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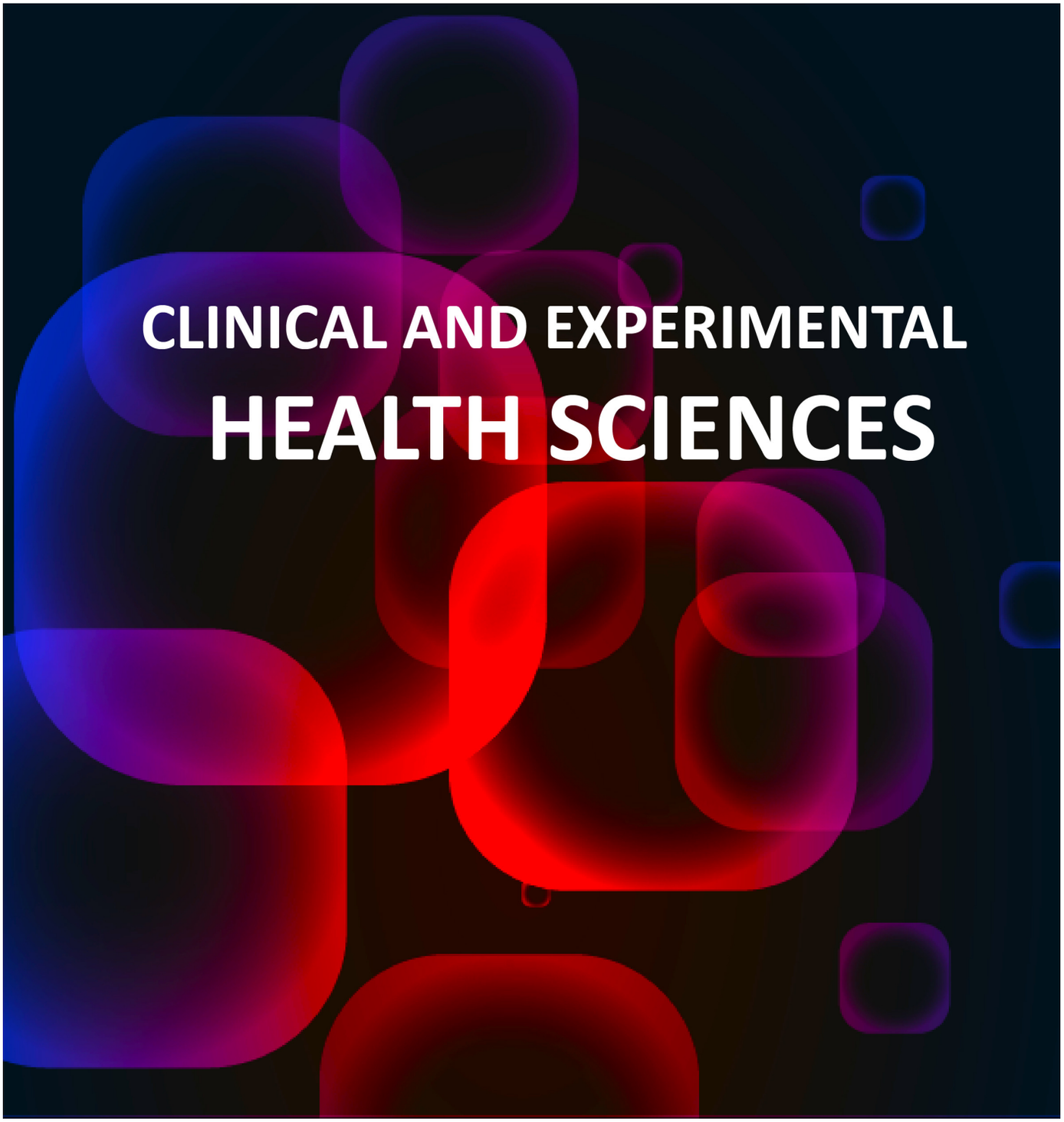

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Clinical and Experimental Health Sciences

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ABOUT

Clinical and Experimental Health Sciences (Clin Exp Health Sci) is an international, scientific, open-access periodical published in accordance with independent, unbiased, and double-blinded peer-review principles.

The journal was published under the name MUSBED until April 22, 2016.

The target audience of the journal includes specialists and medical professionals working in all disciplines of health sciences. The journal is the official publication of Marmara University, Institute of Health Sciences and it is published four times a year in March, June, September, and December. The journal publishes content in English. The journal publishes clinical and experimental research articles, review articles, case reports, letters to the editor, and editorial comments prepared in accordance with ethical guidelines in all disciplines of health sciences.

Aim & Scope

The aim of the journal is to contribute to the literature by publishing clinical and experimental research articles, case reports, letters to the editor, and and editorial comments prepared in accordance with the ethical guidelines in all disciplines of health sciences. The target audience of the journal includes specialists and medical professionals working in all disciplines of health sciences.

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Clinical and Experimental Health Sciences

Contents

ORIGINAL ARTICLES

Contents

Contents

ORIGINAL ARTICLES

REVIEWS

CASE REPORTS

Clinical and Experimental Health Sciences

Evaluation of Self-Care and Self-Efficacy in Patients Experiencing Phantom Pain After Amputation

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ABSTRACT

Objective: This study was conducted with a comparative and descriptive research design to evaluate the self-care agency and self-efficacy of patients who experienced phantom pain after limb amputation.

Method: The population of the study consisted of 54 patients who underwent limb amputation operations in a university hospital in Istanbul between 1 January 2018 and 1 January 2020. Sample selection was not made, all patients included the population were reached by phone after their discharge. When the data were collected, one patient was excluded due to being younger than 18 years of age, 12 patients died, 9 patients had stump pain, and the study was completed with 32 patients. Data were collected using a "Personal Information Form", the Visual Analog Scale, the Exercise of Self-Care Agency Scale and the Self-Efficacy Scale.

Results: The mean Exercise of Self-Care Agency Scale score of the patients was found to be 79.37±21.55. Their mean total Self-Efficacy score was 70.00±16.10. There was no statistically significant relationship between postoperative phantom pain and scale scores.

Conclusion: It was determined that patients who experienced phantom pain after amputation had moderate self-care agency and perceived self-efficacy levels.
Keywords: Amputation, phantom limb pain, self-care, self-efficacy.

1. INTRODUCTION

Amputation is the procedure of removing a damaged extremity that cannot be corrected, by surgically cutting it with its bone out of the body (1,2). Amputation can affect the bio-physical, psychological, and socio-economic dimensions of the individual's life, causing problems in activities of daily living and therefore a decrease in self-care agency. Self-care refers to activities initiated and performed to improve health, protect one's life, health, and well-being, prevent the deterioration of one's health, and maintain one's health (2).

Self-care agency (SCA) is the individual's ability to perform self-care related activities (3,4). According to Orem, SCA is "a multidimensional concept that includes having the necessary motivation, idea generation capacity, energy, and knowledge to carry out self-care activities that maintain health and well-being" (5). To lead a satisfying life, awareness of health-related needs to be strengthened should be raised in people, and behaviors and skills that will enable them to use their health-related skills to the fullest should be developed (6,7). Nurses, who identify the inadequacies in the self-care of individuals, aim to help patients or healthy individuals meet their self-care needs (4).

As defined by Senemoğlu, self-efficacy is one of the cognitive perception factors that affect an individual's behaviors (8,9). Self-efficacy was expressed by Bandura in 1977 as a concept that affects behavior. According to Bandura, "self-efficacy is the individual's own judgment of their capacity to organize and successfully perform the activities necessary to demonstrate a certain performance." In other words, self-efficacy is an individual's judgment and belief about their own regarding the extent to which they can be successful in overcoming difficult situations that they may encounter in the future (8). It is important that nurses, who aim to protect individuals from ill-advised behaviors and help them adopt positive behaviors that improve and maintain health, control the individual's health-related behaviors and have knowledge about this concept (10-12).

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Problems that develop in individuals after amputation cause insufficiency in their activities of daily living from different perspective, causing people to become fully or partially dependent in the physical, financial, and social sense, thus affecting their self-care agency and self-efficacy (13). "Phantom Feeling (PF)", which is defined as feeling as if the amputated extremity is still in place, and "Phantom Pain (PP)", which is seen as pain in the non-existing extremity, are chronic problems that are frequently encountered after amputations (14). The incidence of PP after amputation is reported to be in the range of 49-83% (15,16). The discomforts that the patient may experience during or after the operation or the procedures to be performed (e.g., pain, nausea, vomiting, hypothermia, anxiety), including the experience of PP, which is a very common problem, may be the main reason for a decrease in their self-care agency and self-efficacy levels. A nurse should implement all necessary nursing interventions to reduce or resolve the situations that will cause discomfort in the patient before and after each procedure (17).

2. METHODS

2.1. Research Design and Participants

This study was conducted with a descriptive design to improve the quality of patient care by evaluating self-care agency and self-efficacy in patients who underwent amputation operations and experienced PP, guide nursing interventions, and direct future research on the subject in the field of nursing.

The population of the study consisted of 54 Turkish patients who underwent limb amputation operation in the orthopedics and traumatology clinic of a university hospital in Istanbul between 1 January 2018 and 1 January 2020.

Sample selection was not performed because the number of patients that could be reached was very small. All patients who made up the population were called by phone after their discharge. However, the study was completed with 32 patients between 1 March and 30 April 2020, when the data were collected, because one patient was younger than 18 years old, 12 patients died, and 9 patients had stump pain.

2.1.1. Inclusion criteria: Patients aged 18 years or older, who underwent limb amputation operations between 1 January 2018 and 1 January 2020, experienced PP, and were oriented to person, place, and time were included.

2.1.2. Exclusion criteria: In differentiating PP from stump pain, the location and nature of the patient's pain were questioned. The pain of the patients who described it as a sharp, burning, electric shock-like pain localized in the remaining limb, superficially in the deep tissues at the incision line, or spread to the entire remaining limb, was considered stump pain, and such cases were excluded from the study (18,19).

2.2. Data Collection

The data of the study were collected with a "Personal Information Form", the "Visual Analog Scale", the "Exercise of Self-Care Agency Scale", and the "Self-Efficacy Scale".

2.2.1. Personal Information Form: This form included 21 questions prepared by the researchers in light of the relevant literature, to collect information on the introductory characteristics of the patients and their characteristics related to their disease and surgery. The questioned individual characteristics included sex, age, marital status, educational status, income level, chronic illness, medication use, companion status, and habits. The features of PP and surgery that were questioned included the status of pain before and after the surgery. Questions inquiring about the location and severity of postoperative pain and the use of analgesics were also included among these 21 questions (1,11,13-17).

2.2.2. Visual Analog Scale (VAS): VAS for Pain is a one-dimensional individual pain assessment method and is usually in the form of a 10 cm long line, with "No Pain-Zero (0)" at one end and "Unbearable Pain-Ten (10)" at the other end. It can be used as a horizontal or vertical ruler. It is an easy-to-use scale to evaluate response to treatment and pain (20,21).

2.2.3. Exercise of Self-Care Agency Scale (ESCA): ESCA is a scale developed by Kearney and Fleischer in 1979 and used to determine people's ability to take care of themselves (22). It is a 5-point Likert-type scale consisting of 35 items, the validity and reliability study of which was conducted by Nahcivan in Turkey in 1994, focusing on the self-evaluation of individuals regarding their involvement in self-care activities (6). Each item is scored from 0 to 4. The maximum possible score is 140, and it is accepted that higher scores correspond to higher levels of self-care agency. The scale is based on 4 features. These are an active versus a passive response to situations, motivation, knowledge and information seeking, and self-worth, self-esteem, and self-concept of the individual. In the study, the Cronbach's alpha coefficient of the scale was 0.94, and it was determined to be a highly reliable scale.

2.2.4. Self-Efficacy Scale (SES): SES is a Likert-type scale that measures an individual's effectiveness/competence, it consists of 23 items, and each of its items is scored between 1 and 5. The validity and reliability of the Turkish version of the scale developed by Sherer et al. (1982) (23) were tested by Gözüm and Aksayan in 1999. The minimum and maximum possible scores on the scale are 23 and 115. A high total score indicates a high level of self-efficacy-efficacy perceived by the respondent (24). In the study, the Cronbach's alpha value of the total scale was found to be 0.95, and it was determined to be a highly reliable scale.

Data were collected between 1 March and 30 April 2020. The patients were reached by phone. Verbal consent was obtained by the researcher. Written permission could not be obtained

due to the ongoing COVID-19 pandemic restrictions. Filling out the forms took about 15-20 minutes for each patient.

2.3. Data Analysis

The NCSS (Number Cruncher Statistical System, Kaysville, Utah, USA) program was used for statistical analyses. Descriptive statistical methods (mean, standard deviation, median, frequency, ratio, minimum, maximum) were used to analyze the data. The conformity of the quantitative data to normal distribution was tested with the Kolmogorov-Smirnov test, the Shapiro-Wilk test, and graphical evaluations. The Mann-Whitney U test was used in the comparisons of two groups of data that did not show normal distribution. The Wilcoxon Signed-Rank test was used for intragroup comparisons. Spearman's Correlation Analysis was used to evaluate the relationships between variables. The threshold for statistical significance was accepted as p< .05.

2.4. Ethical Considerations

Before starting the study, ethical approval from Istanbul University-Cerrahpaşa Social Sciences and Humanities Ethics Committee (Decision No: 2019/159) and institutional permission from the relevant hospital (Date: 06/04/2020 and No: 51988) were obtained. The individuals participating in the study were informed about the study, and their verbal consent was obtained. For the use of the scales, permission was obtained via e-mail from the authors who conducted the validity and reliability studies of the scales.

2.5. Limitations

The limitation of the study was that it was conducted as a descriptive study with 32 patients and a single group that could be reached.

2.6. Strengths

The strengths of the study are that there is no other study in the literature on self-care and self-efficacy in patients experiencing PP and that it is the first study on this subject.

3. RESULTS

The distribution of the descriptive characteristics of the patients who were included in the study is shown in Table1. It was determined that 81.2% (n=26) of the patients had Diabetes Mellitus (DM), and 37.5% (n=12) had chronic diseases other than DM. It was found that 81.2% (n=26) of the amputation indications was DM, and 18.8% (n=6) were conditions other than DM. While the time since the amputation operations of the patients was 0-12 months in 28.2%, and it was 13-24 months in 71.8%. Among the amputated limbs of the patients, 62.5% (n=20) were below the knee, 31.3% (n=10) were above the knee, and 6.2% (n=2) were below or above the elbow. Preoperative pain was experienced by 84.4% (n=27) of the patients, while 100% (n=32) had postoperative pain. According to their statements, 96.9% (n=31) had PF after their operation. Painkillers were used by 65.6% (n=21) of the patients (Table2). There was no statistically significant difference between the postoperative PP scores of the patients based on the presence of preoperative pain among them (p> .05) **(Table 1).**

Table 1. *Distribution of descriptive features, diseases and distributions regarding amputation (n=32)*

		Bottom-Top	Avg±SD
Age (years)		38-87	62,94±12,65
		n	**%**
Gender	**Female**	8	25.0
	Male	24	75.0
Marital Status	**Married**	24	75.0
	Single	8	25.0
Education Status	**Primary school and below**	16	50.0
	Secondary school and above	16	50.0
Working Status	**Not working**	25	78.1
	Working	7	21.9
Diabetes mellitus	**No**	6	18.8
	Yes	26	81.2
Other chronic diseases	**No**	20	62.5
	Yes	12	37.5
Time after amputation	**0-12 months**	9	28.2
	13-24 months	23	71.8
Amputation cause	**Diabetes**	26	81.2
	Non-diabetes causes	6	18.8
Amputated limb	**Under the knees**	20	62.5
	Above knee	10	31.3
	Below and above the elbow	2	6.2
Preoperative pain	**No**	5	15.6
	Yes	27	84.4
Post-surgical phantom feeling	**No**	1	3.1
	Yes	31	96.9
Post-surgical phantom pain	**Yes**	32	100.0
Use of painkillers	**No**	11	34.4
	Yes	21	65.6

Avg: *Avarage;* ***SD:****Standard Deviation*

The total ESCA scores of the patients ranged from 52 to 122, with a mean score of 79.37±21.55. The Cronbach's alpha internal consistency coefficient of the scale based on the responses of the patients given to the scale items was 0.94, and the scale was found to have high validity and reliability.

The mean SES scores of the patients were 24.68±6.14 for starting the behavior, 20.77±3.63 for maintaining the behavior, 16.25±5.51 for completing the behavior, and 8.87±2.28 for struggling with obstacles, whereas their mean total SES score was 70.00±16.10. The Cronbach's alpha internal consistency

coefficient of the total scale in this study was found to be 0.95, and it was determined to be a highly reliable scale **(Table 2).**

Table 2: *Self-efficacy scale scores and Cronbach's alpha internal consistency coefficients*

Factors	Number of items	Bottom-Top	Avg±SD	Alpha value
Factor-1: Starting the behavior	8	15-35	24.68±6.14	0.728
Factor-2: Maintaining the behavior	7	14-26	20.77±3.63	0.815
Factor-3: Complete the behavior	5	8-25	16.25±5.51	0.910
Factor-4: Struggling with obstacles	3	4-13	8.87±2.28	0.554
TOTAL: Self-Efficacy	23	47-98	70.00±16.10	0.953

Avg: *Avarage;* ***SD:****Standard Deviation*

The total ESCA scores of the patients were significantly related to their behavior initiation scores (r= .737), their behavior completion scores (r= .754), their struggle with obstacles scores (r= .783), and their total SES scores (r= .752) (p< .001; p< .01). A positive, moderate, and statistically significant correlation was determined between the total ESCA scores of the patients and their SES behavior maintenance subscale scores (r= .602; p= .001; p<0.01). There was no statistically significant correlation between the total ESCA scores of the patients and their preoperative pain or postoperative PP scores (p> .05). There was also no statistically significant correlation between the scores of the patients on the starting the behavior, maintaining the behavior, completing the behavior, and struggling with obstacles subscales and their preoperative pain or postoperative PP scores (p> .05). Moreover, there was no statistically significant relationship between the total SES scores of the patients and their preoperative pain or postoperative PP scores (p> .05) **(Table 3).**

Table 3: *Relationship between scales*

			Self-efficacy					Pain	
		Self-care Agency Total	Starting the behavior	Keeping the behavior	Completting the behavior	Struggling with obstacles	Total	Preopreative	Postoperative PP
Self-care Agency Total	r	1.000	0.737	0.602	0.754	0.783	0.752	-0.147	-0.230
	p	**-**	***.001***	***.001***	***.001***	***.001***	***.001***	***.421***	***.205***
Starting the behavior	r	-	1.000	0.816	0.876	0.781	0.952	-0.111	-0.169
	p	-	-	***.001***	***.001***	***.001***	***.001***	*.544*	***.354***
Keeping the behavior	r	-	-	1.000	0.840	0.638	0.890	-0.131	0.122
	p	-	-	-	***.001***	***.001***	***.001***	***.483***	*.512*
Completting the behavior	r	-	-	-	1.000	0.746	0.950	-0.193	-0.055
	p	-	-	-	-	***.001***	***.001***	***.289***	*.765*
Strugging with obstacles	r	-	-	-	-	1.000	0.821	-0.320	-0.266
	p	-	-	-	-	-	***.001***	***.074***	***.141***
Self-efficacy total	r	-	-	-	-	-	1.000	-0.242	-0.160
	p	-	-	-	-	-	-	***.189***	***.390***
Preoperative Pain	r	-	-	-	-	-	-	1.000	0.581
	p	-	-	-	-	-	-	-	***.001***

r: *Spearman's Correlation Coefficient* ***p< .01 **PP:** Phantom Pain*

The preoperative pain and postoperative PP scores of the patients were determined to not vary significantly in relation to their sex or age (p> .05). There was no significant difference between the male and female patients in terms of the degrees of change in their postoperative PP scores compared to their preoperative pain scores (p> .05).

Furthermore, the education levels of the patients were not found to be significantly associated with their preoperative pain or postoperative PP scores (p> .05). However, the postoperative PP scores of the patients who were primary school graduates increased significantly compared to their preoperative pain scores (p= .019; p< .05).

4. DISCUSSION

In the relevant literature, there is no other study about the self-care agency and self-efficacy levels of amputation patients who experience PP. In this study, which was conducted to evaluate the self-care agency and self-efficacy of patients who experienced PP after limb amputation, PP was found in 32 (76.2%) of 42 patients who underwent limb amputation in the specified dates. In 96.9% (n=31) of the patients, PF, which felt like the amputated limb was still in place after the operation, was also observed. Alsancak and Altınkaynak reported PP in 7 (23%) and PF in 16 (53%) of 30 patients who underwent lower extremity amputation (25).

In a study conducted with 147 individuals with lower and upper extremity amputations, it was reported that 60% of upper extremity amputees had PP, 70.7% had PF, 65.8% of lower extremity amputees had PP, and 75.6% had PF (26). In another study in which 5700 amputation cases were evaluated, it was stated that the prevalence of PP was around 75% (27). It can be thought that the differences in the incidence of PP in the literature are probably due to differences in the times when these studies were carried out, the countries where they conducted, and the methods used during data collection, as well as the low total number of individuals who experienced PP, which was one of the limitations of this study.

Approximately 90% of amputations are performed due to peripheral vascular diseases. Approximately half of the amputations performed for peripheral vascular diseases are in diabetic patients (28). Lower extremity amputations are frequently performed especially for diabetic and vascular reasons, and they are among the operations in which post-surgical pain is the most common (29). Walker stated that in individuals with heart disease, the duration of the disease is important, and newly diagnosed patients have more positive health beliefs than patients who are diagnosed earlier in their lives (30). In the study conducted by Kara and Fesçi with individuals with Type 1 DM, it was reported that as the duration of the disease increased, self-care agency decreased (31). In this study, it was determined that 81.2% (n=26) of the patients had DM, and 37.5% (n=12) had chronic diseases other than DM. This explained the more frequent PP experience in patients who were amputated due to diabetes, similarly to other studies in the literature.

It was reported that approximately 85% of all amputations are performed on the lower extremities (28,32). Nutritional disorders due to vascular causes are mostly seen in the lower extremities (30). In this study, 62.5% (n=20) of the amputation cases were below the knee, 31.3% (n=10) were above the knee, and 6.2% (n=2) were below or above the elbow. Consistent with the literature, most of the patients had amputations at different levels in their lower extremities.

