is associated with the participant's low fatigue level (12). Hand grip strength was evaluated with a dynamometer (Jamar Hydraulic Hand Dynamometer). Patients sat in a chair with their shoulders adducted, their elbows flexed to 90°, and their forearms and wrists in a neutral position. Hand grip strength normally ranges from 30 to 50 kg, depending on age and gender (13). Psychological status was assessed with the Hospital Anxiety and Depression Scale. For anxiety and depression values, 0-7 points are considered normal, 8-10 points are borderline, and 11 points and above are considered abnormal (14). Sleep quality was assessed with the 'Pittsburgh Sleep Quality Index'. The total score in this index ranges from 0 to 21, with a higher score indicating poor sleep quality (14). Participants' functional status was assessed using the 'Revised Fibromyalgia Impact Questionnaire.' A higher score on the questionnaire indicates that fibromyalgia affects function more (15).

Statistical analysis: Appropriate sample size was calculated based on (GSH-Px) from a similar previous study using G*Power 3.1. According to this effect size, for ANOVA: Fixed Effects, omnibus, one-way test analysis, with 95% power and a=0.05, a total of 33 people, 11 people to each group, should be taken (16-17). For continuous variables, one way ANOVA and Tukey's Post Hoc Test were used. Kruskal Wallis test and Wilcoxon signed rank test were used for nonparametric variables.

RESULTS

There were no statistically significant differences between the descriptive data of the groups shown in Table 1.

Table 1. Characteristics of participants

	HIIT (n=11)	MICT (n=11)	CG (n=11)	p
Age (y), mean (SD)	46.82 (9.66)	46.45 (9.12)	51.36 (8.73)	0.38
Gender (M:F), n	1:10	1:10	1:10	1.00
Body mass index (kg/cm^2), mean (SD)	30.29 (6.01)	27.85 (4.11)	27.01 (4.79)	0.30

HIIT= high intensity interval training group, MICT= Moderate intensity continuous training group, CG= Control group, BMI= Body mass index. p<0.05

As a result of the post-study actual power analysis, the study power was determined as 0.78. When the post-treatment biochemical values of the groups were compared, given in Table 2, a significant difference was detected in SOD and MPO values. The significant difference in SOD values is due to the increase in SOD in the HIIT group and the decrease in SOD in the MICT group. The difference in MPO values is also due to the HIIT group. When the intra-group changes before and after treatment were examined, significant changes were found in SOD, MDA, GSH-Px for HIIT, and SOD and MPO values for MICT. The effect of treatment on symptoms is shown in Table 3. In the comparison between the groups after the treatment, a significant difference was found between the groups' right grip strength and fatigue values, and it was determined that this difference was caused by HIIT and CG. When the changes within the group were examined, a significant improvement was found in the pressure pain threshold, right grip and fatigue severity values in the HIIT group, and in the left grip and fatigue severity values in the MICT group (p<0.05).

Table 2. Intra- and intergroup effect of treatment on biochemical parameters

Outcomes	Group	Time period		p*	p** (before)	p** (after)
		Baseline	6 weeks			
Free Trp (ug/ml)	HIIT	15.5(7.9-73.6)	12.3(8.9-63.8)	0.15	**0.03**	0.72
	MICT	10.6(3.8-16.4)	12.1(2.8-14)	0.18		
	CG	11.1(7.5-26.1)	12.1 (6.6-28.4)	0.45		
5-HIIA(ng/L)	HIIT	437(-163-1487)	207(-13-1597)	0.59	0.40	0.37
	MICT	160(53.6-313.6)	153.6(-43-477)	0.72		
	CG	157(73.6-370.3)	207(107-463.6)	0.24		
SOD (U/L)	HIIT	79.9(37.6)-275.2)	109.1(63.2-314)	**0.008**	0.69	**0.003**
	MICT	75.9(39.9-105.8)	60.0(1.02-82.9)	**0.02**		
	CG	76.2(16.2-104.2)	71.5(56.9-335.4)	0.24		
MDA (nmol/ml)	HIIT	10.19(6.1-26.8)	10.2(7.5-36.2)	**0.004**	0.25	0.067
	MICT	8.21(5.2-11.05)	8.71(4.2-12.6)	0.37		
	CG	8.97(6.3-14.8)	9.46(6.2-16.9)	0.72		
25-OH VİT D(ng/ml)	HIIT	6.83(1.4-39.7)	7.36(4.7-39.8)	0.53	**0.02**	0.07
	MICT	5.12(0.5-9.5)	6.13(2.93-7.36)	0.21		
	CG	4.8(2.6-11.4)	5.22(2.6-12.8)	0.53		
MPO (ng/ml)	HIIT	2.98(1.3-8.6)	3.28(1.9-8.2)	0.21	0.08	**0.03**
	MICT	2.23(0.4-3.4)	1.97(0.3-3.2)	**0.02**		
	CG	2.12(1.4-4)	2.14(0.2-3.9)	0.20		
GSH(PX) (U/ml)	HIIT	17.73(1.0-107.7)	17.93(8.5-127.1)	**0.01**	0.18	0.11
	MICT	13.29(1.0-28.3)	12.58(1-27.6)	0.59		
	CG	17.86(1.0-33.7)	16.52(3.8-32.6)	0.47		

**Wilcoxon signed rank test. ** Kruskal Wallis test. HIIT= high intensity interval training group, MICT= moderate intensity continuous training group, CG= control group, SOD= Superoxide dismutase, MDA= Malondialdehyde, GSH-Px= Glutathione peroxidase, MPO= Myeloperoxidase, 5-HIIA= 5-Hydroxyindoleacetic acid, Trp= Tryptophan, OH= Hydroxide, Vit= Vitamin. p<0.05.*

Table 3. Intra- and intergroup effect of treatment on symptoms

Outcomes	Groups	Time period		p*	p** (before)	p** (after)
		Baseline	6 weeks			
Pressure pain threshold	HIIT	4.71±0.85	5.48±1.5	**0.003**	0.72	0.49
	MICT	4.82±1.53	5.20±1.49	**0.009**		
	CG	5.10±1.05	4.77±1.12	**0.03**		
Handgrip strength-R (kg)	HIIT	43.18±7.83	45±7.41	**0.038**	0.08	**0.034**
	MICT	38.64±10.02	38.18±10.55	0.341		
	CG	35.45±4.71	35.91±5.39	0.676		
Handgrip strength-L (kg)	HIIT	35.91±14.97	37.73±12.91	0.10	0.16	0.33
	MICT	27.73±7.86	31.36±10.51	**0.03**		
	CG	33.64±5.04	34.09±4.36	0.72		
Fatigue FSS (0-7)	HIIT	5.64±1.19	4.96±1.15	**0.004**	0.41	**0.031**
	MICT	6.30±0.74	5.84±0.82	**0.016**		
	CG	6.02±1.38	6.12±1.02	0.472		
Sleep quality PSQI (0-21)	HIIT	7.64±2.76	7.82±2.71	0.34	0.47	0.53
	MICT	8.91±4.39	8.64±4.36	0.19		
	CG	9.36±2.76	9.45±2.77	0.34		
HADS-Anxiety (0-21)	HIIT	7.82±4.07	7±3.60	0.28	0.38	0.19
	MICT	9.64±4.88	8.82±5.03	0.09		
	CG	10.45±4.56	10.55±4.61	0.341		
HADS-Depression (0-21)	HIIT	4.36±3.72	4.09±3.72	0.19	0.50	0.53
	MICT	5.82±3.99	5.45±4.18	0.45		
	CG	6.27±4.17	6.64±4.03	0.16		
RFIQ (0-100)	HIIT	51.91±14.30	46.45±14.83	**0.006**	0.24	**0.02**
	MICT	53.18±12.06	50.73±12.14	0.13		
	CG	60.23±9.63	64.45±9.20	0.19		

**Paired Sample T Test, **One Way ANOVA. HIIT= high intensity interval training group, MICT= moderate intensity continuous training group, CG= control group, FSS= Fatigue Severity Scale, PSQI= Pittsburgh Sleep Quality Index, HADS= The Hospital Anxiety and Depression Scale, RFIQ= Revised Fibromyalgia Impact Questionnaire, R: right, L: left. p<0.05*

DISCUSSION

We think that this study we have conducted is the first randomized controlled study to examine the effects of telerehabilitation-based HIIT on biochemical parameters in FMS patients. A study on lower extremity-based HIIT was also conducted by Atan et al. in 2020 in the FMS patient group, but biochemical parameters were not evaluated in this study. In a study evaluating the effect of exercise on 5-HIAA levels, the effect of 20-week aerobic exercise and stretching exercise programs on serotonin metabolites was evaluated, and a significant increase was found in the 5-HIAA levels of the aerobic exercise group. In a study evaluating the effects of different exercise intensities on vitamin D levels, the effects of HIIT on serum vitamin D levels in obese male adolescents were evaluated. Although it has been determined that the 8-week HIIT program improves vitamin D levels in adolescent men, it has been reported that further studies in different age ranges are needed. In the results of our biochemical analysis, no statistically significant difference was observed regarding the serotonergic system and vitamin D, but there were significant differences in oxidative stress in the exercise groups. (5,18).

Oxidative stress is very important in the pathophysiology of FMS. Under normal conditions, regular exercise reduces the level of MDA, which is a sign of oxidative stress, and increases the level of SOD. SOD and GSH-Px constitute the basic line of defense against free radicals in the cell. In the event of excessive exercise, a great increase in oxygen consumption leads to oxidative stress. In our study, in the HIIT group, exercise increased SOD, GSH-Px and MDA after treatment, leading to an increase in both oxidative stress and the antioxidant mechanism that constitutes the primary line of defense. However, MPO levels showed a significant improvement only in the MICT group, but SOD levels showed a significant decrease in this group. Recent studies have reported that oxidative stress plays a role in the pathophysiology of fibromyalgia and that lipid peroxidation products caused by oxidative stress increase in the plasma of patients. It has also been stated that the antioxidant capacity of these patient groups decreases. The reason for the decrease in SOD levels may be related to this situation (19,20,21).

Fatigue, along with pain and sensitivity, is one of the most important symptoms that negatively affect the daily life and psychological state of fibromyalgia patients. In a systematic review evaluating the effect of exercise on fatigue severity in fibromyalgia patients, it was determined that exercise was moderately effective in reducing fatigue. In our study, the effect of aerobic exercise groups on fatigue was found to be significant, while the effect values were higher in the HIIT group. Fatigue severity decreased in all exercise groups, supporting the literature. The fact that the effect size of the HIIT group was higher supports the literature (22).

Publications have reported a decrease in grip strength, especially in fibromyalgia patients. In our study, it was determined that the participants had a grip strength of 30-50 kg, which is expected in the healthy group. However, since it is dominant, it is an expected result that the right grip strength values are higher than the left. In the HIIT group, there was a significant increase in the right hand grip strength and in the MICT group in the left hand grip strength. It is known that factors such as pain intensity, depression, and sleep quality in fibromyalgia can affect a person's grip strength. For this reason, we think that we obtained different results in two separate exercise groups (23).

In relation to the revised fibromyalgia impact questionnaire, different exercise programs have been reported to improve symptoms and provide significant reductions in survey results. Due to the significant improvements in symptoms in our study, the improvements seen in the Revise Fibromyalgia Impact Questionnaire in the HIIT group are expected. Although improvements were observed in parameters such as fatigue and grip strength in the MICT group, the HIIT group developed more significant results. The fact that no significant change was observed in depression, anxiety and sleep quality in our study may be attributed to the fact that FMS is a complex disease and is related to factors such as patients' sleep status and family life.

Telerehabilitation is a widely used approach in treatment processes, especially during the pandemic period. In a study evaluating the acute effects of telerehabilitation and aerobic exercise on the symptoms of the disease in female patients with FMS, significant results were reported especially for pain symptoms. Obtaining similar results with exercise programs and face-to-face treatment programs on many symptoms in our study results is a finding that supports the effectiveness of telerehabilitation and exercise (8).

It is possible to report many limitations in the treatment process. Patients have been using drugs for a long time. They continued to use drugs during the treatment process, but a new drug was not started and the existing drug dose was not changed in order not to affect the treatment results. Another limitation is that we did not clearly assess the physical activity levels in the CG. Patients may have been involved in aerobic exercise activities such as walking and swimming, aerobic exercise is definitely recommended for this patient group. For this reason, it is not possible to restrict aerobic activity. Another shortcoming is that the evaluating therapist is not blind.

CONCLUSION

In a patient group such as FMS, where exercise is recommended as a treatment option without side effects with strong evidence, telerehabilitation-based exercise training both reduces contact and allows patients to complete their exercise processes without disrupting their daily lives. HIIT training can be considered as an effective treatment method because it produces similar results to MICT and requires a shorter treatment time. However, longer-term exercise studies are needed for symptom control and blood analysis.

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RESEARCH ARTICLE

RETROSPECTIVE EVALUATION OF DIET COMPLIANCE ON PLASMA AMINO ACID AND VITAMIN LEVELS IN PATIENTS WITH PHENYLKETONURIA

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ABSTRACT

Background: In this study, it was aimed to compare the plasma amino acid and blood vitamin/mineral levels in patients with classical phenylketonuria and healthy controls.
Methods: 54 patients with classical phenylketonuria and 22 healthy controls (76 children, 47 boys, 61.8%) were included in the study. The patient group was divided into two subgroups as high adherence to phenylalanine-restricted diet (HAD, 16 patients) and low adherence to this diet (LAD, 38 patients) according to the mean plasma phenylalanine level of the patients of the previous year. Anthropometric measurements (body weight and height and standard deviation score values), plasma phenylalanine and other amino acid levels, hemoglobin, vitamin B12, folic acid, vitamin D, zinc, ferritin levels of all groups were recorded.
Results: The mean age of the entire study group was 10.1 ± 3.6 (minimum: 3.5 - maximum: 17) years. There was no significant difference between the phenylketonuria group and the control group in terms of age, gender distribution and anthropometric data. There was a significant difference between the three groups in terms of plasma phenylalanine levels (plasma phenylalanine levels 299.0 ± 77.2; 813.7 ± 356.6 and 47.5 ± 15.9 µmol/L in HAD, LAD and control groups respectively, p= 0.001). Tryptophan was significantly lower in the HAD group than in the LAD and control groups (p= 0.001 and p= 0.006, respectively). Lysine was found to be significantly higher and histidine was lower in the HAD group than the control group (p= 0.016 and p= 0.008, respectively). Hemoglobin, vitamin B12, folic acid and 25-OH vitamin D levels were found to be significantly higher in the PKU patient group compared to healthy children and no difference between zinc and ferritin levels.
Conclusion: As a result, in patients with phenylketonuria who comply with the diet and whose anthropometric data are in the normal range, no significant deterioration in vitamin/mineral and amino acid values is observed. Compliance of the patients with a diet restricted from phenylalanine will both reduce the neurological effects and ensure that the patient is nutritionally balanced.

Keywords: Classical phenylketonuria, phenylalanine-restricted diet, hemoglobin, plasma amino acids, vitamins

INTRODUCTION

Phenylketonuria (PKU, OMIM # 261600) is an autosomal recessive inherited metabolic disease that occurs as a result of deficiency of the phenylalanine hydroxylase (PAH) enzyme that catalyzes the conversion of phenylalanine (Phe) to tyrosine,

cofactor metabolism disorder or activator protein DNAJC12 metabolism disorder [1]. Untreated PKU is characterized by irreversible intellectual disability, microcephaly, motor disorders, eczematous rash, autism, seizures, developmental and psychiatric problems [3].

The goal of treatment of hyperphenylalaninemia is to reduce the blood Phe level to acceptable limits in order to prevent central nervous system damage [4]. All infants with plasma Phe levels above 360 µmol/L should be treated with diet or other options and treatment should be started in the first week after birth [2]. This is achieved by severely reducing protein-containing foods, providing a protein substitute that includes amino acids other than Phe as well as needed macro and micronutrients, and frequent monitoring of blood Phe levels [4]. The low-Phe diets prescribed for patients with classic PKU are limited to food groups, including fruits, most vegetables, sugars, pure fats, and medically modified, low-protein food products [5]. Due to the limited intake of natural protein, micronutrients need to be supplemented in protein substitutes to prevent nutritional deficiencies. Deficiencies in selenium, zinc, and iron have been reported in patients with PKU [6]. Many complications can occur if a Phe-restricted diet is not well managed. During dietary therapy, if the patient does not get enough Phe, an essential amino acid, the blood Phe level drops and neurological symptoms may occur. Problems such as growth retardation, protein-energy malnutrition, skin lesions, anemia, osteopenia or osteoporosis, diarrhea, hair loss may occur when the diet content is not sufficiently balanced, the patient does not follow the diet or does not consume the recommended amino acid formulas regularly. There is also a risk of death in severe cases. Deficiency of nutrients such as selenium, zinc, iron, retinol, long-chain polyunsaturated fatty acids and omega-3 can sometimes be seen in patients following a strict diet [7]. Vitamin B12 deficiency may also occur in patients, which may contribute to the worsening of the neurological picture [8]. This study aimed to investigate the relationship between diet compliance and plasma amino acid and blood vitamin/mineral levels in patients with classical phenylketonuria who were receiving diet therapy.

MATERIALS AND METHODS

Study Design

This study is a cross-sectional descriptive study planned to investigate the relationship between the adherence to Phe-restricted diet and plasma amino acid and blood vitamin/mineral levels in patients with PKU, who are being followed up at Dokuz Eylul University Faculty of Medicine, Division of Pediatric Metabolism and Nutrition. Fifty-four patients aged between 3.5 and 17 years who were being followed up and treated with the diagnosis of classical PKU were included in the study. All patients were referred to our hospital with high Phe levels in the national newborn screening program.

Having a chronic disease that requires other treatment in addition to PKU, having taken vitamin/mineral therapy in the last three months due to micronutrient deficiency, having a disease that will cause malabsorption (lactose intolerance, giardiasis, cystic fibrosis, celiac disease), growth hormone deficiency or hypothyroidism, had severe mental retardation, had uncontrolled epilepsy, and did not regularly come to their outpatient control visits not included in the study. In addition, 3 patients with BH4 metabolism disorder, 50 patients with hyperphenylalaninemia who were followed without diet, 20 patients on large neutral amino acid therapy, and 90 patients with PKU who were receiving sapropterin dihydrochloride in addition to Phe-restricted diet were excluded from the study. Patients who did not give consent to participate in the study were also excluded from the study.

The PKU patient group (54 patients) was divided into two subgroups according to the mean plasma Phe level of the patients in the last year. Those with a mean Phe level between 120 and 360 µmol/L in the last year were considered high adherence to diet (HAD, 16 patients), and those over 360 µmol/L were considered low adherence to diet (LAD, 38 patients). Twenty-two healthy children between the ages of 3.5 and 17 who applied to our clinic for family screening and did not receive any treatment were included in the study as the control group. Demographics (age, gender), anthropometric measurements (body weight and height and standard deviation score values), plasma Phe level and amino acid levels, hemoglobin, vitamin B12, folic acid, vitamin D, zinc, ferritin levels of all groups were measured.

Laboratory Analyses

All measurements were made on a blood sample taken in the morning after an overnight fast. Plasma amino acid levels were determined by HPLC, serum vitamin B12, folic acid and ferritin levels were determined by immunoassay method (Unicel DxI800,

Beckman Coulter, CA. USA), serum zinc levels and hemoglobin levels were determined by spectrophotometry (AU5800 analyzer, Beckman Coulter, CA. USA), 25-OH vitamin D level was analyzed by immunoassay method (Advia Centaur XP Analyzer, Siemens Healthcare Diagnostics, Erlangen, Germany). Serum limit values of micronutrients were determined as follows: B12 vitamin: 200-1900 pg/mL, folic acid: 3-17.5 ng/mL, ferritin: 14-325 ng/mL, 25-OH vitamin D: 20-42 ng/mL, zinc: 65-140 µg/dL (9,10,11).

Ethical Approval

The study protocol was designed in compliance with the Declaration of Helsinki, 1964. Non-interventional Research Ethics Committee of Dokuz Eylul University approved this study (Date: 19.10.2022, Decision No: 2022/33-03). Informed consent was obtained from the families before enrollment in the study.

Statistical analyses

Statistical analysis was performed in SPSS Software 22.0. Categorical data were expressed as numbers and percentages (%), whereas numeric data were expressed as arithmetic mean ± standard deviation. The conformity of the variables to the normal distribution was analyzed with the Kolmogorov-Smirnov test. In comparison of group ratios, Fischer's exact test was used instead of chi-square test when expected values in the cells were below 5. Student t-test was used to compare group means, and Mann Whitney-U test was used if the distribution of groups did not show normal distribution. The difference between the three groups was analyzed with the Kruskal Wallis test, and the Mann Whitney-U test was used as a post hoc test. In all statistical analyzes, a p value below 0.05 was considered significant.

RESULTS

A total of 76 children (47 boys, 61.8%; mean age 10.1 ± 3.6 [3.5-17] years) were included in the study, 54 of whom were PKU patients and 22 healthy controls. There was consanguinity between the parents of 19 patients (35.2%) in the phenylketonuria group and 6 patients (27.2%) in the control group (p = 0.597). There was no significant difference between the PKU group and the control group in terms of age, gender distribution and anthropometric data (Table 1). Physical examination of all patients was performed and no macro or micronutrient deficiency findings such as edema, skin wounds, hair loss, delayed wound healing, rickets, frequent infections

Table 1. Demographic, anthropometric features and, hemoglobin, vitamin and mineral levels of the phenylketonuria group and the control group

Parameter	Phenylketonuria group (n= 54)	Control group (n=22)	p value
Age (years)	10.7 ± 3.7	8.9 ± 2.9	0.056
Gender (M/F)	30/24	17/5	0.118
Body weight (kg)	42.7 ± 17.0	35.9 ± 17.6	0.132
Body weight SDS	0.02 ± 1.02	0.24 ± 1.21	0.460
Height (cm)	145.7 ± 20.2	135.6 ± 21.5	0.068
Height SDS	-0.08 ± 1.11	0.11 ± 1.18	0.521
Hemoglobin (g/dL)	13.2 ± 1.3	12.5 ± 1.3	**0.035**
Vitamin B12 (pg/mL)	388.3 ± 224.3	294.0 ± 116.2	**0.019**
Vitamin B12 (<200 pg/mL), n (%)	10 (18.5)	1 (4.5)	0.085
Folic acid (ng/mL)	15.0 ± 4.0	10.9 ± 4.8	**0.001**
Folic acid (>17.5 ng/mL), n (%)	15 (27.8)	2 (9.1)	**0.050**
25-OH vitamin D (ng/mL)	26.1 ± 9.8	21.1 ± 6.4	**0.012**
25-OH vitamin D (<20 ng/mL), n (%)	16 (29.6)	10 (45.5)	0.192
Zinc (µg/dL)	95.1 ± 16.4	98.6 ± 21.4	0.451
Zinc (<65 µg/dL), n(%)	3 (5.6)	0 (0.0)	*
Ferritin (ng/mL)	30.2 ± 16.8	24.5 ± 15.4	0.176
Ferritin (<16 ng/mL), n (%)	8 (14.8)	7 (31.8)	0.058

* Comparison could not be made because the groups had a value of 0.

Table 2. Plasma amino acid levels of the phenylketonuria group and the control group (µmol/L)

Parameter	Phenylketonuria group (n= 54)	Control group (n=22)	p value
Phenylalanine	580.1 ± 355.5	47.5 ± 15.9	**0.001**
Tyrosine	68.8 ± 48.8	64.6 ± 18.2	0.582
Tryptophan	60.1 ± 26.5	64.0 ± 16.5	0.528
Leucine	120.9 ± 86.3	107.9 ± 35.0	0.496
İzoleucine	73.2 ± 53.5	68.3 ± 26.5	0.683
Valine	258.3 ± 132.8	260.3 ± 90.4	0.947
Methionine	30.2 ± 14.8	27.1 ± 6.3	0.353
Lysine	165.6 ± 66.5	128.5 ± 44.1	**0.019**
Threonine	131.7 ± 53.6	123.3 ± 39.8	0.510
Histidine	76.0 ± 20.1	90.8 ± 18.9	**0.004**
Homocysteine	6.9 ± 3.8	8.5 ± 6.3	0.191

were detected in any patient or child in the control group. There were no signs of protein or essential aminoacid deficiency (skin lesions, diarrhea, edema) in any of the PKU patients.

Hemoglobin, vitamin B12, folic acid and 25-OH vitamin D levels were found to be significantly higher in the PKU patient group compared to healthy children (Table 1). There was no difference between zinc and ferritin levels. The ratio of patients with high folic acid levels in the phenylketonuria group was significantly higher than the control group (p=0.050). Although vitamin B12 levels were significantly higher in the PKU group, the rate of vitamin B12 deficiency was 18.5% in this group and 4.5% in the control group, but no significant difference was detected between the two groups (Table 1). Similarly, plasma homocysteine levels did not show any significant difference between the two groups (Table 2).

When amino acid values were compared between the two groups, a significant difference was found in plasma phenylalanine, lysine and histidine levels (Table 2). Other investigated amino acids were similar between the two groups.

The PKU group (54 patients) was divided into two subgroups according to the mean plasma Phe level of the patients in the last year. When the vitamin and mineral values of the two PKU groups and the control group were compared, a significant difference was found between the three groups in terms of folic acid and ferritin levels (Table 3). When paired group comparisons were made in terms of these parameters, it was found that the folic acid value was higher in the HDA and LDA groups than in the control group (p= 0.003 and p= 0.001, respectively); ferritin level was found to be significantly higher in the HDA group than in the LDA and control group (p= 0.012 and p= 0.004, respectively).

A significant difference was found between the phenylketonuria groups, which were compatible and incompatible with the diet, and the control group in terms of phenylalanine, tryptophan, lysine and histidine values (p= 0.001, p= 0.001, p= 0.008 and p= 0.007, respectively) (Table 4). When paired group comparisons were made in terms of these parameters, tryptophan was significantly lower in the HDA group than in the LDA and control groups (p= 0.001 and p= 0.006, respectively); lysine was found to be significantly higher in the HDA group than the control group (p= 0.016), while histidine was significantly lower in the HDA group than the control group (p= 0.008).

DISCUSSION

The primary approach to treating phenylketonuria (PKU) involves implementing a phenylalanine (Phe)-restricted diet. Compliance with a life-long Phe-restricted diet is difficult. This requires reducing the intake of natural proteins and replacing them with a protein source that lacks Phe, consisting of various amino acids. However, maintaining strict adherence to the PKU diet can be challenging for patients and their families, particularly over the long term. In this study, the effect of Phe-restricted diet compliance on the nutritional parameters and the amino acid of patients with phenylketonuria was investigated. The exclusion of patients who received BH4 or large neutral amino acid therapy in addition to diet therapy enabled us to better analyze whether dietary adherence and the amino acid formulas used had an effect on micronutrients and plasma amino acid levels.

Table 3. Hemoglobin, vitamin and mineral levels of two phenylketonuria groups and the control group

Parameter	PKU group (high adherence to diet) (n= 16)	PKU group (low adherence to diet) (n= 38)	Controls (n=22)	p value
Hemoglobin (g/dL)	13.1 ± 1.7	13.3 ± 1.1	12.5 ± 1.3	0.071
Vitamin B12 (pg/mL)	389.6 ± 201.8	387.8 ± 235.8	294.0 ± 116.2	0.214
Vitamin B12 (<200 pg/mL), n (%)	2 (12.5)	8 (21.1)	1 (4.5)	0.170
Folic acid (ng/mL)	14.8 ± 3.3	15.0 ± 4.3	10.9 ± 4.8	**0.001**
Folic acid (>17.5 ng/mL), n (%)	4 (25.0)	11 (28.9)	2 (9.1)	0.160
25-OH vitamin D (ng/mL)	27.5 ± 9.9	25.5 ± 9.8	21.1 ± 6.4	0.135
25-OH vitamin D (<20 ng/mL), n (%)	4 (25.0)	12 (31.6)	10 (45.5)	0.379
Zinc (µg/dL)	87.7 ± 19.4	98.3 ± 14.2	98.6 ± 21.4	0.172
Zinc (<65 µg/dL), n(%)	3 (18.8)	0 (0.0)	0 (0.0)	*
Ferritin (ng/mL)	40.7 ± 22.2	25.8 ± 11.8	24.5 ± 15.4	**0.011**
Ferritin (<16 ng/mL), n (%)	1 (6.3)	7 (18.4)	7 (31.8)	0.123

* Comparison could not be made because there was a value of 0 in the groups.

Table 4. Plasma amino acid levels of the phenylketonuria groups that were compatible and incompatible with the diet and the control group (µmol/L)

Parameter	PKU group (high adherence to diet) (n= 16)	PKU group (low adherence to diet) (n= 38)	Controls (n=22)	p value
Phenylalanine	299.0 ± 77.2	813.7 ± 356.6	47.5 ± 15.9	**0.001**
Tyrosine	69.1 ± 60.3	68.7 ± 44.0	64.6 ± 18.2	0.547
Tryptophan	44.9 ± 12.7	66.5 ± 28.3	64.0 ± 16.5	**0.001**
Leucine	104.7 ± 57.2	127.7 ± 95.9	107.9 ± 35.0	0.608
İzoleucine	60.8 ± 31.4	78.5 ± 60.1	68.3 ± 26.5	0.534
Valine	237.9 ± 101.0	266.8 ± 144.5	260.3 ± 90.4	0.640
Methionine	28.1 ± 11.1	31.0 ± 16.2	27.1 ± 6.3	0.878
Lysine	143.6 ± 63.6	174.8 ± 66.3	128.5 ± 44.1	**0.008**
Threonine	129.3 ± 46.3	132.7 ± 56.9	123.3 ± 39.8	0.956
Histidine	76.7 ± 15.3	75.7 ± 21.9	90.8 ± 18.9	**0.007**
Homocysteine	6.4 ± 2.6	7.1 ± 4.2	8.5 ± 6.3	0.485

In patients with classical PKU, a Phe-restricted diet is applied to reduce plasma Phe levels and to keep this level in a range that does not harm the brain, and this diet may cause growth retardation in patients [12,13]. In this study, no significant difference was found between the PKU patient groups and the control group in terms of weight and height SDS values. On the other hand, in recent years, it has been found that the prevalence of obesity has increased in this patient group over the years, since patients with PKU have too many Phe-restricted food options [14,15,16]. Since there may be differences in vitamin/mineral

levels in obesity and also malnutrition, there were no patients or controls with obesity as well as moderate-to-severe malnutrition in our study.

In our study, hemoglobin, vitamin B12, folic acid, and 25-OH vitamin D levels were found to be significantly higher in patients with PKU when compared to the control group. Ferritin levels were found to be significantly higher in the HDA patient group than in the LDA and control groups. Since protein-rich natural foods such as meat, chicken, fish, eggs, legumes and nuts are completely excluded from the diet in patients on a Phe-restricted diet, nutritional anemia may occur frequently in this patient group [5,12,17]. Patients with PKU must consume amino acid mixtures or formulas that do not contain Phe to meet their daily protein needs. Especially in the last decades, data on micronutrient deficiency in patients with PKU have been increasing; based on these data, micronutrients are added to these formulas and the contents of the formulas are updated [18,19]. Patients with PKU regularly consume these products, which meet approximately 80% of their daily protein needs, in three or four meals a day, and at the same time, these products complement the patients' vitamin, mineral and essential fatty acid requirements. Because diet-compliant patients consume these formulas regularly, micronutrient deficiencies now rarely occur in the compliant group [19,20,21]. In PKU patients who do not comply with the diet, diet leaks are usually made with foods that are poor in iron, zinc, essential fatty acids, calcium and vitamin B12, such as bread, potatoes, rice. Consuming these foods more than allowed causes the patients to become fat, but on the other hand, the level of phenylalanine increases, the intake of vitamins/minerals decreases and the blood values of these nutrients decrease. In studies conducted in the last decade, 5-14% deficiency rates were found in zinc levels in patients with classical PKU [17,20]. In our previous study, no difference was found between the patients with classical PKU and the healthy control group in terms of serum zinc levels; zinc deficiency was detected at a rate of 0.9% in the patient group and 5.6% in the control group [5]. In this study, zinc deficiency was detected in 3 patients, and there were no patients with deficiency in the control group. This may be due to the small number of individuals in all three groups.

In our study, hemoglobin, vitamin B12, and folic acid were found to be significantly higher in patients with PKU when compared to the control group. In the 1990s, approximately 10% of patients with PKU found folic acid deficiency and 30% vitamin B12 deficiency, while in recent studies, folic acid and vitamin B12 levels were found to be similar to the control group or higher in patients with PKU than in healthy controls [5,18,21,22]. This was due to the fact that the patients consumed more folic acid-rich vegetables and fruits than the control groups, and that the amino acid formulas were rich in folic acid and vitamin B12, resulting in the absence or frequency of folic acid and vitamin B12 deficiency in the patient group [18].

When the PKU group and the control group were compared in terms of plasma amino acid values, the plasma lysine level was found to be significantly higher and histidine level was low in the PKU group. There was no difference between other amino acid levels. When comparing the three groups, tryptophan was significantly lower in the HDA group than in the LDA and control groups; lysine was found to be significantly higher in the HDA group compared to the control group, and histidine was found to be significantly lower in the HDA group than the control group. There are few studies evaluating blood levels of amino acids other than Phe and tyrosine in patients with PKU who are on diet therapy, and there are discrepancies between the results of these studies. In a study conducted in China, tyrosine, alanine, asparagine, glutamine, methionine, arginine, glycine, glutamine, ornithine, and threonine were found to be significantly lower than the control group in the group that included patients with PKU who did and did not comply with the diet; valine, histidine and serine levels were found to be significantly higher [24]. In another study, it was found that arginine, citrulline, valine, methionine levels were high and tyrosine levels were low in patients at the time of initial diagnosis, and citrulline and valine levels remained high, but improvements were observed in other amino acid levels after Phe-restricted diet and amino acid formulas were given to the patients [23,24]. In our study, we did not measure the plasma amino acid levels of infants before treatment, but the results of both studies and our study do not fully overlap. According to another study by Matuszewska and colleagues, 28 different parameters were analyzed in 20 classic phenylketonuria (PKU) patients, and levels of kynurenine, tryptophan, asparagine, and proline were found to be significantly lower compared to the control group. This finding suggests that dietary restriction of phenylalanine in PKU patients can affect not only this amino acid but also the levels of other essential and non-essential amino acids. It is

particularly emphasized that some amino acids and metabolites, such as kynurenine, which are important for immune functions, inflammation, and niacin synthesis, may be affected in this process [26]. . These results suggest that the amino acid balance in the treatment of PKU patients should be carefully monitored.

All these differences may be caused by the treatment compliance of the patients, the content of the given amino acid formulas, the sources of natural proteins, and the differences in the general nutritional status of the patients. In our study, the amounts of amino acids and vitamins/minerals that the patients take with diet were not calculated.

There was no significant difference in plasma tyrosine levels between the whole patient group with PKU and the control group, and also between the PKU groups that were compatible and incompatible with the diet and the control group. Similarly, no correlation was found between plasma Phe and tyrosine levels in groups with PKU. Since patients with classical PKU have complete deficiency of the enzyme phenylalanine hydroxylase, which converts phenylalanine to tyrosine, tyrosine deficiency may occur as a result of failure of tyrosine synthesis in these patients [24]. In fact, since melanin cannot be synthesized from tyrosine, these patients may have light skin, hair and eye color [24,25]. As mentioned earlier, patients with phenylketonuria meet more than 80% of their protein needs from amino acid mixtures that do not contain phenylalanine. In order to prevent secondary tyrosine deficiency in patients with PKU, these amino acid mixture products are enriched with tyrosine. Therefore, tyrosine deficiency is not observed in patients with PKU today. In our study, low tyrosine was not found in patients with PKU. There was no skin or hair color change in the patients included in the study.

The first limitation of our research is that the number of cases in each group is relatively small. Phenylketonuria is a rare disease, so a small number of patients are followed in each center. In addition, since the amino acid levels and micronutrients we used in our study are affected by many conditions, the exclusion criteria were kept in large numbers in our study in order to interpret the findings more accurately. This caused the number of individuals included in the groups to be low. The second limitation is that only the follow-up parameters examined in routine controls were used in our study, since the study was conducted from file information. In prospective studies, in addition to these parameters, studies can be conducted with more micronutrients such as selenium, essential fatty acids, carnitine, vitamins A and E. The third limitation is that since our study was conducted retrospectively, the daily dietary vitamin-mineral levels of the patients could not be calculated.

As a result, in patients with phenylketonuria who comply with the diet and whose anthropometric data are in the normal range, there is no significant deterioration in vitamin/mineral and amino acid values. Therefore, patients with PKU should follow a Phe-restricted diet and regular vitamin/mineral follow-ups are required.

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RESEARCH ARTICLE

DEMENTIA-RELATED KNOWLEDGE, ATTITUDES, AND PRACTICES AMONG FAMILY MEDICINE RESIDENTS

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ABSTRACT

Purpose: Primary care physicians are often the first to encounter dementia patients, highlighting the importance of their roles in early detection and management. This study evaluates the knowledge, attitudes, and practices regarding dementia of family medicine residents (FMRs) in Türkiye.
Materials and Methods: This cross-sectional study was conducted from March to April 2022 using an online questionnaire distributed to family medicine clinics across Türkiye. The questionnaire, consisting of three sections, demographics, the Dementia Knowledge Assessment Scale (DKAS), and the Dementia Attitude Scale (DAS), was distributed using Google Forms.
Results: Three hundred ninety-three FMRs, with a mean age of 28.9±3.0 years, 71.2% of whom were women, participated. The mean DKAS score was 18.33±5.11, and the mean DAS score was 93.48±12.29. Higher knowledge levels were associated with receipt of postgraduate training, experience in dementia care, and willingness to screen for dementia. Positive attitudes were associated with more years in residency, a family history of dementia, and prior dementia-related education.
Conclusion: FMRs demonstrated limited knowledge of, but positive attitudes toward dementia. This study underscores the need for enhanced dementia training in residency programs to improve early detection and management skills among primary care physicians. Addressing gaps in knowledge and confidence can lead to better patient outcomes and more effective dementia care.

Keywords: Alzheimer's disease, attitude, dementia, family medicine, knowledge

INTRODUCTION

Dementia is a complex and debilitating syndrome characterized by a progressive decline in cognitive functions, impacting memory, thinking, and behavior sufficiently to interfere with daily activities (1). Alzheimer's disease is the most prevalent form of dementia, accounting for 60-70% of cases. Globally, approximately 9.9 million new cases of dementia are reported annually, making it the second-most important cause of disability and the seventh-leading cause of death among individuals aged 70 and older (2). This rising prevalence presents a significant challenge, not only to affected individuals and their families, but also to national healthcare systems and economies. This global burden necessitates the involvement of family physicians in timely dementia care.

Primary care physicians (PCPs) frequently serve as the initial point of contact within the healthcare system for patients exhibiting early signs of dementia. Given their longstanding relationships with patients, PCPs are uniquely positioned to observe and identify early cognitive changes (3). The general practice setting provides an ideal environment for early detection and intervention, which is crucial for the effective management of dementia (4). Practical dementia

care in primary settings relies heavily on the implementation of established diagnostic and treatment guidelines, such as those developed in the USA and Canada (5, 6). These guidelines emphasize the importance of early diagnosis and comprehensive management strategies to improve patient outcomes and alleviate the burden on caregivers (6).

Despite the availability of these guidelines, several studies have highlighted significant barriers to optimal dementia care in primary care settings. These barriers include inadequate knowledge, insufficient training, and negative attitudes toward dementia among healthcare professionals (3). PCPs' knowledge and attitudes towards dementia significantly influence their diagnostic and management practices, impacting the quality of care for patients and their families (4). Studies from various countries have revealed that many PCPs lack confidence in diagnosing and managing dementia, underscoring the need for enhanced education and training programs (7-9).

The role of family medicine residents (FMRs) is crucial in the context of dementia care. As future primary care providers, their training and preparedness to handle dementia cases will shape the future nature of dementia care. Understanding the current knowledge, attitudes, and practices regarding dementia of FMRs is essential for developing targeted educational interventions that can bridge existing gaps (10, 11).

Research has shown that well-structured dementia education programs can significantly improve knowledge, attitudes, and self-confidence in dementia care among FMRs (4, 12). These programs typically cover various aspects of dementia care, including early detection, diagnostic criteria, management strategies, and communication with patients and their families (13, 14). By improving FMRs' competency in these areas, such educational initiatives can lead to better patient outcomes, and thus to a more effective healthcare system (12).

To summarize, dementia represents a growing public health challenge with far-reaching implications for individuals, families, and healthcare systems worldwide. PCPs, and particularly FMRs, play a pivotal role in the early detection and management of dementia. Addressing the gaps in knowledge, attitudes, and practices among these healthcare providers through targeted education and training programs is essential for enhancing the quality of dementia care. This study evaluated dementia-related knowledge, attitudes, and practices among family medicine residents, thus providing insights capable of shaping the development of effective educational interventions.

MATERIALS AND METHODS

This cross-sectional study was conducted in Türkiye in March and April 2022 using an online questionnaire. The eligibility criteria were: (1) working as an FMR in Türkiye, (2) being an FMR currently enrolled in a full-time residency program (in addition to the full-time residency program, there is also a contract residency program in family medicine for family physicians working in the field in Türkiye), (3) junior and senior FMRs reflecting different levels of experience and training, and (4) willingness to complete the survey. The exclusion criterion was an incomplete questionnaire. Ethics committee approval was obtained from Ondokuz Mayıs University Clinical Research Ethics Committee (Date: 23.02.2022, Decision No: OMUKAEK 2022/89) before data collection. The study population consisted of FMRs in family medicine clinics at universities and training and research hospitals across Türkiye. The sample size was calculated using OpenEpi, with a confidence level of 95%. Since there are no previous studies on this subject in Türkiye, a 50% probability estimate was employed. Based on this assumption, the minimum sample size was calculated at 384 participants.

Family medicine clinics across Türkiye were selected using a cluster sampling method. The clusters were based on geographical regions to ensure a representative sample. At least four clinics from each region were contacted. A list of medical-dental hospitals with authorized specialty education programs was obtained from the official website "https://tuk.saglik.gov.tr/", a list of institutions authorized to provide training in family medicine. The clinics identified as providing training in this field were then identified, and the chief residents or trainers in these selected clinics were contacted to facilitate questionnaire distribution. Questionnaires were sent to chief assistants or trainers in the selected clinics. The questionnaire was created using Google Forms and was distributed via clinic e-mail groups or clinic WhatsApp groups, ensuring wide distribution and convenience for respondents. Since all resident physicians use smartphones, particular efforts were made to ensure the completion of the online form by contacting the relevant authorities in each department

Table 1. FMRs' characteristics (n=393)

Variables	Category	n (%)
Sex	Female	280 (71.2)
	Male	113 (28.8)
Age (years)	<30	258 (65.6)
	≥30	135 (34.4)
Years in the profession (years)	<5	258 (65.6)
	≥5	135 (34.4)
Residency	First-year	197 (50.1)
	Second-year	59 (15.0)
	Third-year	137 (34.9)
Presence of dementia in the family	Yes	112 (28.5)
	No	281 (71.5)
Encountering a dementia patient during undergraduate education	Yes	347 (88.3)
	No	46 (11.7)
Receipt of education regarding dementia after graduation	Yes	66 (16.8)
	No	327 (83.2)
Involvement in the diagnosis, treatment, or follow-up of dementia during the residency	Yes	110 (28.0)
	No	283 (72.0)
Testing patients for cognitive impairment	Yes	185 (47.2)
	No	207 (52.8)
Evaluating an elderly patient with suspected dementia in terms of cognitive impairment	Yes	317 (80.7)
	No	76 (19.3)
Willing to actively screen for the early symptoms of dementia	Yes	350 (89.1)
	No	43 (10.9)
Wishing to take part in educational sessions to improve one's dementia knowledge and patient management skills	Yes	374 (95.2)
	No	19 (4.8)

as frequently as possible. The questionnaire was completed with a maximum of three reminders being sent to the faculty member or chief assistant contacted. In the event that no response was received from the relevant institution despite three reminders being issued, the survey was continued by passing to another institution in the same region.

In order to ensure the reliability and validity of the results, a pilot study was conducted prior to the main survey. Feedback from the pilot study was used to refine the questionnaire. The questionnaire was based on a review of the previous literature (4, 8, 9, 13, 14) and consisted of three sections:

1. Demographic Data: This section included questions concerning the participant's age, sex, years in the profession, dementia training, working experience with dementia patients, the presence of a patient with dementia in the family, and willingness to attend dementia training sessions.

2. The Dementia Knowledge Assessment Scale (DKAS): Developed by Annear et al., the DKAS is a 17-item five-point Likert-type scale with possible scores ranging from 0 to 34 (15). Higher scores indicate greater knowledge of dementia. A Turkish validity and reliability study was conducted by Akyol et al. to ensure the scale's appropriateness in the Turkish context (16). Permission to use the scale was obtained from the authors of the original research.

3. The Dementia Attitude Scale (DAS): Developed by O'Connor and McFadden (17) and validated in Turkish by Çetinkaya et al., the DAS is a 20-item seven-point Likert-type scale (18). Possible scores range from 20 to 140, with higher scores indicating a more positive attitude toward dementia. The requisite permission to employ the scale was obtained from the authors of the original research.

Data were analyzed using IBM SPSS Statistics version 21.0. Descriptive statistics were used to express the data as numbers, percentages, means, standard deviations, and medians (minimum-maximum values). The normality of distribution was assessed using the Kolmogorov-Smirnov test and graphical methods. The independent-sample t-test and ANOVA were used to compare continuous variables. Pearson's correlation and linear regression analyses were performed to explore relationships between variables. Statistical significance was set at $p < 0.05$.

RESULTS

Three hundred ninety-three FMRs were included in this study. The mean age of the FMRs was 28.9±3.0 years, 71.2% were women, 65.6% were younger than 30, 34.4% had been in the profession for five years or more, 50.1% were in their first years of residency, 28.5% had a family member or relative diagnosed with dementia, 88.3% had encountered a patient with dementia during undergraduate training, 16.8% had received postgraduate training in dementia, and 28.0% had been involved in the diagnosis, treatment, or follow-up of a patient with dementia during their residency. Those with experience of working with individuals with dementia from their family medicine residencies were most likely to work in home care and palliative care. Analysis showed that 47.2% of FMRs reported having administered a cognitive test to patients. While 87.1% of those who used cognitive testing did so when they suspected a cognitive disorder, 32.3% used cognitive testing during routine geriatric examinations. Of the FMRs who reported using a cognitive test, 95.7% used the standardized Mini-Mental State Examination (MMSE), 38.7% applied the Clock Drawing test, and 4.8% employed other tests. When those who did not perform cognitive tests were asked their reasons for this, 68.1% felt that they were not competent to administer and interpret cognitive tests, 61.4% felt sufficiently competent to perform a neuropsychiatric evaluation, 53.1% cited time constraints, and 44.9% stated that tests should be administered by relevant specialists (in the fields of neurology, geriatrics, and psychiatry). In addition, 80.7% of FMRs reported that they would screen older patients with suspected cognitive impairment or dementia for depression, 89.1% wished to screen for early signs of dementia actively, and 95.2% wanted to receive training to improve their dementia knowledge, and patient management skills (Table 1).

Table 2. Relationships between FMRs' knowledge and attitude scores and sociodemographic and descriptive characteristics

Variable	Category	DKAS		DAS	
		Mean±SD	p	Mean±SD	p
Gender	Female	18.34±5.15	0.951	93.67±12.10	0.634
	Male	18.30±5.02		93.02±12.80	
Age (years)	<30	18.54±4.92	0.245	93.67±12.30	0.677
	≥30	17.91±5.43		93.12±12.32	
Years in the professions	<5	18.51±4.89	0.329	92.71±11.95	0.086
	≥5	17.98±5.51		94.56±12.85	
Year of residency	1	17.78±5.43		91.98±12.41	
	2	18.34±4.70	0.063	94.17±10.62	**0.044**
	≥3	19.11±4.71		95.34±12.59	
Presence of dementia in the family	Yes	18.96±4.95	0.118	96.13±12.00	**0.007**
	No	18.07±5.15		92.43±12.27	
Encountering a dementia patient during undergraduate education	Yes	18.41±5.13	0.342	94.07±12.43	**0.009**
	No	17.65±4.96		89.02±10.27	
Receipt of education regarding dementia after graduation	Yes	19.64±5.46	**0.022**	95.39±14.96	0.243
	No	18.06±5.00		93.10±11.67	
Involvement in the diagnosis, treatment, or follow-up of dementia during the residency	Yes	19.22±5.61	**0.043**	95.16±14.08	0.123
	No	17.98±4.86		92.83±11.49	
Testing patients for cognitive impairment	Yes	18.68±5.25	0.208	96.17±13.04	**<0.001**
	No	18.02±4.98		91.01±11.04	
Evaluating an elderly patient with suspected dementia in terms of cognitive impairment	Yes	18.48±5.02	0.223	94.22±12.23	**0.015**
	No	17.68±5.45		90.42±12.17	
Willingness to actively screen for early symptoms of dementia	Yes	18.53±5.05	**0.027**	94.40±12.29	**<0.001**
	No	16.70±5.31		86.00±9.55	
Wishing to take part in educational sessions to improve one's dementia knowledge and patient management skills	Yes	18.36±5.10	0.514	93.83±12.09	**0.012**
	No	17.58±5.34		86.58±14.44	

*SD: Standard deviation; bold values indicate statistical significance. *ANOVA, post hoc LSD test.*

Table 3. Linear regression models predicting dementia knowledge scores

Model	Factors	B (95%CI)	Beta	t	p
1	(Constant)	18.061 (17.509-18.613)		64.299	**<0.001**
	Receipt of education regarding dementia after graduation	1.575 (0.228-2.923)	0.115	2.298	**0.022**
2	(Constant)	16.560 (15.039-18.082)		21.397	**<0.001**
	Receipt of education regarding dementia after graduation	1.475 (0.130-2.820)	0.108	2.156	**0.032**
	Willingness to actively screen for early symptoms of dementia	1.704 (0.093-3.315)	0.104	2.080	**0.038**

B, standardized regression coefficients; Beta, non-standardized regression coefficients
Model 1: F= 5.281, p=0.022, Adjusted R^2=0.011, Model 2: F= 4.825, p=0.009, Adjusted R^2=0.019

Table 4. Linear regression models predicting dementia attitude scores

Model	Factors	B (95% CI)	Beta	t	p
1	(Constant)	86.000 (82.397-89.603)		46.929	**<0.001**
	Willingness to actively screen for early symptoms of dementia	8.361 (4.543-12.179)	0.213	4.305	**<0.001**
2	(Constant)	84.067 (80.415-87.719)		45.258	**<0.001**
	Willingness to actively screen for early symptoms of dementia	7.940 (4.191-11.689)	0.202	4.164	**<0.001**
	Testing patients for cognitive impairment	4.890 (2.543-7.237)	0.199	4.097	**<0.001**
3	(Constant)	82.641 (78.921-86.361)		43.680	**<0.001**
	Willingness to actively screen for early symptoms of dementia	8.117 (4.409-11.826)	0.207	4.303	**<0.001**
	Testing patients for cognitive impairment	5.111 (2.786-7.436)	0.208	4.322	**<0.001**
	Presence of dementia in the family	4.110 (1.537-6.684)	0.151	3.140	**0.002**
4	(Constant)	78.888 (74.079-83.698)		32.248	**<0.001**
	Willingness to actively screen for early symptoms of dementia	8.131 (4.445-11.818)	0.207	4.337	**<0.001**
	Testing patients for cognitive impairment	4.938 (2.623-7.253)	0.201	4.194	**<0.001**
	Presence of dementia in the family	3.988 (1.428-6.548)	0.146	3.063	**0.002**
	Encountering a dementia patient during undergraduate education	4.369 (0.788-7.951)	0.115	2.399	**0.017**

B, standardized regression coefficients; Beta, non-standardized regression coefficients
Model 1: F=18.533, p<0.001, Adjusted R^2=0.043, Model 2: F=18.033, p<0.001, Adjusted R^2=0.080, Model 3: F=15.583, p<0.001, Adjusted R^2=0.101, Model 4: F=13.269, p<0.001, Adjusted R^2=0.112

The FMRs' mean overall DKAS score was 18.33±5.11, and their mean DAS score was 93.48±12.29. DKAS scores were higher among those FMRs who had received postgraduate training in dementia (p=0.022), who had participated in the diagnosis, treatment, and follow-up of a dementia patient (p=0.043), and who actively wished to screen for early signs of dementia (p=0.027). Positive attitudes towards dementia increased in line with the number of years spent in residency (p=0.44). DAS positive attitude scores were higher among FMRs with a relative diagnosed with dementia (p=0.007), who had encountered a patient with dementia during their undergraduate training (p=0.009), who used

cognitive tests to evaluate cognitive disorders in their clinical practice (p<0. 001), who indicated that they would screen a patient with suspected cognitive impairment or dementia for depression (p=0.015), who would actively screen for early signs of dementia (p<0.001), and who would willingly attend training to improve their knowledge and management skills (p=0.012) (Table 2).

A positive relationship was observed between knowledge and attitude scores (r=0.306, p<0.001). In the linear regression model applied between the variables and the DKAS, receipt of training on dementia after graduation and willingness to actively screen for early symptoms of dementia emerged as determinants of attitude (Table 3).

In the linear regression model applied between the variables and the DAS, willingness to actively screen for early symptoms of dementia, testing patients for cognitive impairment, a family history of dementia, and having dealt with a patient with dementia during undergraduate training emerged as determinants of attitude (Table 4).

DISCUSSION

This study assessed the knowledge, attitudes, and practices regarding dementia of FMRs in Türkiye and the factors affecting these. The findings indicate that while FMRs possess generally positive attitudes toward dementia, their knowledge about the condition is limited, and they do not feel competent to perform neurocognitive assessments. This underscores the need for enhanced dementia-related content in residency training programs.

Comparative studies from various countries underscore the variability in dementia training among healthcare professionals. For instance, approximately half the physicians in a study from China reported having received dementia training (8), whereas only one in five participants in a study from Hungary had been given similar training (13). In a study from the United Kingdom, approximately one-quarter of physicians reported receiving dementia training during their residencies (11). The present study aligns well with these findings, showing that only approximately one in five FMRs in Türkiye had received dementia training post-graduation, and that approximately one in four had been involved in dementia patient care during their residencies. This suggests a significant gap in dementia education and is corroborated by previous studies from Canada and France highlighting similar deficiencies in training programs (19, 20).

A study involving community healthcare workers (HCWs) reported that their knowledge levels were associated with age, sex, and experience of caring for patients with dementia (21). Training in geriatrics has been shown to enhance the ability to rapidly diagnose dementia (4). A previous study of general practitioners (GPs) observed that participants who took part in training on the subject of dementia tended, when in doubt, to diagnose more patients in terms of cognitive disorder (13). Another study showed that FMRs with more years of training were more confident in diagnosing dementia (22). No significant association was observed in the present study between dementia knowledge levels and age, time spent as a resident, or years in the profession. However, individuals who had received postgraduate training in dementia exhibited higher levels of knowledge. A study of primary HCWs in Hong Kong described their competence in terms of early diagnosis of dementia as low and concluded that this was due to a paucity of dementia-related experience. HCWs who had the opportunity to work in geriatric/psychogeriatric departments after graduation were reported to be more confident in diagnosing dementia in its early stages (23). In another study, PCPs receiving specialist training also reported feeling more confident on the subject of dementia care (24). Approximately one-quarter of the FMRs in the present study had worked with patients with dementia during their residency training. These participants were most likely to work in home care and palliative care. In addition, knowledge of dementia was greater among those who had received dementia training after graduation, those who had been involved in the diagnosis, treatment, and follow-up of dementia patients during residency, and those who were willing to screen for early signs of dementia actively. Greater professional experience did not contribute to the general level of knowledge concerning dementia, unless specific training on the subject had been received. Theoretical teaching and practical measures should be increased during residency training for PCPs.

A previous study from Canada emphasized the lack of dementia training in family medicine specialization programs (19). A study from France highlighted the lack of instruction regarding Alzheimer's disease in the training of GPs (20). A study from the United Kingdom reported that the majority of trainee GPs

were eager to participate in dementia training sessions (11). Other studies have similarly concluded that most GPs and hospital clinicians also wish to receive dementia-related training (14, 24). Another study showed that dementia workshops for PCPs improve clinicians' ability to manage dementia and also increase their confidence (25). Most FMRs in the present study also wished to participate in dementia-related education. This finding is particularly important because it shows that receipt of postgraduate training regarding dementia is a factor influencing dementia-related knowledge levels. It is also very important that physicians express a willingness to participate in dementia education, which will assist them to improve their knowledge levels and thus be of greater benefit to patients.

A previous study, in which one-third of GPs felt that they lacked competence in diagnosing dementia, described a lack of confidence in the face of contemporary diagnostic approaches, time limitations, and reimbursement difficulties in some countries as obstacles to early diagnosis (26). In another study involving GPs, the reasons cited for not carrying out testing included lack of time, lack of sufficient knowledge concerning test methods, and the idea that testing is best conducted by a specialist (13). The majority of FMRs in the present study did not test patients with cognitive disorders. The reasons for this included the clinicians not regarding themselves as competent in applying and interpreting tests or performing neuropsychiatric evaluations, and time limitations. Another reason may be that FMRs have little contact with patients with dementia during specialist training. However, observation of their populations gives PCPs a better opportunity to become aware of and suspect early cognitive changes in their patients. This results in significant benefits for some patients. In addition, the majority of FMRs reported wishing to screen for the early signs of dementia actively. Improving deficiencies in time and education on this subject will result in more effective protection against dementia in primary care settings.

In terms of the application of tests for cognitive evaluation, a study of PCPs in 25 European countries revealed that the MMSE was the most popular test in all countries except Hungary, followed by the Clock-Drawing test (27). According to a study of PCPs in Germany, most physicians performed a cognitive test when cognitive impairment was suspected, while one in three performed a general geriatric evaluation. The most frequently applied method was the Clock-Drawing test, followed by the MMSE (4). Another study of GPs in Hungary reported that half the participants applied a cognitive test when they suspected cognitive impairment, although the other half did not apply such tests even when such impairment was suspected (13). A study of primary care practitioners and neurologists in the USA also reported that only half applied cognitive tests to patients with suspected cognitive impairment (28).

In another study from Germany, one physician in five simultaneously assessed patients for depression, and one in three also evaluated their daily activities (29). An examination of practices around the world shows that the most commonly used tests are the MMSE and the Clock Drawing test. This may be because these are the best-known tests and because they are easy to administer. The fact that similar tools are used worldwide is also important in terms of standardization. Studies have shown that the proportion of PCPs using cognitive tests is relatively low. Most FMRs in this study used cognitive tests when cognitive impairment was suspected. Improving work experience with these tests during the training process may help increase their application in primary care. Cognitive and mental screening will be highly beneficial due to the importance of early diagnosis of dementia in primary care.

Higher rates of screening patients for depression when cognitive disorders are suspected have been reported among primary care clinicians compared to neurologists (28). In a study involving nurses, the majority of participants correctly answered whether depression could be confused with dementia (30). Another study involving GPs showed that awareness that the evaluation of depression is part of the diagnostic process for dementia was not as high as awareness that the assessment of dementia also requires cognitive evaluation (31). The great majority of the FMRs in the present study also reported evaluating patients with suspected dementia for depression. Research shows that depression is associated with the incidence of dementia through various psychological and physiological mechanisms. Depression also constitutes part of the prodromal and early stages of dementia. Reverse causation is also possible when depressive symptoms derive from the neuropathology of dementia, which occurs years before the onset of clinical dementia. Depression may constitute a risk factor for dementia, although dementia can also cause depression at more

advanced ages (32). In the light of this relationship, the optimal approach should also involve evaluating depression in patients with suspected dementia at an advanced age.

The FMRs in this study exhibited a positive attitude toward dementia. Studies have shown that positive attitudes among healthcare professionals are associated with better patient satisfaction and adherence to treatment (9, 30, 33). A study from Hong Kong also reported positive attitudes toward early diagnosis among physicians (23). Other studies also concluded that physicians exhibited positive attitudes toward patients with dementia (11, 21, 29). A positive attitude toward patients is important, particularly in the context of elderly individuals, since this can affect patient satisfaction and adherence to treatment. A previous study showed that geriatric training positively affected attitudes toward elderly patients (34). A statistically significant association was found between knowledge of dementia and attitudes toward the condition in the present study. A positive attitude may encourage the desire for information on the subject. A previous study involving community HCWs showed a similar association between knowledge and attitude (21). Although primary care provides an opportunity to identify patients with dementia, negative attitudes may discourage patients from freely describing their symptoms and may lead to avoidance behaviors. A positive attitude on the part of physicians toward this disease, which is highly prevalent in old age, will therefore be particularly valuable in terms of a healthy doctor-patient relationship.

This study was limited to family medicine residents, and assessments of family physicians and family medicine specialists working in the field were not conducted. The cross-sectional design also limits our ability to infer causality. The data collection method used in this study, the Google Forms survey, also has certain limitations. In particular, participation in online surveys is subject to volunteer bias, and the pressure to provide correct answers, as well as recall bias and misreporting, may affect the validity of the results. Future research should explore longitudinal designs to assess the impact of enhanced training programs on dementia care practices. Additionally, qualitative studies could provide deeper insights into the barriers and facilitators of dementia care among family medicine residents.

CONCLUSION

A large proportion of the physicians in this study were interested in actively screening for dementia and attending education sessions about the disease. While their knowledge of dementia was limited, their attitudes toward it were positive. However, the level of use of cognitive tests among the physicians was markedly low. Importantly, the desire to screen for early signs of dementia was a strong predictor of both dementia knowledge and attitudes toward the disease.

In order to address these findings, residency programs should integrate more comprehensive dementia training, encompassing both theoretical knowledge and practical skills. Postgraduate training opportunities in dementia care should be expanded to ensure ongoing professional development and competency. The adoption of widely recognized cognitive tests such as the MMSE and Clock Drawing test should be encouraged, with appropriate training being given on their administration and interpretation. Training programs must also emphasize fostering positive attitudes toward dementia to enhance patient care and outcomes.

By addressing these gaps and implementing the recommended training programs, we can improve the quality of dementia care provided by future family physicians, ultimately benefiting both patients and the healthcare system as a whole.

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RESEARCH ARTICLE

INVESTIGATION OF THE EFFECT OF MARAS POWDER (NICOTIANA RUSTICA LINN) USE ON INSULIN RESISTANCE

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ABSTRACT

Purpose: One of the common types of tobacco, which is widely used all over the world and has significant public health concerns, is smokeless tobacco. Maras powder (MP), a type of smokless tobacco, is consumed especially in the Southeastern and Eastern Anatolia regions of Turkiye, and it draws attention due to the lack of sufficient studies on its health effects. This study was conducted for the first time and aimed to determine the effect of MP use on insulin resistance.
Materials and Methods: A total of 58 male subjects (25 MP users and 33 control groups) between the ages of 20-49 years were included in the study. Demographic data, systolic pressure (SBP) and diastotic pressure (DBP) were recorded. For the study, 5 ml of blood was collected from the subjects and serum glucose (mg/dL), total cholesterol (mg/dL), triglycerides (mg/dL), and HDL (mg/dL) were measured via Cobas 6000 series c 501 clinical Biochemistry analyzer. Homeostasis Model Assesment-Insulin Resistance (HOMA-IR) method was used to evaluate insulin resistance.
Results: According to the statistical analysis, triglyceride, glucose, insulin and HOMA-IR levels were significantly higher in MP users compared to the control group ($p<0.05$). In addition, significant correlations were observed between insulin, HOMA-IR, glucose and triglyceride levels in MP users.
Conclusion: In this study, we obtained data indicating that the use of MP with limited information regarding its health effects, may be associated with insulin resistance. This study was conducted for the first time and we believe it will address the existing gap in the literature in this area of research and enhance the public awareness.

Keywords: Insulin resistance, Maras Powder, Tobacco

INTRODUCTION

More than one billion people worldwide are active tobacco users, mostly men in developing countries. Although Nicotiana tabacum (N. tabacum) of tobacco, which has many types, was previously used for actions such as religious rituals, it is now mostly used in the production of commercial cigarettes (1,2).

Smoking is the most common form of tobacco use, but the use of smokeless forms of tobacco is also increasing. Although various forms of smokeless tobacco are consumed in many parts of the world, Maras Powder (MP), a form of smokeless tobacco obtained from Nicotiana rustica Linn (N. rustica L.), is widely used in Turkiye (3). N. tabacum and N. rustica L. plants contain molecules such as nicotine, nornicotine and anatabine. Studies have shown that nicotine binds to nicotinic acetylcholine receptors (nAChR) in the brain and increases dopamine levels, which plays a key role in creating addiction. N. rustica L. contains about seven times more nicotine than N. tabacum and the use of this smokeless tobacco is known to have addictive effects like cigarettes (4).

Today, there are numerous studies proving the negative effects of smoking on health. Studies have shown that N. tabacum type tobacco induces the development of type 2 diabetes mellitus (T2DM) and has effects on the development of diabetes-related complications (5).