Preoperative pain was detected in 84.4% (n=27) of the patients who were included in this study, and postoperative PP was detected in 100% (n=32). The rate of patients who used painkillers was 65.6% (n=21). The relevance of the presence, severity, or duration of pain before surgery to PP is controversial. Severe and especially long-lasting pain before amputation surgery is considered a risk factor for the chronicity of pain (33). In a study involving amputations performed due to vascular diseases, it was noted that while the frequency of preoperative pain was 80%, chronic PP remained at around 60%, and the duration of pain, rather than the presence of pain, was a risk factor for chronicity (34). In a larger series of lower extremity amputations, there was no significant relationship between the presence of preoperative pain and its chronicity (35). In this study, in parallel with the literature, no significant relationship was found between the presence of preoperative pain and the presence of postoperative PP, and it is thought that the usage of painkillers by most of the included patients may have affected the results.

Gül et al. evaluated self-care agency in patients who underwent kidney transplantation, and the mean SCA score of their patients was 108.9±20.1 (36) In their study which included hypertensive patients, Türkcan Düzöz reported a mean SCA score of 100.04±17.62 (37). In a study conducted by Üstündağ and Zengin to determine the self-care agency of patients with head and neck cancers, 38% of the patients were found to have a moderate SCA score of 80.88±11.51, and 62% were found to have a high score of 107.51±12.05 (38). In this study, the mean total ESCA score of the patients was determined as 79.37±21.55. Similar to the literature, the self-care agency of the patients who experienced PP was found to be moderate.

In the study conducted by Üstündağ and Zengin, in which patients with head and neck cancers were included, no significant difference was observed between age groups in terms of their mean scores of self-care agency (38). Türkcan Düzöz stated that the highest self-care agency score was in the group aged 40 and below, and the lowest score was in the group aged 61 and above (37). Similarly, no significant relationship was found between age groups and self-care agency in this study.

Üstündağ and Zengin examined the distribution of the scale scores of patients according to sex and determined that the SCA scores of the male patients (102.62±17.38) were higher than

those of the female patients (89.86±15.41), and the difference between their mean scores was statistically significant (38). The lack of a statistically significant difference between ESCA scores according to sex in this study could have been due to the heterogeneity of the male-female distribution.

It was reported by Üstündağ and Zengin that the self-care agency scores of patients increased as their education levels increased, and this relationship was statistically significant (38). Türkcan Düzöz observed that as the education levels of patients increased, their self-care agency scores also increased (37). In the study conducted by Alemdar and Çınar Pakyüz with hemodialysis patients, it was determined that the self-care agency scores of the patients differed significantly based on their educational status (39). It can be stated that the lack of a significant relationship between education levels and self-care agency in this study was due to the fact that the study was conducted with a small number of people, which was also one of the limitations of the study.

Yılmaz et al. determined in their study on self-efficacy regarding bowel preparation before colonoscopy that the general perceived self-efficacy levels of the patients were moderate, and there was no statistically significant relationship between scale scores and compliance with the pre-colonoscopy preparation instructions (40). In different study, it has been reported that high self-efficacy levels increase disease adjustment (41). In this study, the mean total SES score of the patients was determined as 70.00±16.10. The fact that there was no significant relationship between self-efficacy levels and PP scores suggested that the result on the evaluated variable was due to a chronic condition.

Vatansever and Ünsar reported that there was no statistically significant difference between male and female patients with essential hypertension in terms of their self-efficacy levels, and there was also no significant difference in medication adherence scores based on the educational statuses of the patients (41).

In the study conducted by Karasawa et al. with disabled patients who had chronic pain, chronic pain and self-efficacy scores were found to be independent of the severity of pain, but they had statistically significant relationships to a decrease in self-efficacy scores and disability status (42). These findings provide an idea about the importance of relieving chronic pain, strengthening self-efficacy, and promoting the sense of independence in the individual. The absence of a significant relationship between self-efficacy levels and PP in this study can be interpreted as that experiencing phantom limb pain does not have an effect on self-efficacy.

Studies on self-efficacy have shown that patients with high self-efficacy recover in a shorter time, and their quality-of-life increases (40-42). PP is also a form of chronic pain. It is a serious problem that is frequently seen, especially after amputation, and it hinders the daily activities of individuals. Since there is no other study evaluating PP in addition to self-care agency and self-efficacy in the literature, no generalization can be made about the self-care agency and self-efficacy levels of individuals who experience PP.

Tsay and Healstead and Song et al. stated that patients with high self-care agency also had high self-efficacy, and there was a positive relationship between these two variables (43,44). In their study that included hemodialysis patients, Lew and Owen reported that positive relationships between self-efficacy, self-care, and quality of life (45). In this study, a positive, statistically significant, and moderate correlation was determined between the mean total ESCA scores of the patients and their score on the maintaining the behavior subscale, which is a dimension of SES (r= .602; p= .001; p< .01). The findings of this study were compatible with the information in the literature.

5. CONCLUSION

In this descriptive study, it was determined that patients who experienced PP after amputation had moderate self-care agency and perceived self-efficacy levels. Since generalization cannot be made in line with the results of this study, it is recommended to conduct comparative and experimental studies with larger samples and in different cultures to examine the self-care agency and self-efficacy of patients who experience PP.

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Author Contributions:

Research idea: HBK, NAK
Design of the study: HBK, NAK
Acquisition of data for the study: HBK, NAR, SG
Analysis of data for the study: HBK, NAK, NAR, SG
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Clinical and Experimental Health Sciences

Nicotine Use Frequency and Addiction among Medical Students

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ABSTRACT

Objective: Evaluating the rate of smoking and factors associated with nicotine addiction in university students will provide target-oriented interventions for students. For this reason, this study aims to measure the frequency of nicotine use and nicotine dependence and related factors among medical students.

Methods: In this cross-sectional study, an online questionnaire about nicotine use frequency and dependence was sent to a state university's medical students via mobile phones. No sample size was calculated, instead, the study aimed to reach a total of 1522 medical students. The Fagerström Nicotine Dependence Test was used to measure nicotine dependence levels. $p < .05$ was considered as statistically significance level.

Results: Of the students, 79.3% (n=306) were non-smokers, 14.7% (n=57) were smokers and 6.0% (n=23) of the students were ex-smokers. Male students, students not living with their families, students with a higher income, and those having at least one smoking parent had statistically significantly higher smoking rates *($p < .05$)*. According to the Fagerström Test, of the smoking students, 63.2% (n=36) had low nicotine dependency, 33.3% (n=19) had moderate nicotine dependency, and 3.5% (n=2) showed high nicotine dependency.

Conclusion: Smoking rates were high in medical students, despite the medical knowledge related the negative health outcomes. And students had high level of nicotine dependence. Further studies are needed about the strategies on the prevention of smoking among medical students.

Keywords: Medical students, smokers, nicotine use, nicotine dependence, The Fagerström Nicotine Dependence Test

1. INTRODUCTION

Nicotine dependence which is one of the major addiction problems, is experienced worldwide. It is important to study and understand the frequency of nicotine usage and addiction as according to Benowitz (2010) smoking is the leading cause of preventable death (1). Tobacco use causes more than 7 million deaths per year and the chance that a lifelong smoker will die prematurely from a complication of smoking is approximately 50% (1).

According to the World Health Organization (WHO), the tobacco epidemic is one of the biggest public health threats the world has ever faced (2). Investigation of the smoking prevalence is important to fight the epidemic of smoking. Special populations like students should be also concerned in epidemiological studies. Because it is important to know the challenges before taking action against tobacco usage.

According to the results of the National Household Health Survey 2017 study, the percentage of tobacco use in Turkey was found to be 43.6% for males, and 19.7% for females, and 31.6% for the whole population (3). In the same study, the percentage of current smokers between ages 15-29 years was 44.6% for males, 15.9% for females, and 30.6% for both sexes (3). A study conducted on university students of two different faculties in Turkey (social sciences and health sciences) states that 20.6 % of the students are smoking. In the same study students of health sciences faculties smoke less than social sciences' students (4).

Nicotine dependence can be associated with many factors. A cross-sectional study conducted in Jordan among university students from 3 public and 3 private Jordanian universities states that of the 892 participants, 51.2% were nicotine dependent. Students who started smoking before 18 years of age, had a smoking family member and were studying at governmental universities had higher nicotine dependence levels (5). Another study, a study regarding socio-demographic factors that affect the use of tobacco in 13 low

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and middle-income countries, including Turkey, found that a positive association between the prevalence of tobacco use and education level in Turkey exists (6). According to a recent study conducted on university students in Turkey, 31.4% of the students were moderately and 13.4% were highly dependent on nicotine (7).

Even though nicotine usage has harmful effects on health and well-being, it is still commonly seen among university students. Evaluating the rate of smoking and factors associated with nicotine addiction in university students will provide target-oriented interventions for students. For this reason, this study aims to measure the frequency of nicotine use and nicotine dependence and related factors among medical students.

2. METHODS

2.1. Study Design and Students

The study was conducted from February to May 2021. In this cross-sectional type of study, an online questionnaire prepared using Google Docs about nicotine use frequency and dependence was sent to medical students via WhatsApp application. The study population for this study was a state university's medical students of the academic year 2020-2021. Total number of medical students in this university was 1552 for the 2020-2021 academic year. No sample size was calculated; instead, the study aimed to reach a total of 1522 medical students: 290 1st year students, 255 2nd year students, 236 3rd year students, 265 4th year, 261 5th year, and 216 6th year. Before the students were given access to the questions, informed consent was taken. Students checked the "I have read and agree to the terms of this questionnaire" button. No identity information was requested from the students and the questionnaires were filled anonymously.

2.2. Instruments

The questionnaire consisted of 21 questions: 6 demographic questions, 6 questions from the Fagerström test for nicotine dependence, and 9 questions related to smoking prevalence among students.

The Fagerström Nicotine Dependence Test (FTND) is a standard international tool used to assess the intensity of physical nicotine dependence. It is reliable and validated for the Turkish population (8). FTND provides a measure that evaluates cigarette consumption and nicotine dependence. Each item of the six-item scale is scored between 0 and 3 points, and the range of points that can be obtained from the scale varies between 0 and 10. According to the total scores obtained from the scale, nicotine dependence is graded in three groups low (0-3 points), moderate (4-6 points), and high (7-10 points) (9).

2.3. Statistical Methods

The analysis of the data was done with SPSS (Statistical Package for Social Sciences) for Windows 25.0 program. Descriptive data were presented with median, interquartile range (IQR), number (n) and frequency (%). In the analysis of the data, the Chi-square test was used for categorical variables. The Mann-Whitney U test was used for comparison of continuous variables that did not fit the normal distribution. $p< .05$ was considered as statistically significance level.

2.4. Ethical Concerns

In order to conduct the research, institutional permission was obtained from the university where the study was conducted. In addition, ethics committee approval was obtained from the ethics committee of the studied university (Date: 05.02.2021, decision number= 09.2021.213). After providing information about the study, informed consent was obtained from the students.

3. RESULTS

The total number of students was 386. The median age of the students was 21 years (IQR=2). The median age of onset of smoking was 17 years (IQR=2).

Of the students, 60.9% (n=235) were females, 39.1% (n=151) were males. The majority of students who answered the questionnaire were in the preclinical phase. Most of the students were 2nd year students 28.5% (n=110). Of the students, 67.4% (n=260) lived with their families. Most of the students, 58.0% (n=224), had a monthly income of more than 1000 TL (Table 1).

Table 1. *Sociodemographic features of the students*

Characteristic	n	%
Total	386	100.0
Gender		
Male	151	39.1
Female	235	60.9
Year of education		
1st	86	22.3
2nd	110	28.5
3rd	88	22.8
4th	36	9.3
5th	28	7.3
6th	38	9.8
Place of living		
With family	260	67.4
At a dormitory	26	6.7
Apartment with friends	46	11.9
Apartment alone	54	14.0
Monthly Income		
More than 500 TL	27	7.0
500-1000 TL	135	35.0
More than 1000 TL	224	58.0

Of the students, 79.3% (n=306) were nonsmokers, 14.7% (n=57) were smokers and 6.0% (n=23) of the students were ex-smokers. 64.5% (n=249) of the students' parents were

both non-smokers. 22.3% (n=86) of students indicated that only their father smokes, and 5.2% (n=20) indicated that only their mother smokes. 8.0% (n=31) of the students' parents were both smokers (Table 2). Of the smokers, 59.6% (n=34) smoke less than 10 cigarettes a day, 28.1% (n=16) 11-20 cigarettes per day, 12.3% (n=7) 21-30 cigarettes per day.

Table 2. *Smoking frequency and parental smoking*

Characteristic	n	%
Smoking Status		
Yes	57	14.7
No	306	79.3
Ex-Smoker	23	6.0
Parental Smoking Status		
Both Parents Smoke	31	8.0
Only Mother Smokes	20	5.2
Only Father Smokes	86	22.3
Neither Parent Smokes	249	64.5

Male students, students not living with their families, students with a higher income and having at least one smoking parent had statistically significantly higher smoking rates. There was no significant relationship between smoking and the class of students (p= .760). While 23.8% (n=36) of male students were smoking, 8.9% (n=21) of female students were smokers (p< .001). While 11.5% (n=30) of those living with their family were smoking, 21.4% (n=27) of those not living with their family were smokers (p= .010). 18.8% (n=42) of those with high incomes and 9.3% (n=15) of those with low income were smokers (p= .009). Smoking percentage was 20.4% (n=28) among students having smoking parents and 11.6% (n=29) among students whose parents were not smoking (p= .020) (Table 3).

Table 3. *Smoking and related factors*

	Smoking		Total n (%)	*p* value
	Yes n (%)	No-Quitted n (%)		
Gender				
Male	36 (23.8)	115 (76.2)	151 (100.0)	< .001
Female	21 (8.9)	214 (91.1)	235 (100.0)	
Class				
1-2-3	41 (14.4)	243 (85.6)	284 (100.0)	.760
4-5-6	16 (15.7)	86 (84.3)	102 (100.0)	
Living With Family				
With Family	30 (11.5)	230 (88.5)	260 (100.0)	.010
Other	27 (21.4)	99 (78.6)	126 (100.0)	
Income				
Low (<1000 TL)	15 (9.3)	147 (90.7)	162 (100.0)	.009
High (>1000 TL)	42 (18.8)	182 (81.3)	224 (100.0)	
Parental Smoking				
At least one of them smokes	28 (20.4)	109 (79.6)	137 (100.0)	.020
Neither of them smokes	29 (11.6)	220 (88.4)	249 (100.0)	

Of the smoking students %63.2 (n=36) were in the low-dependency group, 33.3% (n=19) were in moderate-dependency group, and 3.5% (n=2) were in high dependency group on smoking. When the effect of gender on nicotine dependence was examined, 66.7% (n=24) of males showed low nicotine dependence, whereas 57.1% (n=12) of females showed low nicotine dependence. No statistically significant relationship was found between gender and nicotine dependency levels (p= .472). Of the clinical phase students 68.8% (n=11) showed moderate-high dependence on nicotine, whereas this percentage was lower in preclinical phase students as 24.4% (n=10). Clinical phase students had a significantly higher percentage of moderate-high dependence on nicotine when compared with the preclinical phase students (p= .002). Of the students living with their families 76.7% (n=23) showed low nicotine dependence, whereas other smoking students who didn't live with a family 49.1% (n=13) showed low dependence. Low dependence levels were statistically higher in students living with their families (p=0.026). The percentage of moderate-high dependence levels of the students with lower income was 13.3% (n=2), whereas 36.8% (n=19) of the students with higher income had moderate-high dependence levels. Moderate-high dependence was significantly higher in students with high income (p= .028). Of the students having at least one smoking parent, 50.0% (n=14) of them had moderate-high dependence. On the other hand, 24.1% (n=7) of the students whose parents did not smoke showed moderate-high dependence. Moderate-high dependence was significantly higher in students with smoking parents (p= .043) (Table 4).

Table 4. *Dependency levels and related factors*

	Dependency Level		Total n (%)	*p* value
	Low n (%)	Moderate-High n (%)		
Gender				
Male	24 (66.7)	12 (33.3)	36 (100.0)	.472
Female	12 (57.1)	9 (42.9)	21 (100.0)	
Class				
1-2-3	31 (75.6)	10 (24.4)	41 (100.0)	.002
4-5-6	5 (31.3)	11 (68.8)	16 (100.0)	
Living With Family				
With Family	23 (76.7)	7 (23.3)	30 (100.0)	.026
Other	13 (49.1)	14 (51.9)	27 (100.0)	
Income				
Low (<1000 TL)	13 (86.7)	2 (13.3)	15 (100.0)	.028
High (>1000 TL)	23 (63.2)	19 (36.8)	42 (100.0)	
Parental Smoking				
At least one of them smokes	14 (50.0)	14 (50.0)	28 (100.0)	.043
Neither of them smokes	22 (63.2)	7 (24.1)	29 (100.0)	

The median age of low-dependency smokers was calculated to be 21.0 (3.0) and for moderate-high dependency levels 22.0 (4.5). The relationship between age and dependency

levels does not appear to be statistically significant (p= .172). The median age was 18.0 (2.0) for low-dependency smokers to start smoking and 17.0 (2.0) for moderate to high-dependency smokers. There was no statistically significant relationship between the beginning age of smoking and dependency levels (*p*= .917).

Of the students who were smokers 73.7% (n=42) had tried to quit smoking previously. 26.3% (n=15) never tried to quit smoking. Of the smoking students 87.7% (n=50) were thinking about quitting in the future. On the other hand, 12.3% (n=7) of smokers were not thinking about quitting.

4. DISCUSSION

Smoking remains the leading cause of preventable death worldwide. For this reason, investigating the prevalence and dependency levels and the relationship between nicotine dependence and social factors is important to understand how to implement effective smoking-cessation methods.

It is assumed that smoking rates will decrease if awareness regarding the dangers of consuming nicotine can be increased in society. The medical faculty students act as role models as they are a scientifically literate part of the public, in the sense that they are educated on the dangers of smoking tobacco. According to a recent study with 8045 respondents, the rate of tobacco consumption in Turkey is approximately 33.0% (10). Approximately 15.0% of medical students were smokers in our study. This percentage is similar to other studies conducted among university students of health science-related faculties (17.0%-20.0%) (11-14). However, according to a recent study, smoking rates are higher among university students of arts, social sciences, business administration, engineering, and agriculture faculties (smoking rates are between 19.6%-45.0%) (15). These results could be explained by the fact that medical students are expected to have increased awareness regarding the risk of smoking and nicotine consumption, hence smoking prevalence is lower among medical students relative to other university students.

The percentage of male and female smokers in our study was 23.8% and 8.9%, respectively. These findings were mirrored in other medical faculties in Turkey according to Cooper et al. (13) and health profession students in Europe and the Americas as per Sreeramareddy et al. (14). Bozkurt et al. conducted a study about the pattern of smoking behavior and associated factors affecting smoking use in the South-East Anatolian region of Turkey. The findings of the study, concerning gender, indicated that smoking is almost five times more frequent in males compared to females (16). The differences in the frequency between male and female cigarette smoking could stem from cultural and behavioral differences (17).

Although male smoking rates exceed those of females, 42.9% of female smokers exhibited a moderate to high dependency level, in contrast to males, where the moderate to high dependency level was 33.3%. This could be explained by the fact that females experience more stress than males, as is shown in the studies (18, 19). Higher stress levels could explain the increased dependence on nicotine which is seen in females. Dependency levels according to which phase the students were in (preclinical vs. clinical) showed a statistically significant relationship. Moderate-high level of dependency rate of clinical phase students was significantly higher than preclinical students (68.8% vs 24.4%). According to the literature, psychosocial stress is a significant risk factor for cigarette smoking (20). It is reasonable to draw the same conclusion about dependency levels and the increased perceived stress of clinical years of medical school. As indicated in a similar study, medical students may have anxiety due to challenges in clinical practice and lack of experience in performing academic tasks which is associated with the transition to the clinical phase (21).

Our study shows a statistically significant relationship between higher income levels and higher dependency levels, as the percentage of those with higher income who had moderate to high nicotine dependency was 36.8% compared to 13.3% of people with lower income. These results were not consistent with the results of the Chen et al. study, which suggests an association of higher scores on the Fagerström Test for Nicotine Dependence with lower income and job status (22). The possible reason behind these findings could be that higher income means that the students can financially support the smoking habit, as higher cigarette prices were inversely proportional to adult smoking levels in low – and middle-income countries as was shown by the Kostova et al. study that included Turkey as one of the participating countries (23).

The results of our study also show that smokers who had smoking parents were more likely (50.0% vs 24.1%) to show moderate-high dependence levels. It is suggested that parental smoking is a strong predictor that a child will become a smoker in the future (24). Moreover, these smokers likely picked up the habit from social circles such as having smoker family members or friends, since a strong driver to initiation of smoking is close friends and siblings or vulnerability to peer pressure, especially during the ages of 13-17 years as suggested by the studies (25, 26). The low nicotine dependence scores for students who had non-smoker parents also suggest that they are likely capable of dropping the habit more easily compared to those with smoker parents, as they are not as exposed to the habit or second-hand smoke in their home environments, as is concluded by a study (27).

4.1. Limitations and the Strengths of the Study

This study had several limitations. First, since the questionnaire was administered online, participation must have been affected. This is particularly seen in clinical students' return rate which was lower than that of preclinical students. Students who do not have smartphones or who do not use WhatsApp could not be reached. This can cause a biased sampling of our study. Moreover, reporting bias is also possible since the questionnaire was self-administered. Furthermore, while the honesty of the participant could

be called into question, the fact that it was submitted anonymously means there was no fear of reprisal and thus may be no reason to hide the truth. Since our participation rate was not very high, the cross-sectional nature of the study is limited in terms of representing the population.

The study contributes to the literature, giving data on the frequency of smoking and evaluating nicotine dependence with the Fagerström Nicotine Dependence Test, which is a valid measurement tool. The results of the study provide additional data on the frequency of smoking among medical students in Turkey.

5. CONCLUSION

Smoking rates were high among medical students, despite the medical knowledge related to the negative health outcomes. And students had high levels of nicotine dependence. Further studies are needed about the strategies for the prevention of smoking among university students. Prevention programs and campaigns targeting university students should be arranged and available to the students. Provided with the right guidance and tools, medical students can be equipped to permanently quit and aid in the quitting of their patients in the future.