T2DM is a life-threatening metabolic disorder characterized by insulin resistance and pancreatic β-cell dysfunction in peripheral tissues (6). The global prevalence of diabetes has brought this disease to pandemic level (7). According to a study by Sun et al, there are more than 500 million diabetic patients in the world in 2021 and it is predicted that this number will exceed 780 million in 2045 (8). The major contributer to the high prevalence of diabetes is insulin resistance, which is frequently associated with dietary habbits, obesity and physical inactivity (6). Insulin resistance occurs approximately 10-15 years before the diagnosis of T2DM (9). Studies have shown that nicotine found in cigarettes is effective in the development of insulin resistance (10,11).

In the literature, over 20.000 articles on N. tabacum have been published in NCBI so far, while there are only 198 publications on N. rustica L. (12). N. rustica L. is consumed by sucking 1-2 g between the lips and gingiva. Due to its smokeless use, it does not contain CO gas in the content of cigarettes, but due to the high amount of nicotine, it has been reported to play a critical role in the development of oral, esophageal, pancreatic cancer, especially with its carcinogenic, genotoxic effects (13-15). In this study, which was conducted for the first time, we aimed to investigate the relationship between N. rustica L. smokeless tobacco (MP) and insulin resistance in males. Despite being published considerably less than N. Tabacum, the health implications of N. rustica L. remain insuffeciantly understood, especially due to its high nicotine content compared to N. Tabacum. We aimed that our study could contribute to address the literature gap in this topic.

MATERIALS AND METHODS

Study Design

This study was conducted in the Central Laboratory of Kahramanmaraş Necip Fazil City Hospital with the approval of the ethics committee of Kahramanmaras Sutcu Imam University Faculty of Medicine, Clinical Research Ethics Committee (Date: 14.06.2017; Decision No: 04). Between February and October 2017, 25 male volunteers who were non-smokers and used one-pack (approximately 16 g) of MP daily for at least five years were included in the study as the study group and 33 volunteers who were neither smokers nor MP users were enrolled as the control group. Demographic data and 5ml blood samples were collected from all the study participants.

Preparation of Samples

After an overnight fasting, venous blood of all individuals participating in the study was collected in gel tubes. After waiting for 30 minutes for clotting, serum was separated by centrifugation (Hettich Zentrifugen Rotanta 460R, Germany) at 3000 rpm for 10 minutes. Serum samples were stored at -20°C until analyzed.

Measurement of Biochemical Parameters

Serum glucose, triglyceride, total cholesterol and HDL cholesterol levels were measured spectrophotometrically using Cobas 6000 series c 501 clinical biochemistry analyzer (Roche Diagnostics, Germany) and LDL cholesterol levels were calculated by Friedewald formula. Homeostasis Model Assesment-Insulin Resistance (HOMA-IR) method was used to evaluate insulin resistance. HOMA-IR was calculated as [fasting blood glucose (mmol/L/18) x fasting insulin (mU/ml)] divided by 22.5.

Statistical Analysis

IBM SPSS version 22 package program was used for data analysis. In the statistical evaluation, the compatibility of the variables with normal distribution was examined by Shapiro-Wilk test. Independent samples “T test” was used to compare variables with normal distribution between the study groups. Mann-Whitney U test was used to compare variables that did not show normal distribution between the study groups. The relationship between variables was analyzed by Pearson and Spearman correlation tests. Correlation coefficients r >0.89 were considered very strong correlation, as strong correlation r=0.70-0.89, moderate correlation r=0.40-0.69, and weak correlation r=0.10-0.39. Results were expressed as median (min-max). Statistical significance was accepted as $p<0.05$.

RESULTS

A total of 58 male volunteers aged 20-49 years, including MP users (43.1%) and control group (56.9%), participated in the study. Demographic findings, systolic pressure (SBP), diastolic pressure (DBP) and biochemical data are presented in Table

1. According to the statistical analysis, triglyceride, glucose, insulin and HOMA-IR levels were significantly higher in MP users compared to the control group ($p<0.05$). In addition, significant correlations were observed between insulin, HOMA-IR, glucose and triglyceride levels in MP users (Tables 2 and 3).Whereas, a very strong correlation was observed between insulin and HOMA-IR($r=0.973$; Table 2); moderate correlations were observed between insulin and glucose ($r=0.439$) (Table 2) and HOMA-IR with glucose ($r=0.692$) and triglyceride ($r=0.592$) (Table 3).

DISCUSSION

According to studies, tobacco, which is associated with many diseases such as cancer, heart disease, stroke, and diabetes, has approximately 1.5 billion users, with more than one billion cigarette tobacco and over 350 million smokeless tobacco (16). MP, which is a type of smokeless tobacco widely used in Kahramanmaras and surrounding provinces of Turkiye as well as all over the world, is a significant public health issue. Local people prefer MP because it is cheap, easily accessible, has a higher drug effect than cigarettes, and is an alternative substance to quit smoking. However, it has been shown that MP has mutagenic, carcinogenic, genotoxic, and teratogenic effects on cardiovascular system, nervous system, oral health, and gastrointestinal system disorders in addition to creating physiological and psychological addiction (17). These effects are shown by the fact that trace elements such as lead, zinc, copper, manganese and aluminium are high enough to be harmful to health and toxic substances such as nicotine, nornicotine, arsenic which are found in cigarettes, are found much more in MP content (4, 17). In addition, the fact that urinary cotinine level is up to three times higher in MP is one of the evidences showing the negative effects on health (18).

Although it has been reported that more than 650.000 people have lost their lives worldwide due to smokeless tobacco consumption, studies on MP are quite insufficient (17). In a limited number of studies, it has been found that MP has negative effects on the cardiovascular system by decreasing nitric oxide (NO) levels (19). In another study, lesions in the lower lip mucosa of MP users were examined and moderate dysplasia was commonly observed. It was found that the risk of oral cancer was correlated with the duration of MP use and this risk increased significantly in those who used MP for more than 15 years (20). Kurtul et al. demonstrated that MDA level was higher in MP than in control groups and MP increased serum lipid peroxidation level (21). When the effects of MP use on haematological parameters were examined, it was determined that iron and leukocyte levels were higher in MP users compared to the control group, while monocyte and platelet levels were lower. It has been reported that nicotine and tobacco-specific nitrosamine levels found in MP lead to chronic inflammatory changes in systemic circulation, various cells, and organs (22).

Smoking is at higher risk factor for atherosclerosis and cardiovascular disease (23). Some studies comparing non-smokers with smokers have revealed that smokers developed hyperinsulinaemic and insulin resistance and that these changes lead to dyslipidaemia and endothelial dysfunction (24,25). Faccihini et al. showed that smokers developed hyperinsulinaemic and insulin resistance (26). Li et al. showed that nicotine exposure suppresses nuclear

Table 1. Demographic and laboratory data of MP users and non-users (Results are given as Median (Min-Max))

	MP users (n=25)	Control group (n=33)	P value
Age[a]	36.00 (25.00-49.00)	38.00 (20.00-48.00)	0.659
Body Mass Index (BMI)[b]	21.10 (16.30-29.90)	21.10 (12.40-37.00)	0.715
Systolic Pressure (mmHg)[a]	120.00 (100.00-140.00)	120.00 (100.00-140.00)	0.386
Diastolic Pressure (mmHg)[a]	80.00 (60.00-100.00)	80.00 (60.00-90.00)	0.182
Triglyceride (mg/dL)[a]	120.00 (40.00-292.00)	70.00(45.00-290.00)	0.015*
Glucose (mg/dL)[a]	98.00 (72.00-141.00)	91.00 (74.00-143.00)	0.014*
Cholesterol (mg/dL)[a]	161.00 (76.00-270.00)	130.00 (65.00-249.00)	0.101
HDL (mg/dL)[a]	57.00 (29.00-88.00)	67.00 (32.00-81.00)	0.315
LDL (mg/dL)[a]	78.00 (43.00-161.00)	76.00 (11.00-184.00)	0.379
Insulin (µU/L)[a]	15.10 (4.98-40.20)	10.10 (4.40-50.30)	0.047*
HOMA-IR[a]	4.18 (1.15-8.93)	2.34 (0.92-14.28)	0.032*

*[a]Mann-Whitney U test; [b]Independent samples t test; α:0.05; *Statistically significant.*

factor erythroid 2 (NRF2) and causes glucose intolerance and thus insulin resistance (27). In a study conducted in Turkiye it was found that smoking did not affect HOMA-IR in women but decreased it in men (28).

Increased insulin resistance may lead to disorders in carbohydrate body metabolism, hypertension and dyslipidaemia. In our study, the fact that glucose levels in MP users were higher than non-users may be due to the fact that MP use affects blood glucose balance. Elevated triglycerides, decreased HDL and increased LDL levels, also called atherogenic dyslipidaemia, may be associated with insulin resistance. It is known that smokers have high plasma triglyceride and decreased HDL levels29. Since smokers have both insulin resistance and dyslipidaemia, the risk of cardiovascular disease is increased among these individuals (30). In our study, LDL cholesterol was found to be high and HDL cholesterol was found to be low in MP smokers, but the difference between the two groups was not statistically significant. This suggests that increased insulin resistance may cause dyslipidaemia in MP users. Moreover, triglyceride levels were significantly higher in MP users in our study. While triglyceride levels were found to be elevated in smokers in some studies, other studies reported low triglyceride levels among smokers (26). Changes in triglyceride levels in both smokers and MP users may be associated with lifestyle, genetic predisposition, and dietary habits.

The correlation analysis of insulin among MP users revealed a statistically significant very strong positive correlation with HOMA-IR (r=0.973; p<0.001) and a positive moderate correlation with glucose (r=0.439; p<0.011) (Table 2). In addition to our results, moderate correlations of HOMA-IR with glucose (r=0.692; p<0.001) and triglyceride (r=0.592; p<0.001) were observed in MP users (Table 3).

Study Limitations

In our study, urinary cotinine level and the amount of nicotine in the blood were not measured in the study groups in order to objectively determine MP use, the duration of weed use in the MP-using group (5 years/ 1 pack per day) was low, relatively few cases were included, and our data need to be supported by larger-scale studies. In addition, this study was conducted only in male individuals for homogenous distribution and no research was conducted in females.

Table 2. Correlations of insulin and measurement parameters in MP users.

	Insulin	
	r	**p value***
Age	0.031	0.865
Glucose (mg/dL)	0.439	0.011*
Triglyceride (mg/dL)	0.362	0.039*
Cholesterol (mg/dL)	0.065	0.721
HDL (mg/dL)	-0.065	0.718
LDL (mg/dL)	0.029	0.874
HOMA-IR	0.973	0.001*

*Pearson correlation test; α: 0.05; *Statistically significant.*

Table 3. Correlations of HOMA-IR with other parameters in MP users.

	HOMA-IR	
	r	**p value***
Age	0.212	0.237
Glucose (mg/dL)	0.692	<0.001*
Triglyceride (mg/dL)	0.592	<0.001*
Cholesterol (mg/dL)	0.199	0.266
HDL (mg/dL)	-0.166	0.355
LDL (mg/dL)	0.015	0.403

*Pearson correlation test; α: 0.05; *Statistically significant*

CONCLUSION

MP, a smokeless tobacco, is a type of tobacco that is very harmful to health, contrary to what is known. The presence of toxic components such as nicotine, nornicotine, arsenic as well as trace elements that may threaten health in cigarettes causes carcinogenic, mutagenic and genotoxic effects. This study was conducted for the first time, and it revealed that MP may support insulin resistance. We believe that the nicotine substance in MP may affect insulin resistance either through the NRF2 pathway or by increasing catecholamine synthesis as in cigarettes. Studies on MP are very limited, and we anticipate that further research on this tobacco, which may threaten public health, will be highly beneficial.

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Conflict of Interests: The authors state no conflict of interest with respect to the research, authorship, and/or publication of this article.

Ethical Approval: Before the initiation of the study, all participants received an explanation of the procedure and the risks that would later be faced in their participation, and they provided informed

consent to participate in this study. The study was approved by the ethics committee of the Kahramanmaras Sutcu Imam University Faculty of Medicine, Clinical Research Ethics Committee (Date: 14.06.2017; Decision No: 04), and all procedures were in accordance with the Declaration of Helsinki.

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RESEARCH ARTICLE

PERIPHERAL BLOOD CELL CHANGES AND OUTCOMES AFTER PARTIAL SPLENIC EMBOLIZATION FOR HYPERSPLENISM İN CIRRHOTIC PATIENTS

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ABSTRACT

Purpose: Partial splenic embolization is a common treatment for hypersplenism in patients with cirrhosis. In this investigation, we evaluated the effectiveness and safety of partial splenic embolization in patients with cirrhosis.
Materials and Methods: We retrospectively investigated 17 patients with hypersplenism secondary to cirrhosis who underwent partial splenic embolization. Following partial splenic embolization, peripheral blood cell counts were measured at regular intervals over a period of twelve months. Post-procedural complications were recorded.
Results: This study included 17 individuals, with a mean age of 54.5 ± 10 years. Hemoglobin, platelet, neutrophil, lymphocyte, and white blood cell counts showed statistically significant increases. When compared to the pre-procedure levels at each time point (pre-procedure vs. 1st day $p<0.001$; pre-procedure vs. 1st week $p<0.001$; pre-procedure vs. 1st month $p<0.001$; pre-procedure vs. 6th month $p<0.001$; pre-procedure vs. 1st year $p<0.001$). The most common complication was post-embolization syndrome (88.2%), which was managed with conservative treatment. One patient died of esophageal variceal bleeding.
Conclusion: Partial splenic embolization improves long-term hematological parameters with manageable side effects.

Keywords: Partial splenic embolization, cirrhosis, hypersplenism

INTRODUCTION

Splenomegaly and/or hypersplenism are frequent complications of portal hypertension in cirrhosis and are usually asymptomatic. These conditions result in hematological abnormalities, including thrombocytopenia, leukopenia, and anemia, due to the increased splenic sequestration of blood cells. Patients with cirrhosis have a worse prognosis due to these hematological abnormalities since they are more likely to experience bleeding, infections, and other related problems (1-4). Furthermore, this may result in a patient's ineligibility for some therapies,

including interferon therapy, antineoplastic chemotherapy, and major surgery (5-7).

For hypersplenism, surgical splenectomy is a useful therapy option. However, splenectomy is associated with serious complications (8,9). Partial splenic embolization (PSE) has emerged as a significant treatment for hypersplenism in patients with cirrhosis as a viable substitute for surgery (10). This is particularly true in individuals whose surgical risks preclude splenectomy (2,11,12). PSE works by selectively embolizing parts of the spleen, thereby reducing its volume and function and improving peripheral blood cell counts (1). Several investigations have revealed that PSE is safe and effective in this patient population and have reported the benefits of PSE (7,13,14). Another approach for patients who do not adequately respond to the initial procedure is repeated partial splenic embolization (RPSE) (15).

The most common complication of PSE is post-embolization syndrome (PES), which manifests as fever, nausea, and pain (10,16). Conservative therapy, however, can effectively reduce these symptoms (7,15,17). Septicemia and splenic abscess are uncommon but serious side effects that require immediate medical attention (18). PSE improves the hematological parameters in cirrhotic patients. Nevertheless, severe complications such as venous thrombosis, splenic infarction, and death have been published (1,2). The premise of this investigation was that PSE is a safe and efficient treatment for cirrhotic hypersplenism and has the benefit of being a nonsurgical intervention.

This study aimed to evaluate peripheral blood cell changes following PSE in patients with hypersplenism secondary to cirrhosis and investigate the clinical results after PSE.

MATERIALS AND METHODS

Study Design and Patient Population

This retrospective analysis included patients with hypersplenism and cirrhosis who underwent PSE at Dokuz Eylul University Hospital between January 2010 and March 2024. Seventeen patients were included in this study. Patients aged ≥ 18 years, diagnosed with confirmed cirrhosis, with evidence of hypersplenism with splenomegaly, and eligible for PSE based on a multidisciplinary team review were included in the study. The diagnosis of hypersplenism was made using clinical laboratory data, ultrasonography, and computed tomography examinations. Patients with prior splenectomy or splenic embolization, infections or sepsis, or other conditions that would exclude the procedure, such as severe portal vein thrombosis or irreversible coagulopathy, were excluded. Supporting therapies were administered to patients with severe anemia, thrombocytopenia, or coagulation disorders before PSE.

Previous medical records provided information on patient demographics and the etiology of cirrhosis. Biochemical data and peripheral blood cell counts were collected, and the severity of liver disease was evaluated by calculating the Child-Pugh score.

The study confirmed the tenets of the Declaration of Helsinki and was approved by the Non-Invasive Clinical Research Ethics Committee of Dokuz Eylul University (Date: 29/05/2024, Decision Number: 2024/19-16).

Partial Splenic Embolization Procedure

Two experienced interventional radiologists performed PSE under local anesthesia. Embolization was done using a distal technique (19). A catheter was inserted through the femoral artery and carefully placed inside the splenic artery under fluoroscopic guidance. After that, a coaxial microcatheter was placed into at least one lower pole pedicle, and embolization was performed from the microcatheter. Embolization involved injecting an embolic agent, polyvinyl alcohol particles or microspheres, into the branches of the splenic artery with the goal of partially infarcting between 50 and 80 percent of the spleen. The degree of embolization was estimated using pre-procedural imaging and the patient's clinical status. Postprocedural analgesics, antibiotics, and hospital monitoring were administered to all patients to reduce the risk of infection and post-embolization syndrome. Informed consent was obtained from all patients before the procedure.

Data Collection and Follow-up

The patients were hospitalized before the PSE procedure. Following PSE, all patients stayed at the hospital until PES or any other serious problems had disappeared. Subsequently, they were observed at an outpatient clinic. Peripheral blood cell counts (including platelet count, white blood cell count, lymphocyte and hemoglobin levels) were measured before the procedure, at 1 day, 1 week, 1 month, 3-month, 6 month and 1 year after PSE. Liver function tests and abdominal imaging were also repeated

during follow-up visits to monitor for complications such as splenic infarction, abscess formation, or ascites. Any post-procedural complications were recorded.

Statistical Analysis

Compliance with normal distribution was assessed with the Shapiro-Wilk test. Descriptive statistics were used to summarize baseline characteristics. Continuous variables were expressed as mean ± standard deviation (SD) or median-interquartile range (IQR), while categorical variables were expressed as frequencies and percentages. Wilcoxon signed-rank tests were used to compare pre-and post-procedural blood counts. A p-value of <0.05 was considered statistically significant. The data were evaluated and visualized using the SPSS (v29.0) package program.

RESULTS

Patients' characteristics

This cohort included 17 patients (mean age, 54.5 ± 10 years). Of these, 52.9% (n=9) were male and 47.1% (n=8) were female. The most common concomitant disease was diabetes mellitus, present in 23.5% (n=4) of patients. This was followed by hypertension in 17.6% (n=3), chronic kidney disease, and cancer, each present in 11.8% (n=2), while cardiac disease and cerebrovascular disease were observed in 5.9% (n=1) of patients. In terms of cirrhosis etiology, hepatitis B virus (HBV) infection and non-alcoholic fatty liver disease (NAFLD) were each responsible for 23.5% (n=4) of cases. Autoimmune hepatitis was found in 17.6% (n=3), primary biliary cholangitis and alcohol-related liver disease in 11.8% (n=2) of cases each, and HBV + hepatitis D virus (HDV) co-infection and Wilson's disease in 5.9% (n=1) of patients each. The Child-Pugh classifications of these 17 patients were Child A in 7 (41.1%), Child B in 6 (35.2%), and Child C in 4 (23.5%) patients. The patient characteristics are summarized in Table 1.

Efficacy of partial splenic embolization

Hematological parameters were monitored during the first year following PSE. Hemoglobin, platelet, neutrophil, lymphocyte, and white blood cell counts showed statistically significant increases when compared to pre-procedure levels at each time point (p<0.001). Table 2 lists the changes in the hematological parameters.

The median white blood cell count showed a significant increase from a baseline of 2.3 (IQR: 1.35–3.35) 103/uL to 5.4 (IQR: 4.4–10.9) 103/uL on the first-day post-PSE (p <0.001). The count further increased to 10.5 (IQR: 6.4–12.0) 103/uL at the first week and gradually stabilized over time, reaching 6.7 (IQR: 3.6–8.5) 103/uL by the first year (p <0.001). Neutrophil counts followed a similar trend.

Lymphocyte counts were 0.4 (IQR: 0.3–0.7) 103/uL pre-PSE and 1.1 (IQR: 0.6–1.3) 103/uL at the one-year mark (p <0.001), respectively. After a year (p <0.001), hemoglobin levels improved gradually, rising from 9.1 (IQR: 7.8–11.4) g/dL pre-PSE to 10.7 (IQR: 9.2–12.8) g/dL.

The most notable change was observed in platelet counts, which rose sharply from a baseline of 47 (IQR: 34–57) 103/uL to 142 (IQR: 87–187) 103/uL in the first week (p <0.001). Platelet counts continued to increase, peaking at 203 (IQR: 105–42) 103/uL by the first month before reaching 150 (IQR: 87–191) 103/uL by the first year.

The change of platelet count during the 1st year after PSE is demonstrated in Figure.

Complications

To minimize the complication rate, most patients had a splenic infarction rate of 50%-70%. The complications after PSE in our study were varied.

Table 1. Characteristics partial splenic embolizationin 17 patients with cirrhosis

Characteristics	Total patients (n = 17)
Age, years, mean±SD	54.5 ± 10
Sex, n (%)	
Male	9 (52.9)
Female	8 (47.1)
Comorbidities, n (%)	
Cardiac disease,	1 (5.9)
Chronic kidney disease	2 (11.8)
Cerebrovascular disease	1 (5.9)
Diabetes mellitus	4(23.5)
Hypertension	3 (17.6)
Cancer	2 (11.8)
Cirrhosis etiology, n (%)	
HBV	4(23.5)
HBV+HDV	1 (5.9)
Autoimmune hepatitis	3 (17.6)
Primary Biliary Cholangitis	2 (11.8)
Alcohol	2 (11.8)
Wilson	1 (5.9)
NAFLD	4(23.5)
Child's classification, n (%)	
A	7(41.1)
B	6(35.2)
C	4(23.5)

SD: standard deviation, HBV, hepatitis B; HDV, hepatitis D; NAFLD, Non-alcoholic fatty liver disease

Table 2. Changes in hematological parameters in the first year

	Pre-PSE	1st day	1st week	1st month	3rd month	6th month	1st year
WBC (10^3/uL), median (IQR)	2.3 (1.35-3.35)	5.4 (4.4-10.9)	10.5 (6.4-12)	6 (4.9-7.5)	6.5 (4.5-10.3)	5.7 (4.4-7.1)	6.7 (3.6-8.5)
Neu (10^3/uL), median (IQR)	1.2 (0.9-2.1)	3.6 (3.1-9.8)	6.5 (4.3-8.4)	4.4 (2.3-5.5)	3.3 (2.8-7.8)	3.2 (2.5-4.7)	3.9 (1.6-5.9)
Lym (10^3/uL), median (IQR)	0.4 (0.3-0.7)	0.4 (0.3-0.6)	0.7 (0.4-0.9)	0.7 (0.6-1.2)	1 (0.6-1.8)	1.1 (0.8-1.5)	1.1 (0.6-1.3)
Hb (g/dL), median (IQR)	9.1 (7.8-11.4)	10.7 (8.8-11.4)	10.2 (8.4-11.9)	10.3 (9.3-12.3)	11.3 (9.4-12.2)	11.7 (10-12.8)	10.7 (9.2-12.8)
PLT (10^3/uL), median (IQR)	47 (34-57)	72 (60-102)	142 (87-187)	203 (105-42)	136 (100-213)	149 (124-195)	150 (87-191)

PSE: Partial splenic embolization, WBC: White blood cell, Neu: neutrophil, Lym: lymphocytes, Hb: hemoglobin, PLT: platelets
** The change in hematological parameters levels was statistically significant between pre-PSE and all the time points (pre-PSE vs 1st day p<0.001; pre-PSE vs 1st week p<0.001; pre-PSE vs 1st month p<0.001; pre-PSE vs. 6th month p<0.001; pre-PSE vs. 1st year p<0.001). Wilcoxon Signed Rank test was used for statistical analysis and data was provided as median (interquartile range (IQR)). Two-sided p value <0.05 was considered statistically significant.*

PES was the most common complication, affecting 88.2% (n=15) of patients. These symptoms were easily controlled via conservative therapy. Pleural effusion or ascites was seen in 35.2% (n=6), which spontaneously resolved. Splenic hematoma and peritonitis were each observed in 11.8% (n=2). There were no cases of splenic abscess. Hepatic encephalopathy developed in 11.8% (n=2) of patients. 0ne patient (5.9%) developed esophageal variceal bleeding 10 days after PSE and was admitted for endoscopic treatment but died of hypovolemic shock. The mean hospital stay was 13.7 ± 10.8 days. Patient complications are listed in Table 3.

DISCUSSION

This study was conducted on 17 patients who underwent partial splenic embolization with hypersplenism secondary to chronic liver disease. A less invasive therapeutic approach for treating hypersplenism in cirrhotic patients is partial splenic embolization. Hematological indicators were significantly improved in our study as a result of PSE. These results corroborate those of other studies showing that PSE is effective in treating hypersplenic cytopenia (20,21). Postembolization syndrome was the most common side effect; however, it resolved with conservative therapy in almost all patients. However, one patient died from bleeding after PSE.

Table 3. Complications after PSE

Parameter	n:17
PES,n(%)	15(88.2)
Splenic hematoma,n(%)	2(11.8)
Pleural effusion/ascites,n(%)	6(35.2)
Peritonitis,n(%)	2(11.8)
Splenic abscess,n(%)	0 (0)
Length of hospital stay, (day) Mean±SD	13.7±10.8
Hepatic encephalopathy,n(%)	2(11.8)
Variceal bleeding,n(%)	1 (5.9)
Death,n(%)	1 (5.9)

Patients with different cirrhosis stages and comorbidities were included in the study population, which could have affected the results and unfavorable consequences of PSE. The mean age of the cirrhosis patients in our study was 54.5 years, and there were several different causes of the illness. Furthermore, the majority of patients showed reasonably intact liver function based on the distribution of their Child-Pugh classifications. These features provide a basis for understanding the possible impacts of PSE on different patient attributes.

White blood cell and neutrophil counts increased dramatically following the PSE, reaching a peak during the first week. Throughout the year, lymphocyte levels, which had started out low, progressively increased. Moreover, hemoglobin demonstrated consistent improvement, reaching notable heights by the one-year milestone. The most notable difference was in the platelet counts, which increased quickly following the treatment and peaked in the first month before continuing to rise by the end of the year. These results are consistent with those of the previous studies (1,2,4,21). Patients with cirrhosis who have thrombocytopenia have a high risk of bleeding, while those with neutropenia are more

prone to infection. Furthermore, it is more challenging to administer vital drugs such as cancer therapy or antivirals. It is especially crucial that the immune cell and platelet counts are restored after PSE. The assumption that PSE offers long-lasting benefits in treating anemia, a condition that typically worsens the prognosis of patients with cirrhosis, is supported by the long-term stability of hemoglobin levels in this study. PSE contributed to patients with cirrhosis having lower infection rates and improved immune function following PSE.

RPSE has been proposed as a feasible option for patients whose blood counts do not improve following initial PSE. According to a study by Tan et al., patients who underwent repeated PSE treatments demonstrated greater gains in platelet counts than those who underwent a single procedure (15). While RPSE was not used in our investigation, it is important to consider its potential benefits for some patients. Another crucial aspect to consider is the durability of long-term hematological improvements following PSE. Our results show that blood counts at one year are still significantly higher than the pre-PSE values, despite the fact that they frequently peak and then decline. In line with these results, PSE has been demonstrated to result in long-lasting elevations in blood counts, allowing patients to manage cirrhosis-related issues better.

In our study, the most frequent side effect affecting 88.2% of patients was PES, which is characterized by pain, fever, and nausea. PES rates following PSE have been recorded in previous studies, suggesting that this is a common but controllable issue (1,2,22). Pleural effusion or ascites, which are acknowledged consequences of PSE, occurred in 35.2% of patients in our study. Splenic hematoma and peritonitis occurred in two individuals (11.8%) in our group, but both conditions were well treated. One patient had esophageal variceal bleeding and died due to this reason. A study of 23 cases reported one patient developing bacterial peritonitis 12 days after PSE, leading to death from septicemia despite intensive care (1). Similarly, Wu et al. described a patient who died of acute myocardial infarction linked to esophageal bleeding 32 months post-PSE (21). N'Kontchou et al. found splenic necrosis in over 70% of patients who died from septic shock (2), highlighting that serious complications tend to be more common when infarctions in the splenic parenchyma exceed 70% (4). In our study, we tried to keep the splenic infarction rate between 50%-70%. Careful procedure planning is crucial for reducing these hazards.

This study has several limitations. First, the sample size was relatively small, with only 17 patients included in the analysis. Second, this was a retrospective study in which short-term complications were monitored, but the long-term complications of PSE, such as portal vein thrombosis or splenic infarction, were not sufficiently analyzed despite being followed for a year.

CONCLUSION

In conclusion, partial splenic embolization for hypersplenism is a percutaneous interventional method that offers patients with cirrhosis a viable alternative to surgery. PSE provides long-term improvements in the hematological parameters of patients with cirrhosis and hypersplenism. Compared to splenectomy, PSE is a less invasive alternative, particularly suitable for patients at high surgical risk, with advantages such as the ability to perform the procedure under local anesthesia. However, complications, such as post-embolization syndrome and variceal bleeding, should be considered. When it comes to treating hypersplenism in cirrhotic individuals, partial splenic embolization should be the first choice due to its effectiveness and safety

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Conflict of Interest: The authors have no conflict of interest to declare.

Ethical Approval: The study confirmed the tenets of the Declaration of Helsinki and was approved by the Non-Invasive Clinical Research Ethics Committee of Dokuz Eylul University (Date: 29/05/2024, Decision Number: 2024/19-16).

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RESEARCH ARTICLE

INVESTIGATION OF THE EFFECTS OF 8-WEEK TABATA TRAINING ON PHYSICAL PERFORMANCE IN AMPUTEE FOOTBALL PLAYERS

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ABSTRACT

Purpose: The aim of this study was to investigate the effects of 8-week tabata training on physical performance in amputee football players. For this purpose, 24 male football players (age 21.9±5.24, height 174.8±7.62, weight 65.4±8.23) who played in amputee football Super league and had at least 5 years of amputee football history participated in the study voluntarily.
Materials and Methods: The athletes participating in the study were divided into 2 groups of 12 each as control group (CG) and tabata group (TG) by simple random method. While the TG applied an 8-week tabata exercise protocol during the normal football season (6 units per week, total 64 units), the CG continued only football training (6 session per week, 48 total units). Tabata protocol was applied as 4 sets 2 times a week for 8 weeks. Height, weight, standing long jump, vertical jump, 10-20-30 m. sprint and yoyo tests were performed as pre-test before 8 weeks and post-test after 8 weeks. Dependent sample t test was used to determine whether there was a difference between the pre-test and post-test of each group.
Results: According to the pre-post test data between the groups; statistically significant differences were found in the final weight (-1.92±0.38), final VKI (-1.05±0.19), final SLJ (33.91±7.51), final VJ (7.66±1.54), and final Yoyo (1.437±0.26) measurements of TG and KG ($p>0.05$).
Conclusion: Tabata protocol applied for 8 weeks will be very useful to be used more in the development of aerobic and anaerobic endurance skills which are very important in amputee football players.

Keywords: amputee football, endurance, tabata, high-intensity, vertical jump

INTRODUCTION

Football is a team sport that requires coordinating sudden and fast movements with multi-directional movements (1,2). Football is one of the most popular sports in the world and amputee football, beach football and futsal are sports branches inspired by football. Amputee football is one of the sports branches played by disabled individuals. Amputee football is a branch that requires sports performance such as high-level endurance, strength, speed, quickness and strategy, high level control and is played by athletes who do not have a leg using a cane (crutch). An amputee football match is a type of football played for a total of 50 minutes consisting of 25 minutes and 2 halves. Amputee football is played in an area of 60x40 metres with castles at both ends with its own rules (3). Considering the high intensity of the football competition, amputee footballers should be in excellent condition to easily perform all activities with and without the ball while moving with crutches (4). Studies indicate that using crutches is quite tiring (5). Therefore, it can be safely said that,

as with footballers, players should be well prepared for the game not only technically and tactically, but also, most importantly, physically (6). Similarly, in amputee football, the muscular strength and endurance required by the branch and should have an appropriate body composition according to the position played and the physiological and morphological characteristics required by the positions (7). High-intensity interval training (HIIT) models have emerged as a method to improve cardiometabolic function and thus physical performance. Based on this logic, tabata training modelling has been applied to improve physical performance. Tabata involves the completion of short or long repetitive sets of high-intensity exercise with alternating rest periods (8). Tabata is defined as repeated and intense efforts interspersed with periods of low or moderate intensity exercise recovery (active recovery) or complete rest, performed at an intensity that causes >85% of maximum heart rate (HR). The total work duration can be 10 s-6 min and is performed with a 1:1-3:1 work/rest ratio (e.g. 10 s work for 10 s rest or 30 s work for 10 s rest) (8). However, the tabata training model has not been used in amputee footballers and its effect on physical performance is unknown. The aim of this study was to investigate the effects of tabata training on performance in amputee footballers. The performance effects were compared between Tabata Group and Control Group for 8 weeks.

Hypotheses of the study: Does 8 weeks of training with tabata protocol have an effect on vertical jump performance in amputee football players? Does 8 weeks of training with tabata protocol have an effect on long jump performance in amputee football players? Does 8 weeks of training with tabata protocol have an effect on endurance performance in amputee football players?

MATERIALS AND METHODS

Participants

Twenty-four male volunteers aged between 18-40 years, living in Ankara province, regularly following football training for at least the last 1 year, not having any cardiovascular disease, blood disease, alcohol and cigarette consumption participated in the study. Participants were asked to eat at least 2 hours before the tests. According to G-power analyses; Effect size 0.8, α err prob: 0.20, Power (1-β err prob): 0.80, 28 people in total. The participants were divided into 2 groups, each consisting of 12 people, as tabata group (TG) and control group (CG) by simple random sampling method. Height, weight, standing long jump, vertical jump, 10-20-30 m. sprint and yoyo tests were applied to the participants as pre-test before 8 weeks and post-test after 8 weeks. It was approved by Istanbul Nisantası University Ethics Committee (Date: 04.01.2024, No: 2024-01) that the research design was in compliance with the Helsinki Declaration on Ethical Principles in Medical Research on Humans (2023/06).

Research design

The participants were divided into two simple random groups as Tabata Group (TG) and Control Group (CG). Firstly, height-weight and body mass of the participants were taken. Then vertical jump, long jump, 10-20-30 metres sprint and endurance tests were taken. CG continued routine football training for 8 weeks, while TG applied tabata protocol 2 days a week. The study was completed by repeating the post-tests. The values obtained from the pre-test and post-tests were recorded and statistically analysed. The tests applied in the study are the tests applied to the national athletes in the Brazilian amputee national team, as well as the measurements applied for amputee athletes (7).

Inclusion and exclusion criteria of the study;

- It was assumed that all individuals participating in the study understood the importance of the study and the tests.
- It was assumed that all individuals participating in the study performed maximal performance during the tests.
- It was assumed that all individuals participating in the study voluntarily performed the tabata protocol.

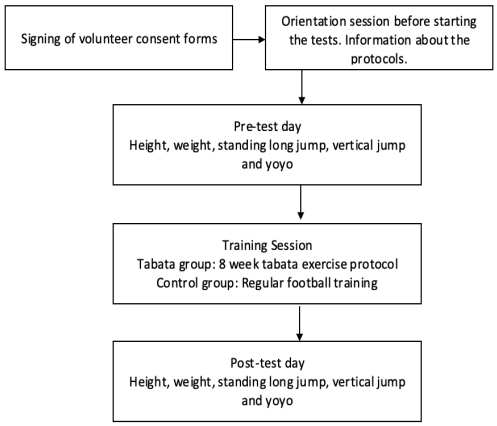

Figure 1. Research diagram

- This study was limited to 24 male athletes between the ages of 18-40 who played licensed football in the Turkish Armed Forces Rehabilitation Centre Disabled Sports Club in Ankara province in the 2023-2024 season.

Height and weight measurement

'The height of the participants was measured in m with a wall-mounted stadiometer (Holtain, UK) with an accuracy of 0.1 cm in an anatomical posture, barefoot, with feet fully on the ground, heels together and in contact with the wall, knees tense and body upright position, with reference to the point where the tip of the head touches the height meter table. Body weights were measured with a digital scale (Seca, Vogel and Halke, Hamburg) with an accuracy of 0.1 kg, while wearing light sportswear consisting of shorts and T-shirt, barefoot and in anatomical posture. Height and body weight were measured twice and averaged (9).

Speed test

10 Metre, 20 Metre and 30 Metre Run (Witty, Italy) The athlete ran with maximal effort on a flat surface with funnels between point A and point B. After complete rest, the test was repeated and the best of the 2 measurements was recorded as a score (9).

Vertical jump

The athlete was asked to jump on a Microgate witty, wirelles trainig timer (China) jump mat with feet apart and upright to the highest point she could reach. The measurement was done 2 times and the best degree was recorded (10).

Standing long jump

The measurement was made between the toe of the participant's foot at the starting line and the heel of the foot where the participant jumped and fell, and the point where the participant fell with a metre was calculated and noted. The test was performed on non-slippery ground, the test was repeated after complete rest and the best degree of the 2 measurements was recorded as a score (10).

Yoyo endurance test

The Yoyo test is a physical fitness test that measures endurance and fitness. Especially popular among athletes, it is used to assess running endurance and speed. The test involves the participant running forwards and backwards along a line and takes place at certain intervals at increasing speeds. The participant starts at one end of a line and runs towards a target at the other end. Then, as soon as he/she reaches the target, he/she turns back and this process is repeated at a certain speed. The speed is indicated by audible signals and the speed increases each time. If the participant fails to reach the target or does not align with the signal sound, the test ends and the last level run is recorded (11).

Data analysis

SPSS 25 package programme (SPSS Inc., Chicago, IL, USA) was used for statistical analysis of the data. Mean, minimum, maximum and standard deviation values were calculated for height, weight, standing long jump, vertical jump, 10-20-30 m. sprint and yoyo tests of all participants. Skewness-Kurtosis test was performed to determine whether the data of the groups were normally distributed, and it was determined that the data of the two groups were normally distributed. Within-group pre-test and post-test comparisons were analysed by paired sample t test and between-group pre-test and post-test comparisons were analysed by independent sample t test. Alpha level $p \leq 0.05$ was taken as the statistical significance level.

RESULTS

When Table 1. is examined, the tabata group data of the participants in the study; mean age 22.3±4.14, mean height 174.1±8.43, body weight 66.1±8.32, body mass index 20.85±5.46, standing long jump 173.9±21.2, vertical jump 27.83±5.06, 10-m. sprint 2.42±0.2, 20-m. sprint 3.95±0.28, 30-m. sprint 5.69±0.38, yoyo 40.05±1.21.

When Table 2. is examined, the control group data of the participants in the study; mean age 21.7±5.50, mean height 175.6±7.34, body weight 65.7±7.79, body mass index 21.3±1.60, standing long jump 147.7±18.5, vertical jump 24.5±5.4, 10-m. sprint 2.37±0.16, 20-m. sprint 3.99±0.36, 30-m. sprint 5.63±0.36, yoyo 39.65±0.91.

When the relationship analiyzed between the groups, statistically significant differences were found in the post test weight (-1.92±0.38), post-test BMI (-1.05±0.19), SLJ post-test (33.91±7.51), VJ post-test (7.66±1.54), and Yoyo post-test (1.437±0.26) measurements of TG and CG ($p>0.05$).

Table 1. Descriptive statistics of the Tabata group and findings related to Skewness and Kurtosis values

Parameters	n	Min.	Max.	Mean±SD	Skewness	Kurtosis	Cohens'd
Age (year)	12	17	30	22.3±4.14	0.527	-0.786	0.32
Height (cm)	12	155	185	174.1±8.43	-0.893	0.637	0.83
Body Weight (kg)	12	55.6	84.6	66.1±8.32	0.892	0.717	1.61
BMI (cm)	12	10	29	20.85±5.46	-.278	-.921	1.43
Standing long jump (cm)	12	131	209	173.9±21.2	-0.455	0.228	-2.76
Vertical jump (cm)	12	16	33	27.83±5.06	-1.176	1.531	-1.63
10-m sprint (sc)	12	2.13	2.78	2.42±0.20	0.835	0.118	-0.25
20-m sprint (sc)	12	3.50	4.43	3.95±0.28	0.386	-0.338	-0.24
30-m sprint (sc)	12	5.08	6.60	5.69±0.38	0.973	2.765	0.20
Yoyo (kg/ml/min)	12	38.75	42.14	40.05±1.21	0.724	-0.958	-1.23

Min: Minimum, Max: Maximum, sc: second, BMI: Body Mass Index, SD: Standart Deviation

Table 2. Descriptive statistics of Control group and findings related to Skewness and Kurtosis values

Parameters	n	Min.	Max.	Mean±SD	Skewness	Kurtosis	Cohens'd
Age (year)	12	16	35	21.7±5.50	1.562	2.226	0.12
Height (cm)	12	157	183	175.6±7.34	-1.524	2.916	0.76
Body Weight (kg)	12	54.2	81	65.7±7.79	0.449	-0.2631	1.10
BMI (cm)	12	17.9	23.6	21.3±1.60	-0.503	0.398	1.94
Standing long jump (cm)	12	118	173	147.7±18.5	-0.161	-0.419	-0.22
Vertical jump (cm)	12	16	36	24.5±5.4	0.458	0.621	-0.36
10-m sprint (sc)	12	2.13	2.67	2.37±0.16	0.406	-0.823	-0.45
20-m sprint (sc)	12	3.56	4.67	3.99±0.36	0.457	-0.979	-0.12
30-m sprint (sc)	12	5.10	6.15	5.63±0.36	-0.020	-1.403	0.12
Yoyo (kg/ml/min)	12	37.7	40.7	39.65±0.91	-0.635	-0.071	-0.40

Min: Minimum, Max: Maximum, sc: second, BMI: Body Mass Index, SD: Standart Deviation

Table 3. Pre-post test values between groups

Parameters		Mean±SD	p	Cohens'd
BMI (cm)	Pre	0.19±0.13	0.195	0.13
	Post	-1.05±0.19	<.001*	0.19
Standing long jump (cm)	Pre	26.16±7.57	<.001*	0.99
	Post	33.91±7.51	0.046	1.30
Vertical jump (cm)	Pre	3.33±1.47	<.001*	0.65
	Post	7.66±1.54	0.960	1.30
10-m sprint (sc)	Pre	-0.03±0.06	0.111	-0.01
	Post	-0.082±0.04	0.662	-0.50
20-m sprint (sc)	Pre	-0.038±0.08	0.888	-0.12
	Post	-0.015±0.10	0.651	-0.04
30-m sprint (sc)	Pre	0.054±0.11	0.623	0.13
	Post	0.041±0.08	0.193	0.14
Yoyo (kg/ml/min)	Pre	0.406±0.29	<.001*	0.40
	Post	1.437±0.26	<.001*	1.59

*Min: Minimum, Max: Maximum, sc: second, BMI: Body Mass Index, SD: Standart Deviation, *: p<0.05*

DISCUSSION

This study was conducted to investigate the effects of 8-week tabata training on physical performance in amputee football players. For this purpose, 24 male football players who played in amputee football super league and had at least 5 years of amputee football history participated in the study voluntarily. An 8-week tabata exercise protocol was applied to the athletes during the normal football season. As a result of the study, it can be hypothesised that tabata practice improves physical performance in amputee footballers.

When the studies on the body composition of the participants were examined; Kayıhan and colleagues

determined the body mass index as 21.9±0.25 kg/m2 in a study conducted on 14 amputee national team football players (12) This study shows that a 4-week basic training programme including exercises for the improvement of physical parameters provided significant improvements in the body composition of amputee football national team athletes. Also, in another study, 12 amputee football players were compared with 14 sedentary below-knee amputees (13,14). According to the body mass index findings, there was a statistically significant difference between amputee football players and sedentary amputees in favour of football players. In a study conducted on 11 amputee footballers in the literature, it was reported that the body mass index was 21.88±2.08 kg/m2. (14). In another study, when the groups of 28 amputee footballers between the ages of 16-45 were compared among themselves, it was emphasised that the body mass index of the footballers who received training decreased more (16). In Yıldız's study, the effects of 6-week preparation period training applied to amputee football players on physical and physiological parameters were examined. The mean age of the players in the study was X=33.27±7.43 years, mean height was X=173.06±7.11cm, mean body weight was X=74.75±11.43 kg for pre-test and X=72.20 ± 9.50 kg for post-test (17). The study showed that there was no significant relationship between age and height and physical-physiological parameters, while there was a significant difference between body weight and parameters. These results indicate that the effect of training programme on body weight is significant (18). However, the unrelated findings of the study with age and height suggest that further research is required to understand the effect of training on these parameters in more depth. In this context, it may be important to conduct more comprehensive and long-term studies to understand how training programmes affect individual physical characteristics (18,20). In addition, it was stated that waist-hip ratios were higher in the training group than in the control group in proportion to body mass indexes. However, this difference was reported to disappear after the training. In our study, statistically significant differences were found in weight (-1.92±0.38) and BMI (-1.05±0.19) measurements (p>0.05).

According to the vertical jump distance classification study, the jump distance in men was defined as 66 cm and above excellent, 50-65 cm good, 40-49 cm average, 33-39 cm poor, 29 cm poor, and centimetres and below this measure were defined as 'very poor'. In our study, when the jump heights of amputee footballers before training were analysed, it was seen that the control and exercise groups were 'very poor' (21,22). When the distance jumped after training was analysed, it was seen that amputee football players in the control group jumped more than amputee football players in the training group, but no difference was observed between the two groups in terms of jump distance. It was noted that the difference in strength between the healthy and amputee sides was higher in amputee footballers than in sedentary amputees (23). The explanation for this result is that amputee footballers use their healthy side extremities more than sedentary amputees and their healthy side extremities are stronger. Mills et al. documented improvements in vertical jump performance in a training group of 20 female athletes aged between 18 and 23 years who participated in 10 weeks of lumbopelvic stabilisation training (24,25). In a 7-week, 3-day-a-week training programme for 19 female high school athletes, plyometric training was compared with dynamic stability and balance training, and both groups had better jump performance and significantly increased in the vertical jump test. Although the increase in dynamic stability and balance training was not an expected result, the researchers attributed this to the application of plyometric training.

This study can be applied for the application of different training techniques in amputee football. Physical performances of athletes can be improved with basic strength exercises to be applied on the field.

CONCLUSION

The analysis of the data revealed that there was a significant difference in body weight values between the teams. Also, significant differences were found between the groups in standing long jump and vertical jump values. As a result, it can be said that strength training plans made with the Tabata protocol applied during the season can contribute to the sportive performance of the teams and play an important role in the challenge against competitors.

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RESEARCH ARTICLE

INVESTIGATION OF THE RELATIONSHIP OF KINESIOPHOBIA AND PHYSICAL ACTIVITY LEVEL WITH DYSPNEA, MUSCLE STRENGTH, AND PROPRIOCEPTION IN PATIENTS WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE: A RETROSPECTIVE STUDY

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ABSTRACT

Purpose: This study aimed to examine the relationship of kinesiophobia and physical activity level with dyspnea, peripheral muscle strength and proprioceptive acuity in patients with chronic obstructive pulmonary disease (COPD).
Materials and Methods: A total of 36 patients with COPD were included in this retrospective cross-sectional study. The patients' demographics, kinesiophobia level, physical activity level, dyspnea severity, peripheral muscle strength, and proprioceptive level were recorded from the patient file.
Results: According to the current results, kinesiophobia level showed a significant relationship with physical activity level and sitting time ($p<0.05$). Additionally, the patients' kinesiophobia level showed a moderate significant relationship with dyspnea severity, quadriceps and tibialis anterior muscle strength ($p<0.05$). In addition, the physical activity level showed a moderate to strong significant correlation with sitting time, dyspnea severity, proprioceptive level, and muscle strength ($p<0.05$).
Conclusion: Dyspnea, peripheral muscle strength, and proprioceptive levels are important interrelated parameters that increase kinesiophobia and limit physical activity in COPD. Therefore, dyspnea, peripheral muscle strength, and proprioception should be evaluated within the scope of pulmonary rehabilitation from the early period, and therapeutic approaches aiming to minimize the effects of these symptoms should be included in the pulmonary rehabilitation program.

Keywords: Lung diseases; dyspnea; physical activity; kinesiophobia; peripheral muscle strength

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) with pulmonary and systemic symptoms is a progressive and treatable disease. The course of COPD is complicated by developing systemic consequences and comorbidities (1). One of the most important pulmonary symptoms of COPD is dyspnea, which increases upon physical exertion (2). Patients with COPD attempt to compensate for the increased dyspnea by reducing their activity level or avoiding activities, resulting in peripheral muscle deconditioning (3). Although activity limitation in COPD is multifactorial, dyspnea related to acute derangements in dynamic respiratory mechanics is the primary activity-limiting factor in most patients with COPD (2, 3).

In addition to the pulmonary conditions, systemic effects of COPD, such as inflammation and hypoxia, also lead to peripheral muscle weakness via impairing neuromuscular activity (4). Approximately 32% of patients with COPD suffer from skeletal muscle weakness and decreased muscle endurance (5). Previous studies reported that the quadriceps muscle weakens most rapidly in patients with COPD, and quadriceps weakness in this patient population is a primary indicator of decreased functional capacity, deteriorated pulmonary function, and increased mortality (6). As a result, in addition to the pulmonary pathologies, extra-pulmonary conditions of COPD provoke physical activity avoidance, which increases fall risk and fractures (6, 7).

Kinesiophobia is an individual's excessive avoidance of physical movement and activity due to the fear of a painful injury or re-injury (8). Pulmonary and extrapulmonary conditions of COPD reduce physical activity levels by exaggerating the fear of movement and thus impair peripheral muscle strength, proprioception, and balance (7, 9, 10). Patients experience a progressive decrease in functional exercise capacity, pulmonary function, and peripheral muscle strength as a consequence of physical inactivity; these symptoms affect each other negatively, thus leading to a vicious cycle (3, 11). Additionally, physical inactivity seriously impairs the self-care and mobility functions, and social life of patients with COPD (3). In the existing literature, there are a limited number of studies examining the relation of COPD-specific pain- and dyspnea-related kinesiophobia to physical activity level (12-14). However, no study investigated the association between extrapulmonary conditions (i.e., peripheral muscle weakness, neuromuscular control, proprioception) and kinesiophobia in patients with COPD. Studies are needed to establish to what extent kinesiophobia, resulting from pulmonary and extrapulmonary conditions of COPD, is related to activity and functional limitation. Such studies can help to determine appropriate pulmonary rehabilitation for patients with COPD. Therefore, this study aimed to determine the relationship of kinesiophobia and physical activity level with dyspnea, peripheral muscle strength and proprioception in patients with COPD.

MATERIALS AND METHODS

Study Design and Participants

In this retrospective cross-sectional study, the files of patients with COPD included in pulmonary rehabilitation between 2017 and 2020 in the Pulmonary Rehabilitation Unit of the Department of Chest Diseases of Dokuz Eylül University were evaluated. The files of patients aged 40 years and over who were diagnosed with COPD according to the "Global Initiative for Chronic Obstructive Lung Disease (GOLD)" criteria were included in the study to obtain clinical data (15). Patients' files recorded incompletely regarding the evaluation parameters or files of patient with musculoskeletal or neuromuscular disorders, previous lung surgery, unstable angina, malignancy, congestive heart failure, uncontrolled hypertension, and a history of pulmonary diseases other than COPD were not included in the study.

Ethical Approval

Prior to the study, local ethics committee approval for the retrospective use of databases recorded in patients' files for the current study was obtained from the Non-Invasive Clinical Research Ethics Committee of Dokuz Eylul University (Date: 09.11.2022, Decision No: 2022/36-20). This study was conducted in accordance with the Declaration of Helsinki.

Assessments

The demographics and clinical data (age, gender, height, smoking history, disease duration) were recorded from patients' files. Additionally, forced expiratory volume in one second (FEV1), forced vital capacity (FVC), FEV1/FVC ratio, and their percentages according to predicted values according to the American Thoracic Society/European Respiratory Society criteria, the airflow limitation stage in COPD according to the GOLD criteria

(Mild/Stage 1; FEV1≥80%, Moderate/Stage 2; 50%≤FEV1<80%, Severe/Stage 3; 30%≤FEV1<50% and Very severe/Stage 4; FEV1<30%), the presence of respiratory symptoms (cough, sputum, and dyspnea severity) were recorded from the patients' files to be used in this study. Furthermore, the patients' kinesiophobia and physical activity levels, perceived dyspnea severity, peripheral muscle strength, and proprioception levels were also recorded from the patients' files.

The evaluating methods for these variables are explained below;

Kinesiophobia (fear of movement): The Tampa Scale of Kinesiophobia (TSK), a reliable and valid 17-item self-reported questionnaire with a 4-point Likert scale, was used to determine the kinesiophobia level (16). The TSK is based on the fear-avoidance, fear of movement, and fear of reinjury model. The total score of the TSK ranges from 17 to 68, with higher scores representing a high degree of kinesiophobia. A TSK score at or above the cut-off value (≥ 37) is considered a high degree of kinesiophobia (17).

Physical activity level: The short form of the International Physical Activity Questionnaire (IPAQ), a valid and reliable scale, is frequently used for assessing physical activity levels. This scale consists of 7 questions and provides information about the time spent in walking, moderate- and vigorous-intensity activities in the last 7 days (for last week). The total physical activity score is obtained by multiplying the duration (minutes) and frequency (days) values of walking, moderate and vigorous activity with the different metabolic equivalent of task (MET) coefficient values for each activity and then calculating a score of the "MET-minutes/week" type by adding these three activity scores. The time spent sitting is evaluated as a separate question and its score is calculated separately. The physical activity level is classified as inactive (<600 MET-min/week), low (600-3000 MET-min/week), and sufficient activity level (>3000 MET-min/week) (18).

Dyspnea level: The modified Medical Research Council (mMRC) dyspnea scale is frequently preferred for evaluating patients' perceived dyspnea levels during daily activities. This scale grades the patient's perceived dyspnea in various physical activities for five levels (range 0-4) (19).

Muscle strength: Isometric muscle strength measures using Hand-held dynamometer (HHD) is a reliable, valid, and simple method (20). The quadriceps, iliopsoas and tibialis anterior muscle strength scores that had been evaluated with HHD (MicroFET2®, Hoggan Health Industries, Inc., UT, USA) were recorded from the patients' files for analysis.

Proprioceptive measurement: The target angle reproduction test is a valid and reliable method to assess joint position sense acuity (21). For knee proprioception assessment, subjects are seated on a standard chair or examination table; then, the lower leg is passively placed at the target knee flexion angle to allow the subjects to memorize the target angle. Following the familiarization session, subjects are blindfolded and asked to replicate the target angle by actively moving their leg from the starting position to the target angle. The difference between the target and reproduced angles represents the knee proprioception acuity. Knee proprioception that had been measured for the knee flexion angle of 60˚ was recorded from patients' files.

Statistical Analysis

The IBM® SPSS® (ver. 26.0; IBM Corp., Armonk, NY, USA) package program for Windows software was used for analysis of data. The normal distribution of the data was determined using the Shapiro-Wilk test. Descriptive statistics were presented as mean (standard deviation) for parametric variables, median (interquartile range) for non-parametric variables, and frequencies and percentages for categorical variables. The correlations were assessed using the Pearson's and Spearman correlation coefficient for parametric and non-parametric conditions, respectively. The correlation level was considered negligible if the coefficient was less than 0.10, low if it was between 0.10 to 0.39, moderate if it was between 0.40 to 0.69, and strong if it was greater than 0.70 (22). A value of $p<0.05$ was considered statistically significant.

An a priori power analysis identified a sample size of 36 that was needed to achieve 90% statistical power with a probability of a 2-tailed type I error of 0.05 and an effect size of 0.50 in the correlation analysis (23). In order to reach the determined sample size, a total of 62 patients' files were screened for eligibility. A total of 26 patients' files were excluded because they did not meet the inclusion criteria.

RESULTS

The mean age of the patients was 66.94 ± 10.40 years, and 14 (38.9%) of the participants were female. Most of the patients (72.2%) in stage-2 according to GOLD, and they had FEV1% of 42.41 ±

Table 1. Demographic and clinical characteristics of patients with COPD

Variables	Mean ± SD Median (IQR_{25-75}) n (%)	Min. – Max.
Sex (male/female), n (%)	14 (38.9) / 22 (61.1)	–
Age (years)	66.94 ± 10.40	46.00 – 81.00
Height (cm)	162.00 (155.00/172.00)	150.00 – 190.00
Weight (kg)	71.41 ± 13.29	48.10 – 95.60
BMI (kg/m^2)	27.00 (22.50/30.38)	19.20 – 35.30
Duration of disease (years)	11.00 (10.00/16.50)	.25 – 20.00
GOLD stage		
Stage 1 (Mild), n (%)	2 (5.6)	–
Stage 2 (Moderate), n (%)	26 (72.2)	–
Stage 3 (Severe), n (%)	8 (22.2)	–
Smoking history		
No smokers, n (%)	6 (16.7)	–
Former smokers, n (%)	9 (25.0)	–
Current smokers, n (%)	21 (58.3)	–
Smoking consumption (pack/years)	20.00 (2.00/92.50)	.00 – 360.00
Regular exercise habit		
Yes, n (%)	7 (19.4)	–
No, n (%)	29 (80.6)	–
Respiratory symptoms		
Shortness of breath, n (%)	28 (77.8)	–
Cough, n (%)	26 (72.2)	–
Sputum, n (%)	25 (69.4)	–
Pulmonary function tests		
FEV1 (%pred)	42.41 ± 7.12	29.00 – 56.00
FVC (%pred)	60.00 ± 10.99	42.00 – 88.00
FEV1/FVC (%pred)	51.84 ± 7.34	36.45 – 63.39

COPD: chronic obstructive pulmonary disease, SD: Standard deviation, IQR: Interquartile range, n: number, Min: Minimum, Max: Maximum, BMI: Body Mass Index, pred: predicted, GOLD: Global Initiative for Chronic Obstructive Lung Disease, FEV1 forced expiratory volume in one second, FVC forced vital capacity

7.12 percent predicted. In addition, 77.8%, 72.2%, and 69.4% of participants had dyspnea, cough, and sputum, respectively. The median value of disease duration was 11.00 (10.00/16.50) years. Demographics and clinical characteristics of the patients are presented in Table 1.

Most of the patients with COPD (77.8%) had a high level of kinesiophobia (≥ 37 points) according to their mean TSK score (43.94 ± 11.12). The participants' physical activity level (66.00 (.00/240.00) MET-min/week) was low, and their sitting time (10.00 (6.00/18.00) hour/week) was considerably prolonged. In addition, the quadriceps, iliopsoas and tibialis anterior muscle strengths of the individuals were 7.47 ± 2.40 kg, 7.34 ± 2.06 kg, 8.93 (7.10/9.60) kg, respectively. The median value of the mMRC score was 4.00 (3.00/5.00), which is greater than the threshold value of mMRC ≥2. The knee proprioceptive acuity of participants was determined as 3.50 (1.33/4.16) degrees. The kinesiophobia, physical activity level, sitting times, muscle strength scores, dyspnea severity, and knee proprioception of patients with COPD are presented in Table 2.

According to the correlation analysis; a moderate correlation of kinesiophobia with physical activity level (r=-609 p<0.001), and with sitting time (r=674 p<0.001). The kinesiophobia level of the patients showed a significantly moderate association with the dyspnea severity (r=446 p=0.006), with quadriceps muscle strength (r=-519 p=0.001), and with tibialis

anterior muscle strength (r=-413 p=0.012). A significant association of the physical activity level, ranging from moderate to strong, was found with sitting time, mMRC, proprioception, lower extremity muscle strength (p<0.05). A significant association of participants' sitting time, ranging from weak to moderate, was determined with mMRC, quadriceps and tibialis anterior muscle strength, proprioceptive scores (p<0.05). The relationship between kinesiophobia level, physical activity level, sitting time, dyspnea level, lower extremity muscle strength, and proprioception of patients with COPD are presented in Table 3.

DISCUSSION

According to our results, the majority of patients with COPD exhibited high levels of kinesiophobia, physical inactivity, and prolonged sitting times. Nevertheless, kinesiophobia, physical activity level, and sitting time showed a low to strong significant association with perceived dyspnea severity, peripheral muscle strength, and proprioception in patients with COPD.

Skeletal muscle deconditioning, as one of the common systemic effects of COPD, is associated with airflow limitation, exercise intolerance, physical inactivity, and mortality (24, 25). Lower extremity muscle weakness, due to the systemic effects of COPD, considerably increases exercise intolerance, fatigue, and fall risk, initiating a vicious cycle that limits physical activity participation (26). Therefore, exercise rehabilitation focusing on muscle strengthening is an important component of the pulmonary rehabilitation program. In COPD, muscle

Table 2. Scores of kinesiophobia, physical activity level, sitting time, perceived dyspnea level, muscle strength, and proprioception in patients with COPD

Variables	Mean ± SD Median (IQR_{25-75})	Min. – Max.
Kinesiophobia	43.94 ± 11.12	27.00 – 66.00
TPA (MET-min/week)	66.00 (.00/240.00)	.00 – 5892.00
Sitting time (hour/week)	10.00 (6.00/18.00)	3.00 – 18.00
mMRC score	3.00 (2.00/4.00)	.00 – 4.00
Quadriceps strength (kg)	7.47 ± 2.40	3.40 – 12.20
Iliopsoas strength (kg)	7.34 ± 2.06	3.70 – 12.27
Tibialis anterior strength (kg)	8.93 (7.10/9.60)	5.23 – 10.87
Proprioception (degree)	3.50 (1.33/4.16)	.67 – 7.33

COPD: chronic obstructive pulmonary disease, SD: Standard deviation, IQR: Interquartile range, Min: Minimum, Max: Maximum, kg: kilogram, TPA: total physical activity, MET-min/week: metabolic equivalent minutes per week, mMRC: modified Medical Research Council

Table 3. Correlation of kinesiophobia and physical activity level with perceived dyspnea level, muscle strength, and proprioception in patients with COPD

	Kinesiophobia (r)	Physical Activity Level	
		TPA (r)	Sitting time (r)
TPA (MET-min/week)	-.609***	–	-.783***
Sitting time (hour/week)	.674***	-.783***	–
mMRC score	.446**	-.477**	.474**
Quadriceps strength (kg)	-.519**	.586***	-.471**
Iliopsoas strength (kg)	-.306	.436**	-.375*
Tibialis anterior strength (kg)	-.413*	.612***	-.496**
Proprioception (degree)	.258	-.573***	.419*

COPD: chronic obstructive pulmonary disease, r: Pearson's correlation coefficient, kg: kilogram, TPA: total physical activity, MET-min/week: metabolic equivalent minutes per week, mMRC: modified Medical Research Council *0,01<p≤0,05, **0,001<p≤0,01, ***p≤0,001

mass and strength loss are predominantly observed in the lower extremity muscles, especially in the quadriceps muscle (6, 27). The quadriceps muscle is the most commonly studied muscle group in patients with COPD because it is primarily affected compared to other muscle groups and determinant of ambulation and functioning and provides prognostic data (24). Studies have shown that patients with COPD experience a decrease in quadriceps strength ranging from 30 to 80% compared to healthy controls, depending on the severity of the disease (24, 28). Consistent with the literature, the mean value of quadriceps muscle strength of patients with COPD in the current study is similar to those reported in previous studies (28). In addition, the mean value of quadriceps strength of patients with COPD in the current study is considerably lower than those of healthy individuals presented in the previous study (28). In the existing literature, a limited number of studies evaluated lower extremity muscle groups other than the quadriceps specific to the COPD patient group (7, 27). Our findings are similar to the results of the previous studies regarding the tibialis anterior and iliopsoas muscle strength levels of patients with COPD. However, our patients' tibialis anterior and iliopsoas strength levels are considerably lower than those of healthy individuals presented in the previous study (7, 27).

Although quadriceps muscle weakness and associated factors in patients with COPD have been extensively studied, no study investigated the relationship between kinesiophobia and quadriceps muscle strength in patients with COPD in the existing literature. Additionally, a limited number of studies have evaluated lower extremity muscle groups (i.e., tibialis anterior, iliopsoas) other than the quadriceps muscle in patients with COPD and examined their relationship with COPD-specific symptoms (7, 27). Nonetheless, previous studies conducted on different patient populations have reported significant associations between kinesiophobia and lower extremity muscle strength (29, 30), and similarly, our findings showed a moderately significant association between kinesiophobia and lower extremity muscle strength in patients with COPD, as well. Consequently, our results suggest that including the kinesiophobia and peripheral muscle strength assessment into the routine clinical evaluations of patients with COPD from an early stage is necessary to determine an appropriate pulmonary rehabilitation program.