There is a need for qualitative studies to be conducted in this area in order to understand the reasons that lead medical school students to smoke, the factors that may be related to nicotine addiction, and the barriers to quitting. There is a need for educational updates in the curriculum of the students that will draw attention to the harmful effects of smoking. Students should be informed about the practices that support smoking cessation, such as smoking cessation polyclinics. Implementing practices that prevent smoking, such as restricting smoking areas within the faculty, are among the practices that can reduce smoking rates among students. In faculties, psychological counselling should be planned especially for students with high nicotine dependence. In summary, we think that effective smoking-cessation methods among medical students can be implemented with the help of ongoing psychological, environmental, and educational support.

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Clinical and Experimental Health Sciences

Investigation of The Incidence and Risk Factors of Falls, An Undesirable Incident in Hospitals: A Retrospective Study of Eight Years

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ABSTRACT

Objective: Falls are undesirable incidents that must be reported in hospitals. Duration of hospital stay and cost of treatment may increase due to post-fall injuries. Therefore, it is very important to investigate the incidence and risk factors of falls to prevent them. The aim of the study was to investigate the incidence and risk factors of falls.

Methods: The retrospective descriptive study was conducted in one of the largest medical faculty hospitals located in the western of Türkiye. The study data of 160,119 cases of falls between 2012 and 2019 were examined retrospectively using a "Socio-demographic Information Form" and "HARIZMI" and "ITAKI" Fall Risk Scales.

Results: Statistically significant differences were found between fall rates by year ($p < .05$). It was detected that the risk of falls in adults was 2.3 times higher than in children (95% CI: 1.86-2.89); the risk of falls in men was 1.2 times higher than in women (95% CI: 1.04-1.43), and the risk of falls in internal medicine units was 4.2 times higher than in surgical units (95% CI: 3.57-5.06).

Conclusion: The falls mostly occurred in internal medicine units. The risk of falls was higher in internal medicine units than in surgical units. The risk of falls was higher in adults than in children and men than in women. Necessary precautions should be taken for adult ve men patients who are at risk of falls. Institutional procedures and flowcharts should be established to rapidly evaluate cases of falls and make the necessary interventions. Warning signs should be placed in the rooms of patients who are at risk of falling.

Keywords: Care quality, fall, fall incidence, fall risk, nursing, patient safety.

1. INTRODUCTION

A fall is defined as "an event that results in a person coming to rest inadvertently on the ground or floor or other lower levels" by the World Health Organization (WHO). Globally, falls are a major problem of public health. Fall injuries can be fatal or often non-fatal. An estimated 684,000 fatal falls are known to occur each year and falls are counted as the second leading cause of unintentional injury deaths, after road traffic injuries (1). Approximately 30-50% of falls harm patients in some way and the most severe cases can result in death (2). Falls in hospital environments cause worsening in patients' clinical conditions, prolonging hospital stay due to patients' limitations and physical disabilities, increased cost of treatment, and ethical and legal problems for the institution (2,3).

More than 80% of deaths due to falls occur in middle or low-income countries. Sixty percent of these deaths occur in the West Pacific and South East Asia. All over the world, fall-related death rates are highest among adults aged over 60 years (1). The financial burdens resulting from fall injuries are significant. The average healthcare cost per fall injury for people aged 65 years or older in the Republic of Finland and Australia is 3611 USD and 1049 USD, respectively (1). Baris et al. (4) calculated the additional hospital cost and labor loss in Turkey caused by severe fall injuries as 3,303,60 USD and 14.61 days. Therefore, the prevention of falls is a worldwide concern and also one of WHO's international patient safety goals. Countries around the world have started prevention programs to decrease the risk of patient harm caused by falls (2). A fall is an indicator of nursing care and preventing them is one of the primary responsibilities of nurses.

Inpatient falls are among the most common undesirable incidents in hospitals that must be reported (3,5). Causes of falls are categorized into two groups related to internal and external factors (3,6). Internal factors include a history of falls,

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agitation, stroke, neurologic-cardiac problems, problems in walking and movement, and urinary incontinence. External factors include an increase in urinary frequency, not removing bed frames, insufficient lighting, wet or slippery floors, and difficult access to the call bell (7).

The presence of additional chronic diseases along with falls leads to serious problems due to being dependent on others for physical activities, especially at older ages. The incidence of falls in hospitals worldwide is reported to be approximately 2-1.7% per patient/day (7).

Identifying the incidence and risk factors of falls, which cause severe harm in hospitalized patients, is significantly important to shorten hospitalization times by eliminating the risk of falls, decreasing the cost of fall-related treatments, and reducing labor loss and the workload of healthcare professionals. Based on these facts, answers to the following questions were sought in the study:

- What is the distribution of the number of falls according to demographic characteristics, ward, year and month of hospitalization?
- What are the characteristics related to fall?
- What are the characteristics related to the time of the fall and the practices performed after the fall?
- What are the risk factors that affect falls?

2. METHODS

2.1. Ethical Considerations

The Human Rights Declaration of Helsinki was abided by throughout the study. Written ethical board consent was obtained from İstanbul University İstanbul Faculty of Medicine Ethics Committee (permission date and no. 18/12/2020-31).

2.2. Design and Aim of The Study

This retrospective, descriptive study was conducted to investigate the incidence and risk factors of falls.

2.3. Population and Sample

The study was conducted in one of the largest medical faculty hospitals located in the western Turkey. The study was conducted with 160,119 patients who fell during hospitalization in clinics between 2012 and 2019.

2.4. Measures

In the hospital where the research was conducted, all patients admitted to inpatient units are evaluated for the risk of falls, with no exception. For evaluating the risk of falls, the HARIZMI Fall Risk Scale was used for the patients aged 0-16 years, and the ITAKI Fall Risk Scale was used for patients aged over 16 years. A socio-demographic information form, and the HARIZMI and ITAKI Fall Risk Scales were used for data collection.

2.4.1. Socio-demographic and Fall-related Information Form: This form investigates socio-demographic information such as age, sex, inpatient clinic, fall risk score, fall status, number of falls, the time between falling and being admitted to the clinic, time of fall, day of fall, site of fall, place/moment of fall, type of fall, mental health status before fall, presence of a companion during the fall, interventions after the fall, use of drugs that increase fall risk, fall risk evaluation, and fall-related information such as implementation of fall protocols, being in the fall protocol, presence of a healthcare professional at the time of the fall, and whether the fall occurred when the room was lit.

2.4.2. HARIZMI Fall Risk Scale: Thescale was developed by the Ministry of Health in Turkey (8). It consists of nine items questioning the risk of falls (neurologic diseases/symptoms, change in oxygenation, specific disease/symptom in terms of fall risk, being in a suitable bed, visual impairment, equipment connected to the patient, need for physical support while walking/standing, being in the post-op period and use of risky drugs). Two risk levels, low and high, were determined over the total score obtained by evaluating the risk factors. If the total score was below 15, the fall risk of the patient was considered low, and if the total score was 15 and above, the fall risk of the patient is considered high (9). The scale is used for the assessment of fall risk in pediatric patients.

2.4.3. ITAKI Fall Risk Scale: The scale was developed by the Ministry of Health in Turkey (8). It consists of 19 risk factors that can cause patients to fall. Risk factors are categorized as minor and major; minor risk factors are given 1 point and major risk factors are given 5 points. There are a total of 11 minor and eight major risk factors. Two risk levels, low and high, were determined over the total score obtained by evaluating the risk factors. If the total score is below 5, the fall risk of the patient is considered low, and if the total score is 5, the fall risk of the patient is considered high (10). The scale is used for the assessment of fall risk in adult patients.

2.5. Data Collection

In the hospital where the research was conducted, following the risk evaluation, all the adult patients with an ITAKI fall risk score of 5 and above and all the pediatric patients with a HARIZMI fall risk score of 15 and above are accepted as at risk of fall. Fall risk is evaluated in four different situations (the postoperative period, when the patient falls, in case of a change of unit, and a change in the patient's conditions). Therefore, patients are evaluated for each admission to the hospital and the changes are recorded. Data related to the patients are sent to the Department of Nursing Services at the end of each month and analyses are performed by the department. The research was conducted using data

obtained from the patients accepted as at-risk and patients who were not at-risk at admission but fell nonetheless. The data of the research were obtained from the data sent to the Department of Nursing Services.

2.6. Data Analyses

The NCSS (Number Cruncher Statistical System) 2007 (Kaysville, Utah, USA) program was used for statistical analyses. Descriptive statistical methods (average, standard deviation, median, frequency, rate, minimum, maximum) were used for evaluating the study data. Pearson's Chi-square test and the Fisher-Freeman-Halton test were used for the comparison of qualitative data. Logistic regression analysis was used to investigate the risk factors that affect falls. The significance level was accepted as $p < .05$.

3. RESULTS

The results of the research were investigated under four headings:

3.1. Distribution of The Number of Falls According to Demographic Characteristics, Ward, Year and Month of Hospitalization

Accordingly, no statistically significant differences were found between patients who fell once or more than twice in terms of age groups, sex, hospitalization unit, ward unit, and the month in which the fall occurred ($p > .05$). However, a statistically significant difference was detected between patients who fell in terms of the years in which the falls occurred ($p < .05$). When the fall rates were compared by years, statistically significant differences were found between fall rates by years ($p < .05$). It was determined that there was a decrease in falls in the period from 2012 to 2019 (Table 1).

When the distribution of the fall rates according to the department and ward unit was investigated, it was determined that falls mostly occurred in internal medicine units (Table 1).

3.2. Distribution of Characteristics Related to Falls

It was detected that 0.04% (n=616) of the patients included in the assessment fell and the average number of falls was 1.12±0.57 (Min=1, Max=6). It was also detected that the majority of the falls occurred between 00:00-08:00 (n=234, 38%) and within working hours during weekdays (n=447, 72.6%), in patient rooms (n=384, 62.3%), while the patients were on the movement (n=351, 57.0%), and in the form of fainting/collapsing (n=258, 41.9%) (Table 2).

The average fall risk score of patients who fell was 13.14±6.77 (Min=0, Max=45). The mental status of the majority of the patients who fell was oriented/cooperate (n=514, 83.4%), 64.9% (n=400) of the patients did not have a companion at the time of the fall, 73.5% (n=453) were given medical attention following the fall, and 56.8% (n=350) of the patients did not use drugs that increase the risk of falling (Table 2).

Table 1. *Distribution of the number of falls according to demographic characteristics, ward, year and month of hospitalization*

Variables		1 fall (n=571)	≥2 falls (n=45)	%
Age group 1	Newborn	2 (100)	0 (0)	[b].526
	1 month-1 year	22 (100)	0 (0)	
	1-5 years	51 (92.7)	4 (7.3)	
	6-10 years	14 (100)	0 (0)	
	11-16 years	8 (100)	0 (0)	
	17-25 years	21 (95.5)	1 (4.5)	
	26-35 years	33 (100)	0 (0)	
	36-45 years	44 (95.7)	2 (4.3)	
	46-55 years	68 (88.3)	9 (11.7)	
	56-65 years	114 (92.7)	9 (7.3)	
	66 years and above	194 (90.7)	20 (9.3)	
Age group 2	Newborn	2 (100)	0 (0)	[b].317
	Child	95 (96.0)	4 (4.0)	
	Adult	474 (92.0)	41 (8.0)	
Sex	Female	246 (95.0)	13 (5.0)	[a].063
	Male	325 (91.0)	32 (9.0)	
Unit	Internal	393 (92.7)	31 (7.3)	[a].993
	Surgical	178 (92.7)	14 (7.3)	
Ward-Unit	Emergency	33 (97.1)	1 (2.9)	[b].529
	Intensive care	27 (90.0)	3 (10.0)	
	Ward	511 (92.6)	41 (7.4)	
Year of hospitalization	2012	94 (92.2)	8 (7.8)	[b].006**
	2013	85 (89.5)	10 (10.5)	
	2014	97 (100)	0 (0)	
	2015	72 (88.9)	9 (11.1)	
	2016	65 (91.5)	6 (8.5)	
	2017	58 (87.9)	8 (12.1)	
	2018	60 (96.8)	2 (3.2)	
	2019	40 (95.2)	2 (4.8)	
Month of hospitalization	January	58 (96.7)	2 (3.3)	[b].729
	February	48 (90.6)	5 (9.4)	
	March	61 (95.3)	3 (4.7)	
	April	57 (91.9)	5 (8.1)	
	May	45 (93.8)	3 (6.3)	
	June	46 (92.0)	4 (8.0)	
	July	42 (97.7)	1 (2.3)	
	August	41 (89.1)	5 (10.9)	
	September	39 (92.9)	3 (7.1)	
	October	49 (94.2)	3 (5.8)	
	November	42 (89.4)	5 (10.6)	
	December	43 (87.8)	6 (12.2)	

[a]*Pearson Chi-Square Test* [b]*Fisher-Freeman-Halton Test*

Table 2. *Distribution of characteristics related to falls (N=160.119)*

		n	%
Status of fall	Yes	616	0.4
	No	159,503	99.6
Number of falls (n=616)	*Min-Max (Median)*	1-6 (1)	
	Avg±SD	1.12±0.57	
	1 time	571	92.7
	≥2 times	45	7.3
Time between fall and hospitalization (days) (n=616)	*Min-Max (Median)*	1-240 (8)	
	Avg±SD	14.24±20.87	
Time of fall (n=616)	08:00-16:00	196	31.8
	16:00-24:00	186	30.2
	00:00-08:00	234	38.0
Day of fall (n=616)	Weekday	447	72.6
	Weekend/Public Holiday	169	27.4
Area of fall (n=616)	Patient room	384	62.3
	Hallway	56	9.1
	Bathroom/Toilet	137	22.3
	On the way to surgery	11	1.8
	Place of surgery	26	4.2
	Intensive care	2	0.3
Place/moment of fall (n=616)	From bed/stretcher/table	220	35.7
	While on the move	351	57.0
	During replacement/ transfer	45	7.3
Type of fall (n=616)	Slipping	50	8.1
	Tripping	40	6.5
	Fainting/Collapsing	258	41.9
	Mistakes during transfer	50	8.1
	Falling from high	218	35.4
Fall risk score (n=616)	*Min-Max (Median)*	0-45 (12)	
	Avg±SD	13.14±6.77	
Mental status before fall (n=616)	Oriented/Cooperative	514	83.4
	Disoriented	56	9.1
	Confused	46	7.5
Presence of an accompanist at the moment of fall (n=616)	Yes	216	35.1
	No	400	64.9
Intervention after fall (n=616)	Yes	453	73.5
	No	163	26.5
Use of medication that increase the risk of fall (n=616)	Yes	266	43.2
	No	350	56.8

**Total number of fall cases reported*

3.3. Characteristics Related to The Time of The Fall and Distribution of The Practices Performed After The Fall

The distribution of the characteristics regarding fall risk, the moment of fall, and surgeries performed following the fall and its consequences are given in Table 3. According to Table 3, 95.5% (n=588) of the patients did not have a healthcare professional with them at the time of the fall, 84.7% (n=522) had the lights turned off in their room, and the floor was not wet or slippery in 98.2% (n=605) of the patients' rooms. Radiologic procedures were performed following 38.5% (n=237) of the falls, 3.2% (n=20) of the patients underwent blood tests, 47.6% (n=293) were monitored for vital signs, 7.1% (n=44) were asked for consultation after falls, and 50.5% (n=53) had minor injuries due to the fall (Table 3).

Table 3. *Characteristics related to the time of the fall and distribution of the practices performed after the fall (N=616)*

Variables		n	%
Fall risk assessment	Yes	475	77.1
	No	141	22.9
Fall protocol application	Yes	220	35.7
	No	396	64.3
Characteristics of patients at the time of fall			
Presence of a healthcare professional	Yes	28	4.5
	No	588	95.5
Lights being turned on in the room	Yes	94	15.3
	No	522	84.7
Wet or slippery room floors	Yes	11	1.8
	No	605	98.2
Characteristics of the applications performed after falls			
Radiological operations	Yes	237	38.5
	No	379	61.5
Blood test	Yes	20	3.2
	No	596	96.8
Vital sign monitoring	Yes	293	47.6
	No	323	52.4
Consultation	Yes	44	7.1
	No	572	92.9
Fall-related problems	Yes	105	17.0
	No	511	83.0
Level of problem*	Severe damages	36	34.3
	Intermediate damages	16	15.2
	Minor damages	53	50.5

**Calculations were made on 105 patients who developed fall-related problems.*

3.4. Logistic Regression Analysis Results of The Risk Factors Affecting Falls

Among the risk factors affecting falls, department, age group (2), and sex were evaluated using enter logistic regression analysis. The explanatory coefficient of the model was found to be 99.6%, which indicates a good level. According to the model, unit, age, and sex had significant effects on falls. Fall risk was 4.2 times higher in internal medicine units than in surgical units (95% CI: 3.6-5.1). Fall risk in adults was 2.3 times higher than in children (95% CI: 1.9-2.9). Fall risk was 1.2 times higher in males than in females (95% CI: 1.04-1.43) (Table 4).

Tablo 4. *Logistic regression analysis of risk factors affecting falls*

	%	ODDS RATIO	95% CI	
			Lower	Upper
Unit (Internal)	.001**	4.251	3.569	5.064
Age group (Child)	.001**			
Age group (Newborn)	.352	1.948	0.478	7.935
Age group (Adult)	.001**	2.322	1.862	2.895
Sex (Male)	.015*	1.220	1.039	1.432

**p< .05 **p< .01*

4. DISCUSSION

In the study, falls, which are one of the quality indicators and undesirable events that must be reported in inpatient treatment institutions, were retrospectively analyzed. Among a total of 160,119 patients who were hospitalized between 2012 and 2019, a total of 616 patients were found to have fallen. In another study conducted in Brazil, the incidence of falls between 2011 and 2015 was found as 1.7 per 1000 patients per day (11).

In the study, it was found that most of the falls occurred in female patients, patients aged over 66 years, and in internal medicine units. Similar results were obtained in another study (7). In a study conducted by Lee et al. (12), it was found that in a risk group of 2227 patients with an average age of 61±15.04 years, 309 of them fell. It is stated that fall risk increases with age due to comorbidities (7,13). However, another study stated that demographic variables such as age and sex were not risk factors for falls (14). It has also been found that the majority of falls occur in emergency units and psychiatric clinics (11).

By investigating cases of falls in our study by year, a significant decrease was observed in the number of falls between 2012 and 2019. This decrease may be related to the fact that the quality indicator studies in the research hospital have not accelerated since 2012 and records of falls have been kept more regularly since then.

Similar to some studies in the literatüre (7,14,15), our study found that most falls occurred during night shifts. Contrary to these results, some studies stated that fall incidence was higher during day shifts because more personnel caused increased activity among patients (16,17). As stated by Türkmen (18), the number of patients per nurse on night shift is quite high in Turkey (1:25). Therefore, this result of our study may be associated with the inability to closely monitor patients due to the limited healthcare personnel working night shifts.

In the study, it was detected that falls occurred in the patient's room while patients were on the movement and in the form of fainting/collapsing. Contrary to the result of our study, some other studies (14,19) reported that patients mostly fell from a chair/couch or a bed.

In the study, similar to other studies in the literatüre (14,20), it was found that most of the patients who fell were alone at the time of the fall and there were no accompanists or healthcare personnel with them (21). Therefore, interventions are suggested such as scheduled toilet times, regular nurse rounds, admitting fall-prone patients to rooms that are directly visible from or close to the nursing stations (14), routine hourly nurse visits, fall alarm sensors in beds, and installing video cameras in patient rooms (22). As a result, nursing activities can be planned to prevent patient falls. In another study conducted on this subject, it was found that the majority of nurses' preventive nursing activities for individual falls included assessing the patient's visual status (99.2%), reviewing the medications used by the patient (99.2%), evaluating the patient's fall history (99%), communicating effectively with the healthcare team (99%) and knowing the risky medications used by the patient (99%). It was determined that the intervention that the nurses stated that they practiced the least was having the patient perform walking-balance-strengthening exercises (58.5%) (23).

In the study, the mental status of the majority of patients who fell was good. Similar to the result of our study, Najafpour et al. (14) stated that most patients who fell were conscious and well-oriented; disorientation, confusion, or agitation did not increase the risk of falling. In another study, a strong relationship was found between concentration difficulties and falls (22). Relationships between mental disorders and concentration difficulties can be further investigated in studies examining falls.

In the study, it was found that the patients who fell were not using any medication that would increase the risk of falling. It was stated that there was no relationship between medication and fall risk (24). Contrary to this, in another study, it was emphasized that medication played an important role in in-hospital falls and the Medication Fall Risk Score could be used to identify patients who are at high risk of falling (25). In the study by Najafpour et al. (14) the use of sedative drugs, anti-convulsant drugs, anti-diabetic drugs, benzodiazepine, angiotensin-converting enzyme (ACE) inhibitors, anti-infective agents, antihistamine, and chemotherapy drugs was associated with a higher probability of falling. In the study, the relationship between the fall risk and the medication used by patients who fell was not tested. Further studies should investigate the relationships between medication use and fall risk.

In the study, the ITAKI and HARIZMI Fall Risk Scales were used to determine fall risk. According to the results of our study, the average fall risk score of the patients who fell was normal for the adult patients and high for the pediatric patients. In the present study, patients with a score of five and above on the fall risk scale were included in the evaluation. The primary purpose of fall risk assessment is to identify patients with a high risk of falling and prevent them from falling by making interventions specific to the risk factors of the patients (9). Therefore, it is very important to use valid and reliable risk assessment tools in all inpatient treatment institutions.

In the study, it was identified that the lights were mostly off in the patients' rooms and the floor was not wet or slippery at the time of falling. Visual impairment is seen as a factor

contributing to falls in hospitals (26). It may be beneficial to perform eye examinations on patients by nurses and to warn staff about patients who are at risk of falling due to poor vision to implement preventive measures. On the other hand, it is known that the floor in the patient room is an extrinsic factor that increases the risk of falls (27-29). Therefore, attention should be paid to ensuring that the patient's room and other places used by the patient (e.g. toilet, bathroom) are not wet or slippery. In this regard, regular training should be given to cleaning personnel in the hospital. Employees should be warned about the patient is at risk of falling by placing warning symbols in the rooms of patients at risk of falling.

In the study, it was identified that patients who fell usually developed minor fall-related injuries, and 34.3% (n=36) had severe injuries. Hitch et al. (20) reported that 42% of falls resulted in injuries. In another study, it was found that 8% (n=109) of all falls resulted in severe injuries and the most common were fractures, concussion, lacerations, and hematoma (3). Moreover, other studies also stated that more than 63% of falls were associated with mild to severe injuries (14). Serious health problems may develop after a fall. Patient falls both increases the time of hospitalization and the cost of care, and lawsuits may be filed as a result of complaints due to injuries. Therefore, it can be suggested to broadly examine injuries after a fall and investigate how the fall occurred and its related factors by conducting qualitative interviews with the patients to facilitate developing specific preventive measures in hospitals.

In the study, it was determined that vital signs follow-up and blood tests were performed, radiologic procedures were performed, and consultation was requested in patients who fell. Patients who fall should be evaluated for their consciousness levels, vital signs, presence of pain, and visible injuries in line with the local policy and incident/accident intervention procedures, without being moved after the fall. Nurses should be aware of the patients' medical history, laboratory results, and prescription drugs that can increase the risk of injury (e.g. warfarin) due to falls (28). In addition, after a fall, patients with cardiovascular disease should undergo electrocardiography (ECG) and their peripheral oxygen saturation levels should be checked. Blood glucose levels of patients with diabetes should be measured. Anti-coagulant or anti-thrombotic drugs are particularly risky for patients with head trauma after a fall because of the risk of cerebral hemorrhage or other types of internal bleeding (30,31). Patients who fall should be evaluated rapidly through physical examinations and consultation should be requested from other disciplines if needed.

In the study, it was found that age groups, sex, and the unit of hospitalization had significant effects on falls. One study reported that the male sex was a significant predictor of severe fall injuries (3). By contrast, another study found that patients who fell were mostly female (7). Similar to our study, age, sex, and unit were reported among the factors that affected falls (12).

The major limitations of the research included conducting the research at a single center and obtaining data retrospectively from fall report forms. In addition, not being able to examine injuries in detail following falls and not being able to obtain qualitative data related to falls were among the limitations of the research. Another limitation is that pediatric and adult patients were evaluated in the same study.

5. CONCLUSION

As a result of this retrospective study, which included a total of 8 years, falls decreased in number by the year. The majority of the falls occurred during night shifts, in the patients' rooms, when the patient was on the movement, and when they were alone. In addition, male sex, adult patients and internal medicine units were significant factors that increased the risk of falls. To prevent falls, it is very important to conduct further studies to investigate the incidence of falls and their risk factors. The prevention of falls is an important subject that needs to be handled seriously in healthcare institutions. The functionality and quality of life of patients can change substantially as a result of falling. Nurses play an important role in the prevention of falls. Nurses should evaluate each patient in terms of fall risk using a valid and reliable scale. To prevent falling, necessary safety precautions should be taken, especially for male adult patients, and patients in unaccompanied departments. Night lamps should be used in patient rooms during the night.

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Clinical and Experimental Health Sciences

The Effect of Progressive Relaxation Exercises on Fatigue, Nausea and Vomiting in Patients with Breast Cancer Receiving Chemotherapy

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ABSTRACT

Objective: The research was designed experimentally to evaluate the effectiveness of progressive relaxation exercises (PRE) in the prevention of chemotherapy-induced fatigue, nausea and vomiting in patients diagnosed with breast cancer.

Methods: 50 patients diagnosed with breast cancer received doxorubicin and taxane-based treatment who applied to the chemotherapy unit of a private hospital were participated in the study. 24 patients were included in the experimental group, and 26 patients were included in the control group, who were selected by simple random method and agreed to want to get in the research. The participants in the intevention group were provided with one-to-one relaxation training accompanied by Relaxation Exercises CD of the Turkish Psychological Association before the initiation of treatment. Data were collected using "Patient Identification Form", "Piper Fatigue Scale (PFS)" and "Rhodes Index of Nausea, Vomiting and Retching (RINVR)" forms.

Results: Most of the patients who attended in the research were married (76%) and the mean age was 52.72±10.17. A total of 54% of the participants received doxorubicin-based treatment and 46% of them was on taxane-based treatment. When we investigated the effect of PRE on fatigue, nausea and vomiting, it was found out that fatigue and "nausea, vomiting and retching (NVR) experience, occurrence and distress" scores on the day of treatment were significantly lower than those of the other five days, but there wasn't difference in five-day symptom scores between the groups.

Conclusion: It was concluded that PRE were not an effective approach in decreasing the symptoms of fatigue, nausea, vomiting in the research group patients with breast cancer and receiving chemotherapy.

Keywords: Muscle relaxation, fatigue, nausea, vomiting, chemotherapy.

1. INTRODUCTION

Cancer and cancer-related mortality rates are increasing day by day. According to the World Cancer Report published in 2012 by the "International Agency for Cancer Research (IARC)"; It was determined that 14.1 million people were newly diagnosed with cancer and 8.2 million people died from cancer (1).

Most widespread cause of death in women is breast cancer in the world and in our country. Radiotherapy and surgery are used as local methods to treat breast cancer, but chemotherapy, as a systemic treatment, has a great place and importance. Chemotherapy has many side effects, depending on treatment regimen used. The most widespread side effects are neutropenia, nausea, fatigue, vomiting, costıpation nausea, vomiting, anemia and peripheral neuropathy (2, 3).

Fatigue in patients receiving chemotherapy has an extremely negative effect on quality of life of patients and activities of daily living. "The National Comprehensive Cancer Network (NCCN)" describes fatigue with cancer as "an uncommon, progressive and subjective sense of exhaustion that prevents daily life activites of a person, which occurs due to cancer or cancer treatment". It has been reported in the literature that the prevalenceof fatigue in patients receiving chemotherapy is between 40-100% (4). Fatigue is normally a subjective symptom that increases with activity and goes away after rest. However, fatigue in cancer patients is different. There is fatigue that does not go away after rest, independent ofthe activity (5).

Pharmacological and non-pharmacological approaches are used to manage fatigue. Pharmacological approaches are often used to treat symptoms developing due to fatigue (6). There are studies supporting the effectiveness of psycho-educational approaches (7) and exercise from non-pharmacological approaches. However, it has been emphasized in the literature that energy conservation approaches, sleep, massage and reflexology, relaxation exercises and imagery therapy, music therapy, painting, yoga, acupuncture, hypnosis, reiki and therapeutic touch may be effective but further studies are needed (8).

Other side effects that affect the quality of life and also daily living activities of patients receiving chemotherapy are

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nausea and vomiting. Nausea and vomiting usually begin one to two hours after chemotherapy and last for six to twelve hours. Some patients may experience nausea and vomiting a few days after chemotherapy. It has been reported that the frequency of nausea and vomiting in patients treated with chemotherapy ranges from 66% to 91% (9).

Pharmacological or non-pharmacological methods are also used in the treatment of nausea and vomiting caused by chemotherapy. The effectiveness of antiemetics from pharmacological methods has been proven. It has been emphasized that non-pharmacological approaches such as patient education, music therapy, hypnosis, relaxation exercise, massage, aromatherapy, and yoga, acupuncture, acupressure may also be effective (10).

One of the methods which are recommended for the treatment of chemotherapy-related fatigue and nausea-vomiting, and whose effectiveness is controversial is also progressive relaxation exercises (PRE). PRE is defined as relaxation exercise consisting of stretching and loosening of large skeletal muscle groups, respectively. This exercise is aimed at relaxation to reduce the feeling of tension and the level of perceived stress. It is emphasized that PRE reduces the stimulation of the autonomic and central nervous system and increases the activity of the parasympathetic nervous system (11).

Progressive relaxation exercises are a cost-effective technique that can be easily taught by clinical psychologists, nurses, yoga instructors and other complementary medicine practitioners. Training is carried out in one or several sessions in group or one-to-one with a CD or voice recorder. Relaxation training is recommended before, after or during medical treatment or procedure. Relaxation sessions take 20 to 30 minutes, but this is not a standard time. Muscle groups involved in processing and deep breathing techniques affect this time (11).

There are insufficent studies evaluating the effectiveness of PRE on problems such as pain, anxiety, depression, sleep disorders, nausea, vomiting, fatigue patients with cancer, due to chemotherapy and there is no sufficient evidence. The studies have been reported to be methodologically poor (11). Kwekkeboom et al. indicated in their reserch on 40 hospitalized cancer patients, progressive relaxation exercises were not effective on pain (12). In another study by Isa et al., it was carried out that relaxation exercises had positive effects on anxiety, but the same effect was not observed on depression (13). In a study by Simeit et al., it was determined that the effects of autogenic training and relaxation exercises on sleep were not different (14).

Pathak et al. carried out a study to determine efficiency of PRE on pain and fatigue in 100 participants receiving radiotherapy, and identified that relaxation exercises in combination with standard treatment reduced pain and fatigue (4).

Cancer patients are going through a difficult process either because of the effect of the disease or the side effects of the treatment methods. When advanced, simple, cost-effective and reliable nursing practices such as progressive relaxation training are provided to these patients in addition to standard pharmacological treatment, it is considered that they will increase symptom control and improve life quality.

2. METHODS

2.1. Ethical Considerations

The written permission of "Marmara University Faculty of Medicine Ethics Committee" numbered with 09.2016.540 was obtained. A written consent was obtained from the private hospital where the research was carried out.

Written informed permission was taken from the participants included in the research after explanations were made. Progressive relaxation exercises, standard tretment and standard care were applied to the intervention group. The control group received standard treatment and standard care. The control group was taught progressive relaxation exercises five days after treatment, if desired.

2.2. Patients and Data Collection

This research was designed to evaluate the efficacy of PRE in reducing chemotherapy-induced fatigue, nausea, and vomiting in patients treated with chemotherapy for breast cancer. The research is a randomized controlled trial conducted in a single center. Between March 2017 and February 2018, it was conducted inthe outpatient chemotherapy unit in a private hospital. 50 patients in total who applied to the outpatient chemotherapy units to receive their treatments on the dates specified and were selected by simple random method were included in the sample. 24 and 26 patients were respectively included in experimental and control groups with simple random selection method in accordance with randomization list. According to the power analysis to determine the trial sample (assuming Δ: 0.928, Power:0.80, β: 0.05 and α: 0.05), the sample number of the experimental/control groups was determined as 20 patients for each and 40 in total. Inclusion criteria for the research; to have been diagnosed with breast cancer, to receive Doxorubicin or Taxane-based treatments, to not have a communication problem that would prevent participation in the research, and to wa nt to get in the research after necessary explanation was given.

After the trial was explained and the participant's oral and written permission were obtained, 26 of the participants were get involved in the intervention group and 24 of them were get involved in the control group according to the prepared randomization list. Simple randomization method was used to select patients. The randomization list was created using a computer program for the study. After determining the patients who met the inclusion criteria for the trial, their consent was obtained and they were included in the experimental or control group according to the list. Before initiating treatment in participants in the intervention group, the "Patient Identification Form", the PFS and the RINVR forms were filled in face-to-face interviews and the participant was taken to a quiet place and was

given one-to-one relaxation training throughthe Relaxation Exercises CD of the Turkish Psychological Association. The participant to be given relaxation training was taken to a quiet place and was provided to sit comfortably. She was asked to close her eyes. She was recommended to perform relaxation exercises at least once a day for one week after the end of treatment, and she was given a brochure of relaxation exercises. The PFS and the RINVR forms were given to the participant to fill them in for five days after treatment. The patient interview and training lasted about 30-40 minutes. On the sixth day after the treatment, the participant was contacted by telephone to inquire whether she did regular relaxation exercises. The results of the PFS and RINVR forms were obtained and the telephone call lasted 5-10 minutes. In the first week after chemotherapy, patients were evaluated for five days, as intense fatigue, nausea and vomiting were observed.

After the participant in the control group was informed about the study, she was told that relaxation exercises could be taught at the end of the study if desired, and the participants were taught five days later upon their request. Before initiating treatment in patients, the "Patient Identification Form", the PFS and the RINVR forms were filled in one-to-one. The PFS and the RINVR forms were given to the participant to fill them in for five days after treatment. The interview lasted 15-20 minutes in total. On the sixth day after the treatment, the patient was contacted by telephone and the results of the PFS and RINVR forms were obtained and the telephone call lasted 5-10 minutes. Standard antiemetic treatment was also administered to both groups. Standard antiemetic therapy included 5-HT3 receptor antagonist, NK-1 receptor antagonist, and corticosteroid drugs, depending on the ematogenicity levels of the chemotherapy treatment protocols. Participants were evaluated in a single cure.

2.2.1. Patient Identification Form: The Patient Identification Form, formed in accordance with the literatüre (15, 16) and including important variables related to the study, consists of two parts and twenty-six items including sociodemographic characteristics of the patient and chemotherapy medicines and disease-specific characteristics.

Piper Fatigue Scale (PFS): It was created by Piper et al. (17). The Turkish version of the scale's validity and reliability was carried out by Can. The scale consists of twenty-seven items. Twenty-three of these items are considered as 0-10 point(s) of VAS (Visual Analog Scale) and four items are considered as open-ended. These items are divided into four sub-dimensions, "being fatigue behavior/severity sub-dimension", "affect sub-dimension", "sensory sub-dimension" and "cognitive/mental sub-dimension". Cronbach's alpha (α) reliability coefficient was found to be between 0.92-0.96 for the subgroups and 0.97 for the total scale (18).

Rhodes Index of Nausea, Vomiting and Retching (RINVR): It was created by Rhodes et al (19). The Turkish version of scale's validity and reliability was carried out by Tan and Genc. This scale includes a total of eight items assessing "nausea, vomiting and retching experience", "the incidence of nausea, vomiting and retching", and" nausea, vomiting and retching distress". Responses for each item were classified by using appropriate expressions and scoring the severity of nausea, vomiting and retching experience from 0 to 4. Cronbach's alpha (α) reliability coefficient was found to be between 0.81-0.95 for the subgroups and 0.95 for the total scale (20).

2.3. Data Analysis

A statistical program was used to evaluate the study findings. The "Shapiro Wilks" test was used to evaluate conformity of variables to normal distribution. Descriptive statistical methods (mean, standard deviation, frequency) and "Mann Whitney U" test were used to evaluate quantitative data between two groups. The Friedman test was used for repeated measures analysis. "Wilcoxon Signed Ranks" test was used to evaluate the measurement time that caused the difference. Significance value was accepted as $p< .05$.

3. RESULTS

3.1. Sociodemographic and Disease-Specific Characteristics

Mean age of the patients was 52.72±10.17 and the study was conducted with 50 females. A total of 48% (n=24) of the participants were in the intervention group and 52% (n=26) were in the control group.

In the intervention group, 79.2% (n=19) of the participants were married and 20.8% of them (n=5) were single. Most of the patients (70.8%) were housewives and 37.5% of them were primary school graduates. Generally, non-smokers (54.2%) did not also use alcohol and their income was moderately good (66.7%). Although 70.8% of the patients were not working, 87.5% had health insurance.

Considering the intervention group in terms of disease characteristics, all participants were diagnosed with breast malignant neoplasm. Most of the patients (62.5%) received doxorubicin-based treatment; 37.5% of them were on taxane-based treatment and 95.8% had previous surgical treatment.

In the control group, most of the participants were married (79.2%), 76.9% of the participants were housewives, 53.8% were primary school graduates; most of participants were non-smokers (80.8%) and did not consume alcohol. Of most participants with moderate income (65.4%), 76.9% did not work and 96.2% had health insurance.

If the disease characteristics of the participants in the control group were examined, all patients were diagnosed with breast malignant neoplasm; 46.1% of the patients were on doxorubicin-based treatment, 53.8% of them were on taxane-based treatment and most of them (76.9%) had previous surgical treatment.

The body mass index of the control group was more than the experimental group (p=.001).

Sociodemographic and other disease-specific descriptive characteristics of the groups were similar (p>.05) (Table 1).

Table 1. *Sociodemographic and disease-specific characteristics of patients in experimental and control groups*

Descriptive characteristics		Experimental (n=24)	Control (n=26)	Total (n=50)	Z/χ^2	p
Age (year)	Avg±SD (Median)	50.38±9.92 (50)	54.88±10.09 (55)	*52.72±10.17 (53.5)*	[1]-1.507	.132
BMI (kg/m^2)	Avg±SD (Median)	25.70±3.58 (25.87)	31.23±7.05 (30.12)	*28.59±6.26 (27.87)*	[1]**-3.214**	**.001****
Number of cures (n)	Avg±SD (Median)	2.00±1.02 (2)	2.38±0.90 (2)	*2.20±0.97 (2)*	[1]-1.461	.144
Marital status	Married	19 (79.2%)	19 (73.1%)	*39 (76%)*	[2]0.254	.745
	Single/widow	5 (20.8%)	7 (26.9%)	*12 (24%)*		
Educational background	Elementary school and less	10 (41.7%)	15 (57.7%)	*25 (50%)*	[2]0.721	.396
	Secondary school and higher	14 (58.3%)	11 (42.3%)	*25 (50%)*		
Profession	Housewife	17 (70.8%)	20 (76.9%)	*37 (74%)*	[2]0.028	.867
	Self-employed	7 (29.2%)	6 (23.1%)	*13 (26%)*		
Smoking	Smoker/Ex-Smoker	11 (45.8%)	5 (19.2%)	*16 (32%)*	[2]2.928	.087
	Non-smoker	13 (54.2%)	21 (80.8%)	*34 (68%)*		
Treatment	Doxorubicin-based	15 (62.5%)	12 (46.2%)	*27 (54%)*	[2]0.765	.382
	Taxane-based	9 (37.5%)	14 (53.8%)	*23 (46%)*		

Z: Mann-Whitney U Test *χ^2: Continuity (Yates) Correction and Fisher's Exact Chi-Square Test* **p=.001*

3.2. The Effect of Progressive Relaxation Exercises on Fatigue

When we examined the five-day fatigue levels of the patients with and without progressive relaxation exercises, it was found in both groups that fatigue on the treatment day was lower than that of the other five days (p=.001), but the groups were similar in terms of five-day fatigue scores. (p>.05). It was determined that PRE was not an effective application in reducing fatigue.

As a result of the post hoc comparison, done for the determination of the day on which difference occurred between the groups, it was identified in both groups that fatigue on the first day was less than that of the third day and fatigue on the third day was more than that of the fifth day (p=.001) (Table 2).

Table 2. *Comparison of the effect of progressive relaxation exercises on fatigue in experimental and control groups*

Piper Fatigue Scale		Experimental (n=24)	Control (n=26)	Z	p
		Avg±SD (Median)	Avg±SD (Median)		
Total	Day 0	0.28±0.54 (0)	0.90±1.85 (0)	-0.725	.469
	Day 1	4.44±3.39 (5)	3.12±2.95 (2.5)	-1.460	.144
	Day 2	5.43±3.25 (7)	5.01±3.08 (5)	-0.546	.585
	Day 3	5.62±3.36 (8)	6.22±2.93 (7)	-0.713	.476
	Day 4	5.11±3.15 (5.75)	5.46±3.04 (5.32)	-0.545	.586
	Day 5	4.78±3.11 (4.94)	4.61±2.81 (3.67)	-0.332	.740
	Post-hoc	1*3 3*5	1*3 3*5		
	χ^2	55.184	64.907		
	p	.001**	.001**		

Z: Mann Whitney U Test *χ^2: Friedman Test* **p=.00*

3.3. The Effect of Progressive Relaxation Exercises on Nausea-Vomiting-Retching Experience, Occurrence and Distress

When we examined the five-day "nausea, vomiting, retching experience, occurrence and distress" of the patients with and without progressive relaxation exercises, it was detected that "nausea, vomiting, retching experience, occurrence and distress" on the day of treatment were lower than those of the next five days in the both groups (p=.003; p=.001), but there wasn't difference between the groups in terms of five-day "nausea, vomiting, retching experience, occurrence and distress" (p>.05). PRE were identified not to be an effective approach in reducing "nausea, vomiting, retching experience, occurrence and distress".

Table 3. *Comparison of the effect of progressive relaxation exercises on nausea-vomiting-retching experience, occurrence and distress in experimental and control groups*

Nausea-Vomiting-Retching Experience, Occurrence, Distress		Experimental (n=24)	Control (n=26)	Z	P
		Avg±SD (Median)	Avg±SD (Median)		
NVR Experience	Day 0	0.46±1.28 (0)	1.00±4.20 (0)	-0.466	.641
	Day 1	3.42±5.22 (0)	2.42±5.17 (0)	-1.312	.190
	Day 2	3.54±5.80 (0)	4.65±8.49 (0)	-0.130	.897
	Day 3	3.88±5.64 (3)	5.58±9.10 (0)	-0.220	.826
	Day 4	3.25±5.23 (0)	5.15±8.04 (0)	-0.487	.626
	Day 5	3.46±6.15 (0)	3.12±4.19 (0)	-0.252	.801
	Post-hoc		1*3		
	χ^2	17.749	35.649		
	p	0.003**	0.001**		
NVR Occurrence	Day 0	0.29±0.81 (0)	0.58±2.40 (0)	-0.466	.641
	Day 1	2.13±3.22 (0)	1.42±3.05 (0)	-1.347	.178
	Day 2	2.21±3.54 (0)	2.77±5.13 (0)	-0.163	.871
	Day 3	2.42±3.43 (2)	3.31±5.50 (0)	-0.284	.777
	Day 4	2.04±3.20 (0)	3.00±4.85 (0)	-0.401	.688
	Day 5	2.13±3.59 (0)	1.69±2.22 (0)	-0.077	.939
	Post-hoc		1*3		
	χ^2	17.749	25.649		
	p	.003**	.001**		
NVR Distress	Day 0	0.17±0.48 (0)	0.42±1.79 (0)	-0.466	.641
	Day 1	1.29±2.01 (0)	1.00±2.14 (0)	-1.302	.193
	Day 2	1.33±2.28 (0)	1.88±3.39 (0)	-0.108	.914
	Day 3	1.46±2.23 (1)	2.27±3.63 (0)	-0.179	.858
	Day 4	1.21±2.04 (0)	2.15±3.23 (0)	-0.564	.573
	Day 5	1.33±2.58 (0)	1.42±2.00 (0)	-0.473	.636
	Post-hoc		1*3		
	χ^2	17.749	26.693		
	p	.003**	.001**		

Z: Mann Whitney U Test *χ^2: Friedman Test* **p< .01*

As a result of the post hoc comparison, done for the determination of the day on which difference occurred within the group, it was identified in the control group that "nausea, vomiting, retching experience, occurrence and distress" on the first day were lower than those of the third day (p=.001) (Table 3).