Kinesiophobia reduces compliance not only to musculoskeletal rehabilitation but also to pulmonary rehabilitation (9, 13) since it increases the tendency to avoid physical activity. Fear of movement complicates exercise and physical activity participation in patients with COPD (9, 13). According to the American College of Sports Medicine (ACSM) and the American Heart Association (AHA) guidelines, adults are recommended "to engage in at least 30 min of moderate-intensity physical activity for five days or at least 20 minutes of vigorous-intensity physical activity for three days in a week" (31). However, patients with COPD in our study represent considerably lower physical activity levels than indicated by these physical activity guidelines. According to the IPAQ scores of the patients with COPD in the current study, 77.8% were inactive (<600 MET-min/week), and 11.1% had a low physical activity level (600-3000 MET-min/week), which is an important finding indicating a decrease in their physical activity levels. Similar to our finding, previous studies have shown that patients with COPD have lower daily physical activity and higher kinesiophobia levels compared to healthy individuals (13, 32, 33). On the other hand, only one study, in the existing literature, examined the relationship between physical activity level and kinesiophobia in patients with COPD (33). Although authors of this study used the breathlessness beliefs questionnaire (BBQ) for the kinesiophobia assessment, they reported similar results to our findings (33). In addition, in line with the current data in the literature, 77.8% of the patients with COPD had high kinesiophobia (cut-off value ≥ 37 points) (17), and a significantly moderate and inverse correlation was observed between physical activity and kinesiophobia levels in the current study.

Patients with COPD reduce their exercise and physical activity participation due to concerns that symptoms will be triggered (9). In addition, compared to their healthy peers, patients with COPD perceive their disease as an obstacle to participating in physical activity and, therefore, reduce their daily physical activities, such as climbing stairs and walking, which increases the risk of comorbidities and leads to more inactivity (9, 13). In fact, a limited number of studies have shown that uncontrolled disease symptoms such as pain, fatigue, and dyspnea result in increased kinesiophobia in patients with COPD (13, 14, 32). On the other hand, searching the existing literature, according to our knowledge, no study examined the relationship between muscle

strength and kinesiophobia level in patients with COPD; however, according to our findings, muscle strength (quadriceps and tibialis anterior) showed a moderately and inversely significant correlation with kinesiophobia level. Moreover, the positive significant relationship obtained in the current study between lower extremity muscle strength, especially the quadriceps muscle, and physical activity level suggests that muscle strength is important in maintaining functional independence in patients with COPD. Although cross-sectional correlational analysis, used in this study, limits our ability to comment on causality and effect, our findings support the view that adding muscle strengthening exercises, particularly in the lower extremity muscle group, to pulmonary rehabilitation from an early stage can improve physical activity level and kinesiophobia in patients with COPD.

Recent studies have reported that approximately one-third of patients with COPD have experienced at least one fall in the last six months due to loss of postural balance (34). Falls in patients with COPD lead to complications such as morbidity and mortality, increasing healthcare costs (34, 35). Adequate postural balance requires the interaction of visual, vestibular, and proprioceptive inputs, which provides appropriate processing time for motor responses (10). However, the most important determinant of postural control is accurate and appropriate proprioceptive sensory inputs. Proprioceptive sensory inputs, increasing body awareness, enable safer and more appropriate movements in daily life activities; such movement patterns are closely related to motor control and functional performance (10, 34). A few studies showed proprioceptive impairments in patients with COPD (10, 36); nonetheless, according to our knowledge, no study examined the association between proprioception and COPD-specific symptoms.

According to our findings, proprioceptive level showed a moderately significant correlation with physical activity level and sitting time, but no significant correlation was found between proprioceptive level and kinesiophobia. COPD-specific symptoms, such as increased respiratory demand and decreased activity level, debilitate proprioceptive sensory input and eventually impair postural stability (7, 34). In patients with COPD, the perceived dyspnea level and supplemental oxygen requirement increase with both vigorous physical activity and especially at the advanced stages of the disease. Increased dyspnea level and supplemental oxygen requirement oxygen are closely associated with reduced exercise capacity, increased postural imbalance, and falls (7). Regular and adequate physical activity increases the proprioceptive level and, therefore, neuromuscular control by contributing to morphological adaptations in muscle fibers, muscle spindles, and mechanoreceptors that provide proprioceptive sensory input (37). On the other hand, previous studies have shown evidence that patients with COPD have a sedentary lifestyle and tend to gradually reduce physical activity to cope with the increased respiratory demand (7, 37). This further increases inactivity and thus reduces lower extremity muscle strength and proprioceptive level. As a result, patients with COPD experience impairment in producing maximum voluntary contraction and in integration of sensory input compared to healthy peers (7, 10, 37). These suggestions are in line with our findings that proprioception showed a significant correlation with physical activity level and sitting time. Consequently, our findings postulate that proprioceptive exercises added to the pulmonary rehabilitation of patients with COPD can improve physical activity levels and, therefore, muscle strength and kinesiophobia.

Dyspnea, as a subjective experience and a core symptom of COPD, is a significant determinant of quality of life, exercise compliance and tolerance, and mortality (3). The mMRC is commonly used to evaluate dyspnea severity, and the cut-off point for severe dyspnea has been specified as mMRC score ≥ 2 (38). In the current study, the mMRC score of patients with COPD was above the specified cut-off value (mMRC ≥ 2) and showed a significant correlation with physical activity level and sitting time. Patients with COPD reduce their exercise/activity participation to diminish perceived dyspnea severity; this weakens the lower extremity muscles (especially the quadriceps) more than the upper extremity and respiratory muscles (39). The mechanism of activity avoidance due to respiratory symptoms in COPD may explain the findings of our study. More, in the current study, the median disease duration of COPD was 11 years; muscle strength might have been impaired as a result of long-term exposure to dyspnea and activity avoidance during this long period. Besides, dyspnea severity was significantly correlated with the kinesiophobia level, in the current study. Patients with COPD experience increased dyspnea during daily activities and even at rest, depending on the severity

level of the disease. When individuals perceive breathless during activity or at rest, cortical regions associated with their previous dyspnea experience are activated, which stimulates cortical regions processing emotions, such as the insula, anterior cingulate cortex, and amygdala, through neural pathways, thereby triggering the dyspnea-related fear (40). Showing a low-to-moderate significant correlation between kinesiophobia and dyspnea severity, previous studies advocated that dyspnea-related fear in patients with COPD is the main reason for exercise and physical activity avoidance (14, 33). While the BBQ, which includes questions about dyspnea-related kinesiophobia, was used in these previous studies (14, 33), the TSK scale, most items of which are related to pain, was used for kinesiophobia assessment in the current study. Although a different questionnaire was used in the current study, our results were similar to those of previous studies. Dyspnea and pain are multidimensional and subjective experiences with common neural pathways stimulating similar cortical areas. Therefore, the perception of pain and dyspnea may trigger similar effects on kinesiophobia (40, 41). This mechanism could be a possible reason for our finding regarding the significant correlation between dyspnea and kinesiophobia.

Limitations

The main limitation is that no healthy controls were included in the current study. Nevertheless, we compared our findings with those of both patient and healthy groups of previous studies conducted in the same patient population and also interpreted our findings by considering the cut-off values of the evaluation parameters. Second, the retrospective design of the study is another limitation. Third, the generalizability of our results for all COPD stages is limited by the fact that not all GOLD stages were included into the current study. Additionally, conducting the current study in a single-centered clinic may also influence the generalizability of our findings. The fourth limitation is that the TSK questionnaire is not disease-specific, and its items mostly relate to pain. Using a dyspnea-specific kinesiophobia questionnaire in addition to the TSK could have provided more comprehensive inferences. Lastly, the parameters such as patients' anxiety which possibly affect kinesiophobia in patients with COPD were not evaluated. Therefore, future studies with a prospective design, including healthy controls and evaluating different parameters that may affect kinesiophobia (i.e., anxiety level) should be warranted. Despite these limitations, our study, comprehensively examining the relationship between kinesiophobia, physical activity, dyspnea, muscle strength, and proprioceptive level in patients with COPD, provided conclusive results, which can be a resource for clinicians and researchers.

CONCLUSION

Our study showed that increased kinesiophobia and decreased physical activity levels in COPD are associated with increased dyspnea severity, decreased muscle strength, and proprioceptive level. These findings obtained in the current study suggests that increased dyspnea severity, decreased muscle strength and proprioceptive level in patients with COPD may trigger activity avoidance. Therefore, our results emphasize the importance of evaluating these parameters from an early stage of pulmonary rehabilitation. In conclusion, a pulmonary rehabilitation program should include therapeutic approaches aiming to decrease kinesiophobia level and maintain physical activity participation in patients with COPD by improving dyspnea, muscle strength, and proprioceptive levels.

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RESEARCH ARTICLE

INVESTIGATION OF EPIDERMAL GROWTH FACTOR RECEPTOR PATHWAY RELATED, EXOSOMAL miRNAS IN NON-SMALL CELL LUNG CANCER CASES

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ABSTRACT

Purpose: The Epidermal Growth Factor Receptor (EGFR) gene and its associated pathways have recently emerged as promising targets in precision medicine for Non-Small Cell Lung Cancer (NSCLC). This study serves as a proof of concept, leveraging exosomal miRNAs as a cost-effective and minimally invasive liquid biopsy method. We aim to investigate four exosomal miRNAs: miR-22-3p, miR-221-3p, miR-30b and miR-30c and with miR-1288 serving as control. These miRNAs have been previously defined in the literature.
Materials and Methods: A total of thirty-six samples from distinct Non-Small Cell Lung Cancer (NSCLC) cases are included. Exosomes are derived from the patients' plasma, followed by miRNA isolation, cDNA synthesis, and quantitative polymerase chain reactions. The Δ/Δ Ct approach is employed for quantification of miRNAs.
Results: The controls: miRNA and miR-1288 are expressed in almost all samples. One sample is an exception. The two target miRNAs, miR-30c and miR-22-3p, generated successful polymerase chain reaction (PCR) curves. However, the remaining two miRNAs, miR-221-3p and miR-30b produced PCR curves with low amplitude.
Conclusion: miR-30b, miR-30c, miR-221-3p, miR-22-3p have previously been reported to be associated with lung cancer, but they have not been studied in a patient series until now. In our study, exosomal miR-22-3p and miR-30c were isolated from the cancer patients' plasma, suggesting that they could serve as potential lung cancer biomarkers.

Keywords: EGFR, exosome, liquid biopsy, miRNA, Non-Small Cell Lung Cancer (NSCLC).

INTRODUCTION

Among the world, lung cancer is the leading cause cancer related deaths. World Health Organization reported the incidence for lung cancer cases is about 2 million per year (1). In Turkey, the incidence is 29,314. Majority of those cases are male patients and the mean age of patients at the time of lung cancer diagnosis is 60. The 80.7% of them have Non-Small Cell Lung Cancer (NSCLC) type. At the time of diagnosis, about 47% of them are in the advanced

stage (2). Patients diagnosed at an early stage have a chance of cure with surgery, but patients diagnosed at a late stage do not have this chance. Therefore, new treatment approaches are needed.

Emerging therapeutic approaches are being developed to address the genetic profile of tumors. In clinical settings, key biomarkers for NSCLC are the driver mutations associated with the EGFR, KRAS, ALK, ROS1 and MET genes. About 15% of NSCLC patients have mutations in the Epidermal Growth Factor Receptor (EGFR) gene. Exon 19 deletions and the L858R mutation of EGFR enhance the tyrosine kinase activity. Medications such as erlotinib, gefitinib, and afatinib, which target these EGFR mutations, improve progression-free survival (3). However, in 50% of cases, additional EGFR mutations (such as T790M) lead to drug resistance (4). Examining EGFR mutations has become a standard procedure in advanced-stage NSCLC cases. Somatic EGFR mutations need to be monitored intermittently throughout the cancer process and the genetic profile in different metastatic foci needs to be determined. Liquid biopsy has provided the answer to this need.

Liquid biopsy, includes four main concepts: Extracellular Vesicles (EVs), Tumor-educated platelets (TEPs), Circulating tumor cells (CTCs), Cell-free DNA (cfDNA). Exosomes are subclass of EVs, and they are stable in plasma (5). Exosomes are membrane vesicles and they are 40-150 nm in diameter. Exosomes may be detected in plasma, ascites and other body fluids and. Recent studies suggest that those vesicles function as communicators between tumor cells and far body sites. Exosomes released by tumors contribute to tumor progression and play key roles in immunomodulation, angiogenesis, and drug resistance (6). They transport active genetic material, with miRNA notably influencing the pre-metastatic niche (7). It is proposed that exosomal miRNAs may have relations with EGFR variants, making them potential biomarkers for NSCLC cases (8). Thus, we aim to examine four miRNAs associated with the exosomal EGFR pathway in metastatic NSCLC patients tested for EGFR variants through liquid biopsy.

MATERIALS AND METHODS

Participants

Four miRNA targets associated with the EGFR pathway were used for analysis: miR-221-3p, miR-22-3p miR-30b and miR-30c. The miR-1228 and the housekeeping gene SNORD48 were analyzed as internal controls. Patients diagnosed with advanced stage NSCLC participated in the study. EGFR somatic mutation research results of solid biopsy samples taken at the time of diagnosis and liquid biopsy samples taken during follow-up are used in the study. Since there are no guidelines regarding the sampling criteria and when liquid biopsy samples should be taken during routine patient follow-up, the results of available liquid biopsy samples are used.

Study Group

The study group included NSCLC patients who underwent EGFR mutation testing via liquid/solid biopsies and were managed at the Department of Pulmonary Medicine, Ege University Hospital, and the Genetic Evaluation Center, Tepecik Training and Research Hospital. The study has been approved by the ethics committee of Dokuz Eylul University, Non-invasive Clinical Research Ethical Committee (Date: 13.02.2019, Decision no: 2019/03-72).

RNA Isolation and Sample Collection

Blood samples were collected from 36 cases, and plasma was separated within 24 hours. Plasma samples were kept at -80°C until exosome extraction. Sequentially, exosome extraction, miRNA isolation (50 µL total), and cDNA synthesis were performed using commercial kits as per the manufacturers' protocols [Midi Kit for Plasma/Serum Exosome Purification and RNA Isolation (58500), Norgen, Canada].

Quantitative Polymerase Chain Reaction (Q-PCR)

The cDNA samples are diluted to a concentration 80 times lower than the original. PCR reactions are carried out using the “LightCycler® 480 SYBR Green I Master” (Roche Molecular Systems, USA) along with specific primers targeting the desired miRNAs and reference genes. The Δ/ΔCt approach is applied to normalize the Ct values of miRNAs and perform relative quantification (9).

Statistical Analysis

The statistical analyses were performed to assess the frequency and distribution of mutations detected in solid and liquid biopsies, as well as the association between mutation status and patient response to targeted therapies. Descriptive statistics, including frequencies and percentages, were used to

summarize categorical data. Continuous variables were analyzed using mean and standard deviation values where applicable. For the evaluation of mutation prevalence in solid biopsies, the data revealed a significant distribution across different mutation types. Chi-square tests were employed to determine whether the observed differences in mutation frequencies (e.g., Exon 19 deletion, Exon 21 mutation, and combined mutations) were statistically significant. Similarly, comparisons were made between the mutation detection rates in solid biopsies and the first liquid biopsies to evaluate concordance and sensitivity. A second liquid biopsy is performed in patients with decreased response to targeted therapy to investigate the presence of mutations associated with drug resistance. In cases requiring a second liquid biopsy, mutation dynamics were analyzed to assess the emergence of drug resistance-associated mutations. The proportional differences in mutation detection rates between the first and second liquid biopsies were evaluated using Fisher's exact test, given the small sample size. This approach allowed for robust statistical evaluation of the emergence of T790M mutations and other resistance-related genetic alterations. All statistical analyses were conducted using [software name, e.g., SPSS, R], and a p-value of <0.05 was considered statistically significant. Results are reported with 95% confidence intervals to ensure the reliability of the findings.

RESULTS

The descriptive characteristics of 36 patients are as follows: Twenty males (55.6%) males and 16 females (44.4%). The mean age of the patients was 60.1±11.7 and the median was 58.0 [38.0-85.0]. When the distribution of patients according to smoking status is evaluated: 11.1% (n=4) are smokers (active smokers), 19.4% (n=7) are non-smokers, and 25.0% (n=9) are ex-smokers (former smokers). When the distribution of patients according to survival status is evaluated: 33.3% (n=12) are dead and 30.6% (n=11) are alive (Table I). The patient samples are previously investigated for somatic, driver EGFR mutations as routine workup of NSCLC cases by Roche Cobas (USA) system according to the producer's recommendations.

When the mutation detection status of the patients in solid biopsy is evaluated: No mutation is detected in 47.2% (n=17) of the cases, Exon 19 deletion is detected in 36.1% (n=13) of the cases, Exon 21 mutation in 5.6% (n=2), Exon 19 deletion and L858R mutation in 8.3% (n=3). In the first liquid biopsy of patients, Exon 19 deletion mutation is detected in 8.3% (n=3) of the cases, Exon 20 insertion is detected in 2.8% (n=1), and no mutation is detected in 88.9% of the cases. In the second liquid biopsy of patients Exon 19 deletion is found in 25.0% (n=1), Exon 19 deletion and T790M mutation in 50.0% (n=2), and Exon 20 insertion in 25.0% (n=1) of patients (Table 1). While there are 13 cases in which Exon 19 deletion is detected in solid biopsy; and 2 of these cases could be detected in the first liquid biopsy (Table 2).

Table 1. Descriptive characteristics of 36 patients.

	No	**%**
Gender		
Male	20	55.6
Female	16	44.4
Total	36	100.0
	Median ± SD	**Median [min-max]**
Age	60.1±11.7	58.0 [38.0-85.0]
Smoking Status		
Unknown	16	44.4
Ex smoker	9	25.0
Non-smoker	7	19.4
Smoker	4	11.1
Total	36	100.0
Survival		
Death	12	33.3
Alive	11	30.6
Unknown	13	36.1
Total	36	100.0
Solid Biopsy Result		
No mutation	17	47.2
Exon19 deletion	13	36.1
Exon 21	2	5.6
Exon 20 insertion	1	2.8
Exon 19 deletion & L858R	3	8.3
Total	36	100.0
Liquid Biopsy (first)		
Exon 19 deletion & T790M	3	8.3
Exon 20 insertion	1	2.8
No mutation	27	88.9
Total	31	100.0
Liquid Biopsy (second)		
Exon 19 deletion	1	25.0
Exon 19 deletion & T790M	2	50.0
Exon 20 insertion	1	25.0
Total	4	100.0

Table 2. The mutation status of patients by solid biopsy and first liquid biopsy.

Solid Biopsy Results and First Liquid Biopsy Results, Crosstabulation						
			Liquid Bx 1			Total n (%)
			Exon 19 deletion and T790M n (%)	Exon 20 insertion n (%)	No mutation n (%)	
Solid Biopsy	Exon 19 deletion n (%)	n	2	0	11	13
		% within Solid Biopsy	15.4%	0.0%	84.6%	100.0%
		% within First Liquid Biopsy	66.7%	0.0%	34.4%	36.1%
	Exon 19 deletion and L858R mutation n (%)	n	0	0	3	3
		% within Solid Biopsy	0.0%	0.0%	100.0%	100.0%
		% within First Liquid Biopsy	0.0%	0.0%	9.4%	8.3%
	Exon 20 insertion n (%)	n	0	1	0	1
		% within Solid Biopsy	0.0%	100.0%	0.0%	100.0%
		% within First Liquid Biopsy	0.0%	100.0%	0.0%	2.8%
	Exon 21 mutation n (%)	n	0	0	2	2
		% within Solid Biopsy	0.0%	0.0%	100.0%	100.0%
		% within First Liquid Biopsy	0.0%	0.0%	6.3%	5.6%
	No mutation (%)	n	1	0	16	17
		% within Solid Biopsy	5.9%	0.0%	94.1%	100.0%
		% within First Liquid Biopsy	33.3%	0.0%	50.0%	47.2%
Total n (%)		n	3	1	32	36
		% within Solid Biopsy	8.3%	2.8%	88.9%	100.0%
		% within First Liquid Biopsy	100.0%	100.0%	100.0%	100.0%

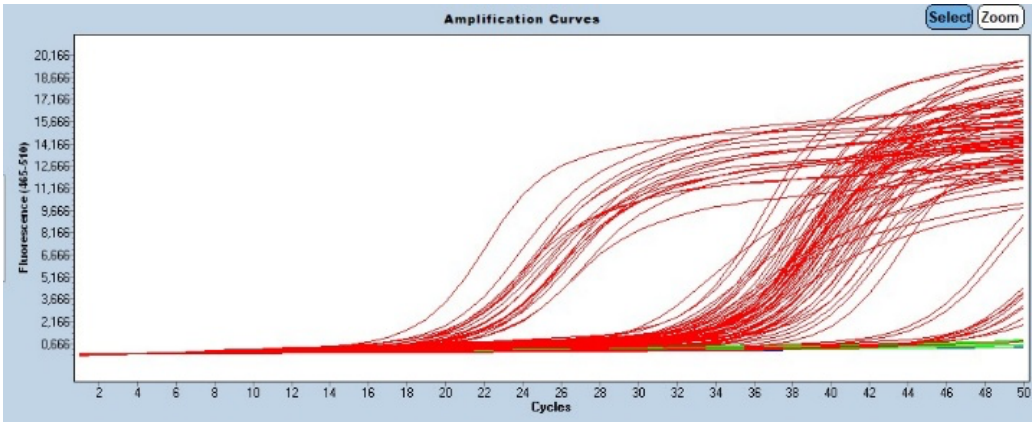

Figure 1. Amplification curves of the control and target probes. The control probe miR1288 has Ct values between 16-21 cycles (left side). The mean Ct values for miR-22-3p and miR-30c are 30.8 and 38.4, respectively (center panel). In contrast, two of the miRNAs, miR-221-3p and miR-30b, exhibit low PCR amplification curves, with Ct values exceeding 40 (right panel).

The control miR1288 was identified in every sample, except for one, whereas SNORD48 did not amplify in seven samples. This indicates that miR1288 is a reliable Q-PCR control. Two target miRNAs, miR-221-3p and miR-30b, yield insufficient PCR amplicons. Because of the limited quantity of extracted miRNA (50 µL), these targets cannot be reassessed and are therefore excluded from the study. The remaining miRNAs, miR-22-3p and miR-30c, generate PCR amplification curves, with average Ct values of 30.8 for miR-22-3p and 38.4 for miR-30c (Figure 1).

Among the samples analyzed, four cases showing amplification for miR-22-3p and miR-30c also exhibit EGFR mutations in their initial liquid biopsy: Three cases involve exon 19 deletions combined with the T790M mutation, and one case presents with an exon 20 insertion.

DISCUSSION

The EGFR gene is the most widely investigated target for personalized new therapies in routine oncology practice. Drug-sensitive and resistant variants are determining the management of NSCLC cases (11-13). Academic research is also focused on EGFR-related pathways and looking for genetic and epigenetic variations (14-15). Tumor tissues from the solid biopsies have the advantage great amount of tumor DNA but the disadvantages are also present: Resampling and simultaneous monitorization of tumor genotype evolution in all metastatic foci are almost impossible in most of the NSCLC cases. Monitorization is mandatory to realize the therapeutic targets. Therefore, the liquid biopsies from tumor-derived molecules such as ctDNA in the plasma get their role in clinical management. Moreover, other liquid biopsy techniques are emerging. One of them is based on EVs.

miRNAs are promising targets due to their potential as predictive biomarkers and their prospective role as therapeutic agents. Recently, several miRNAs have been investigated in the context of lung cancer, including microRNA-133b, microRNA-135, miR-181a, miR-223, microRNA-155, microRNA-200c, miR-30a-5p, microRNA-106a, miR-26a, miRNA-212, miR-486-5p, miR-3127-5p, miR-130a, miR-138-5p, microRNA-137, and the cluster miR-134/487b/655 (16–32).

Two of the targeted miRNAs, namely miR-221-3p and miR-30b, are omitted from the study. We consider that if the first miRNA isolate is adequate, we may have the opportunity to get proper PCR curves for them also. Starting with a minimum 100ul miRNA sample, would make a difference in success rates because of the exclusive lung cancer patient group, most of the time it is not possible to resample. SNORD48 does not appear to be an appropriate control for exosomal miRNA research, but miR1288 just failed in one sample so it could be preferred as a control.

A comprehensive study with 845 cases of EGFR mutation status of lung adenocarcinoma patients from our region is previously reported elsewhere (10). Therefore, the presented study does not focus on EGFR mutations but briefly: The most frequent EGFR mutation is exon 19 deletion in our study group, and it is found in 36.1% of solid biopsies, 8.3% of first liquid biopsies and 25% of second biopsies (Table II). The distinct positive mutation rates seem to be related to patient selection for testing. Because the same laboratory and methods are used for all samples (Roche Cobas, California, USA).

CONCLUSION

As a conclusion, in literature, EVs and miRNAs are investigated as potential promising biomarkers separately because of the challenging patient group, microvesicle, miRNA extraction, and Q-PCR protocols. Extracellular vesicles (EVs) carry a representative sample of cancer cell contents, including miRNAs (5), making exosomal miRNAs clinically significant. In our proof-of-concept study, exosomal miR-30c and miR-22-3p were successfully isolated from the NSCLC cases' plasma in a cost-effective manner, highlighting their potential as biomarkers for these patients. Among the cases analyzed, four exhibit EGFR mutations, with average Ct values of 30.8 for miR-22-3p and 38.4 for miR-30c. These values are comparable to those from non-mutant samples and fall within acceptable ranges for routine laboratory use. We consider that the investigation of the presented and recently reported lung cancer-associated miRNAs by our approach in exosomes would reveal a clinically significant genotype-phenotype correlation in NSCLC cases (16-32).

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Conflict of Interest: The authors declare that there is no conflict of interest for the presented study. The authors alone are responsible for the content and writing of this article.

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RESEARCH ARTICLE

PREDICTORS OF MORTALITY IN GRAM-NEGATIVE BLOODSTREAM INFECTIONS

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ABSTRACT

Purpose: Bloodstream infection (BSI) is the most common healthcare-associated infection in intensive care units (ICUs) and is associated with high mortality rates. In this study, we aimed to evaluate the etiological pathogens and susceptibility distribution and factors affecting mortality in patients followed up in the ICU with the diagnosis of healthcare-associated gram-negative BSI.
Material and Methods: This study was designed as a retrospective cohort study. Patients diagnosed with healthcare-associated BSI during ICU follow-up were included in the study. Patients demographic data, source of BSI, causative microorganisms and their antimicrobial susceptibility and mortality (any cause) rates were collected retrospectively from patient files and patient information sheets. Patients were divided into survival and non- survival groups according to the prognosis and differences in clinical data between the two groups were compared.
Results: The study included 162 patients with gram-negative BSI, of whom 85 (52.5%) died during their ICU stay. The three most common pathogens detected in patients were; *Klebsiella pneumoniae* [60/162(37%)], *Acinetobacter baumannii* [32/162(19.75%)] and *Stenotrophomonas maltophilia* [25/162(15.43%)]. The highest carbapenem resistance rates belonged to *A. baumannii* and *K. pneumoniae* with 93.75% and 81.66%, respectively. Multivariate logistic regression analysis identified, patients requiring invasive mechanical ventilation (IMV) had over three times the odds of death (OR: 3.10, 95% CI: 1.23–7.80, P = 0.016). Septic shock was associated with a nearly threefold increased risk of mortality (OR: 2.78, 95% CI: 1.29–6.00, P = 0.009), and continuous renal replacement therapy also significantly increased mortality risk (OR: 2.52, 95% CI: 1.11–5.71, P = 0.026).
Conclusion: IMV, septic shock, and the need for CRRT during ICU follow-up are risk factors for mortality in gram-negative BSI patients followed in the ICU. Among the etiologic pathogens, the highest resistance rates were found in *A. baumannii* and *K. pneumoniae,* respectively.

Keywords: Bloodstream infectious, intensive care unit, mortality, risk factors

INTRODUCTION

Bloodstream infection (BSI) is the most common healthcare-associated infection in intensive care units (ICUs) and is associated with high mortality rates (1,2). BSIs due to gram-negative pathogens have been observed with increasing frequency over the years (3). The management of gram-negative BSIs presents numerous challenges. Of particular concern is the rapid emergence and spread of carbapenem resistance in isolates, as these bacteria are frequently resistant to many other classes of antibiotics (4). This results in a high rate of clinically inappropriate initial treatment and/or the use of less effective drugs. The increasing carbapenem resistance rates in gram-

negative bacteria is a serious public health problem of global concern. According to the World Health Organization (WHO) 'Antimicrobial resistance surveillance in Europe 2023' report, the carbapenem resistance rate for *Acinetobacter spp.* in Turkey was reported as 91.5% in 2017 and 93.3% in 2021; the carbapenem resistance rate for *Klebsiella spp*. was reported as 32.5% in 2017 and 49.1% in 2021 (5). Knowledge of local epidemiology is useful for determining etiological distributions of antibiotic resistance, factors associated with mortality, guiding infection control, antimicrobial stewardship policies and informing clinicians about the best treatment approach (4). In this study, we aimed to evaluate the etiological pathogens and susceptibility distribution and factors affecting mortality in patients followed up in the ICU with a diagnosis of healthcare-associated gram-negative BSI.

MATERIALS AND METHODS

Study population

This study was designed as a retrospective cohort study. Ethics committee approval was obtained from Non-Pharmaceutical Clinical Research Ethics Committee of Izmir Health Sciences University, Dr.Suat Seren Chest Diseases and Surgery Training and Research Hospital (Date: 04/12/2024, Decision No: 2024/16-10). Patients aged ≥18 years with positive blood cultures for gram-negative pathogens between January 2022 and June 2024 were included in the study. Healthcare-associated BSI was defined as a positive blood culture obtained from a peripheral vein or central catheter at least 48 hours after hospitalization. Clinical signs of bacteremia were considered as the presence of at least one of hypotension, fever or chills. Only the first episode of patients who had more than one BSI during ICU follow-up was included in the study. Patients demographic data, source of BSI, causative microorganisms and their antimicrobial susceptibility, choice of empirical antibiotic therapy, details of any surgery within the previous month, and mortality (any cause) rates were collected retrospectively from patient files and patient information sheets. Patients were divided into survival and non- survival groups according to the prognosis and differences in clinical data between the two groups were compared.

Microbiological data-Blood Culture

The blood culture bottle, which was appropriately taken and delivered to the laboratory, was loaded into the liquid automated blood culture (*BacT Alert, bioMérieux, Marcy l'Etoile, France*) device. When a positive growth signal was obtained, the blood culture bottle was removed from the device and gram staining was performed for microscopic examination, and at the same time, each bottle was passaged into 5% sheep blood, chocolate, and eosin methylene blue (EMB) agar and incubated at 37°C for 18-24 hours. Pure colonies were selected from the growth detected plates and bacterial identification at species level was performed using both classical traditional methods and automated systems (*Phoenix, Becton Dickinson Instrument Systems, Sparks, MD, USA*), and drug susceptibility tests were performed according to the recommendations of the European Committee on Antimicrobial Susceptibility Testing (EUCAST).

Statistical Analysis

The normality of the data was assessed using the Kolmogrov-Smirnov test. Continuous variables were presented as medians and interquartile ranges (IQR) and compared between survivors and non-survivors using the Mann-Whitney U test due to non-normal distributions. Categorical variables were presented as frequencies and percentages, and group comparisons were performed using the chi-square test or Fisher's exact test as appropriate.

Multivariate logistic regression analysis was conducted to identify independent factors associated with mortality. Variables with a *P* value of less than 0.2 in univariate analyses were included in the multivariate model, along with clinically significant variables regardless of their univariate significance. Results were reported as odds ratios (OR) with 95% confidence intervals (CI).

All statistical analyses were performed using Statistical Package for the Social Sciences (SPSS, Inc., Chicago, IL, USA), version 26. A P value of <0.05 was considered statistically significant.