4. DISCUSSION

Exercise, psychotherapy, yoga, acupuncture, aromatherapy, foot bath, reflexology, energy conservation and activity management, training, massage, therapeutic touch are among non-pharmacological methods used in the management of fatigue in patients in patients treated with chemotherapy (8).

Depending on the pharmacological ematogenicity level of the treatment protocol, dopamine receptor antagonists, benzodiazepines, cannabinoids, 5-HT3 receptor antagonists, antacids, NK-1 receptor antagonists, corticosteroids, as well as non-pharmacological methods are most commonly used for the reducing nausea and vomiting caused by chemotherapy (15, 21, 22). According to the guidelines of the American Cancer Society, the use of NK-1 receptor antagonists and corticosteroids in addition to 5-HT3 receptor antagonists is recommended for chemotherapy protocols with high levels of ematogenicity. The use of corticosteroids with palonosetron is recommended for chemotherapy protocols with moderate levels of ematogenicity (23). 5-HT3 receptor antagonists, corticosteroids, NK-1 receptor antagonists used in standard antiemetic protocols depending on the pharmacological ematogenicity levels of treatment protocols were also used to prevent nausea in our study. Methods such as acupuncture, acupressure, acustimulation, electroacupuncture, exercise, ginger, yoga, music therapy, imagery therapy and aromatherapy are also the most commonly used non-pharmacological methods for the reducing of nausea, vomiting (24,25). In this research we investigated the efficiency of PRE in the literature on preventing chemotherapy-induced, nausea, vomiting, fatigue.

There are few researches in the literature, which address the efficiency of PRE on fatigue, nausea, vomiting in patients treated with chemotherapy in our country and abroad, and the results of these studies are controversial (11). Demiralp et al. indicated in their study, evaluating 27 patients with breast cancer to determine the efficiency of relaxation on sleep quality and fatigue, which evaluated the effectiveness of PRE related to chemotherapy-related fatigue and sleep disorders in our country that relaxation exercises improved sleep quality and reduce fatigue (26). In their research on 70 patients diagnosed with breast and colorectal cancer receiving chemotherapy Dogan et al. asked the experimental group to perform progressive relaxation exercises for 20 minutes every day and the patients were followed up for three cycles. Fatigue and depression scores were lower and life quality was higher in the experimental group (27). Dikmen and Terzioğlu's study on 80 gynecological cancer patients showed that progressive relaxation exercises and reflexology reduced pain and fatigue and increase quality of life (28). When we evaluated the efficiency of progressive relaxation exercises on reducing chemotherapy-related fatigue using the Piper Fatigue Scale in our study, it was found that fatigue scores increased, compared to the day of treatment and there was no difference between the groups. In contrast to above-mentioned studies, our study it was determined that PGE was not an effective method in reducing fatigue. We consider that this result arises from the fact that progressive relaxation exercises are not widely used in our society. The groups were found to have a lower total fatigue score on the first day than that of the third day and a higher fatigue score on the third day than that of the fifth day (Table 2). We think the reason is that the effect of chemotherapy medicines was intense in the first week after the treatment and increased towards the third day and gradually decreased at the end of the week.

Considering the studies that evaluated the efficiency of relaxation exercises in preventing chemotherapy-related nausea, vomiting, Soliman et al. indicated in another randomized-controlled research on 74 patients receiving chemotherapy to define the efficiency of PRE on anxiety, nausea, vomiting, that relaxation exercises decreased nausea, vomiting and retching scores (29). In various studies carried out by Carvalho et al., Molassiotis et al. , Gupta et al. , Tian et al. on patients receiving chemotherapy to define the efficiency of progressive relaxation exercises significantly decreased nausea and vomiting levels (9, 30-32). Song et al. carried out a randomized-controlled research on 100 patients with breast cancer, and applied breathing and PRE as well as standard nursing care for the experimental group, while performing only standard nursing care for the control group. Following chemotherapy, side effects (fatigue, nausea, mouth ulcers, acid reflux, cough, pain, anorexia) were detected to be lower in the experimental group (33). Kurt et al. made a research on 49 patients with breast cancer and identified that the severity of symptoms such as pain, nausea, sadness, anxiety, insomnia, shortness of breath, fatigue, anorexia, changes in skin and nails, feeling bad, mouth sores was significantly lower in the intervention group and the severity of all symptoms increased in the control group (34). When we investigated the efficiency of PRE on reducing nausea and vomiting due to chemotherapy using the RINVR, there was little difference between the intervention and control groups in terms of "nausea, vomiting, retching experience, occurrence and distress" scores on the third and fourth days, but this difference was not statistically significant. It was identified in both groups that "nausea-vomiting-retching experience, occurrence and distress" significantly increased compared to those of the day of treatment and there was no difference between the groups. In contrast to above-mentioned studies, our study showed that PRE were ineffective method in decreasing nausea and vomiting (Table 3).

There are also studies in the literature, which address the effectiveness of progressive relaxation exercises, and the results of those researches are in parallel with our research. Arakawa's study on eight Japanese patients receiving chemotherapy showed no difference in the nausea, vomiting

scores between the groups. However, there was a decrease in anxiety scores (35). Young and Nam conducted a study on 74 patients receiving chemotherapy and determined that the participants who applied PRE had lower levels of anxiety and depression., but there wasn't any difference in the levels of nausea and vomiting, fatigue between the both participants (36).

5. CONCLUSION

We determined the efficiency of PRE on nausea and vomiting, fatigue in patients with breast cancer treated with chemotherapy. The research showed that PRE were not an effective approach in decreasing chemotherapy-related fatigue and "nausea, vomiting, retching experience, occurrence and distress" in breast cancer women in this study group. To confirm the effectiveness of progressive relaxation exercises, more research should be conducted on large patient groups following similar metodology.

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Clinical and Experimental Health Sciences

Effects of Functional Pinealectomy on Immunity, Hematopoietic, Gastrointestinal and Urinary Systems in Experimentally Malnutritioned Rats

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ABSTRACT

Objective: The aim of this study was to demonstrate morphological changes in immunity, hematopoietic, gastrointestinal and urinary systems in different melatonin (MEL) release situations in a rat model of protein energy malnutrition (PEM).

Method: A total of 32 adult male Wistar rats were assigned into four equal groups: normal control; PEM light/dark; PEM light, called functional pinealectomy (Px); and PEM dark. PEM was produced with a 50% restricted diet, Px was produced by keeping rats in continuous light environment for 24 hours, and complete blood count and serum albumin level were analyzed at the end of the 6-week experimental period. Measurements of weights of body and some visceral organs were obtained, biochemical and morphological parameters were analyzed in addition to measurements of malondialdehyde (MDA), total glutatione (GSH), tumor necrosis factor (TNF)-α and interleukin-10 (IL-10) in tissue samples.

Results: A reduction in the weights of body and visceral organs of animals in the PEM groups was accompanied by hypoalbuminemia, anemia, leukopenia and lymphopenia, and higher MDA, GSH, TNF-α and IL-10 levels in visceral tissues. There was a significant decrease in parenchymal cells of the liver and spleen, duodenal villi, lymphoid structures and kidney glomeruli, but there was an increase in the spleen capsule thickness and renal Bowman's space, sinusoidal congestion and fat accumulation in the liver. Importantly, these findings were milder in the PEM dark group, while they were prominent in the PEM light group.

Conclusion: This study suggests that MEL has a protective role in reducing the negative effects of PEM, making it a potential therapeutic agent for further investigation.
Keywords: functional pinealectomy, malnutrition, melatonin, protein energy malnutrition, rat.

1. INTRODUCTION

Protein energy malnutrition (PEM) occurs due to decreased intake of macro and micro nutrients such as protein, carbohydrate and fat in less than their required amount (1,2,3,4). Even today, it is a serious health problem and an important cause of morbidity and mortality for all ages, especially in individuals who are hospitalized in intensive care units and had comorbid diseases, from developing countries (3,4,5).

Numerous studies revealed that PEM causes decreased numbers of hematopoietic cells, such as macrophages, lymphocytes and neutrophils, atrophic changes in the spleen, thymus and lymph nodes, blunting of intestinal microvilli and decreased IgA secretion; thus resulting in various visceral dysfunctions (6,7). Moreover, immunomodulatory cytokines released from macrophages, such as tumor necrosis factor (TNF)-α and interleukin (IL), play a regulatory role in inflammation (8), while the free radicals superoxide and hydrogen peroxide result in oxidative stress (1). It is well-known that some molecules which include glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) have vital roles in the antioxidative defense system, although malondialdehyde (MDA) is an indicator of lipid peroxidation. Theoretically, disruption of the critical balance between oxidative and antioxidative systems or accumulation of free radicals results in protein damage and DNA breaks (1).

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Many authors have reported that PEM causes an increased incidence of infections of the gastrointestinal, respiratory or urinary tract, due to oxidative stress, immunodeficiency and hypersensitivity to infectious agents (9). Clinically, it is important to reduce the economic losses associated with long hospitalizations, and the high morbidity and mortality rates related to infections, especially in people with PEM, who are hospitalized for various reasons in intensive care units. Otherwise, recent studies have demonstrated that melatonin (MEL) plays a role in the activation and regulation of T and B lymphocytes which are critical for immunity (10,11). Today, it is a well-known fact that MEL release was found to be in a distinct circadian rhythm that is 5-15 times higher at night than during the day (12). Thus, one could suggest that the antioxidants will become stronger with decreased free radicals due to increased MEL release in a dark environment.

The aim of the present work was to test the hypothesis that keeping adult individuals with PEM in a continuous light environment, as are found in the intensive care units of hospitals, will have negative effects on the health of patients. Therefore, the potential effects of different levels of MEL release provided by keeping malnourished adult rats in different light/dark cycle environments upon some biochemical parameters and histology of immune, hematopoietic, gastrointestinal and urinary systems was investigated.

2. METHODS

2.1. Ethical Statement

The animal experiments were performed according to the Guide for the Care and Use of Laboratory Animals principles (13). The design of the experiment was approved by the ethics committee of Animal Experiments of Aydın Adnan Menderes University (Protocol No. 64583101/2020/087 of 17/09/2020).

2.2. Animals, Diets, and Experimental Design

A total of 32 male Wistar rats of 2-month-old, weighing between 240 and 310 g were used and they were kept in standard cages with 2 rats in each cage. During the experiment, standard laboratory conditions were provided (22°C room temperature, 40-60% humidity). As described by Leite et al. (14), the animals were acclimated for a 3-day adaptation period to determine the daily energy/calorie needs under laboratory conditions.

The rats were randomly divided into four groups for 6 weeks: normal control, i.e. intact or naive control, group (n=8) kept in 12-hour light/12-hour dark environment and fed standard diet; PEM light/dark group (n=8) kept in 12-hour light/12-hour dark environment and fed PEM diet; PEM light, functional pinealectomy (Px), group (n=8) kept in 24 hours of light and fed PEM diet; and PEM dark group (n=8) kept in 24 hour dark environment and fed PEM diet. At the end of the experiment, blood and tissue samples were taken from the rats for biochemical and morphological analyses.

2.3. Functional Pinealectomy and Malnutrition Diet

Functional Px was produced by keeping rats in a continuous light environment for 24 hours (15). A standard rat diet was given without restriction according to the requirements for laboratory animals by the National Research Council (16). For the control group, the daily calorie requirements of the rats were calculated as 25-35 kcal/kg. The PEM diet was provided with a diet that was 50% restricted compared to control rats, as previously described (14,17,18). The water consumption of the rats was unrestricted in all groups.

2.4. Sample Collection

At the end of the experiment before sacrificing the animals by cervical dislocation under anesthesia by intramuscular administration of drug combination consisting of 40 mg/kg ketamine (Ketalar, Eczacıbaşı, Turkey) and 5 mg/kg xylazine (Rompum, Bayer, Germany), blood samples from all rats were taken by the intracardiac route using a 5 cc syringe and they were placed in tubes with ethylenediamine tetraacetic acid (EDTA) and without anticoagulant to be studied simultaneously. The samples were centrifuged at 1000 g for 10 minutes. The complete blood count (CBC) tests, analysing hemoglobin, erythrocyte, leukocyte and lymphocyte counts, were performed from the whole blood samples with EDTA, while serum albumin levels were obtained from the samples. The serum albumin level was studied spectrophotometrically with a routine biochemistry autoanalyzer (Architect C8000, Abbott, IL, USA). The CBC was studied with Mindray BC 6800 (Nanshan, Shenzhen, China) analyzer. Visceral organs were removed from each of the animals for macroscopic evaluation, and then frozen at – 85 degrees without adding any additive for biochemical analysis. After the tissues were removed from the deep freezer and thawed, they were mixed with phosphate buffer at pH: 7.4 at 1/10 volume ratio and homogenized with a homogenizer (PRO 250 Scientology Inc., Monreo, CT USA). The hemogenate was centrifuged at 10000 g for 15 minutes. MDA, total GSH, TNF-α and IL-10 measurements were taken from the supernatant on the same day, as described below.

2.5. Tissue Analysis

MDA production was evaluated by the method of Ohkawa et al. (19). MDA formed a pink complex at high temperature in the presence of thiobarbituric acid and this color was read spectrophotometrically at a wavelength of 532 nm. Tetraethoxypropane was used as a standard and the results are given in nmol/mg protein.

GSH content in tissue supernatants was measured according to the method of Beutler et al. (20). Absorbance was measured at 412 nm using the Shimadzu UV-160 spectrophotometer (Shimadzu UV-160). The GSH concentration was determined using the standards of GSH. Results are expressed as nmol/mg protein.

TNF-α and IL-10 levels in tissue were determined by the immunosorbent assay (ELISA) kit (SunRed Biological Technology, Jufengyuan Road Baoshan, District, Shanghai,

China). Measurements were made with an ELISA microplate reader (DAR 800, Diagnostic Automation, CA 91302, USA). According to the TNF-α kit content, the sensitivity of the test is 4.752 pg/mL, the working range is 5-15000 pg/mL, the CV within and between experiments is < 9 and 11%. The sensitivity of the test according to the IL-10 kit contents is given as 3.002 pg/mL, working range 5-9000 pg/mL, intra-experimental and inter-experiment CV < 9 and 11%.

The levels of protein in the tissue were determined by the Lowry method, which is based on thSSe principle that copper ion (Cu+2) forms a complex with the peptide bonds in proteins and is reduced to Cu+1 in alkaline medium (21).

2.6. Macroscopic Evaluation

Body weight (BW) of each of the rats in the study was measured and recorded once a week from the beginning of the experiment and macroscopic photographs were taken. At the end of the experiment, the abdominal and thoracic cavities of each animal sacrificed were exposed and the visceral organs including liver, spleen, kidney (right and left) and first part of small intestine (duodenum) were removed separately from each animal for weight measurement.

2.7. Microscopic Evaluation

Visceral organ samples from the animals in the control and PEM groups were fixed in neutral buffered formaldehyde and a routine tissue preparation process was performed. 5 µm histological sections of tissues embedded in paraffin blocks were placed on polylysine slides and then they were stained with hematoxylin & eosin (HE) and Mallory's trichrome. These sections were visualized with an Olympus BX-51 light microscope and Olympus DP72 digital camera after staining. Morphometric analyses were performed on the photographs taken by two blinded histologists (Y.U. and C.T.) using CellSensEntry 3.1 (Japan). A total of five different measurements were taken from the structures analyzed in 25 different areas in 25 different sections randomly selected from all animals.

2.8. Statistical Analysis

The assumption of normal distribution of the data was checked with the Kolmogorov-Smirnov test. Descriptive statistics are given as means ± standart error of the mean (SEM). One-way ANOVA was used for group comparisons, while repeated ANOVA measures were used to compare repeated measures. Tukey test was applied as a post hoc method and paired t-test was used to compare dependent measures. Statistical significance was considered as p<0.05.

3. RESULTS

Measurement results of mean weekly BWs in control and PEM groups are shown in Figure 1. There was no significant difference in BWs of rats between the groups at the start of the study, but the change in weekly measurement results during the experiment was statistically significant (p<0.001). Rats in the control group showed a 25% BW gain, whereas rats from PEM groups given a restricted diet showed a progressive reduction in BW, especially in those from the PEM light group, where loss of BW was close to 30%, resulting in a clear difference between their macroscopic appearances (Fig. 2). Furthermore, hair thinning and shedding, broken nails, muscle atrophy, apathy and agitation were observed in the animals in the PEM groups. The differences between the weights of the visceral organs of the animals in PEM groups and those of the control were found to be significant (Table 1). Amongst the PEM groups, it was noted that visceral atrophy was more prominent in animals from the PEM light group, while atrophy was milder in those from the PEM dark group.

Table 1. *Results of visceral organ weight measurement*

	Liver (g)	Spleen (g)	Duodenum) (g)	Kidney (g)	
				Right†	Left†
Normal control (intact or naive control)	14.07±0.97	1.05±0.15	1.25±0.16	2.14±0.35	2.04±0.24
PEM light/ dark	6.47±0.33*	0.45±0.02*	0.70±0.05*	1.10±0.11*	1.11±0.13#
PEM light	6.29±0.52*	0.43±0.06*	0.56±0.16*	0.99±0.11*	1.01±0.10*
PEM dark	7.50±0.76	0.53±0.06*	0.87±0.11*,§	1.14±0.06*	1.17±0.09

*Differences between study groups for liver, spleen, duodenum, and right and left kidney weights (mean ± SEM) are significant (p<0.001); n=8/ group. *Significant difference with normal control (p<0.001). §Significant difference with PEM light (p<0.001). #Significant difference with control (p<0.05). †Differences between right kidney weight measurements and left kidney weight measurements are significant (p<0.05 for control and PEM light groups, p<0.005 for PEM dark group)*

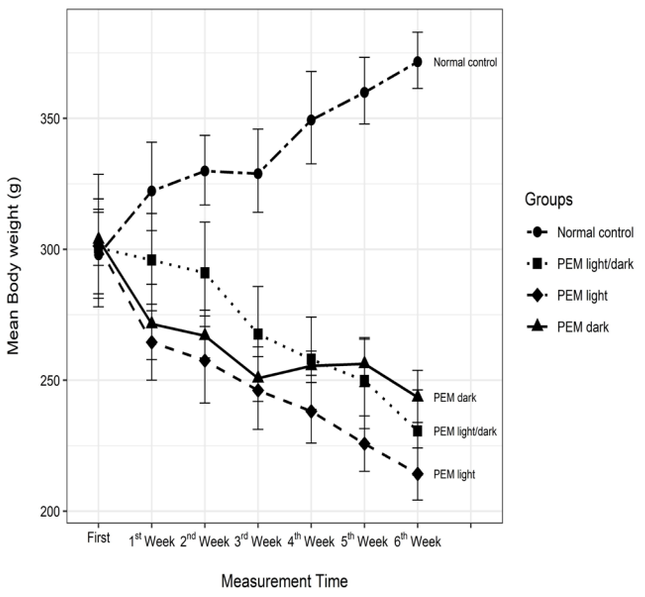

Figure 1. *Weekly change in body weight in rats of the study groups over the course of the experiment (mean ± standard error of the mean). The differences between the normal control (A) and experimental PEM groups (B, C and D) are significant (p<0.001). PEM, protein energy malnutrition.*

Serum albumin levels of PEM groups were found to be lower than those of the control group (p<0.001) and CBC results from animals with PEM clearly demonstrated the presence of anemia, leukopenia, and lymphopenia (Table 2). Hematological findings related to PEM, especially the leukocyte count, were milder in the PEM dark group. MDA, GSH, TNF-α and IL-10 levels in the liver, spleen, duodenum and kidney were also higher in PEM groups compared to the control group (Figs. 3, 4, 5, 6). On the other hand, there was a significant difference between the PEM light group and the PEM dark group in terms of MDA levels of liver and kidney, GSH levels of liver and spleen, TNF-α levels of spleen and duodenum, and the IL-10 level of duodenum (Figs. 3, 4, 5, 6).