RESULTS

The study included 162 patients with BSI, of whom 85 (52.5%) died during their ICU stay. The median age of all patients was 68 years (IQR: 56–75), with non-survivors being significantly older than survivors (71 vs. 64 years, $P = 0.028$). The majority of patients were male (63.6%), though gender distribution was not statistically different between groups ($P = 0.051$). Body mass index was comparable between groups (median: 24.5 kg/m², $P = 0.948$). Common

Table 1. Demographic and clinical characteristics of patients

	All Patients (n=162)	Survivors (n=77)	Non-survivors (n=85)	P value
Age, median (IQR), years	68.0 (56.0 – 75.0)	64 (53 – 71)	71 (58 – 79)	**0.028**
Gender, male, n (%)	103 (63.6)	43 (55.8)	60 (70.6)	0.051
Body mass index, median (IQR), kg/m^2	24.5 (22.2 – 27.7)	24.2 (22.2 – 27.8)	24.5 (22.3 – 27.7)	0.948
Comorbidities, n (%)				
Diabetes mellitus	44 (27.3)	25 (32.5)	19 (22.4)	0.148
COPD	56 (34.6)	31 (40.3)	25 (29.4)	0.147
Congestive heart failure	22 (13.6)	9 (11.7)	13 (15.3)	0.503
Coronary artery disease	9 (5.6)	3 (3.9)	6 (7.1)	0.380
End-stage kidney disease	11 (6.8)	4 (5.2)	7 (8.2)	0.442
Solid organ tumors	35 (21.6)	14 (18.2)	21 (24.7)	0.314
Hematologic malignancies	4 (2.5)	1 (1.3)	3 (3.5)	0.265
Cerebrovascular diseases	6 (3.7)	2 (2.6)	4 (4.7)	0.478
Respiratory support, n (%)				
NIMV	7 (4.3)	1 (1.3)	6 (7.1)	0.072
IMV	124 (76.5)	49 (63.6)	75 (88.2)	**<0.001**
Source of bacteremia, n (%)				
CLA-BSI	121 (74.4)	61 (79.2)	60 (70.6)	N/A
Pneumonia	5 (2.1)	1 (1.3)	4 (4.7)	
Unknown	36 (22.2)	16 (20.8)	20 (23.5)	
Antimicrobial treatment, n (%)				
Carbapenems	79 (48.8)	34 (44.2)	45 (52.9)	N/A
Cephalosporins	35 (21.6)	20 (26.0)	15 (17.6)	
Quinolones	34 (21.0)	15 (19.5)	19 (22.4)	
Piperacillin+Tazobactam	30 (18.5)	14 (18.2)	16 (18.8)	
Vancomycin	11 (6.8)	3 (3.9)	8 (9.4)	
Antifungals	11 (6.8)	8 (10.4)	3 (3.5)	
Sepsis and Septic Shock, n (%)	58 (35.8)	19 (24.7)	39 (45.9)	**0.005**
Continue renal replacement therapy, n (%)	50 (30.9)	16 (20.8)	34 (40.0)	**0.008**
ECMO, n (%)	8 (4.9)	4 (5.2)	4 (4.7)	0.886

APACHE, Acute Physiology and Chronic Health Evaluation; CLA-BSI, Central Line Associated Bloodstream Infection; COPD, Chronic Obstructive Pulmonary Disease; COVID-19, Coronavirus Disease-19; ECMO, Extracorporeal Membrane Oxygenation; IMV, Invasive Mechanical Ventilation; IQR, Interquartile Range; NIMV, Non-Invasive Mechanical Ventilation

comorbidities included chronic obstructive pulmonary disease (34.6%), diabetes mellitus (27.3%), and solid organ tumors (21.6%), with no significant differences between survivors and non-survivors. Key demographic and clinical characteristics of patients were summarized in Table 1.

The three most common pathogens detected in patients followed up with a diagnosis of gram-negative BSI in the ICU were; *Klebsiella pneumoniae* [60/162(37%)], *Acinetobacter baumannii* [32/162(19.75%)] and *Stenotrophomonas maltophilia* [25/162(15.43%)], respectively. The highest resistance rate was detected in *A. baumannii*. Carbapenem, fluoroquinolone, and third-generation cephalosporin resistance was 93.75% in *A. baumannii*. The second highest carbapenem resistance was detected in *K. pneumoniae* with a rate of 81.66%. In *K. Pneumoniae*, quinolone and third-generation cephalosporin resistance (91.66%) and gentamicin resistance (53.33%) were detected. Resistance rates were lower in *Escherichia coli* and *Pseudomonas aeruginosa*. All detected pathogens and resistance rates are shown in Table 2.

The primary source of bacteremia was central line-associated bloodstream infections (74.4%), followed by pneumonia (2.1%). In 22.2% of cases, the source was unidentified. The most frequently used empirical antimicrobials were carbapenems (48.8%), cephalosporins (21.6%), and quinolones (21.0%). Other empirical treatments initiated for the patients are shown in Table 1. Invasive mechanical ventilation (IMV) was more prevalent among non-survivors (88.2% vs. 63.6%, $P < 0.001$). Septic shock occurred in 35.8% of patients and was significantly more common in non-survivors (45.9% vs. 24.7%, $P = 0.005$). Non-survivors were also more likely to require

Table 2. Pathogens and resistance rates

Pathogens	AMP resistance n (%)	GN resistance n (%)	MEM resistance n (%)	CIP resistance n (%)	TZP resistance n (%)	CRO resistance n (%)	CAZ resistance n (%)
Klebsiella spp. (n=60)	35 (58,33)	32 (53,33)	49 (81,66)	55 (91,66)	55 (91,66)	55 (91,66)	53 (88,33)
Escherichia coli (n=14)	0	3 (21,42)	0	10 (71,4)	7 (50)	9 (64,28)	9 (64,28)
Proteus Mirabilis (n=2)	0	1 (50)	0	1 (50)	0	1 (50)	0
Serratia (n=1)	0	0	0	0	0	0	0
Acinetobacter baumannii (n=32)	30 (93,75)	-	30 (93,75)	30 (93,75)	30 (93,75)	-	30 (93,75)
Pseudomonas aeruginosa (n=15)	1 (6,6)	1 (6,6)	4 (26,66)	4 (26,66)	5 (30)	5 (30)	5 (30)
Enterobacter cloacae (n=3)	1 (33,33)	1 (33,33)	1 (33,33)	1 (33,33)	1 (33,33)	1 (33,33)	1 (33,33)
Stenotrophomonas maltophilia (n=25)*	-	-	-	-	-	-	-
Burkholderia Cepacia (n=10)	0	0	0	0	0	0	0

*AMP: Ampicillin, GN: Gentamicin, MEM: Meropenem, CIP: Ciprofloxacin, TZP: Piperacillin tazobactam, CRO: ceftriaxone, CAZ: Ceftazidime. *All agents of Stenotrophomonas maltophilia were sensitive to trimethoprim and sulfamethoxazole.*

continuous renal replacement therapy (CRRT) (40.0% vs. 20.8%, *P* = 0.008). Multivariate logistic regression identified several factors independently associated with mortality. Patients requiring IMV had over three times the odds of death (OR: 3.10, 95% CI: 1.23–7.80, *P* = 0.016). Septic shock was associated with a nearly threefold increased risk of mortality (OR: 2.78, 95% CI: 1.29–6.00, *P* = 0.009), and CRRT also significantly increased mortality risk (OR: 2.52, 95% CI: 1.11–5.71, *P* = 0.026) (Table-3).

DISCUSSION

Evaluation of in-hospital mortality risk factors in gram-negative BSIs, which carry a high risk of morbidity and mortality due to increasing resistance rates, is important to improve outcomes of BSIs. The most important finding in this study was that IMV, presence of septic shock, and the need for CRRT during ICU follow-up increased mortality in gram-negative BSIs. In addition, when the distribution of pathogens was analyzed, the most common pathogens following *K. pneumonia*, *A. baumannii* and *S. maltophilia.*

Table 3. Multivariate logistic regression analysis for mortality in patients with bacteremia

	OR	95%, CI	P Value
Age	1.02	0.99 – 1.05	0.125
Gender, male	1.30	0.61 – 2.79	0.501
Invasive mechanical ventilation	3.10	1.23 – 7.80	0.016
Sepsis and Septic Shock	2.78	1.29 – 6.00	0.009
Continue renal replacement therapy	2.52	1.11 – 5.71	0.026

CI, Confidence Interval; OR, Odd Ratio

In our study, the fatality rate of gram-negative BSI was reported as 52.5%. In the study conducted by Ergönül et al., the fatality rate in healthcare-associated BSIs was reported as 44%, and in the study conducted by Kaye et al., it was reported as 49% (6,7). In our study, the number of patients over 65 years of age was statistically significantly higher in the non-survival group than in the survival group (*P* = 0.028). In many studies, mortality in BSIs has been found to be significantly higher in patients over 65 years of age compared to the younger population (6,8). In one study, it was suggested that septic shock, respiratory failure, and multiorgan failure were more common in the elderly population and therefore mortality was higher (8). In our study, although the frequency of advanced age was higher in the mortality group, consistent with the literature, it was found that it did not predict mortality in the multivariate regression analysis. It was thought that this situation may be due to the limited number of patients included in the study.

In our study, similar to the literature, no difference was found in mortality rates in gram-negative BSIs according to gender (9).

In this study, the three most common pathogens were *K. pneumoniae, A. baumannii,* and *S. Maltophilia*, respectively. It was especially striking that *S. Maltophilia* was one of the three most common pathogens. The highest carbapenem resistance rates belonged to *A. baumannii* and *K. pneumoniae* with 93.75% and 81.66%, respectively. In a prospective observational multicontinental cohort study, carbapenem resistance was reported as 90.4% in *Acinetobacter spp*., 53.1% in *Klebsiella spp.* and 48.8% in *Pseudomonas spp.* (10).

In this study, IMV was significantly more frequent in the non-surviving patient group and increased the risk of death threefold. In a retrospective cohort study, similar to our study, mechanical ventilation was significantly associated with death and poor outcome in patients with gram negative BSI (11). Another retrospective cohort study reported that mortality in BSIs was associated with age (P = .034), ICU hospitalization (P = .04), and invasive procedures (P < .001) (12). In a retrospective cohort study including 433 patients, mechanical ventilation was similarly reported as a risk factor for mortality in BSI patients (13).

Sepsis and septic shock are independent risk factors for mortality in the ICU (14). Although the incidence of gram-positive bacteria in the etiology of sepsis has shown an increasing trend over the last decade, gram-negative bacteria remain the predominant pathogen and have a higher ICU mortality rate in sepsis patients compared to gram-positive bacterial infection (15,16). In a retrospective cross-sectional analysis, a significantly higher SOFA score was reported in patients with gram-negative BSI in the mortality patient group than in the survival group (p<0.0001) (17). In a meta-analysis, septic shock, need for mechanical ventilation, indwelling central venous catheter, neutropenia, concomitant hematological malignancies, chronic kidney disease, inappropriate antimicrobial therapy, and previous antibiotic use were reported as risk factors for mortality (18).

CONCLUSION

IMV, septic shock, and the need for CRRT during ICU follow-up are risk factors for mortality in gram-negative BSI patients followed in the ICU. Among the etiologic pathogens, the highest resistance rates were found in *A. baumannii* and *K. pneumoniae,* respectively.

Limitations

The most important limitation of the study is the relatively small number of patients participating in the study and the fact that it was conducted in a single center. Other limitations were the retrospective design of the study and the fact that carbapenemase genes could not be analyzed for carbapenem-resistant strains.

Author contribution: Study design: TTK Data collection:TTK. Data analysis: SY Study supervision: SY Manuscript writing: TTK, SY Critical revisions for important intellectual content: TTK, SY.
Conflict of interests: The authors declare no competing interests.
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RESEARCH ARTICLE

MECHANOSENSITIVE TRPV4 TRAFFICKING DRIVES TGF-B-MEDIATED MESENCHYMAL TRANSITION IN COLORECTAL CANCER

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ABSTRACT

Background: Epithelial-to-mesenchymal transition (EMT) enhances the invasive potential of cancers, significantly affecting survival rates in metastatic disease. TGF-β, a potent EMT regulator enriched in colon cancer (CRC), is influenced by bioelectric and biophysical forces. While some ion channels and mechanical forces are linked, TGF-β-coupled mechanosensing mechanisms in CRC remain poorly understood. This study investigates the mechanosensitive ion channel TRPV4 and its role in TGF-β-induced EMT, focusing on channel trafficking and its functional implications in CRC.
Methods: We analyzed mechanosensitive ion channels mRNA expressions in CRC stages and evaluated their association with survival through Kaplan-Meier analysis. Correlations were analyzed with mesenchymal gene sets, soluble factors, and TGF-β signaling. Immunofluorescence was used to visualize TRPV4 localization in untreated and 10 ng/mL TGF-β1-treated colon cell lines. Functional studies involved co-stimulation with TGF-β1 and TRPV4 modulators (GSK101 and HC-067047) to assess EMT-related changes.
Results: TRPV4 mRNA is elevated in CRC, with TRPV4-001 as the predominant isoform. High expression correlated with poor survival, EMT signatures, and TGF-β1 signaling. TGF-β1 induced out-of-nucleus TRPV4 translocation. TRPV4 inhibition reduced TGF-β-induced N-cadherin expression, mitigating EMT.
Conclusion: TRPV4 regulates TGF-β-induced EMT through trafficking mechanisms. Its inhibition presents anti-metastatic potential, identifying TRPV4 as a therapeutic target in CRC.

Keywords: TRPV4, TGF-β, EMT, Translocation, Ion channel, Mechanosensitive

INTRODUCTION

Colorectal cancer is the most common cancer in men and the second most common in women under the age of 50. Furthermore, its diagnosis in younger populations has been steadily increasing since 1991 (1). While the five-year survival rates for localized and regional stages are relatively high (91.3% and 71.7%, respectively), survival drops sharply to 15.7% in advanced cases with distant metastases (SEER). Epithelial-to-mesenchymal transition (EMT) is a well-known process that enhances the migratory and invasive capabilities of these malignancies (2). Moreover, the mesenchymal phenotype is associated with drug resistance and disease relapse (3,4). Thus, controlling the mesenchymal phenotype of tumors remains a critical objective in cancer treatment.

TGF-β is a well-known cytokine closely linked to metastatic colon cancer. This infamous cytokine promotes mesenchymal phenotype and increases cancer cell stemness. TGF-β receptor inhibitors and antibodies that neutralize soluble TGF-β ligands, offering potential combination therapies to overcome

metastatic disease and drug resistance (5–7). The TGF-β response is related to multiple signaling pathways, including TGF-β/SMAD, WNT/β-catenin, Notch, and receptor tyrosine kinase, can be induced by physical stimuli from the tumor microenvironment. Transcriptional factors such as snail, slug, ERK, and STAT3 are activated in response to the downstream, and signal for degradation of epithelial markers whereas increase the synthesis of mesenchymal markers such like N-cadherin and vimentin (8).

Communication through bioelectric signaling among cells is essential and widely studied for developmental biology. Tumors exhibit a higher resting membrane potential than healthy cells, ranging from −30 to −20 mV, similar to proliferative cells (9). Studies in the last decade indicate that altered bioelectricity is also very important for cancer progression and metastasis (10). Also, gap junctional coupling results in a multi-cellular network in bioelectrical state which is related to tissue level invasive behavior (11). Oncogene expression depolarizes cells that form tumor-like structures, but is unable to form tumors if this depolarization is artificially prevented by misexpression of hyperpolarizing ion channels (12). Recent studies investigate dysfunction of ion channelopathies and their role as both oncogenes and tumor suppressors (13,14).

Ca^{2+} influx acts as a modulator of TGF-β signaling affecting cellular plasticity and metastasis (15–17). Dysregulation of calcium and potassium channels enhance the invasive potential of cancer cells and may contribute to therapeutic resistance (18–20). TGF-β signaling also regulates ion channel expression and translocation in some diseases however the influence on translocation of ion channels in cancer is poorly understood (21,22).

TGF-β signaling is regulated by mechanical forces, such as shear stress and substrate stiffness (23–25). For example, cells sense the stiffness of their extracellular matrix, with changes in substrate elasticity influencing TGF-β signaling dynamics, such as during the fibroblast-to-myofibroblast transition (26). Moreover, physiologic range shear stress also induces endothelial generation of biologically active TGF-β1 coupled with K+ channel currents (27).

As tumor mass increases and tumor microenvironment undergoes alterations during cancer progression, the mechanical forces within the tissue become imbalanced, creating a high-pressure environment (28–30). Recent studies have shown that elevated

pressure in the cancer microenvironment impacts tumorigenesis, cancer progression, drug resistance, and metastasis (31–33). Mechanosensitive ion channels, which act as receptors sensing pathophysiological pressure, respond to mechanical forces such as tension, compression, and shear stress. These responses promote critical processes such as cell division, survival, invasion, and migration (30,34). Notably, PIEZO and TRPV4 channels are linked to increased cell proliferation and the acquisition of mesenchymal traits in various cancers, including colon cancer (35–37).

While studies have highlighted the importance of bioelectric signaling and physical forces in modulating TGF-β signaling, this process remains poorly understood. Furthermore, although TGF-β is known to regulate ion channels in various diseases, the mechanistic significance of these processes in colon cancer progression is unclear. This study establishes a mechanistic link between mechanosensitive ion channels and TGF-β-induced EMT in colon cancer, focusing on TRPV4 trafficking and function. Through a combination of transcriptomic analysis, functional assays, and immunofluorescence-based localization studies, we identify TRPV4 as a key mediator in TGF-β signaling. Additionally, we explore how TGF-β-induced TRPV4 translocation contributes to mesenchymal transition, providing new insights into the biophysical regulation of TRPV4 and its therapeutic potential in metastatic colon cancer.

MATERIALS AND METHODS

Dataset Selection and Normalization

The TCGA-COAD (The Cancer Genome Atlas-Colorectal Adenocarcinoma) RNA sequencing data were used as the primary patient cohort. Samples classified as ‘Normal’, ‘Primary Solid Tumor’, and cancer stages (stages 1–4) were selected from the available patient samples. Duplicate samples with the same patient ID were excluded. The data were downloaded using the TCGAbiolinks package. The downloaded data were prepared for analysis using the TCGAanalyze_Preprocessing function. A symmetric Spearman correlation matrix was computed (cor.cut=0.6) to identify low-correlation outlier samples among the dataset. Normalization of the counts within and across groups was performed using the TCGAanalyze_normalization function with the default parameters of the EDASeq R package.

Differential Gene Experission Analysis

Genes of mechanosensitive ion channels expressed and their isoforms in homo sapiens were retrieved based on the gene ontology (GO) molecular function term "mechanosensitive ion channel activity" [GO:8381]. The data used in this study were obtained from the colorectal cancer dataset of The Cancer Genome Atlas (TCGA) via the Genomics Data Commons (GDC) cancer data portal, which provides open access. Differential gene expression analyses were performed using the edgeR method. Genes with statistically significant expression changes were identified. An adjusted p-value of <0.05 was considered significant, and a fold-change threshold of |log2FC| ≥ 1 was applied. Significant expression changes were calculated while comparing gene expression levels between the selected groups.

Geneset Scoring and Correlation

Geneset scoring and correlation analyses were performed using a non-logarithmic scale for calculation, while a log-scale axis was used for visualization. The Pearson correlation between TRPV4 expression and various biomarkers, soluble factors, or geneset signature scores was calculated (supplemantary table 1). The mean value of log2(TPM + 1) was used as the signature score for each geneset. The following genesets were included in the analysis: epithelial-mesenchymal transition (EMT) genesets (HALLMARK_EPITHELIAL_MESENCHYMAL_TRANSITION, KOHN_EMT_MESENCHYMAL, KOHN_EMT_EPITHELIAL) and the TGF-B signaling geneset from MSigDB (HALLMARK_TGF_BETA_SIGNALING) (38). GEPIA2 webtool were used for the analysis and visualization (39).

Survival Analysis

Kaplan-Meier analysis was used to study how TRPV4 gene expression affects survival in the microarray data sets collected and normalized by Balázs Győrffy . Both overall survival (OS) and disease-free survival (DFS) were analyzed. Patients were divided into low and high TRPV4 expression groups based on the median expression level. Differences between the groups were tested using the log-rank test (Mantel-Cox test), and exact p-values were provided. Hazard ratios (HR) were calculated using the Cox Proportional Hazards (Cox PH) model. Kaplan-Meier curves were created, with different colors showing low and high TRPV4 expression groups, and p-values were included on the plots (40).

Cell Culture

Colorectal cancer cell lines, HCT-116 (ATCC® CCL-247), HT-29 (ATCC® HTB-38), and LOVO (ATCC® CCL-229), were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 1% penicillin-streptomycin and 10% fetal bovine serum (FBS). The cells were maintained in a humidified incubator at 37°C with 5% CO_2. The culture medium was replaced every 2-3 days, and cells were passaged when they reached approximately 70-80% confluence.

Immunofluorescence Staining

EMT markers N-cadherin and E-cadherin, and TRPV4 channel were stained. Cells were fixed with 4% paraformaldehyde for 20 minutes at room temperature. After fixation, cells were permeabilized with 0.3% Triton X-100 in PBS and incubated overnight at 4°C with primary antibodies: anti-mouse monoclonal anti-E-cadherin (Thermofisher Sc., USA) and anti-rabbit monoclonal anti-N-cadherin (Thermofisher Sc., USA) (dilution according to manufacturer's recommendation) for EMT, and anti-rabbit polyclonal anti-TRPV4 antibody (Thermofisher Sc., USA) for TRPV4 expression. Following incubation, cells were washed twice with PBS. Secondary antibodies Alexa Fluor-488 goat anti-mouse (Thermofisher Sc., USA) and Alexa Fluor-568 goat anti-rabbit (Thermofisher Sc., USA) were applied for 2 hours at room temperature. DNA was stained with DAPI for 5 minutes. After washing, cells were visualized using a fluorescence microscope (Zeiss, AxioVert.A1, Germany) with appropriate wavelengths (Alexa Fluor-488 488 nm, Alexa Fluor-568 568 nm).

TGF-β Treatment and Translocation Scoring

To investigate the effect of TRPV4 on the TGF-β-induced EMT process, HCT-116 were seeded at a density of 10,000 cells per 100 µl in a 96-well plate. The cells were incubated with 10 ng/ml TGF-β (R&D, USA) for 18 hours. Following the incubation, five images per well were captured, and the experiments were repeated in triplicates. The following groups were analyzed using immunofluorescence staining: TRPV4 control group, EMT control group, TGF-β and GSK101 (SelleckChem, USA) co-stimulation group (EMT group), TGF-β and HC-067047 (SelleckChem, USA) treatment group (EMT group), and TGF-β-only

treatment group (TRPV4 & EMT group). After immunofluorescence staining, fluorescence intensity was measured for both nuclear and non-nuclear regions. The nuclear region was identified based on the DAPI signal.

Statistical Analysis

The data generated from in vitro experiments were analyzed using IBM SPSS 24.0 software. The measurements were expressed as standard deviation, median, and minimum-maximum values. For comparing the measurement data, if at least 30 replicates per variable were collected in the experimental groups, the normality of the data was assessed using the Kolmogorov-Smirnov test. For experimental groups with fewer than 30 replicates per variable, non-parametric methods were used without testing for normality. In this case, the Kruskal-Wallis test was used to assess significant differences between multiple groups, and the Mann-Whitney U test was used to evaluate differences between two groups. A p-value of <0.05 was considered statistically significant.

RESULTS

TRPV4 have higher expression level in colorectal cancer patients beginning with early stages

The transcriptomic data of colorectal cancer patients were compared with healthy tissue. Significant changes in the expression of seven ion channels among the 17 analyzed were observed. Notably, the expression of CSCL1 (0.97-fold), FAM155B (1.44-fold), and TRPV4 (1.42-fold) was significantly upregulated, while PIEZO2 (-1.18-fold), TMC4 (-1.17-fold), CSCL2 (-1.06-fold), and TMC8 (-0.5-fold) showed significant downregulation. Furthermore, TMC7, FAM155A, and TRPV4 expressions increase with fold changes of 0.59, 0.7, and 0.8, respectively, in stage IV (metastatic disease primer region) compared to stage II (not spread to any lymph nodes yet) (Figure 1a). Considering mechanisms influencing the metastatic characteristics of cancer are expressed from the onset of carcinogenesis, the ion channels TMC7, CSCL1, FAM155B, FAM155A, and TRPV4 might play a role in supporting colorectal cancer progression and metastasis. Many of these upregulated ion channels are associated with upregulating calcium signaling, a secondary messenger pathway linked to metastatic malignancies and TGF-β signaling.

TRPV4 mRNA expression was found to be elevated in colorectal cancer compared to healthy tissue. TRPV4 has six isoforms, and identifying the isoforms specific to colorectal cancer is crucial for predicting functionality and ensuring selectivity in drug development. RNA sequencing data revealed that the TRPV4-001 isoform (ENST00000261740.6) predominates over the other five isoforms in colorectal cancer (Figure 1b).

Given the principles of cancer evolution, these expression changes are likely to provide a selective advantage to cancer cells. To investigate the impact of TRPV4 on cancer progression or metastasis, Kaplan-Meier survival curves were generated using TCGA data to correlate mRNA expression levels with overall survival and disease-free survival. High TRPV4 expression was inversely associated with both overall, post-progression and disease-free survival (Figure 1c). Hazard ratios suggest that TRPV4 high patients have 1.35 (1.1 - 1.65, FDR = 0.5) times higher risk of death, 1.58 (1.17-2.14, FDR=0.2) times higher risk of progress, and 1.58 (1.27-1.97, FDR=0.01) times higher risk for recurrent disease.

Expression of TRPV4 correlated with EMT and TGF-β signaling

Next, we investigated the correlation of mesenchymal phenotype, infamous transition required for metastasis, and TRPV4 expression (supplemantary table 1). TRPV4 correlates moderately with mesenchymal markers such as N-cadherin ($r = 0.44$, $p = 3.1e-14$), Vimentin ($r = 0.48$, $p < 0.001$), Fibronectin ($r = 0.48$, $p < 0.001$), and Laminin V ($r = 0.49$, $p < 0.001$), all showing moderate positive correlations ($r > 0.4$) and significant p-values. Nevertheless, both the kohn mesenchymal geneset ($r = 0.46$, $p = 1.1e-15$) (Figure 2c) and hallmarks of epithelial to mesenchymal transition geneset (Figure 2a) also show a moderate positive correlation with TRPV4 ($r=0.47$, $p < 0.001$), supporting the link between TRPV4 and mesenchymal features. On the other hand, TRPV4 expression is negatively correlated with epithelial markers like E-Cadherin ($r = -0.12$, $p = 0.043$) and EPCAM ($r = -0.17$, $p = 0.002$). Even though the correlations are weak ($r < 0.3$), the p-values are statistically significant. The epithelial geneset (Figure 2e) also shows a weak negative correlation with TRPV4 ($r = -0.15$, $p = 0.013$), further reinforcing the trend that TRPV4 could be associated with mesenchymal rather than epithelial traits.

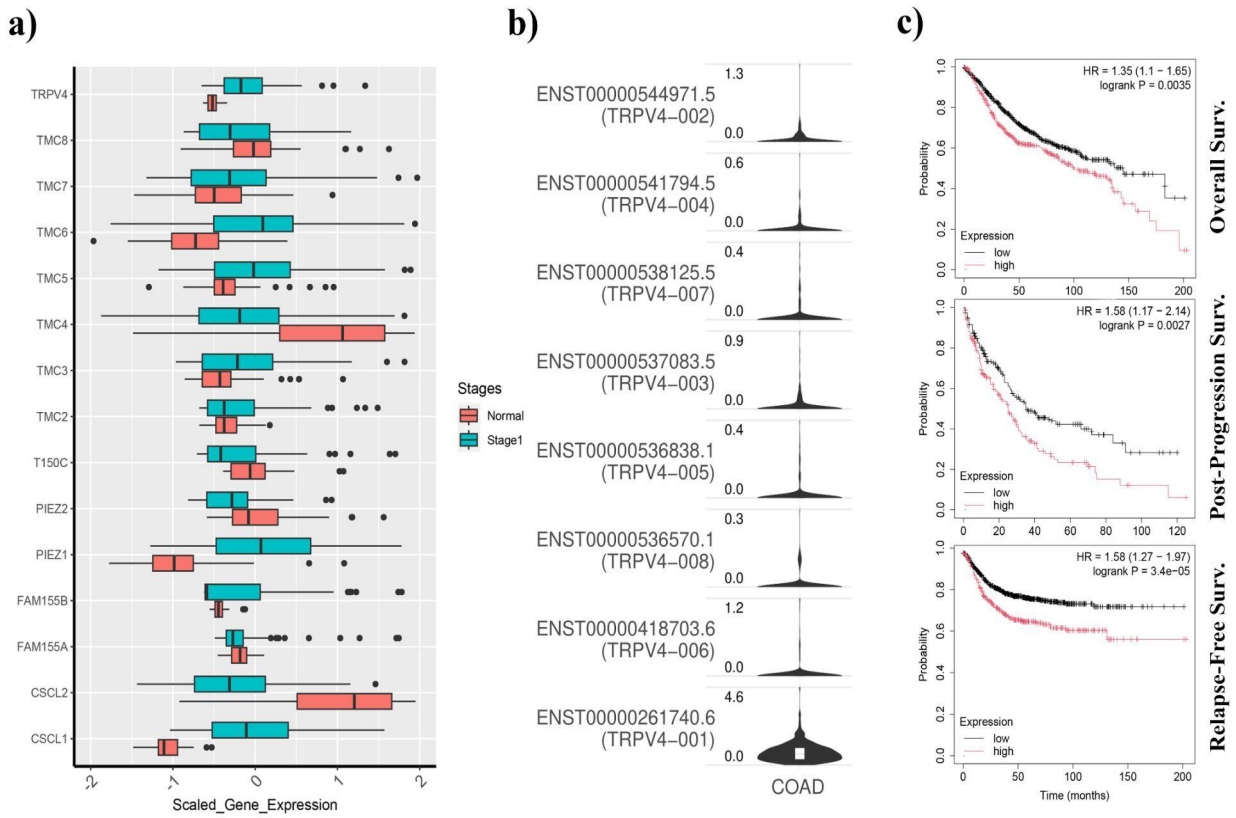

Figure 1. Expression levels of 17 mechanosensitive ion channels in normal colon tissue and stage 1 colorectal cancer (CRC) are shown (a), along with various TRPV4 isoforms (b). Kaplan-Meier survival analysis (c) compares low TRPV4 expression (black) and high TRPV4 expression (red).

To understand the mechanism by which TRPV4 influences EMT, we investigated the relationship between EMT inducer soluble factors and TRPV4 in the tumor microenvironment. The expression of TRPV4 shows a significant positive correlation with TGF-β1 expression (r = 0.5, p = 0) (Figure 2b), TGF-β receptor expression (r = 0.43, p = 1.3e-13) (Figure 2d), and TGF-β signaling (r = 0.41, p = 1.1e-12) (Figure 2f), suggesting a moderate positive relationship with these key components of TGF-β pathways. This indicates that TRPV4 may have a role in modulating TGF-β-related signaling in the context of tumor progression.

TGF-β treatment induce translocation of TRPV4

According to TCGA data, mRNA expression of TRPV4 isoforms in colorectal cancer has been shown. However, to discuss the impact of TRPV4 on colorectal cancer, it is necessary to demonstrate the translation of mRNA expression levels into protein expression, verify membrane localization, and show functionality upon activation. To this end, immunofluorescent methodology was used to visualize the TRPV4 channel through confocal microscopy, allowing for the simultaneous demonstration of expression and localization.

TRPV4 expression was significantly expressed in Duke grade 4 colorectal cancer cell lines HCT-116 and HT-29, as well as in LOVO cells originating from lymphatic metastasis of colorectal cancer (Supplemantary Figure 1). However, in all three colorectal cancer cell types, TRPV4 expression was primarily localized in the nucleus and vesicular structures surrounding the nucleus. While the accumulation around the nucleus could potentially be related to the Golgi apparatus or endoplasmic reticulum, no direct evidence for this is available.

Functional TRPV4 is expected to localize outside the nucleus, as cells regulate its activity through post-translational modifications that control its trafficking from intracellular compartments to the membrane (41,42). Given the increased expression levels observed in stage IV colorectal cancer and its correlation with the mesenchymal phenotype and TGF-β signaling, we hypothesized that TRPV4 trafficking might be regulated by TGF-β.