Table 2. *Results of serum albumin level and complete blood count (CBC)*

	Albumin (g/L)	Hemoglobin (g/dL)	Erythrocyte count (106)	Leukocyte count (103)	Lymphocyte (%)
Normal control (intact or naive control)	36.65±0.97	16.46±0.59	10.32±0.87	5.95±1.29	82.51±6.25
PEM light/ dark	31.60±0.88*	15.83±0.48	8.91±0.64‡	2.76±0.30*	76.36±9.31
PEM light	30.60±1.00*	15.36±1.15#	8.08±1.05*	2.25±0.33*	70.81±4.84#
PEM dark	31.99±1.17*,§	15.96±0.52	9.05±0.29#	3.82±1.06*	77.98±6.56

*The data are presented as mean ± SEM; n=8/group. Differences between study groups for albumin levels as well as erythrocyte and leukocyte counts are significant (p<0.001), while those between both hemoglobin levels and lymphocyte counts are significant (p<0.05). *Significant difference with normal control (p<0.001). §Significant difference with PEM light (p<0.05). #Significant difference with the control (p<0.05). ‡Significant difference with the control (p<0.01)*

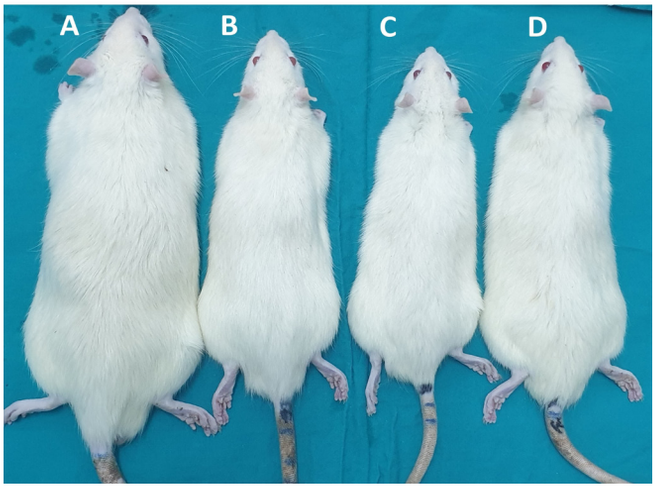

Figure 2. *Photographs of the macroscopic appearances of the animals in the normal control (A) and PEM groups (B, C and D) taken at the end of the experimental study period are shown. Note that among animals in experimental PEM groups, the animal in the PEM dark group (D) is more macroscopically similar to the animal of the control (A). PEM, protein energy malnutrition.*

Table 3. *Histological changes in liver, duodenum and kidney tissue*

Tissues Groups		Normal control (intact or naive control) (n=8)	PEM light/ dark (n=8)	PEM light (n=8)	PEM dark (n=8)
Histological changes in liver					
Parenchyma	Intracytoplasmic edema	None	++	+++	+
	Nuclear hypertrophy	Normal	++	+++	+
	Vena centralis dilatation	Normal	++	+++	+
Stroma	Sinusoidal dilation	Normal	++	+++	+
	Portal triad dilatation	Normal	++	+++	+
Histological changes in duodenum					
Change in epithelial type		N	++	+++	+
Villus atrophy		N	++	+++	+
Reduction in villus size		N	++	+++	+
Reduction in intestinal (duodenum) wall thickness		N	++	+++	+
PMNL infiltration in lamina propria		N	++	+++	+
Degeneration of Lieberkühn and Brünner glands		N	++	+++	+
Histological changes in kidney					
Degeneration of renal corpuscles		N	++	+++	+
Degeneration in podocytes and edema		N	++	+++	+
Cystic degeneration in renal corpuscles		N	++	+++	+
Dilation and congestion in peritubular vessels		N	++	+++	+
Vacuolization in proximal tubules		N	++	+++	+
Epithelial changes in the distal tubules		N	++	+++	+

Abbreviations: N: normal histological structure; +: low level of change; ++: moderate level of change; +++: high level of change

Both number and size of the hepatocyte cells in the liver of the individuals in the PEM groups were found to be decreased in contrast to those of the control animals (Figs. 7, 8). In PEM groups, vacuolization, mononuclear cell infiltration, fat accumulation and sinusoidal congestion and dilatation of the periportal hepatocytes were prominent, whereas they were at a minimal level in PEM dark group (Figs. 7, 8) (Table 3). There was also a statistical difference between the morphometric results of the liver sinusoid and v. centralis diameters of the groups (p<0.001) (Figs. 9, 10, 11).

There was a decrease in the splenic capsule thickness in samples belonging to the PEM light/dark and PEM light groups compared to those of controls (p<0.001) (Figs. 7, 8, 9, 12). Furthermore, narrowing in the a. centralis, disappearance of the lymph node-like structure, and the fibrotic foci in the red pulp were also noted in those of PEM groups (Figs. 7, 7, 9, 12). In the PEM dark group, however, the capsule was similar to that in the PEM light/dark group and there was also a decrease in cord cells, as well as narrowing in the a. centralis structure and the fibrotic foci in the red pulp (Figs. 7, 8, 9, 12).

Group	Liver	Spleen	Intestine (duodenum)	Kidney
Normal control	109.84 ± 8.36*	153.58 ± 12.51	167.04 ± 79.36	145.7 ± 4.46*
PEM light/dark	122.61 ± 7.33	177.15 ± 16.88	219.35 ± 133.33	151.55 ± 8.77
PEM light	145.01 ± 32.19	215.64 ± 113.21	266.52 ± 88.46	160.87 ± 10.66
PEM dark	114.99 ± 7.05*	165.49 ± 43.28	195.57 ± 64.31	147.77 ± 8.00*

* Represent significant difference with PEM light

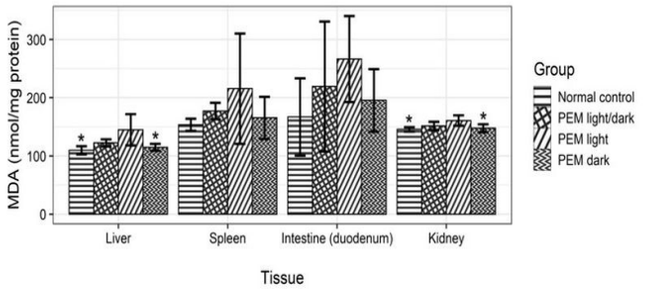

Figure 3. *Results of MDA levels in tissue homogenates taken from the animals in the control and experimental PEM groups taken at the end of the study period are shown. The data are presented as mean ± SEM; n=8/group. *p<0.05between the indicated groups in each tissue sample analysis. MDA, malondialdehyde; PEM, protein energy malnutrition.*

Group	Liver	Spleen	Intestine (duodenum)	Kidney
Normal control	15.34 ± 5.39*	8.01 ± 4.74*	18.1 ± 9.51	13.73 ± 5.37
PEM light/dark	20.04 ± 6.84*	16.09 ± 3.88*#	33.84 ± 47.77	16.02 ± 4.24
PEM light	31.25 ± 12.54	25.63 ± 6.67	46.91 ± 39.94	20.44 ± 6.94
PEM dark	17.93 ± 4.89*	12.24 ± 3.66*	21.28 ± 15.21	14.73 ± 6.02

* Represent significant difference with PEM light; # Represent significant difference with Normal control

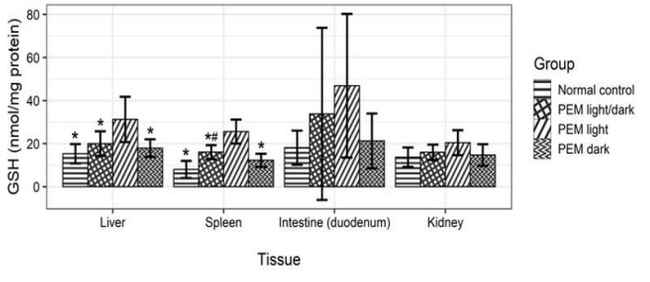

Figure 4. *Results of GSH levels in tissue homogenates taken from the animals in the control and experimental PEM groups taken at the end of the study period are shown. The data are presented as mean ± SEM; n=8/group. *p<0.05 between the indicated groups in each tissue sample analysis. GSH, glutathione; PEM, protein energy malnutrition.*

Group	Liver	Spleen	Intestine (duodenum)	Kidney
Normal control	225.88 ± 55.07	283.94 ± 55.05*	295.77 ± 13.51*	238.72 ± 40.03
PEM light/dark	254.27 ± 38.37	310.49 ± 43.2*	379.56 ± 98.75	257.8 ± 34.79
PEM light	273.6 ± 73.4	401.52 ± 88.03	442.09 ± 132.37	301.3 ± 75.34
PEM dark	243.29 ± 32.51	291.4 ± 67.98*	298.04 ± 55.25*	248.41 ± 37.68

* Represent significant difference with PEM light

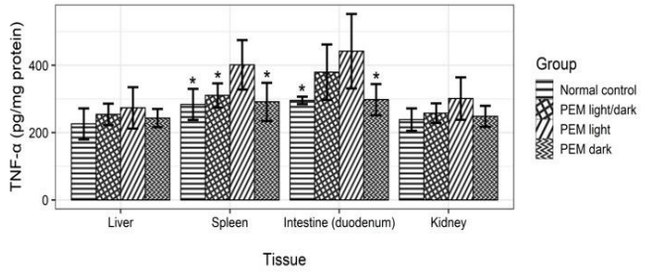

Figure 5. *Results of TNF-α levels in tissue homogenates taken from the animals in the control and experimental PEM groups taken at the end of the study period are shown. The data are presented as mean ± SEM; n=8/group. *p<0.05 between the indicated groups in each tissue sample analysis. PEM, protein energy malnutrition; TNF-α, tumor necrosis factor-α.*

Group	Liver	Spleen	Intestine (duodenum)	Kidney
Normal control	204.06 ± 14.66	246.01 ± 39.54*	230.54 ± 42.31*	252.64 ± 16.47*
PEM light/dark	227.5 ± 32.23	275.63 ± 27.48	264.23 ± 49.87*	259.95 ± 41.71
PEM light	238.65 ± 30.95	297.00 ± 40.84	349.15 ± 97.38	293.78 ± 36.17
PEM dark	226.47 ± 42.65	264.88 ± 16.8	240.08 ± 13.41*	254.94 ± 17.9

* Represent significant difference with PEM light

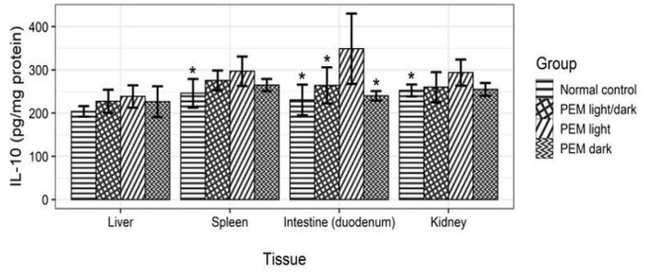

Figure 6. *Results of IL-10 levels in tissue homogenates taken from the animals in the control and experimental PEM groups taken at the end of the study period are shown. The data are presented as mean ± SEM; n=8/group. *p<0.05 between the indicated groups in each tissue sample analysis. IL-10, interleukin-10; PEM, protein energy malnutrition.*

In PEM groups, both number and length of the duodenal villi were decreased, and atrophy was identified in Lieberkuhn and Brunner's glands (Figs. 7, 8, 9, 13) (Table 3). Thinning and single-layered cuboidal epithelium, indistinguishable epithelial-connective tissue boundary, and increased collagen fibers in the lamina propria and duodenal PMNL infiltration

were observed in the PEM light/dark group, although the PEM dark group was similar to that of the control group (Figs. 7, 8, 9, 13) (Table 3).

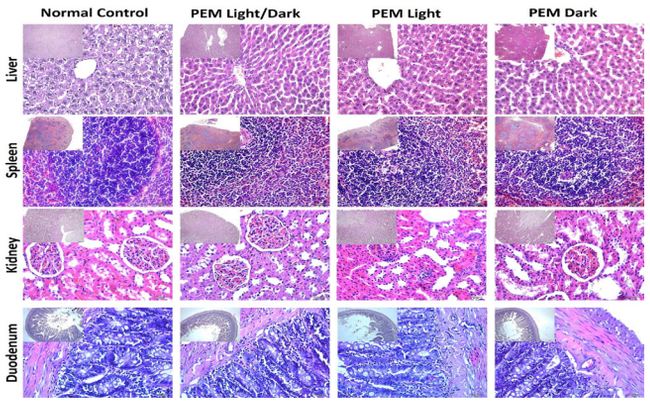

Figure 7. *Representative microscope images of H&E-stained visceral organs (liver, spleen, duodenum, and kidney) in the study groups. Small photos 4x magnification (magnification bar: 20 µm); large photos 40x magnification (magnification bar: 50 µm).*

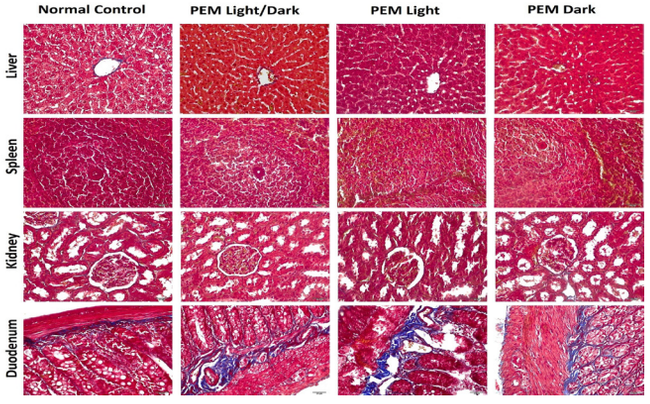

Figure 8. *Representative microscope images of Mallory's trichrome-stained visceral organs (liver, spleen, duodenum, and kidney) in the study groups. Small photos 4x magnification (magnification bar: 20 µm); large photos 40x magnification (magnification bar: 50 µm).*

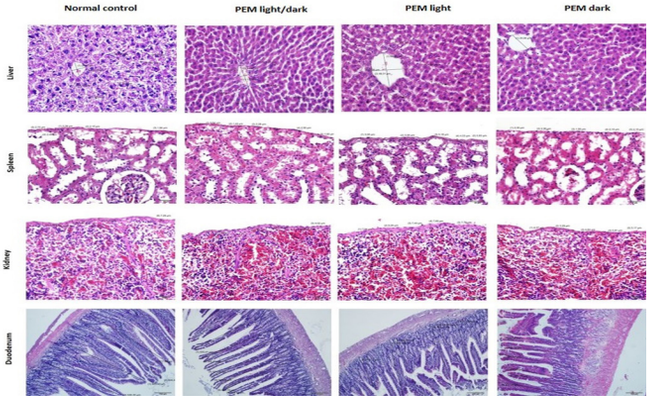

Figure 9. *Histopathology of the tissues of visceral organs (liver, spleen, duodenum, and kidney) in the study groups. 40x magnification (magnification bar: 50 µm). Images of morphometric analyzes made with CellSensEntry 3.1 digital analysis program. Five different measurements were taken from the structures analyzed in 25 different areas and in 25 different sections randomly selected from all animals in each group.*

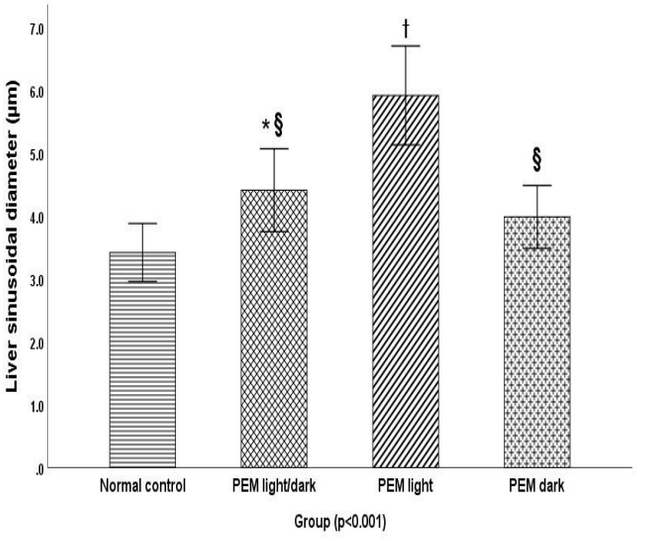

Figure 10. *Results of morphometric measurement of liver tissue sinusoid diameter in the study groups are shown comparatively. The data are presented as mean ± SEM; n=8/group. *Significant difference with control (p<0.05). †Significant difference with control (p<0.01). §Significant difference with PEM light (p<0.01).*

In the PEM groups, there was decreased size, volume, and glomeruli number of the kidney, while there was increased collagen in the kidney, resulting in glomerulosclerosis and interstitial fibrosis (Figs. 7, 8, 9, 14, 15) (Table 3). Likewise, vacuolization, cystic dilatation, vasodilatation and tubular congestion, and edematous glomerular mesangial cells were also observed (Table 3). However, the findings in the PEM dark group were similar to those of control animals (Figs. 7, 8, 9, 14, 15). Morphometrically, the group having the greatest capsule thickness was the PEM light/dark group (Fig. 14), while the group with the largest space in Bowman's capsule was the PEM light group (Fig. 15).

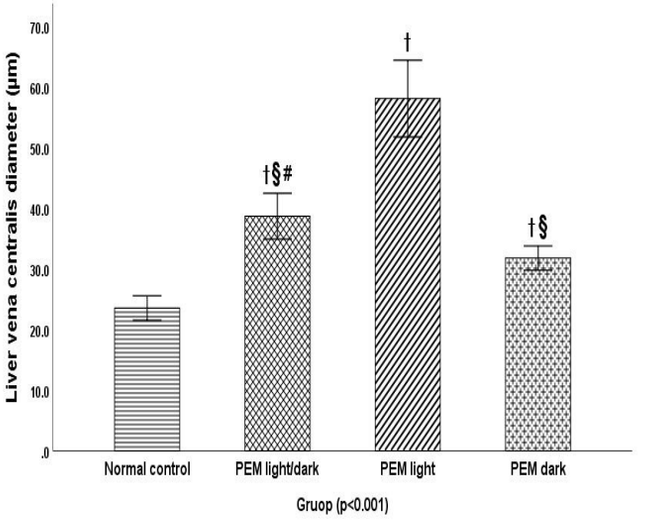

Figure 11. *Results of morphometric measurement of liver tissue vena centralis diameter in the study groups are shown comparatively. The data are presented as mean ± SEM; n=8/group. †Significant difference with control (p<0.01). §Significant difference with PEM light (p<0.01). #Significant difference with PEM dark (p<0.05).*

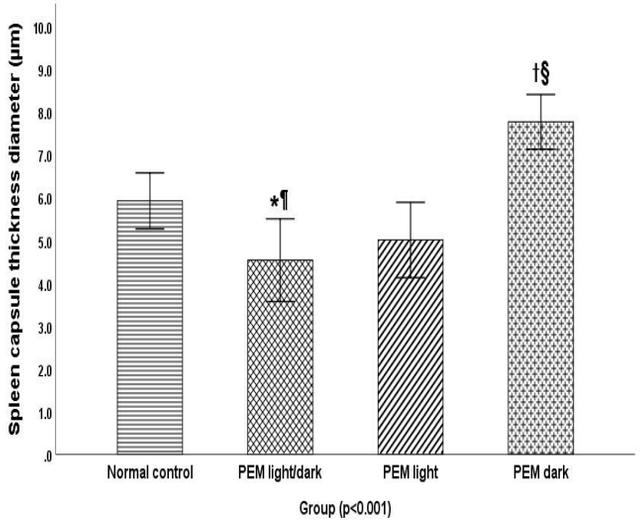

Figure 12. *Results of morphometric measurement of the spleen tissue capsule thickness in the study groups are shown comparatively. The data are presented as mean ± SEM; n=8/group. *Significant difference with control (p<0.05). †Significant difference with control (p<0.01). §Significant difference with PEM light (p<0.01). ¶Significant difference with PEM dark (p<0.01).*

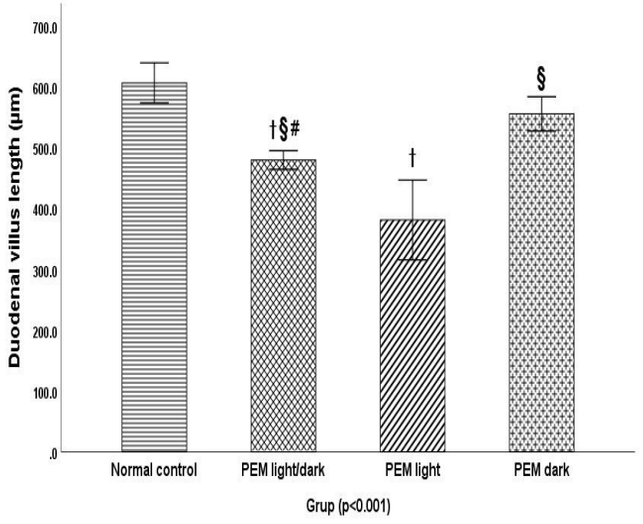

Figure 13. *Results of morphometric measurement of duodenal villus length in the study groups are shown comparatively. The data are presented as mean ± SEM; n=8/group. †Significant difference with control (p<0.01). §Significant difference with PEM light (p<0.01). #Significant difference with PEM dark (p<0.05).*

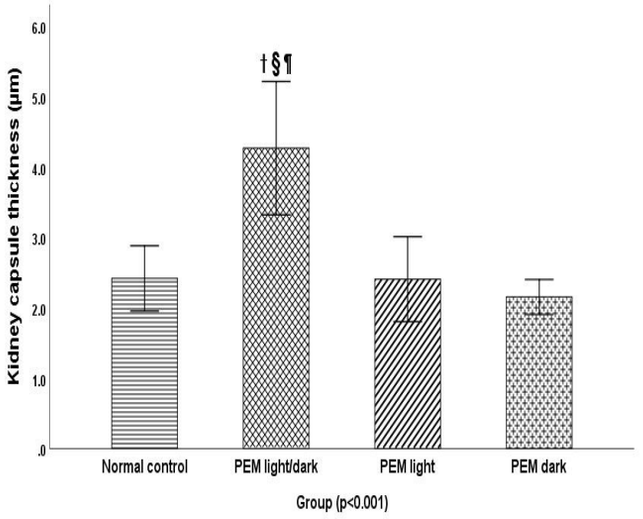

Figure 14. *Results of morphometric measurement of kidney capsule thickness in the study groups are shown comparatively. The data are presented as mean ± SEM; n=8/group. †Significant difference with control (p<0.01). §Significant difference with PEM light (p<0.01). ¶Significant difference with PEM dark (p<0.01).*

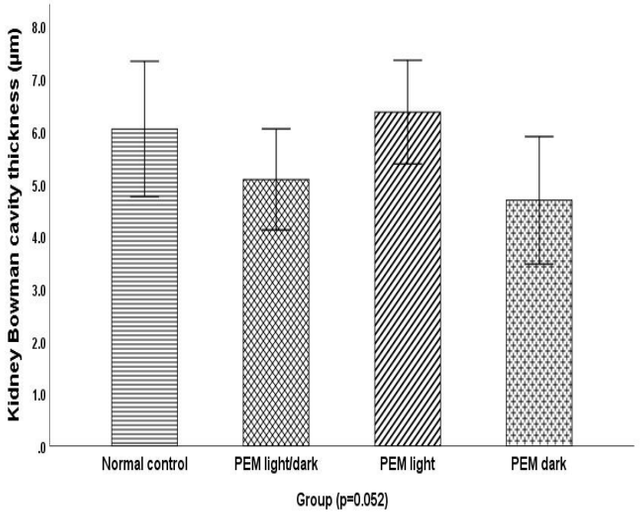

Figure 15. *Results of morphometric measurement of kidney tissue Bowman capsule cavity in the study groups are shown comparatively. The data are presented as mean ± SEM; n=8/group.*

4. DISCUSSION

In this study, we found that the biochemical and morphological findings were milder in the PEM dark group, while they were most prominent in the functional Px group, suggesting that there are potential effects of different light/dark cycles on immunity, hematopoietic, gastrointestinal and urinary systems.