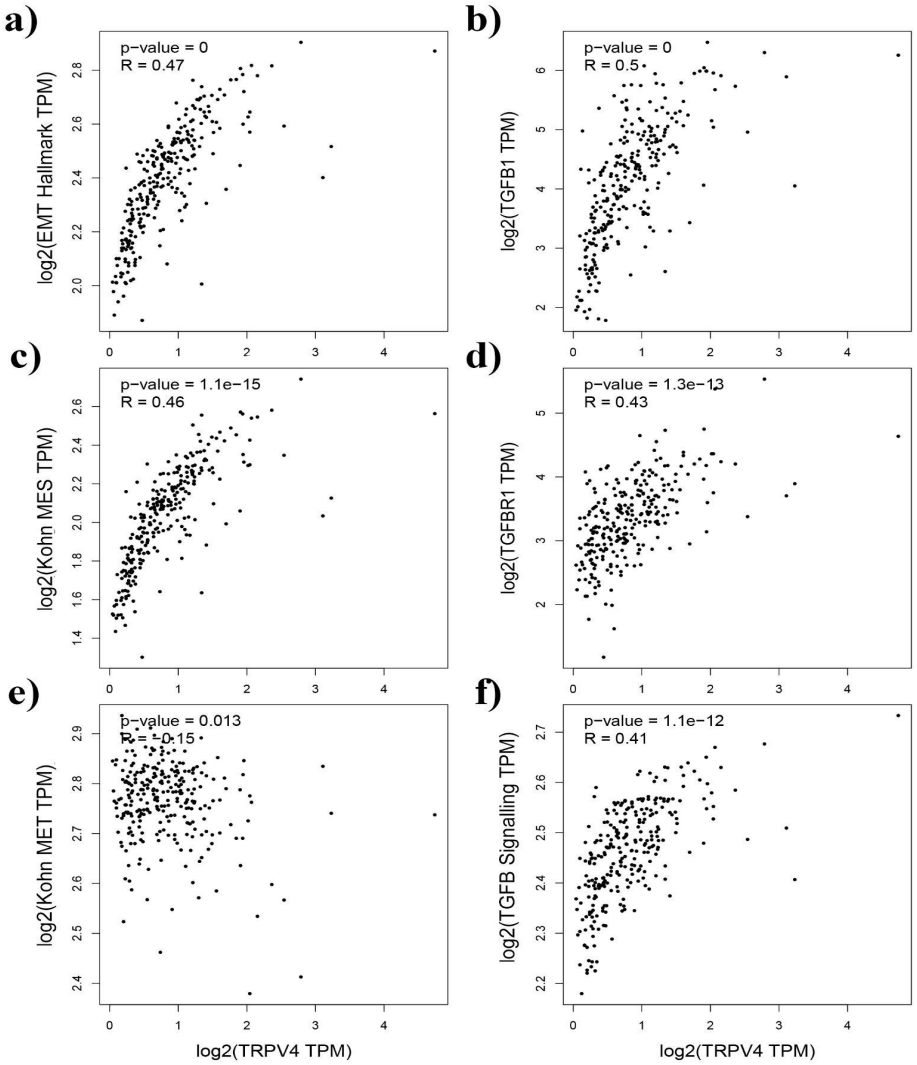

Figure 2. Correlation analysis between TRPV4 expression (log2 TPM) and EMT-associated signatures, mesenchymal markers, and TGF-β signaling pathways. Scatter plot showing a positive correlation between TRPV4 expression and the EMT hallmark gene signature (a), TGFB1 expression (b), the Kohn mesenchymal (MES) signature (c), TGFBR1 expression (d), the Kohn MET signature (e), and TRPV4 expression and the TGF-β signaling gene signature (f).

To test this, the HCT-116 cell line was treated with 10 ng/mL TGF-β1 for 18 hours prior to fixation. A significant decrease in nuclear TRPV4 levels was observed, accompanied by increased cytosolic and membrane expression (Figure 3a, 3b, 3e). These findings suggest that TGF-β signaling plays a critical role in controlling TRPV4 trafficking and potentially contributes to a gain-of-function.

TRPV4 inhibition attuned TGF-β dependent N-Cadherin expression

In TGF-β-stimulated HCT-116 cells, TRPV4 expression was observed to decrease around the perinuclear region while redistributing throughout the cytoplasm and membrane.

To investigate whether this trafficking of TRPV4 plays a role in the epithelial-mesenchymal transition (EMT) process, two experimental groups were subjected to co-stimulation with TGF-β. One group was treated with the TRPV4 activator GSK101, and the other with the TRPV4 inhibitor HC-067047. Following incubation, it was observed that the TRPV4 inhibitor reduced N-cadherin expression (Figure 3d) compared to the TGF-β-only stimulated control group (Figure 3c). These results suggest that TRPV4 contributes to the TGF-β-mediated mesenchymal transition mechanism in colon cancer.

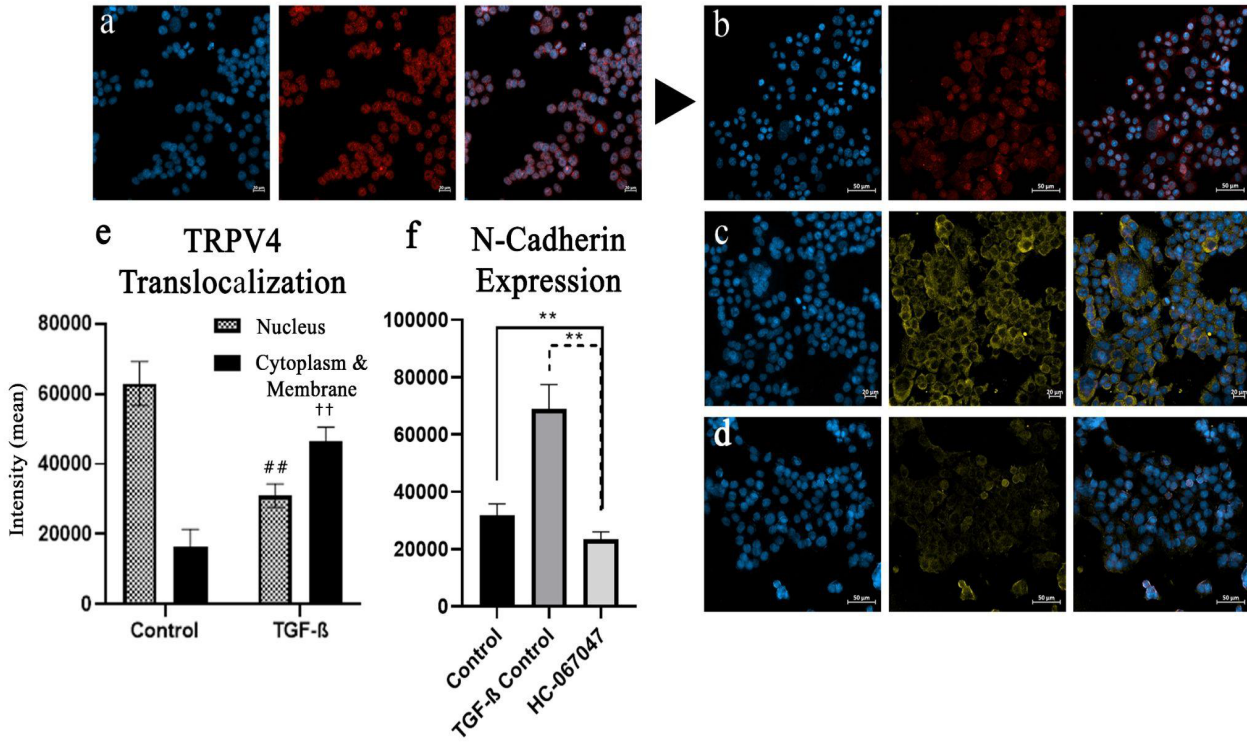

Figure 3. TRPV4 expression & localization in native HCT-116 (a) and TGF-β induced condition (b) TRPV4 translocalization is graphed in (e). All nucleus are blue, TRPV4 is red, and N-Cadherin is green. # and † symbols respectively indicate that statistical significance comparing to corresponding location in control group. N-cadherin expression in TGF-β treated (c) and HC-067047 & TGF-β co-treated cells (d). Alterations in N-Cadherin expression have been showed in (f). ##, ††, ** mean $p<0.01$.

DISCUSSION

This study investigated the mechanosensitive relationship between TGF-β-induced epithelial-mesenchymal transition (EMT) and 17 mechanosensitive ion channels, identifying TRPV4 as a key candidate. TRPV4 was found to have higher expression in progressive colon cancer and exhibited correlations with EMT and TGF-β signaling pathways. While predominantly localized in the nucleus in grade 3 and 4 colon cancer cell lines, TRPV4 shifted to the cytoplasm and membrane in TGF-β-stimulated HCT-116 cells, indicating that TGF-β regulates TRPV4 trafficking, potentially enhancing its functional activity. Moreover, inhibiting TRPV4 significantly reduced TGF-β-induced N-cadherin expression compared to TGF-β treatment alone. These findings suggest that TRPV4 responds early to TGF-β signaling, contributing to its mechanistic role in promoting mesenchymal transition.

TRPV4 has been a regulator in cancer progression through EMT and cytoskeletal remodeling across various cancer types. In colorectal cancer, TRPV4 contributes to invasiveness by stimulating ZEB1 expression and promoting the EMT process via AKT signaling, while its inhibition activates the PTEN pathway, suppressing tumor development (43). Similarly, TRPV4 regulates cytoskeletal dynamics through the RhoA/ROCK1 pathway, enhancing metastasis in endometrial cancer (44). In nasopharyngeal carcinoma, TRPV4 activation enhances tumorigenesis via NFAT4 signaling and its pharmacological inhibition reduces EMT and ERK activation, demonstrating antitumor effects in hepatocellular carcinoma (45,46). There are various other studies showing similar results in several cancers (47,48). These findings align with our study, however, while studying the effect of TRPV4 activation, these studies focused on exogenous activators rather than pathophysiologic channel activity.

TRPV4 function is regulated by endogenous factors like arachidonic acid, endocannabinoids, mechanical forces (e.g., increased matrix stiffness, osmolarity, and shear stress), and cell crowding in the microenvironment (49,50). It also responds to physiologic movements, such as bladder stretch, due to its localization in adherence junctions of urothelial cell membranes. Similarly, the channel expressed on

the basolateral side of epithelial cells and its over-expression related to chronic constipation (51,52). Importantly, TRPV4 translocation appears to be a key regulatory mechanism for its activity (41,42,53). Our study further demonstrates that TRPV4 trafficking as a player regulated by TGF-β and promotes EMT, which could explain a mechanism for its pathophysiological activation in colon cancer.

TRPV4 crosstalks and interacts with soluble factors in both pro-inflammatory and anti-inflammatory in various tissues and pathologies (54–56). The channel mediates MAPK downstream to regulate cytokines and potentiate EGF response (54,57,58). Similarly, TRPV4 involves TGF-β signaling regulation in non-cancer contexts, such as ventricular fibroblasts and chondrocytes (59,60). Our findings indicate that TRPV4 plays a key role in TGF-β-induced epithelial-mesenchymal transition (EMT), with trafficking to the cell membrane enhancing its functional activity in colon cancer. Our observation of TRPV4's role in EMT and TGF-β signaling expands on these findings by a mechanistic link between TRPV4 out-of-nucleus trafficking and its regulatory role in TGF-β-induced mesenchymal transition in colon cancer.

TRPV4 emerges as a promising mechanoreceptor capable of mediating TGF-β1 regulation and function. While our results establish a TGF-β1 dependence on TRPV4, the role of physical forces such as matrix stiffness or shear stress in this mechanism was not investigated. Nonetheless, evidence from non-cancer contexts suggests that physical forces can regulate both the function and trafficking of TRPV4, providing indirect support for our hypothesis (61,62). Notably, in HUVEC cells, TRPV4 activation in response to 12 dyne/cm² shear stress led to a reduction in the VE-cadherin layer, which forms adherens junctions, demonstrating mechanosensitive TRPV4 translocation and functional activation (63). This aligns with our observation of TRPV4 translocation to gain function and supports the notion that physical forces may modulate TGF-β1 activity via TRPV4. Further investigation is needed to explore this mechanistic link in cancer-related contexts, particularly under conditions of altered matrix stiffness and shear stress.

CONCLUSION

Our findings highlight TRPV4 as a critical regulator of TGF-β1-mediated mesenchymal transition in the colon microenvironment through an induced translocation mechanism. The channel's function is facilitated by TGF-β1 induced translocation out-of-nucleus upon stimulation. In this context, we demonstrate that TRPV4 inhibition reduces TGF-β-induced EMT, suggesting potential anti-metastatic benefits. Although not explored in this study, TRPV4's regulation by lipid metabolites and its mechanosensitive properties present new research interest in how tumor cells respond to TGF-β signaling under pathophysiological conditions. Furthermore, its correlation with measurable ionized calcium levels in the blood may provide personalized treatment to calcium signaling (64). Additionally, TRPV4's potential therapeutic relevance is supported by its roles in pain treatment and anti-angiogenic therapies (65,66). Pharmacophore studies targeting active isoforms and splice variants of TRPV4 could support individualized treatments to slow EMT in patients with high TRPV4 expression, paving the way for tailored therapeutic strategies.

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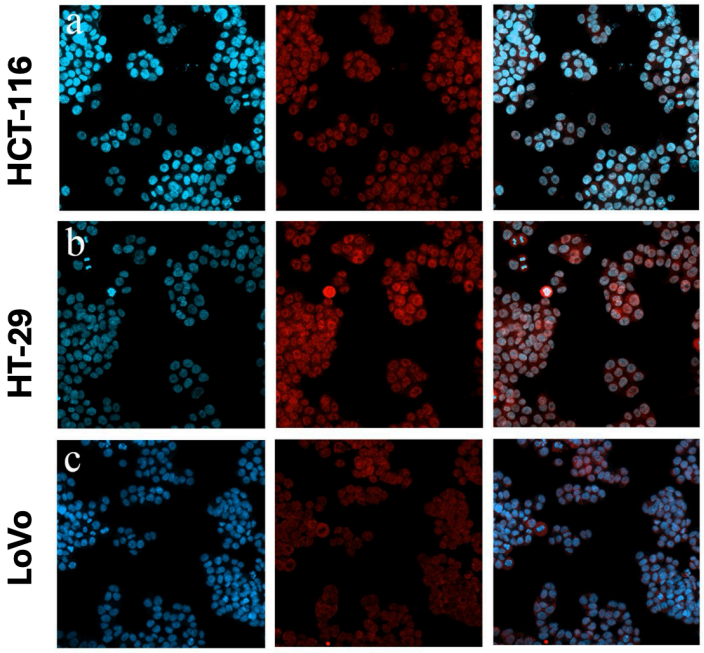
SUPPLEMENTARY MATERIALS



Supplemantary Figure 1. TRPV4 expression and localization in HCT-116 (a), HT-29 (b), LOVO (c). Nuclei are stained blue, and TRPV4 expression is marked in red. Images were captured at 20x magnification.

Supplemantary Table 1. TRPV4 and Gene/Geneset correlations

Category	Gene/Geneset	Pearson (r)	p-value
Epithelial Markers	E-Cadherin	-0.12	0.043
	EPCAM	-0.17	0.0039
Mesenchymal Markers	N-cadherin	0.44	3.1e-14
	Vimentin	0.48	0
	Fibronectin	0.48	0
	Laminin V	0.49	0
EMT Genesets	HALLMARK_EPITHELIAL_MESENCHYMAL_TRANSITION	0.47	0
	KOHN_EMT_MESENCHYMAL	0.46	1.1e-15
	KOHN_EMT_EPITHELIAL	-0.15	0.013
TGF-B1 Signaling	HALLMARK_TGF_BETA_SIGNALING	0.41	1.1e-12
Soluble Factors	TGF-B1	0.5	0
	TGF-B1 receptor	0.43	1.3e-13
	CCL2	0.35	3.3e-09
	CXCL12	0.31	2.2e-07
	IL-1B	0.21	0
	WNT1	0.045	0.45
	WNT2	0.35	1.7e-09
	CXCL8	0.28	3.2e-06
	CXCL6	0.34	5.5e-09
	EGF	0.05	0.4
	HGF	0.24	4.2e-05
	FGF9	0.12	0.056
	FGF10	0.29	1.0e-06
	FGF18	2,70E+01	0.65

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RESEARCH ARTICLE

DIFFERENTIAL EFFECTS OF SERTRALINE AND PENFLURIDOL ON EMT AND ECM REMODELING IN GLIOBLASTOMA CELL LINES

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ABSTRACT

Purpose: Glioblastoma multiforme (GBM) is an aggressive brain tumor with poor prognosis due to rapid recurrence, chemoresistance, and limited efficacy of standard therapies. Epithelial-to-mesenchymal transition (EMT) and matrix metalloproteinase (MMP)-mediated extracellular matrix (ECM) remodeling are critical processes in GBM progression and metastasis. The aim of this study is to examine the potential effects of sertraline and penfluridol on the EMT process and gelatinase activity in human glioblastoma cell lines.
Material and Methods: U87 and U251 human glioblastoma cells were treated with sertraline and penfluridol at previously identified IC50 doses. Protein levels of EMT markers, E-cadherin, vimentin, Snail, Slug, Twist1, phospho-Akt (p-Akt), and tissue inhibitor of metalloproteinases-2 (TIMP-2), were evaluated using Western blotting. Additionally, the impact of sertraline and penfluridol on the release and activity of MMP-2 and MMP-9 were assessed through gelatin zymography.
Results: Both sertraline and penfluridol significantly reduced vimentin expression in U251 cells, indicating inhibition of the mesenchymal phenotype. Conversely, these drugs increased vimentin levels in U87 cells, highlighting cell line-specific differences. Sertraline and penfluridol also increased TIMP-2 levels in U251 cells but not in U87 cells. Neither drug altered MMP-2 or MMP-9 activity in either cell line, suggesting that their effects on ECM remodeling may be mediated through TIMP-2 upregulation rather than direct modulation of gelatinase activity.
Conclusion: These findings suggest that sertraline and penfluridol potentially inhibit EMT and reduce ECM degradation in U251 cells but exert contrasting effects in U87 cells. This highlights the heterogeneity of GBM tumors and the importance of personalized therapeutic approaches.

Keywords: Sertraline, Penfluridol, Glioblastoma, Epithelial-mesenchymal transition, Matrix metalloproteinases

INTRODUCTION

Glioblastoma multiforme (GBM), also known as astrocytoma grade IV, is the most common and aggressive primary brain neoplasm in adults. GBM arises from diffuse astrocytomas or anaplastic astrocytomas and is typically located in deep white matter, the basal ganglia, or the thalamus. Despite multimodal treatment involving surgical resection, radiotherapy, and chemotherapy with temozolomide (TMZ), the prognosis for GBM remains poor, with an average survival of less than 15 months. The limited efficacy of standard GBM treatments is attributed to

several factors, including rapid tumor recurrence, the development of chemoresistance, and the inability of most chemotherapeutic agents to cross the blood-brain barrier (BBB) (1). These limitations underscore the urgent need for novel therapeutic strategies in the treatment of GBM.

Repositioning FDA-approved antipsychotic and antidepressant agents, commonly used in the management of psychiatric disorders, shows potential for the treatment of GBM, due to their long clinical history, proven reliability, and ability to cross the blood-brain barrier (BBB). Sertraline, a selective serotonin reuptake inhibitor (SSRI) antidepressant is widely used to treat depression, anxiety, and obsessive-compulsive disorder. Sertraline, in particular, has minimal side effect profile and is commonly utilized as a reliable and effective antidepressant/anxiolytic agent for patients with metastatic cancer who concurrently exhibit depression (2-4). Penfluridol, a long acting antipsychotic medication used in the treatment of schizophrenia (5, 6). Both sertraline and penfluridol have emerged as promising candidates for cancer therapy due to their ability to exhibit anti-tumorigenic activities by inhibiting the cell cycle, suppressing tumor growth, and promoting apoptosis in various cancer types, including glioblastoma (7), colon (8), liver (9), breast (10, 11), and pancreatic cancers (12, 13). Sertraline has been shown to inhibit the translationally controlled tumor protein (TCTP), an intracellular chaperone that contributes to GBM drug resistance by impairing p53 function (14). Penfluridol, on the other hand, has been shown to induce apoptosis in glioblastoma cells through the inhibition of Akt-mediated GLI1 expression, which is crucial for GBM cell survival and proliferation (7). Despite the promising anti-cancer properties of sertraline and penfluridol observed in various cancer types, their specific effects on EMT and ECM remodeling in GBM remain largely unexplored.

Epithelial-mesenchymal transition (EMT) is a critical process in cancer metastasis, facilitates tumor cell migration and invasion. During EMT, epithelial markers such as E-cadherin are downregulated, while mesenchymal markers such as vimentin are upregulated, driven by transcription factors (Snail, Slug, Twist1, ZEB1/2) (15). EMT is often accompanied by extracellular matrix (ECM) remodeling mediated by matrix metalloproteinases (MMPs) (16). Dysregulation of MMPs, particularly MMP-2 and MMP-9, and their endogenous inhibitors, tissue inhibitors of metalloproteinases (TIMPs) correlate with tumor aggressiveness, promoting angiogenesis, metastasis, and therapy resistance (17).

GBM is characterized by its highly invasive nature, which is closely linked to EMT-like processes. While GBM cells do not undergo a classic EMT due to their non-epithelial origin, they do exhibit EMT-like changes that contribute to their aggressive behavior (18). The ability of sertraline and penfluridol to penetrate the BBB positions them uniquely to potentially modulate these processes directly within the brain microenvironment. If these drugs can inhibit EMT-like changes and MMP activity in GBM, they could enhance the efficacy of existing treatments by reducing tumor invasiveness and therapy resistance. This study aims to investigate potential inhibitory effects of sertraline and penfluridol on EMT process and MMP activity in glioblastoma cells lines (U251 and U87). In this context, we evaluated key EMT markers (E-cadherin, vimentin, Snail, Slug, Twist1) and ECM modulators (MMP-2, MMP-9, TIMP-2) to elucidate their roles in glioblastoma progression. By focusing on these molecular targets, we aim to provide a mechanistic understanding of how sertraline and penfluridol might modulate GBM cell behavior, potentially opening new avenues for therapeutic intervention.

MATERIALS AND METHODS

Cell Culture and Drug Preparation

The human glioblastoma cell lines, U87 and U251, (kindly provided by Dr. Erdoğan Pekcan Erkan) was cultured in Dulbecco's Modified Eagle Medium (DMEM) (Gibco, US) supplemented with 10% fetal bovine serum (FBS) (Gibco, US), 2mM L-glutamine (Gibco, US), 1mM sodium pyruvate (Gibco, US), and100U/mL penicillin - 100µg/mL streptomycin (Gibco, US). The cells were maintained at 37°C in a humidified incubator with a 5% CO_2 atmosphere.

Sertraline (Cat: S6319, Sigma, US) and penfluridol (Cat: P3371, Sigma, US), supplied in lyophilized form, were each dissolved in DMSO to create a primary stock solution at 38 mM and stored at -20°C. Intermediate stock solutions of 2 mM sertraline or penfluridol were prepared by diluting the primary stock solutions with DMSO. For all experiments, 0.1 % DMSO corresponding to the applied maximum doses served as the solvent control (DMSO control).

Cell Viability

The WST-1 cell viability assay was carried out to confirm previously determined the IC_{50} doses of sertraline and penfluridol in U251 and U87 cell lines (19). In the prior study, the effects of these drugs on cell viability were assessed by treating the cells with concentrations ranging from 1 μM to 40 μM and incubating for 24, 48, or 72 hours to establish the IC50 values. In this study, we specifically seeded 2.5 x 10^4 cells into a 96-well plate and allowed them to adhere for 24 hours. Cells were treated with sertraline (12μM for U251, 10μM for U87), penfluridol (5μM for U251, 6.5μM for U87) and solvent control containing 0.1 % DMSO for 72h. Following treatment, WST-1 reagent (Roche Diagnostics, US) was added, and absorbance was obtained at 450 nm (background correction: 620 nm). The percentage of cell viability was calculated relative to the solvent control group.

Western Blotting

After treatment with sertraline, penfluridol and solvent control (0.1 % DMSO) for 72h, the expression levels of target proteins (E-cadherin, vimentin, Slug, Snail, Twist1, pAkt, and TIMP-2) were evaluated using Western blotting. At the end of the treatment period, the cells were lysed in RIPA buffer (Cell Signaling, US) containing protease/phosphatase inhibitors (2 μg/ml aprotinin, 5 μg/ml leupeptin, 1 μg/ml pepstatin A, 1 mM PMSF, 10 mM NaF and 1mM sodium orthovanadate). The total protein concentration was determined using a Bicinchoninic Acid (BCA) total protein assay kit (Pierce, US). Next, 30 μg of total protein was separated in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred to a polyvinylidene fluoride (PVDF) membrane. After blocking the membranes in 1X TBS-T containing 5% non-fat dry milk for 1 hour at room temperature, they were incubated overnight at +4°C with primary antibodies against E-cadherin (1:1000) (Cat: 3195, Cell Signaling, US), vimentin (1:3000) (Cat: 5741, Cell Signaling, US), Snail (1:1000) (Cat: 3895, Cell Signaling, US), Slug (1:1000) (Cat: 9585, Cell Signaling, US), Twist1 (1:1000) (Cat: 46702, Cell Signaling, US), phosho-Akt (p-Akt - Ser473) (1:1000) (Cat: 4060, Cell Signaling, US), TIMP-2 (1:1000) (Cat: 5738, Cell Signaling, US), and α-actinin (1:5000) (Cat: 3134, Cell Signaling, US). Following washings with 1X TBS-T, the membranes were incubated for 1 hour at room temperature with HRP-conjugated secondary antibodies. Images were captured using an enhanced chemiluminescence (ECL) solution (Merck, US) and a UVP gel documentation system (UVP Ltd, UK). The densitometric analyses were performed using the UVP Bioimaging system with LabWorks 4.6 Image Acquisition software (UVP Ltd, UK), and each target protein was normalized to the corresponding reference protein, α-actinin.

Gelatin Zymography

The activity levels of MMP-2 and MMP-9, released from cells treated with sertraline and penfluridol, were evaluated using gelatin zymography. Following 48 hours of treatment with the drugs, the cells were transferred to serum-free media and incubated for an additional 24 hours. The media was then collected and concentrated using concentrator tubes (Millipore, US) at 4°C. The total protein concentration in the concentrated media was determined with BCA total protein analysis method (Pierce, US). Subsequently, 15 μg of the concentrated media was applied to polyacrylamide gels containing 1 mg/ml gelatin, and electrophoresis was performed at a constant 110V at +4°C. After electrophoresis, the gels were washed twice with Triton X-100 and incubated in an activation buffer (50 mM Tris-base, 50 mM NaCl, 1 mM $CaCl_2$, and 0.05% Brij 35 (pH 7.6)) at 37°C for 48 hours. Following Coomassie Blue staining and several washings with distilled water, the gels were visualized using a white light UVP gel documentation system (UVP Ltd., UK), and the densities of the lytic bands was determined using the UVP Bioimaging system with LabWorks 4.6 Image Acquisition software (UVP Ltd, UK). The gelatinolytic activity was calculated based on the formula "band density (area (mm^2) x optical density (O.D/mm^2))/ μg protein"

Statistical Analysis

Data were analyzed using Graph Pad Prism 10.2.3 software (Graph Pad Inc, US) and represented as means ± standard deviations (SD). Experiments were conducted as at least three independent biological replicates. First, the normality of the data distribution was confirmed with the Shapiro–Wilk test. Next, statistical significance was assessed with one-way ANOVA with Tukey test for the multiple group comparisons. For continuous data that do not follow a normal distribution, the Kruskal-Wallis test was utilized for comparisons among three or more groups, with Dunn's test employed for post hoc analyses.

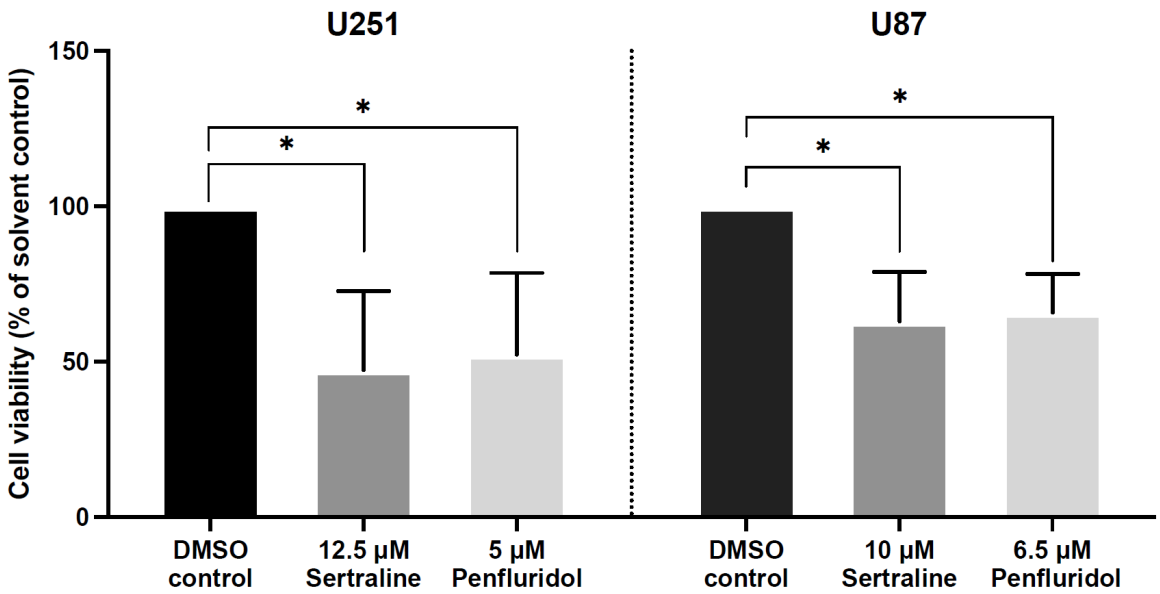

Figure 1. Cell viability of U251 and U87 glioblastoma cells treated with sertraline and penfluridol. U251 and U87 cells were treated with sertraline (12.5 µM for U251, 10 µM for U87) or penfluridol (5 µM for U251, 6.5 µM for U87) for 72 hours. Cell viability was assessed using WST-1 assay. Data are presented as percentage of viable cells compared to DMSO control (%0.1 DMSO). Bars represent mean ± SD (n=3).

Statistical significance was defined as follows: *p < 0.05; **p < 0.01.

RESULTS

Effects of Sertraline and Penfluridol on EMT Markers in U87 and U251 Cells.

In our previous study (19), we established the 72-hour IC_{50} values for sertraline (12.5 µM for U251, 10 µM for U87) and penfluridol (5 µM for U251, 6.5 µM for U87) in glioblastoma cell lines. To confirm these values, we conducted WST-1 cell viability assays. Our results demonstrated that sertraline at 12.5 µM reduced U251 cell viability to 47.3%, while penfluridol at 5 µM decreased it to 52.4% (p< 0.05, n= 3, Figure 1). In U87 cells, 10 µM sertraline and 6.5 µM penfluridol reduced viability to 63.3% and 65.97%, respectively (p< 0.05, n= 3, Figure 1). These concentrations were subsequently used in further experiments.

Next, we investigated the effects of sertraline and penfluridol on EMT markers in human glioblastoma cell lines. Western blot analysis revealed that both drugs significantly decreased vimentin levels in U251 cells (p < 0.01, n = 3, Figure 2a, b), suggesting a suppression of mesenchymal phenotype. However, pAkt (Ser473) levels were slightly reduced, but this decrease was not statistically significant (p > 0.05, n = 3, Figure 2a, b). Additionally, Slug, Snail and Twist1 levels remained unchanged (p > 0.05, n = 3, Figure 2a, b). Due to E-cadherin being undetectable or barely detectable in U251, we could not able to analyze the effects of agents on this marker in U251 cells (Figure 2a).

Conversely, a significant increase in vimentin levels was observed in U87 cells treated with either sertraline or penfluridol (p < 0.05, n = 3, Figure 2c, d). E-cadherin expression showed a slight but non-significant decreased, while Snail, Slug, Twist1, and pAkt (Ser473) levels were unaffected in response to both drugs in U87 cell lines (p > 0.05, n = 3, Figure 2c, d).

These results indicate that sertraline and penfluridol exert opposing effects on vimentin expression in U251 and U87 cells, highlighting cell line-specific responses. Further studies are required to elucidate the molecular mechanisms driving these differential responses and their potential relevance to glioblastoma therapy.

Effects of Sertraline and Penfluridol on Gelatinase Activity in U87 and U251 Cells.