Amongst the factors affecting the development of the human body, the most important one is nutrition and PEM can lead to various infectious diseases by affecting the immunity and

inflammatory response (22,23). In the case of PEM, various morphological and functional changes related to the visceral organs like the liver, spleen, intestine and kidney in addition to hematopoietic and immune systems may occur (24). There is an increase in lipid peroxidation and weakening of the antioxidant defense system, resulting in morphological and functional changes in the visceral organs of individuals with PEM (25). It has also been reported that macrophage activation, cytokine production, dysfunction of T-lymphocytes and cell death occur as a result of apoptosis and cell necrosis in immunity-related organs (4,6,26,27,28,29). Cortes-Barberena et al. (27) reported reduced spleen/body weight ratio, T-lymphocyte count, and cell number and proliferation in different phases of the cell cycle in the spleen of rats with PEM. We found severe atrophic changes in the splenic and intestinal lymphoid structures and a prominent lymphocytopenia in the blood of PEM groups, although the changes were milder in the animals from the PEM dark group, possibly due to endogenous MEL secretion.

Further studies have revealed reduced cell proliferation in the bone marrow and increased DNA damage in lymphoid tissues, including the spleen and circulating lymphocytes (27,30). Ortiz et al. (4) suggested that splenic atrophy occurs due to increase in spontaneous apoptosis in the rat PEM model. Likewise, Santos et al. (31) reported hematopoietic alterations associated with bone marrow atrophy, and impaired homeostasis and immune response. Cortés-Barberena et al. (27) claimed that PEM increased the time for DNA synthesis and the total cycle of the splenic cell. We determined an increased thickness in the splenic capsule but decreased cords in the red pulp in all PEM groups, consistent with previous studies (5,28,31). More importantly, the changes were milder in the animals in the PEM dark group, suggesting a mitigating effect on the aforementioned effects.

In acute and chronic PEM, the lymphopoiesis is impaired due to apoptosis (4,28). Mello et al. (32) found an increase in both the percentage of T-cells and the production of anti-inflammatory IL-10. Similarly, an elevated IL-10 level was found in serum of newly weaned mice fed a low-protein diet (33). Our results confirm a defense mechanism acts to control inflammation in rats with PEM, especially in the functional Px group, although it is difficult to say whether the increase is due to protein or energy/calorie deficiency. Furthermore, lower serum albumin levels related with protein metabolism and an antioxidant defense system in PEM groups are consistent with reports in the literature (3,14,34). We think that the partial increase in the albumin level of the animals in the PEM dark group may be associated with the reparative effects of endogenous MEL on the liver.

In individuals with PEM, normochromic normocytic anemia occurs due to various reasons (5,26) and in addition to energy and/or protein deficiency, deficiency of iron or micronutrients such as vitamins and trace elements cause certain negative effects on erythropoiesis (35). In PEM, erythrocyte and reticulocyte counts are low due to decreased oxygen consumption and erythropoietin production as well as impaired erythroblast maturation (34,36,37). It is widely accepted that PEM affects the hematopoietic system, resulting with hypoplasia/atrophy and decreased myeloid/erythroid cell proliferation in the bone marrow (5,26,31). The hemopoietic tissues have high rates of self-renewal and proliferationand the coexistence of anemia and leukopenia in individuals with PEM confirms the high protein requirement for hematopoiesis (5,26). We think that low values of the erythrocyte count and hemoglobin in PEM groups is associated with suppression of erythropoiesis and the short life span of erythrocytes. However, animals in the PEM dark group had higher CBC values compared to other PEM groups, suggesting a stimulatory effect of endogenous MEL on erythropoiesis. Similarly, splenic hypocellularity and decreased erythrocyte count in the animals from PEM groups, in addition to an increased splenic capsule thickness, suggest a negative effect on the hematopoietic system.

Even today, the morphological and functional changes in the digestive tract, including the liver and duodenum, are still debated (34,38). Previously, Miguel Parra et al. (39) reported decreased weights of visceral organs such as the liver, spleen, and kidney in acute PEM, in addition to a decreased size and number of their parenchymal cells. Comparably, the intestinal mucosa is markedly thinned, with a decrease in the height/number of villi with structural blunting and lymphocyte infiltration (34,38). In contrast, some authors found a partial increase in liver cell ballooning and steatosis, edema, hypoalbuminemia, and anemia in PEM animal models (40,41). It has been reported that mice exposed to PEM in the neonatal period have increased inflammation and oxidative stress in liver (41). In an acute PEM piglet model, Lykke et al. (34) found vacuolization and lipid droplets in hepatocytes, in addition to decreased BW, intestinal atrophy and anemia.

Recently, chronic inflammation and immune dysfunction in the intestines of animals with PEM have been reported (42). Some authors reported an increased level of IL-10 in the liver and jejunum as a response to systemic inflammation to reduce the effects of IL-1β and TNF-α secreted by monocytes and macrophages (43,44). Similarly, Dewan et al. (45) reported an increase in serum TNF-α and IL-10 levels in children with PEM. In our study, MDA, TNF-α, GSH and IL-10 activities were found to be high in the liver and duodenum in all PEM groups, but there was a significant improvement in those of the PEM dark group, suggesting anti-inflammatory effects of MEL upon the gastrointestinal system. However, it is difficult to make a comment regarding the functional features of the duodenums of rats exposed to PEM, such as the wall permeability, motility and enzyme activities. Moreover, the effects of severe PEM on the intestinal tract are well-known, but those related with mild or moderate PEM are not clear (46). Therefore, our experimental model is invaluable, because it demonstrates the changes in the small intestine consistent with mild to moderate PEM. Our morphological findings were most prominent in the functional Px group, while they were minimal in the PEM dark group, suggesting a hepatoprotective effect of MEL. Additionally, an increase in Bowman's capsule space due to the presence of interstitial fibrosis and glomerular degeneration, but a decrease in both weight and volume of the kidney occur in individuals with PEM, resulting in clinical

hypertension and chronic renal failure (24). Santoso et al. (24) demonstrated a close relationship between PEM and the immunological status of the kidney by assessing macrophages producing proinflammatory cytokines such as TNF-α, IL-6, and TGF-β cytokines in mice, as also seen in our study. Based on our findings, we expect an improvement in the renal function of animals in the PEM dark group, although confirmation of this idea will only be possible with additional studies in future.

This experimental study is the first in the literature to evaluate the effects of functional Px on immunity, hematopoietic, gastrointestinal, and urinary systems in malnourished rats. Furthermore, it is worth emphasizing that the rat PEM model is easy, applicable, and reliable, but there are some limitations regarding the applicability of the results obtained in this model to humans, and the measurement of the serum level of MEL and levels of pro-inflammatory/anti-inflammatory cytokines. In this study, we found that animals with PEM with reduced BWs and weights of visceral organs had hypoalbuminemia, anemia, leukopenia, and lymphopenia, and higher MDA, GSH, TNF-α and IL-10 levels in tissue samples. Our morphological findings also revealed a reduction in parenchymal cell count of the visceral organs, lymphoid structures of the spleen and intestine, number and height of intestinal villi, kidney glomeruli count, but an increase in the spleen capsule thickness and kidney Bowman's capsule cavity, and sinusoidal congestion and fat accumulation in the liver. Importantly, these findings were mild in the PEM dark group, in contrast to those of the functional Px group. In summary, we speculate that keeping adult patients hospitalized, especially in intensive care units, with PEM in a continuous light environment will have negative effects on their health.

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Analysis of data for the study: YBT, YU, CT, MY, ÖÇ

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Clinical and Experimental Health Sciences

Salivary pH Changes and Biofilm Formation During Active Orthodontic Treatment with Clear Aligners and Fixed Appliancese

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ABSTRACT

Objective: To examine salivary pH changes and the plaque formation in two different orthodontic treatments clinically.

Methods: The study sample included 40 patients, who were divided into two groups according to the type of orthodontic appliance: Group CA, (n=15) clear aligners; Group FT, (n=25) fixed appliances. Group FT received both metal (FT/SS) and elastic ligatures (FT/EM) for 2 weeks respectively to test the effect of ligature type also. Salivary pH values, plaque index, and plaque percentage were measured at T0 (after scaling and polishing) and T1 (after 2 weeks). pH was measured with a digital caliper (HI 2211 pH/ORP Meter) and plaque was identified by a discoloring agent (Tri Plaque ID Gel). The Paired t-test, Independent t-test, Anova test and Pearson Correlation tests were used in the statistical analysis.

Results: There was significant decrease in salivary pH values after two weeks of metal ligature in FT/SS. Plaque index and plaque percentage parameters showed significant incremental changes between groups with the least increase in CA, followed by FT/SS and FT/EM respectively.

Conclusion: Different types of orthodontic treatment and ligatures significantly effected salivary pH and the amount of plaque formation during orthodontic treatment. Aligners had the least effect on salivary pH and plaque formation while fixed treatment with elastic ligature affected the most. Therefore, aligner treatment may be more beneficial for patients with compromised oral hygiene.

Keywords: Orthodontic appliances, corrective orthodontics, saliva, dental plaque

1. INTRODUCTION

Orthodontic treatment has become increasingly popular in both adolescent and adult patients (1). Fixed appliances constitute the conventional treatment method in orthodontics to restore esthetics and function (2). However fixed appliances can impede brushing and decrease self-cleansing by the saliva, mastication and tongue (1,3). This consequently results in plaque accumulation, which impairs gingival health (4). It has been reported that comprehensive orthodontic treatment on average requires a mean treatment time of 20 months, with a wide range of treatment durations such as 14-33 months (5). Excessive treatment duration and fixed appliances have been associated with a greater susceptibility to adverse effects, including root resorption (6) and plaque-induced conditions, primarily demineralization (7) and microbial changes (8). Therefore, it has been recommended for clinicians to consider the effects that orthodontic treatment, including appliance type, may have on periodontal health (9).

Clear aligners (CA) are removable orthodontic appliances, which are considered as esthetic and comfortable alternatives to fixed treatment (10). Their removable nature makes it easier to maintain dental hygiene than fixed appliances (11). In order to prevent periodontitis, clear aligners have been recommended in orthodontic treatment plans (11-14) and they have been linked to better periodontal health and lower levels of periodontopathic bacteria (11). However, some investigators emphasize that it is important to have a sound judgment regarding the periodontal effects of clear aligners considering that they cover teeth and keratinized gingiva for most of the day (12,15).

Whole saliva, which contains oral germs and food particles, is a complex mixture of fluids produced by the major and minor salivary glands as well as the gingival cervical fluid (16). pH is one of the qualitative properties of saliva (17). Along with other qualitative (salivary protein content, viscosity and buffer capacity) and quantitative properties (the flow rate), salivary pH aid in the equilibrium between demineralization and demineralization of enamel (17). The average pH of whole, unstimulated saliva is typically between 6.75 and 7.25, controlling the pH of the majority of oral surfaces (16).

Dental plaque is a highly complex organization in a biofilm form (1) and is considered the main causative factor in dental caries and periodontal disease (1,18). The pH of plaque is about 6.7. Enamel demineralization occurs when the pH falls below 5.5, which is considered essential (19). This decrease aids in the

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development of white spot lesions, which are said to affect 50% of orthodontic patients (20). Streptococcus mutans and Lactobacilli, two bacteria that produce acid, are the primary produced colonies of particular interest (21). It has been found that the type of orthodontic materials such as the type of archwire ligation material has an impact on plaque harboring surrounding the brackets (21,22). According to most studies, stainless steel (SS) ligatures showed less plaque retention than elastomeric modules (EM) whereas; higher concentrations of acidogenic bacteria can be found with EM ligatures, most notably Streptococcus mutans and Lactobacilli (3,22,23).

The aim of this study was to assess changes on biofilm accumulation and oral cavity pH in different treatment modalities (clear aligners and fixed appliances with metal or elastic ligation) during active orthodontic treatment, an aspect which has not been studied previously; under the null hypothesis that there were no significant differences between different orthodontic appliances on plaque accumulation and saliva pH during active treatment.

2. METHODS

This randomized, cross-sectional clinical trial was approved by the Marmara University Clinical Research Ethics Committee, on 21.02.2022 and with the number 09.2022.297. Two groups of active orthodontic treatment patients who have been under treatment for at least three months in the clinic of Orthodontic Department, Collage of Dentistry, Marmara University, were selected randomly from the active treatment patients' list. All patients or their guardians had provided signed informed consent forms. It was calculated that to have 80% power to detect an effect size, it would be sufficient to have a total sample size of n = 30.

Inclusion criteria were: having non-extraction orthodontic treatment either with fixed appliances or clear aligner treatment for 3-6 months, skeletal and dental Class I malocclusion (SNA: 82°, SNB: 80°) with mild to moderate crowding (3-7 mm) and normal vertical growth pattern (mandibular plane angle: 25°, sum of inner angles: 396°, maxillary height: 60°), age between 16-24 years, orthognathic profile with lip competency, good oral hygiene, no drug usage, same brand and series of brackets in fixed treatment, same manufacturer in clear aligner treatment, permanent dentition. Exclusion criteria were: having less than three months of treatment, single arch undergoing orthodontic treatment, having additional palatal or lingual appliances or attachments (i.e. hyrax screw, transpalatal arch, lingual button etc.), presence of systemic diseases or mouth-breathing, pregnancy, smoking, poor oral hygiene, interrupt or discontinue treatment, previous orthodontic treatment, crown restorations, active periodontal disease or caries. Also, for CA group, all subjects had a minimum of 9 attachments per jaw between 1st molars. 2nd molars were omitted in this regard since FT patients were also bonded between the 1st molars.

The clear aligner group (CA) included 15 aligner orthodontic treatment patients (9 males and 6 females; mean age 17.5 years). The fixed treatment group (FT), contained 25 fixed orthodontic treatment patients (13 males and 12 females; mean age 18.2 years) who went through 2 phases during study: metal ligation for 2 weeks and elastomeric ligation for 2 weeks, respectively. Demographic characteristics of the patients are shown in Table 1.

***Table 1**. Demographic characteristics of the enrolled patients*

Characteristics		CA	F FT	Total
Number of subjects (n)		15	25	40
Gender [A]	Male	9 (60%)	13 (52%)	22 (55%)
	Female	6 (40%)	12 (48%)	18 (45%)
Age (Years) [B]	Male	17.9	17.6	17.7
	Female	16.9	18.8	18.2
Total		17.5	18.2	17.9
pH measurement at T0 [B]		7.08 ± 0.29	7.18 ± 0.27	7.13 ± 0.28
Plaque index and percentage		00	00	00

CA: Clear Aligners; FT: Fixed treatment; [A]: Qualitative data expressed as frequency and percentage. [B]: Continuous data expressed as mean ± standard deviation

Prior to treatment, all patients were referred to Periodontology Department and underwent meticulous phase 1 periodontal therapy. At this stage, patients received oral hygiene instructions. Additionally, at the bonding appointment, all patients received a second instructive session on how to maintain good oral hygiene with orthodontic appliances. During orthodontic treatment, two investigators observed all patients and confirmed that subjects had acceptable oral hygiene habits throughout.

At the time of measurements, patients were asked not drink or eat anything except water over the night until their morning appointment. Periodontal scaling and polishing (Prophylaxis) was done. Tri Plaque ID Gel (GC Corporation, Tokyo, Japan) was used to identify all plaque areas. Scaling was done with ultrasonic and hand instruments to make sure that all the plaque has been removed and the plaque score was made 0 by the same researcher. In proximity of the bonded attachments or brackets, additional care was taken in order not to cause debonding. Hand instruments were preferred in these areas. Prophy cups and brushes were also used. As T0 measurements, salivary pH, plaque index and plaque percentage were recorded. 2 weeks after (T1), patients were asked to come again for the follow up measurements.

At both time-points, firstly the unstimulated saliva samples were collected in the morning between 9 A.M. and 12 P.M. Before taking the saliva sample, patients were asked to rinse their mouth with distilled water and asked to swallow the remnants till they felt their mouth dry. Then the patients pooled the unstimulated saliva in their mouth for 2 minutes, and were asked to drool 5 ml of the pooled saliva passively in 10 ml plastic lab tube. Saliva pH was measured with HI 2211 pH/ORP Meter (Hanna Instruments Inc, USA) with PH Probe Composite Electrode (Elprico, Shenzhen, China). The probe sensor was allowed to equilibrate with the environment before each measurement and was rinsed with distilled water spray and wiped dry gently. pH 4 and pH 7 buffering agents

were used for calibration. Samples were measured in the same order of the appointments. Between each sample, the pH probe was rinsed with distilled water and wiped gently to dry.

After the saliva sample has been collected, Tri Plaque ID Gel was applied on the distal, labial and mesial surfaces from tooth #17 to tooth #47. Patients were asked to rinse their mouth for 2 minutes for the discoloring gel to color the biofilm formed on the teeth surfaces. At T0, the prophylaxis procedure provided that the plaque scores were set 0 as the baseline. At T1, plaque index scores were given depending on the plaque mean index standards: 0, meaning there is no plaque; 1: there is biofilm but a very shallow amount of immature plaque that was formed newly in the past 48 hours and the gel dyes it in pink color; 2: moderate amount of biofilm which the gel dyes in purple color; and 3: mature thick biofilm identified as the acidic plaque pH (< pH 4,5) that has the tendency of enamel demineralization and caries formation, gel dyes it in light blue color. These scores were determined by two investigators and recorded on a periodontal chart and then transferred to an excel sheet and photos were taken. For plaque percentage measurements, the total number of teeth surfaces that presented plaque was divided over to total number of teeth surfaces that were examined. These percentages were also noted in the excel data sheet.

In FT group, first phase included the T0 and T1 measurements with 0.010-inch stainless steel ligatures placed (FT/SS). The second phase included prophylaxis, T0 and T1 measurements with elastomeric ligatures placed in the same patient group (FT/EM). All FT patients had nickel titanium wires with 0.022x0.025-inch slot brackets (Rocky Mountain Orthodontics, Franklin, USA) for more standardization. At the end of the study patients were shown the areas that they need to clean more properly; oral hygiene instructions were elaborated.

Statistical Analysis

The data was analyzed using IBM SPSS (Statistical Package of Social Sciences; IBM Corp, NY, USA) software for Windows, version 28.0.

Statistical methods were used to analyze the data, including the calculation of descriptive statistics such as the frequency and percentage for categorical variables, and the mean, the standard deviation (SD), and the minimum and maximum for the continuous variables.

The Shapiro-Wilk test of normality was applied to evaluate the normal distribution of the parameters, and since the data was found to support parametric assumptions, a paired-samples t-test was performed to compare between the before and after scaling and polishing with two weeks of orthodontic treatment measurements for the same group of patients in fixed group and for patients with aligner treatments. Additionally, the paired t-test was performed to compare the measurements between T0 and T1. Pearson correlation test was performed to evaluate the associations between specific corresponding variables. ANOVA test was performed to evaluate if different types of orthodontic treatment have a significant effect on the pH change and plaque accumulation. An alpha level of .05 was used for all statistical tests and all were two-tailed.

3. RESULTS

The total sample was 40 patients. The majority of the sample was male (n=22, 55%) (Table 1).

In the CA group, the pH change was insignificant at T1-T0 ($p > .05$); while a statistically significant increase in the mean of plaque index and plaque percentage values ($p < .001$) were observed (Table 2). At T1, the highest dental plaque accumulation was observed at the upper right quadrant whereas; the least plaque accumulation was on the lower left quadrant (Figure 1).

***Table 2**. Evaluation of the changes in Clear Aligner (CA) group*

	T0	T1	Mean Difference	P value#
	Mean ± SD	Mean ± SD		
pH	7.081 ± 0.286	7.028 ± 0.332	0.053	.575
Plaque index	00 ± 00	0.205 ± 0.103	-0.205	.00*
Plaque percentage	00 ± 00	20.509± 10.383	-20.509	.00*

*Paired-samples t-test. *: The mean difference is significant at the .05 level; SD: standard deviation.*

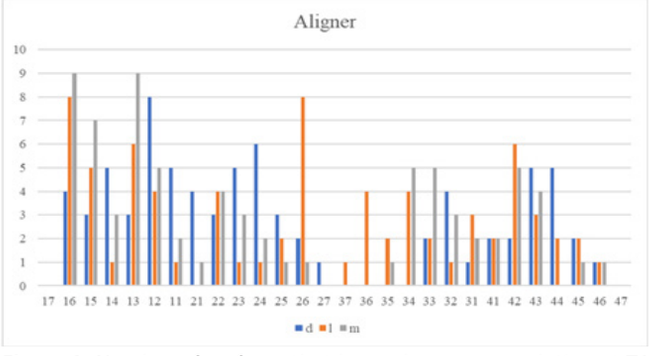

***Figure 1.** Number of surfaces that have plaque accumulation at T1 of CA. d: distal, l: labial and m: mesial.*

For the FT group; FT/SS showed a statistically significant decrease in the mean pH value ($p < .05$). At T1, plaque index and plaque percentage means were significantly higher than the mean at T0 as shown in Table 3. The highest dental plaque accumulation was observed on the labial/buccal tooth surfaces at T1 (Figure 2).

***Table 3.** Evaluation of the changes in FT/SS group*

	T0	T1	Mean Difference	P value#
	Mean ± SD	Mean ± SD		
pH	7.192 ± 0.312	6.956 ± 0.351	0.236	.027*
Plaque index	00 ± 00	0.481± 0.151	-0.481	.00*
Plaque percentage	00 ± 00	41.104± 10.946	-41.104	.00*

*Paired-samples t-test. *: The mean difference is significant at the .05 level; SD: standard deviation.*

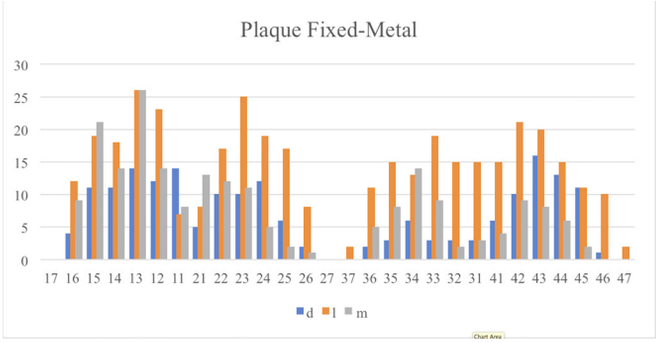

***Figure 2**. Number of surfaces that have plaque accumulation at T1 of FT/SS. d: distal, l: labial and m: mesial.*

In FT/EM group, there was a statistically insignificant decrease in the mean pH value ($p > .05$). The plaque index and plaque percentage means were significantly higher at T1 than the mean at T0 ($p < .001$) (Table 4). Similar to FT/SS, the highest dental plaque accumulation was observed on the labial/buccal tooth surfaces at T1 (Figure 3).