In this study, we focused on the effects of sertraline and penfluridol on key regulators of ECM remodeling, including TIMP-2, MMP-2 and MMP-9 in human

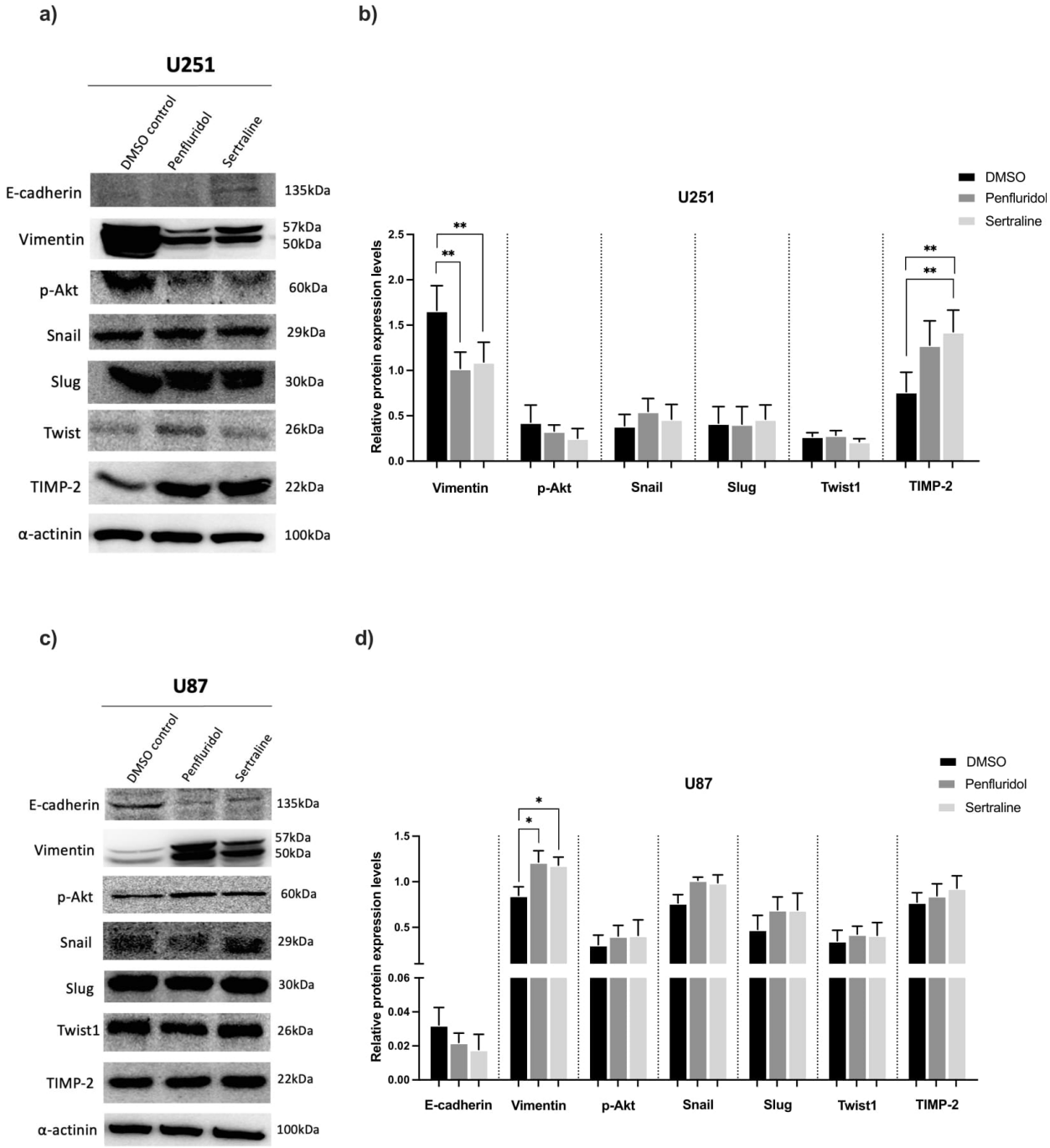

Figure 2. Effects of sertraline and penfluridol on EMT markers and TIMP-2 in U251 and U87 glioblastoma cells. (a) Representative Western blot images of EMT markers and TIMP-2 in U251 cells treated with sertraline (12.5 μM) or penfluridol (5 μM) for 72 hours. (b) Quantification of protein levels in U251 cells. Data are presented as mean ± SD (n=3). **p < 0.01 compared DMSO control (%0.1 DMSO). (c) Representative Western blot images of EMT markers and TIMP-2 in U87 cells treated with sertraline (10 μM) or penfluridol (6.5 μM) for 72 hours. (d) Quantification of protein levels in U87 cells. Data are presented as mean ± SD (n=3). *p < 0.05 compared to DMSO control (%0.1 DMSO).

glioblastoma cell lines. Western blot analysis revealed a significant increase in TIMP-2 protein levels in U251 cells treated with both sertraline ($p < 0.05$, $n = 4$, Figure 2a, b) and penfluridol ($p < 0.05$, $n = 4$, Figure 2a, b). In contrast, neither drug elicited statistically significant change in TIMP-2 levels in U87 cells ($p > 0.05$, $n = 4$, Figure 2c, d).

To further assess the impact on ECM degradation, we examined the activity levels of gelatinases, MMP-2 and MMP-9, using gelatin zymography. Our results

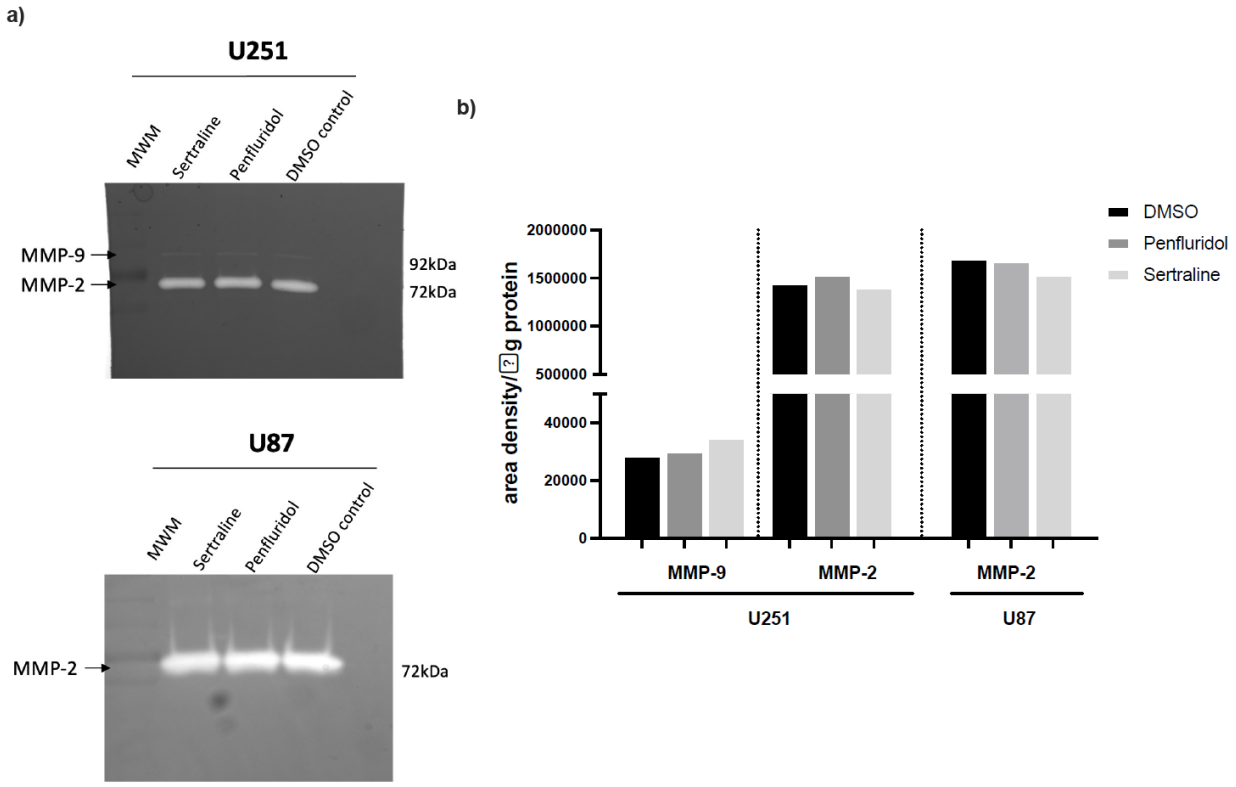

Figure 3. Effects of sertraline and penfluridol on MMP-2 and MMP-9 activity in U251 and U87 glioblastoma cells. Representative gelatin zymography images showing MMP-2 and MMP-9 activity in (a) U251 and (b) U87 cells treated with sertraline or penfluridol for 72 hours (n=1).

indicated that neither sertraline nor penfluridol altered the enzymatic activity of MMP-2 or MMP-9 in either cell line (n = 1, Figure 3). However, this experiment was replicated only once, limiting the statistical power of the findings.

These results suggest that sertraline and penfluridol may modulate ECM dynamics in glioblastoma cells via the upregulation of TIMP-2, particularly in U251 cells, rather than through direct suppression of gelatinase activity.

DISCUSSION

Epithelial-to-mesenchymal transition (EMT) is critical driver of cancer metastasis, enabling epithelial cells to lose polarity and adhesion, acquiring invasive and migratory capacities characteristic of mesenchymal cells. EMT often involves the upregulation of matrix metalloproteinases (MMPs), which degrade extracellular matrix (ECM) components, further enhancing invasiveness (20). This study explored the effects of sertraline and penfluridol on EMT markers and ECM remodeling in glioblastoma cell lines U251 and U87.

Our results demonstrate that both sertraline and penfluridol treatments led to a significant reduction in vimentin levels in U251 cells, suggesting suppression of mesenchymal characteristics. The absence or minimal detection of E-cadherin in U251 cells is characteristic of this mesenchymal-like glioblastoma subtype (21, 22), which presents a limitation of this study by restricting our ability to evaluate the EMT status in these cells. Therefore, we recommend employing more sensitive techniques, such as immunofluorescence (IF), to better evaluate E-cadherin expression in future studies.

Conversely, U87 cells exhibited a paradoxical response: a significant increase in vimentin, and a slight but not significant decrease in E-cadherin. This divergence is particularly noteworthy given our previous observations of enhanced invasiveness in U87 cells compared to U251 cells (19). Moreover, U87 cells are characterized as more proliferative and display a neuronal-like phenotype, while U251 cells have a slower proliferation rate and exhibit mesenchymal-like features (23).

To further explore these differences, we suggest conducting genomic and epigenomic profiling of the sertraline- or penfluridol-treated U87 and U251 cell lines, incorporating whole-exome sequencing, RNA-sequencing, and methylation analyses. This multi-omics approach will help identify key genetic and epigenetic differences between the cell lines and allow for correlations between these findings and the observed variations in their responsiveness to sertraline and penfluridol.

The differential responses observed between U251 and U87 cells underscore the intrinsic heterogeneity of glioblastoma tumors and their variable responsiveness to therapeutic interventions, emphasizing the necessity for personalized treatment strategies. These discrepancies suggest fundamental variations in EMT signaling cascades between these cell lines, warranting further mechanistic investigations. Additionally, the largely unchanged levels of EMT markers, Snail, Slug, and Twist1, indicate that these transcription factors may not be central to the observed effects. Future studies could explore alternative factors, such as Zeb1 and Zeb2, which could provide deeper insights into the mechanisms driving vimentin expression and EMT in glioblastoma. Investigating other EMT and mesenchymal-epithelial transition (MET) related factors, including ZO-1, fibronectin, and N-cadherin, could further enrich our understanding of these processes.

We also evaluated the role of sertraline and penfluridol on ECM remodeling in glioblastoma by assessing TIMP-2, MMP-2, and MMP-9. Neither sertraline nor penfluridol affected the activity of MMP-2 or MMP-9 in either cell line, suggesting that the anti-invasive effects of drugs may not involve direct modulation of gelatinase activity. It is important to note that this result is based on a single biological replicate; thus, further studies are necessary to validate these findings and to explore the potential involvement of other MMPs in ECM remodeling. Notably, both drugs significantly upregulated TIMP-2 levels in U251 cells, suggesting a potential role for this endogenous MMP inhibitor in mediating ECM dynamics. In contrast, TIMP-2 levels remained unchanged in U87 cells, highlighting the cell line-specific responses to sertraline and penfluridol.

To our knowledge, this is the first study to demonstrate the modulatory effects of sertraline and penfluridol on TIMP-2 expression in glioblastoma cell lines. These findings underscore the complexity of glioblastoma ECM regulation and suggest that the drugs' effects on tumor invasiveness may depend on distinct molecular contexts within different cell types.

Previous studies corroborate our findings by highlighting the anti-invasive and anti-metastatic potential of penfluridol in various cancer types, including breast (10, 24), lung (25), and glioblastoma (7) cancer. Hung et al. demonstrated that penfluridol suppresses uPA/uPAR/TGF-β signaling, resulted in reduced MMP-12 activity and EMT inhibition in lung adenocarcinoma cells (26). Additionally, penfluridol decreased MMP-9 expression in vivo and in vitro and inhibited invasion and migration in A549 lung cancer cells (25). In patient specific glioma sphere-forming cells, penfluridol downregulates stemness and invasion markers, including Integrin α6, Zeb-1, N-cadherin (27).

Limited studies have reported the potential of sertraline as an anti-invasive agent in cancer, but its effects in the context of glioblastoma have not been well-explored. Sertraline has been shown to lateral cell motility in breast cancer cells (28), and to target EMT markers in prostate cancer stem cells by downregulating transcription factors such as TCF8 and LEF1 (29). In HT-29 colon cancer cells, sertraline was reported to induce the expression of EMT-related markers, including β-catenin, and, E-cadherin (30). These findings from other cancer types appear to align with our observation of reduced mesenchymal marker expression in the U251 glioblastoma cell line following sertraline treatment.

However, it is important to note that the regulation of EMT in glioblastoma is known to be highly complex and context-dependent, potentially differing from the mechanisms observed in other cancer models. While our study is the first to reveal the effect of sertraline on the invasive process in glioblastoma, further investigation is required to elucidate the precise molecular mechanisms by which sertraline may differentially modulate EMT regulation in distinct glioblastoma subtypes.

CONCLUSION

This study demonstrates the potential of sertraline and penfluridol to modulate EMT and ECM dynamics in glioblastoma cells, with significant inhibitory effects observed in U251 cells. However, the contrasting effects in U87 cells highlight the critical need for personalized therapeutic strategies tailored to the molecular characteristics of individual tumors. It is essential to conduct thorough molecular

characterization of GBM subtypes to better understand these differential responses, as they may arise from distinct molecular profiles, variations in EMT signaling pathways, or intrinsic differences in drug uptake and metabolism.

Future research should prioritize the development of combination therapies that target complementary pathways to enhance treatment efficacy and overcome resistance in glioblastoma. Specifically, integrating sertraline and penfluridol with existing therapies could provide significant benefits. By incorporating molecular profiling into these therapeutic strategies, we can better understand the unique characteristics of individual tumors, ultimately advancing the clinical applicability of these drugs and improving clinical outcomes for patients with glioblastoma.

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Author contributions: DK: Conception, Design, Biological Material Responsibility, Data Collecton and Processing, Analysis - Interpretation, Literature Review, Writing. MS: Design, Biological Material Responsibility, Data Collecton and Processing, Analysis – Interpretatio, Literature Review. ŞİS: Biological Material Responsibility, Data Collecton and Processing, Analysis – Interpretation, Literature Review. GO: Supervision, Fundings, Biological Material Responsibility, Analysis – Interpretation, Literature Review, Critical Review. All authors reviewed and approved the final version.

Conflict of interest: The authors declare no conflict of interest.

Ethical approval: Ethical approval was obtained from the Health Sciences Research Ethics Committee of Izmir University of Economics, Izmir, Turkey (Date: 17/09/2024, No: B.30.2.İEÜSB.0.05.05-20-320).

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RESEARCH ARTICLE

PROLONGED β-HYDROXYBUTYRATE-MEDIATED KETOSIS ENHANCES PONATINIB RESPONSE OF K562 CHRONIC MYELOID LEUKAEMIA CELLS.

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ABSTRACT

Purpose: Ketosis is a metabolic state characterized by production of ketone bodies, including acetoacetate, β-hydroxybutyrate (BHB), and acetone, in response to reduced blood glucose levels. BHB stands out as the principal ketone body in nutritional ketosis which has diverse therapeutic implications for metabolic, nondegenerative and neoplastic disorders. In current study we investigated the impact of β-hydroxybutyrate mediated ketosis on viability and ponatinib response of K562 chronic myeloid leukaemia cells.

Materials and Methods: We investigated the impact of BHB-mediated ketosis on the viability of K562 cells, an *in vitro* model of chronic myeloid leukaemia (CML), and explored the influence of BHB on the sensitivity of these cells to ponatinib, a multi-targeted tyrosine kinase inhibitor used in CML treatment. We used MTT assay to measure cell viability and Hoechst/PI assay to measure cell death.

Results: Our findings reveal that BHB concentrations ranging from 1 mM to 5 mM, which fall within the physiological range of ketosis, elicit a minimal yet concentration-dependent reduction in cell viability. We also observed that while a 24-hour pre-treatment with BHB did not enhance the response of K562 cells to ponatinib, prolonged ketosis (4 days) improved response of cells to the drug by decreasing final cell viability from 25.15% to 13.12%. The primary mode of viability inhibition by ponatinib was cell death which was further intensified by exposure to prolonged ketosis.

Conclusion: Ketosis induced by ketogenic diet of ketone body supplementation is considered as safe and effective adjuvant cancer therapy options and here, we report its potential effectiveness in the context of CML.

Keywords: Ketogenic diet, ketosis, chronic myeloid leukaemia, β-hydroxybutyrate, Ponatinib

INTRODUCTION

Ketosis is the metabolic shift from catabolism of carbohydrates to lipids as a response to decreased blood glucose levels, leading to an increased rate of fatty acid β-oxidation within the liver. Excessive acetyl-CoA produced by β-oxidation serves as a substrate for the synthesis of a class of water-soluble acidic metabolites known as ketone bodies (acetoacetate, β-hydroxybutyrate, and acetone) in a process called ketogenesis within the mitochondrial matrix (1). Acetoacetate and β-hydroxybutyrate (BHB) can subsequently be catabolized back into

acetyl-CoA molecules, which are further oxidized via the Krebs cycle in extrahepatic tissues, providing an alternative energy source to glucose. BHB, in particular, emerges as the principal ketone body during natural ketosis (2) and can be employed as a supplement to induce a controlled state of artificial ketosis (3). Which will be referred as BHB-mediated ketosis in the current study.

Physiological or nutritional ketosis arises as a response to dietary modifications and is predominantly characterized by reduced carbohydrate intake. This results in elevation of ketone body levels, notably plasma BHB concentrations rising up to 7-8 millimolar (mM) range (4). This form of ketosis can also be achieved through ketone body supplementation and has been shown to exhibit multiple health benefits for patients with epilepsy, Alzheimer's disease, Parkinson's disease, and metabolic syndrome (3).

Therapeutic potential of ketosis for treatment of cancers is currently under investigation (5). Ketogenic diet has been demonstrated to sensitize most cancers to standard treatments by interfering with the regular metabolic functions of cancer cells (6,7). This anti-tumour activity often leads to an improved drug response and increased survival rate which has been observed across various cancer types, including glioblastoma, pancreatic, colorectal, breast, liver, and lung cancers, among others (5). However, data remains scarce for certain cancers, such as leukaemia. Notably, there are no preclinical or clinical studies focusing on the impact of ketosis on chronic myeloid leukaemia (CML) patients and/or cells. In current study we decided to investigate the effects of BHB-mediated ketosis on the viability of CML cells as well as the drug response of the cells in ketosis against ponatinib, a multi-targeted tyrosine kinase inhibitor primarily employed in the treatment of CML (8).

MATERIALS AND METHODS

Cell Culture Maintenance

K562 (ATCC, #CCL-243) CML cell line was used in the study. K562 cells were maintained in RPMI-1640 (Biowest, #MS015H) medium (complete medium) containing 10% fetal bovine serum (FBS) (Cytiva, #SV30160.03), 1% (2 mM) L-Gln (Capricorn, #GLN-B) and 1% (100 U/mL) Penicillin/streptomycin (Pan Biotech, #P06-07100) in a humid incubator at 37ºC 5% CO_2 pressure.

Chemical Treatments

β-hydroxybutyrate (Sigma, #166898) was dissolved in absolute Ethanol (EtOH) and subsequently diluted with cell culture media to attain varying concentrations to simulate physiological ketosis. The highest treatment concentrations contained 1% EtOH and since this concentration exceeds the recommended threshold (9) we included vehicle controls to assess and clarify any potential impact of ethanol on our study outcomes. Ponatinib (Selleck chemicals) was dissolved in dimethyl sulfoxide (DMSO) and further diluted with cell culture media to achieve different concentrations for experimental treatments. The highest treatment concentrations contained less than 0.1% DMSO.

Cell Viability Measurement

The viability of K562 cells was assessed using the MTT (3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide, Abcam) assay to evaluate the impact of ponatinib, β-hydroxybutyrate (BHB), and their combination. Briefly, a total of $3x10^3$ K562 cells were seeded in 50 µL of culture medium into the wells of 96-well plates. After an overnight incubation, cells in the experimental wells were treated either with ponatinib (1-14 µM) for 24-hours or BHB (0.01-5 mM) for 48-hours in a total volume of 100 µL. For longer incubation periods (BHB for 6 days, ponatinib for 2 days), 1000 K562 cells were seeded into the wells of 24-well plates in a total volume of 300 µL of culture medium and total volume was 600 µL after the treatment. The non-treated (NT) and vehicle control (VC, %1 EtOH) wells received an equivalent volume of complete medium as the treatment groups. Upon completion of the treatment, 10% MTT solution with a final concentration of 500 µg/mL was added to all wells. The plates were incubated in darkness for 4 hours. Subsequently, a 10% SDS solution (dissolved in 0.01 M HCl) in a 1:1 ratio was added to all wells treated with MTT and incubated overnight. A spectrophotometric reading at 570 nm was conducted using a BMG Labtech LUMIStar Omega instrument. Cell viability in the non-treated group was considered as 100%, and the viability of cells in the treatment groups was calculated accordingly. Each experiment contained at least five (96-well plate experiments) or three (24-well plate experiments) replicate wells and the average viability and standard deviation calculated for these cells were used in graphical representations which were plotted using GraphPad Prism v9. The data is uploaded as a supporting file.

Cell Death Measurement

The Hoechst/Propidium iodide (PI) staining method was employed to assess the impact of ponatinib, β-hydroxybutyrate (BHB), and their combination on cell death in K562 cells. K562 cells were seeded in 24-well plates at a density of 1000 cells per well in a 300 µL culture medium. Following an overnight incubation, the cells were treated either with ponatinib (6 µM) or BHB (5 mM) in 600 µL total volume (BHB for 6 days, ponatinib for 2 days). Upon completing the treatment, the cells were collected and pelleted by centrifugation at 300 g for 5 minutes. Following centrifugation, the supernatant was carefully aspirated, and the cell pellet was resuspended in a phosphate-buffered saline (PBS) solution containing Hoechst-33342 (0.25 µg/mL) (ChemCruz, #sc-495790) and PI (1 µg/mL) (Biolegend, #421301) fluorescent dyes. The suspension was incubated at room temperature for 20 minutes in the dark. After the incubation period, the number of Hoechst-positive cells, representing the entire cell population, and PI-positive cells, indicative of dying cells, were quantified using fluorescence microscopy (Zeiss, AxioScope Z1). Subsequently, the cell death rate was computed based on these counts. For each sample, at least three different cell suspensions were assessed as technical replicates, and the resulting average cell death rate values and standard deviation were used to generate graphical representations which were plotted using GraphPad Prism v9. The data is uploaded as a supporting file. The study was evaluated by the Human Ethical Research Committee of Istinye University.

RESULTS

Ponatinib reduced K562 cell viability at the micromolar level. Cells were treated with different concentrations of ponatinib (ranging from 1 µM to 14 µM) for 24 hours to identify an effective concentration for subsequent experiments. MTT assay results demonstrated a concentration-dependent inhibition of K562 cell viability induced by ponatinib (Figure 1A). 6 µM ponatinib was the lowest concentration to inhibit the cell viability by 50% (specifically 51.5%) and subsequently, was selected as the optimal concentration for further investigations.

β-hydroxybutyrate treatment caused a modest reduction in K562 cell viability.

We have treated the cells for 48 hours with increasing concentrations of BHB (ranging from 10 µM to 5 mM) to assess the impact of ketosis on cell viability. Notably, the highest BHB concentration used to mimic ketosis remained below the upper physiological limit (<8 mM). MTT assay results revealed that BHB-mediated ketosis had a minimal yet concentration-dependent effect on K562 cell viability, reducing it to a minimum of 80% (Figure 1B). Consequently, we chose to proceed with BHB concentrations of 1, 3, and 5 mM for further investigations. Short-term ketosis mediated by BHB did not influence ponatinib response in K562 cells. In addition to the standard 48-hour BHB treatment (at concentrations of 1, 3, or 5 mM) and 24-hour ponatinib treatment (at 6 µM), select wells were pre-treated with BHB for 24 hours before ponatinib exposure, allowing us to elucidate the influence of ketosis on ponatinib sensitivity. The MTT assay results suggested that the 24-hour pre-treatment did not enhance the response of K562 cells to ponatinib (Figure 1C). As a result, we opted to extend our investigations to longer incubation periods using only the highest BHB concentration (at 5 mM).

Prolonged exposure to BHB-mediated ketosis enhanced ponatinib response of K562 cells. Cells were subjected to BHB treatment (at 5 mM) for 6 days, ponatinib treatment (at 6 µM) for 2 days, and a combination regimen in which cells were pre-treated with BHB for 4 days, followed by treatment with ponatinib for 2 days. MTT assay results indicated that the 4-day BHB pre-treatment improved the response of K562 cells to ponatinib, reducing final viability from 25.15% to 13.12% (Figure 1D). Nonetheless, it is worth noting that BHB itself reduced cell viability by 32.48%, suggesting that this effect may be additive rather than synergistic. We should also note that, unlike previous findings, EtOH (%1, as vehicle control) also caused a decrease in viability (79%), albeit to a lesser extent compared to BHB.

Prolonged BHB-mediated ketosis led to a mild increase in the rate of ponatinib induced K562 cell death.

In order to elucidate the mechanism of the viability inhibition, we assessed cell death levels in samples previously examined for viability (6 days of BHB treatment, 2 days of ponatinib treatment, and a combined treatment of 4 days of BHB followed by 2 days of ponatinib). Results from the Hoechst/PI assay revealed cell death as the primary mechanism of viability inhibition induced by ponatinib as 80% of the cells were PI positive. Prolonged exposure to BHB-mediated ketosis further increased the cell death to 89% (Figure 1E).

which may initially resist such changes due to their

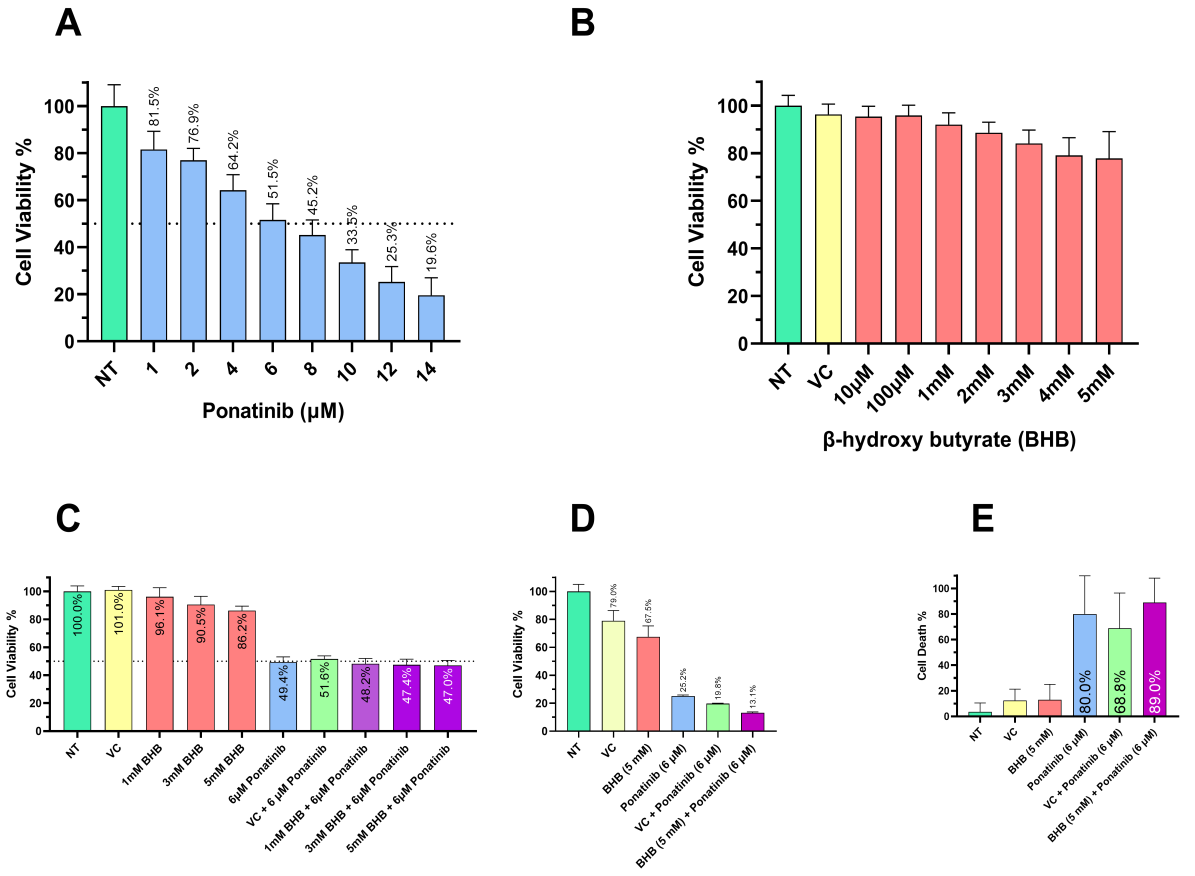

Figure 1. Effects of BHB-mediated ketosis on K562 cells. (A, B, C and D) Cell viability results according to the MTT assay. Non-treated cell viability (NT) was considered as 100% and all the others were calculated accordingly. Vehicle control is 1% ethanol, the solvent for BHB. Each value represents the average viability of at least five samples and error bars represent standard deviation. (A) Effects of 24-hour ponatinib treatment. (B) Effects of 48-hour BHB treatment. (C) Effects of combination treatment (24 hours for ponatinib, 48 hours for BHB). (D) Effects of prolonged exposure to combination treatment (2 days for ponatinib, 6 days for BHB). (E) The percentage of PI positive cells according to the Hoechst/PI assay after prolonged exposure to combination treatment (2 days for ponatinib, 6 days for BHB). Each value represents the average of at least three different samples and error bars represent standard deviation.

DISCUSSION

The utilization of the health benefits associated with ketosis achieved via ketogenic diet or ketone body supplementation, has garnered increasing interest in recent years. Notably, the ketogenic diet has been proposed as a safe and potentially beneficial approach for cancer patients (10,11). Although there are numerous studies focusing on different cancers to elucidate the impact of ketosis *in vitro*, *in vivo* and in clinical settings, the data is still scarce for some cancers including leukaemia. This lack of studies on the impact of ketosis in leukaemia is particularly intriguing because leukemic cells, by nature, are more susceptible to ketosis due to their immediate exposure to the ketogenic environment within the bloodstream. This is in contrast to solid tumours, erratic vasculature and hypoxic regions where the blood cannot reach effectively.

A noteworthy study examining the influence of insulin feedback suppression on drug responses in mouse models of different cancers also included acute myeloid leukaemia (AML) to their study design (12) and found that ketogenic diet enhanced the efficacy of phosphatidylinositol-3 kinase (PI3K) inhibitors. Here we report for the first time that the prolonged ketosis inhibits CML cell viability and enhances ponatinib response *in vitro*. Since physiological ketosis is easily and safely achievable in patients, ketogenic diet or ketone-body induced ketosis could be considered as adjuvant cancer therapy for a spectrum of malignancies including CML. However, it is important to emphasize that while these findings

are compelling, further investigations in clinical settings are imperative to fully elucidate the precise impact of ketosis on patient health and drug response in the context of CML.

Ponatinib is a third-generation kinase inhibitor which can act on BCR-ABL1 kinase and several ABL1 mutations making it an effective treatment for CML patients (8). It is applicable across all phases of the disease and serves as a rescue therapy in cases of resistance or intolerance to dasatinib or nilotinib, as well as in instances where imatinib proves ineffective (13). However, cardiovascular adverse events have emerged as concerningly common complications of ponatinib treatment (14). Since ponatinib-associated cardiovascular toxicity been found to be dose-dependent (15), exploring the combination of ponatinib with other adjuvant therapies, such as a ketogenic diet, to reduce the required therapeutic dose to mitigate the associated side effects is an intriguing and potentially effective strategy. This approach is further supported by the known beneficial effects of the ketogenic diet on cardiovascular risk factors (16).

CONCLUSION

Our study highlights the potential of ketosis as an adjuvant therapy for chronic myeloid leukemia (CML). Nonetheless, it is important to acknowledge that the current research focused on a single cell line and future research should explore cell-line-specific effects of BHB. Finally, more research is warranted to validate these findings in clinical settings.

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