***Table 4.** Evaluation of the changes in FT/EM group*

	T0	T1	Mean Difference	P value#
	Mean ± SD	Mean ± SD		
pH	7.166± 0.230	7.054± 0.341	0.112	.143
plaque index	00 ± 00	0.610± 0.149	-0.610	.00*
plaque percentage	00 ± 00	55.886± 11.729	-55.886	.00*

*Paired-samples t-test. *: The mean difference is significant at the .05 level. SD: standard deviation.*

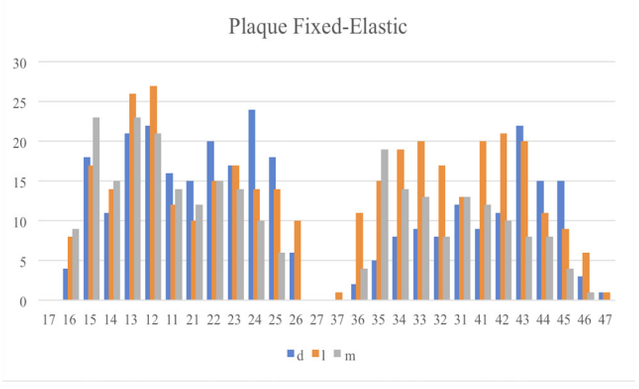

***Figure 3.** Number of surfaces that have plaque accumulation at T1 of FT/EM. d: distal, l= labial and m= mesial.*

Intergroup comparisons revealed a significant difference between all groups in plaque index and percentage parameters (Table 5). For the salivary pH, a significant difference was found between the means of pH change between CA and FT/SS groups, FT/SS and FT/EM groups; while insignificant difference was found between CA and FT/EM groups.

***Table 5.** Intergroup comparisons for all parameters.*

Parameters	Groups	P value		
		CA	FT/SS	FT/EM
PH	CA		.0004	.527
	FT/SS	.0004		.0001
	FT/EM	.527	.0001	
Plaque index	CA		.0005	.0001
	FT/SS	.0005		.0001
	FT/EM	.0001	.0001	
Plaque percentage	CA		.0002	.0006
	FT/SS	.0002		.0001
	FT/EM	.0006	.0001	

CA: Clear aligner; FT/SS: Fixed treatment with stainless steel ligation; FT/EM: Fixed treatment with elastomeric module ligation

Plaque index and pH correlations within groups are shown in Table 6. In all groups, the two parameters were found to be correlated with a weak negative relation and the correlations were statistically insignificant.

***Table 6.** pH and plaque index correlations in three groups.*

		Plaque index		
		CA	FT/SS	FT/EM
pH	Pearson Correlation Coefficient	-0.322	-0.168	-0.36
	P value	.242	.421	.255

CA: Clear aligner; FT/SS: Fixed treatment with stainless steel ligation; FT/EM: Fixed treatment with elastomeric module ligation

4. DISCUSSION

Orthodontic patients go through different kinds and methods of treatments that have the same goal of improving the patients' esthetics and functions by reaching the ideal occlusal relationships and alignment (1). As clinicians, we needed to consider the side effects of the orthodontic treatment that may impair treatment outcome and the patient's oral health (1,6-9). Decrease in the salivary pH and biofilm formation can lead to serious oral health problems like dental caries and periodontal tissue inflammations, which lead to shortening in the tooth longevity and esthetics (1).

Primary aim of this randomized clinical study was to evaluate the changes that happen in the oral cavity (regarding salivary pH and biofilm formation) during active treatment with different kinds of orthodontic appliances: clear aligners and fixed appliances with two ligation methods (elastic and stainless steel). The secondary aim was to assess if changes in oral biofilm formation were associated with the change of salivary pH. Several aspects of orthodontic appliances on oral environment such as their impact on tooth wear (19), demineralization (20), oral microbiome (21-23), salivary properties (24,25) have been assessed in a number of earlier researches. However, the impact of the present three kinds of orthodontic materials on salivary pH and biofilm development has not been examined previously.

In the present study, plaque index and plaque percentage parameters showed significant incremental changes between groups with the least increase in CA, followed by FT/SS and FT/EM respectively. However, pH values did not show a parallel increment with the plaque parameters and there was no significant correlation between pH and plaque accumulation. Actually, the pH values that were obtained at both time points can be considered within the average range (pH 6.2-7.6) of saliva (26). This finding can be a result of the study design. Although initial pH values were measured after a prophylaxis application, patients were already undergoing the treatment at that time-point. And at the second measurement, patients were still undergoing the treatment. The change in pH could be more significant if the pretreatment pH values were available to evaluate the effect of treatment modality on the salivary pH in a prospective study design. Therefore, it can be stated that the pH of the unstimulated saliva was independent of plaque accumulation parameters while undergoing treatment. Several investigators reported similar results to our findings regarding the salivary pH and stated that salivary pH did not significantly change between the studied time points during fixed orthodontic treatment (27-29).

However, there is a lack of consensus on this issue. Alshahrani et al (25) evaluated the alterations in salivary parameters prospectively in patients undergoing fixed orthodontic therapy. They found a significant reduction in salivary pH, total protein concentration, and calcium level in saliva of fixed orthodontic appliances group. On the contrary, Chang et al (30) reported a significant increase in stimulated salivary pH, flow rate, buffer capacity, plaque index scores, and in the levels of streptococcus mutans and lactobacilli after three months of active treatment. However, these studies did not specify the type of ligation material. Al-Haifi et al (31) compared the short-term effect of SS and EM ligatures and concluded that EM ligatures showed a significant decrease in salivary pH. This result was contradictory to our findings, where the SS group decreased more than EM. These studies differ from the present study in methodology, since they compared the pH values of before and after treatment in a prospective design. Another contributory factor in this difference can be the limitations of salivary pH as a diagnostic bio-meter. Several uncontrolled factors such as the diet, lifestyle, and salivary flow rate can affect its value (26). In this study, these factors were aimed to be controlled by having the values of T0 and T1 from each patient at the same hour of the day, without any influence on their lifestyle or diets. Patients were asked not to eat or drink anything before the appointment overnight, and unstimulated (resting) whole salivary samples were collected in a separate room with a quite environment to prevent mechanical or chemical stimuli as in previous studies (24,25).

The increase in plaque accumulation is a direct consequence of impeded oral hygiene procedures (13). Plaque accumulation can favor the transition of the microbial biofilm to a more aggressive periodontopathogenic flora (1). The current findings regarding plaque index and percentage are in accordance with the literature (13,14). A meta-analysis by Jiang et al (14) compared periodontal health in patients undergoing orthodontic treatment with clear aligners with that of those undergoing orthodontic treatment with fixed appliances. They concluded that clear aligners were better for periodontal health, including plaque index, gingival index, and probing depth than were fixed appliances (14). This expected result can be explained by the removable nature of CA, which provides an ease of access to dental and interdental surfaces during brushing (13). Also, retentive areas are much less in CA compared to fixed appliances for two reasons. Firstly, not all teeth receive attachments; in many cases one or two teeth can be free of them. In the present group, all posterior teeth including 1st molars and the premolars had attachments mainly for retentive purposes and for premolar rotations while one or two of anterior teeth did not require attachment placement. Secondly, their surface designs are either convex or flat, whereas brackets have indentations, concavities and undercuts (13).

Regarding SS and EM ligatures, the differences in surface topography, organic content and inertness of these materials are considered as factors that cause different bacterial colonization patterns (32); which is enhanced in EM (3,22,23) due to its organic and porous surface. EM ligature materials are thicker in dimension; therefore, blocking the teeth surface more and creating narrow areas that cannot be cleaned. The current findings are consistent with those of Forsberg et al (23), who found that EM ligatures had greater levels biofilm formation. They also reported increased Streptococcus mutans and Lactobacillus colonization with EM than SS ligatures and recommended to avoid using EM on individuals who did not maintain good dental hygiene. Turkkahraman et al (3) reported that elastomeric rings were more likely to cause bleeding than steel ligatures, but they did not find any appreciable differences in bleeding upon probing or plaque index values. On the other hand, Souza et al (33) related elastomeric rings, as opposed to steel ligatures, to higher scores for bleeding on probing and plaque index. These results indicate that patients treated with fixed appliances are more likely susceptible to gingival inflammation (13).

As a general observation, it was noticeable that the first quadrant of the mouth (upper right) had the most plaque accumulation, concentrated between the lateral incisor and canine area. But the teeth that had most plaque accumulated on its surfaces was the right maxillary first molar, which is due to the reason that most of the sample size were right-handed people and they could not use the correct brushing technique due to the difficulty of accessing the area. Lower left quadrant had the least biofilm formation, which can be explained by contentious salivary flush plus the muscles movements during speech and mastication providing as a physical rub for the buccal surfaces of the teeth in addition to the easier access for tooth brushes and other oral hygiene instruments. Despite that, still plaque accumulated on the buccal surfaces of these teeth. The short follow-up period and limited number of subjects were limitations of this study. A longer observational time in a larger sample and including other periodontal indices can be more informative for an evaluation of the periodontal outcomes of orthodontic

treatment modalities. Another limitation can be considered as the lack of attachment standardization in CA group. It is not possible to have same attachments on the same teeth in all aligner patients due to the nature of orthodontic therapy with aligners. These cases were designed uniquely, according to the individual needs of the malocclusion. However, this issue was tried to be controlled by the inclusion criteria. All subjects in CA group had attachments on a minimum of 9 teeth per jaw.

Orthodontic therapy is specific to patient. Both fixed and removable appliances are vital tools of orthodontic treatment. Each material has its specific properties, designed to achieve the primary goals of orthodontic therapy; while the clinician holds the priority to choose the most beneficial modality for the oral and general wellbeing of the patient. Clinicians should take periodontal effects of appliances into consideration while making this choice, especially in compromised cases.

5. CONCLUSION

The null hypothesis was rejected. The type of orthodontic materials affected the mean plaque index and percentage. The aligner group had the least levels of plaque index, followed by fixed treatment with SS and EM ligatures respectively. Orthodontic treatment with clear aligners can be more beneficial for periodontally compromised patients.

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Author Contributions:
Research idea: YYMAE
Design of the study: YYMAE, YBA
Acquisition of data for the study: YYMAE
Analysis of data for the study: YYMAE
Interpretation of data for the study: YYMAE, YBA
Drafting the manuscript: YYMAE, YBA
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Clinical and Experimental Health Sciences

Turkish Adaptation of Healthy Habits Questionnaire for Adolescents: Diet, Physical Activity, Screen Time, and Sleep Habits

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ABSTRACT

Objective: The main purpose of this study was to adapt the adolescent Healthy Habits Questionnaire (HHQ) to the Turkish language and to test its validity and reliability. The study was also examined the diet, physical activity, screen time, and sleep habits of adolescents.

Methods: This study is a methodological and descriptive design. The sample of the research consists of 320 students. The validity of the HHQ was evaluated by the content validity index, the reliability by test-retest correlations, and the kappa coefficient of agreement. Number and percentage values were used for descriptive features.

Results: The content validity index of HHQ was found to be 0.96. The test-retest correlations of questions containing continuous variables were found to be between 0.44 and 0.91. Kappa values of questions containing categorical variables were between 0.7 and 0.94. It was found that 91.8% of the adolescents consumed less than three servings of vegetables, 76.9% of them consumed less than 2 servings of fruit, and 80.5% of them consumed less than 2 glasses of milk. In addition, it was determined 40.2% of the adolescents used screen use more than two hours, 47.6% did less than one hour of physical activity and 30.2% slept less than eight hours.

Conclusion: HHQ was found as a valid and reliable measurement tool. It was determined that the adolescents did not consume enough vegetables, fruits, water, and milk and did not engage in sufficient physical activity.

Keywords: Adolescence, fruit, screen time, sleeping, vegetable.

1. INTRODUCTION

Adolescence is a period between the ages of 10-19, from childhood to adulthood a rapid change is experienced in terms of physical, psychosocial, and cognitive aspects (1). This period is very significant when adolescents are open to preventable health problems and determine their health behaviors (1,2).

Many chronic health problems such as obesity, Type 2 diabetes, hypertension, and cardiovascular diseases are associated with sedentary life and unhealthy eating habits. There are approximately 41 million adolescents in the world (3). According to the data from the Centers for Disease Control and Prevention obesity rate increased from 15% to 19% (4). Parallel to the increase in obesity, the incidence of diseases such as Type 2 diabetes and hypertension in children and adolescents is increasing (3). In a study, it is stated that 20% of overweight and obese adolescents have lipidemia and 25% have diabetes in the literature (5).

It is quite significant to determine sedentary life and unhealthy eating habits in childhood and adolescence to prevent and reduce chronic health problems (6). Valid and reliable measurement tools that determine healthy habits are needed in the Turkish literature. One of these measurement tools is the Healthy Habits Questionnaire (HHQ). There are English and Spanish versions of the questionnaire including diet, physical activity, screen time, and sleep habits. There are two different forms of the questionnaire used for ages 2-9 and ages 10 and older (7). The HHQ can be used in clinics, family health centers, and schools. It has been stated that the questionnaire, which has been used in many studies abroad, is a measurement tool that is easy to use and can be adapted to all segments of society (8–11). The main purpose of this study was to adapt the HHQ (10 ages and over) to the Turkish language and culture and to test its validity and reliability. The study also examined the diet, physical activity, screen time, and sleep habits of adolescents.

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2. METHODS

This study is a methodological in terms of adapting HHQ to the Turkish language and a descriptive study in terms of examining the healthy habits of adolescents. Permission was obtained via e-mail from the author who developed the HHQ for the study. Ethical approval was obtained from the University Health Sciences Institute Ethics Committee, written permission from the Istanbul Provincial Directorate of National Education, and consent from the students' parents.

The population of the research consisted of students studying in a secondary school located in the Gaziosmanpaşa district of Istanbul. The sample of the study was students are 5th 6th 7th and 8th grades that were chosen from eight classrooms by using the cluster sampling method. It consisted of 320 students studying in eight classes randomly selected from the 5th and 8th grades.

In the study, a pilot test was applied to 118 students with similar sample characteristics. Students who underwent the pilot test were not included in the study.

Data were collected in their classrooms over a period of about 10 minutes based on students' self-report. Data collection tools are sociodemographic form consisting of gender, age, class questions, and HHQ.

2.1. Healthy Habits Questionnaire (HHQ)

HHQ is a measurement tool that evaluates diet, physical activity, screen time and sleep habits in adolescents aged 10 and over that is developed by the Maine Health Organization in the United States, the validity and reliability analysis findings of the questionnaire are not available. The questionnaire is significant in terms of evaluating obesity risk factors (8). It is easy to use in public health fields and schools (8,12,13). HHQ consists of 10 questions. The questionnaire includes five questions about nutrition, one question about sleep, two questions about screen time, and one question that measures physical activity. Eight of these questions are open-ended questions containing numerical values, and one of them is the type of question with two options (There is/no TV, tablet, or smartphone in the bedroom). The tenth question is a multiple-choice question about the habits that the adolescents want to change in their own life, and the adolescent is asked to mark the habit that wants to change. Survey questions are evaluated with percentage and median values, there is no scoring scale. In this study, in line with the literature, for adolescents; daily three servings of vegetables, two servings of fruit (14) eight glasses of water (15), and two glasses of milk (16) are determined as the limit. In addition, screen time is determined as maximum two hours, physical activity duration at least one hour, and sleep duration at least 8 hours (17,18). It is recommended that daily sugary drinks (fruit juice, carbonated drinks) should not be consumed at all (19).

2.1.1. Translation and Cultural Adaptation

The English form of Healthy Habits Questionnaire was translated into Turkish by two linguists. The Turkish forms were evaluated by the researchers and they were found to be similar in meaning. It was sent to two linguists independent of the first for back-translation. The back-translated English form was compared with the original questionnaire and found to be similar in meaning.

2.1.2. Content Validity

For content validity, 10 experts from the fields of public health nursing and nutrition dietetics were consulted. Experts rated each item on a scale of 1-4 (1: not appropriate, 2: somewhat appropriate, items \ expressions need to be adjusted properly, 3: quite appropriate, but minor changes required, 4: very appropriate).

2.1.3. Reliability

The test-retest method was used to assess time stability on questionnaire. The test-retest was conducted with 104 students at two – week intervals.

2.2. Data Analysis

Statistical Package for the Social Sciences (SPSS) 20 program was used for data analysis. The validity of the questionnaire was evaluated with the content validity index (CVI), and its reliability was evaluated with the test-retest. Test-retest evaluation was done with the kappa test for categorical variables and Spearman correlation for continuous variables.

Adolescents' habits were evaluated with median, interquartile range, and percentage.

3. RESULTS

3.1. Findings Related to Validity

It was determined that 10 experts, whose opinions were taken for content validity, gave three and four points to all items and the CVI was 0.96. In line with the suggestions of the experts, corrections were made in questions 1, 2, 3, and 4 without changing the meaning.

3.2. Pilot Test

After the pilot test with 118 students, it was determined that the questionnaire was filled in approximately five minutes and there were no questions left blank. Except for the two questions in the questionnaire, it was determined that the questions were understood. Two questions that needed editing were questions one and six. For a better understanding of the students, examples of fruit and vegetable (F&V) servings were added to the explanation part of the first question asking about daily F&V consumption. In addition, the question was arranged as two separate questions, 1a vegetable and 1b fruit,

and six options (0: does not eat at all-5: five servings or more) to limit the extreme values. The sixth question, asking about the presence of a screen in the bedroom, was asked separately as TV, computer, tablet, and smart phone.

3.3. Findings Related to Reliability

The test-retest correlations of questions containing continuous variables were found to be between 0.44 and 0.91. Kappa values of questions containing categorical variables were found to be between 0.7 and 0.94 (Table 1).

Table 1. *Test-retest results of categorical and continuous variables in the Healthy Habits Questionnaire*

Variables		r	Kappa	P
M1a	Number of fruit servings consumed per day	0.58	-	<.001
M1b	Number of fruit servings consumed per day	0.44	-	<.001
M2	Number of dinners with family in a week	0.96	-	<.001
M3	Number of breakfasts in a week	0.77	-	<.001
M4	Number of meals taken out in a week	0.91	-	<.001
M5	Screen time per day	0.66	-	<.001
M6a	TV in the bedroom	-	0.70	<.001
M6b	Computer in bedroom	-	0.86	<.001
M6c	Tablet in the bedroom	-	0.94	<.001
M6d	Smartphone in the bedroom	-	0.78	<.001
M7	Night time sleep duration	0.49	-	<.001
M8	Daily physical activity time	0.50	-	<.001
M9a	100% fruit juice consumed daily	0.68	-	<.001
M9b	Daily water consumed	0.88	-	<.001
M9c	Daily consumed instant/canned fruit juice and sports drink	0.77	-	<.001
M9d	Daily consumed milk	0.73	-	<.001
M9e	Fizzy drinks or fruit cocktails consumed daily	0.52	-	<.001
M9f	Daily consumption of skim milk	0.62	-	<.001
*p<.001 r: Spearman's correlation				

3.4. Findings on Introductory Features and Health Habits

The mean age of the adolescents was 12.5±1.16 and 60.3% were male. 26.3% of the adolescents were in the 8th grade (Table 2).

Table 2 *Adolescents' sociodemographic characteristics*

Variables		min-max	Average
Age		10-14	12.5±1.16
		n	%
Gender	Female	127	39.7
	Male	193	60.3
Class	5th grade	81	25.3
	6th grade	80	25
	7th grade	75	23.4
	8th grade	84	26.3

The median fruit servings consumed daily by adolescents was two, and the median vegetables was one. The median eating dinner and having breakfast with the family within a week was found to be seven. The median 100% fruit juice, water, whole milk, and skim milk per day was determined as one glass (Table 3).

Table 3. *Adolescents' servings of fruits & vegetables, drink, consumed daily and eating habits according to HHQ*

Variables	Median (IAA difference)
Number of fruit servings consumed per day (min 0 max 5)	2 (1 serving)
Number of vegetable servings consumed per day (min 0 max 5)	1 (1 serving)
Daily consumed vegetables and fruits (min 0 max 14)	3 (1 serving)
Number of dinners with family in a week (min 0 max . 7)	7 (2 times)
Number of breakfasts in a week (min 0 max 7)	7 (5 times)
Number of meals taken from outside in a week (min 0 max 8)	1 (1 time)
Daily drink (glass of water)	
100% juice (min 0 max 9)	1 (1 glass)
Water (min 0 max 10)	6 (5 glasses)
Ready / canned fruit juice and sports drink (min 0 max 9)	0 (1 glass)
Whole milk (min 0 max 9)	1 (1 glass)
Fizzy drink or fruit cocktail (min 0 max 10)	1 (1 glass)
Skimmed, low-fat (1%) or reduced-fat (2%) milk (min 0 max 5)	1 (1 glass)
HHQ: Healthy Habits Questionnaire *IAA: Interquartile range difference (0.75-0.25)* *(1 glass=200 ml)*	

The median daily screen time was two hours. It was determined that 73.4% of the students had an electronic media device in their bedroom (Table 4).

Table 4. *Adolescents' physical activity, screen and sleep time, and habits they want to change, according to HHQ*

Variables	Median (IAA difference)	
Daily screen time (min 0 max 10)	2 (1.5 hours)	
Daily physical activity time (min 0 max . 8)	1 (1 hour)	
Night time sleep duration (min 0 max 10)	8 (2 hours)	
No electronic media device in the bedroom (television, tablet, computer, smartphone)	n	%
Yes	235	73.4
No	85	26.6
The habit they want to change		
Sleeping more	74	24.8
Being more active – exercising more	62	20.8
Using TV or a tablet/smartphone	49	16.4
Drinking more water	38	12.8
Eating more often with your family	26	8.7
Eating more fruits and vegetables	24	8.1
Eating less takeout	15	5
Drinking fewer sodas, instant juices, or fruit cocktails	10	3,4
HHQ: Healthy Habits Questionnaire *IAA: Interquartile range difference (0.75-0.25)* *(1 glass=200 ml)*		

When asked about the habits they want to change; 24.8% of the adolescents stated that they want to sleep more, 20.8% stated that they want to be more active-exercise more,

and 16.4% spend less time watching TV or using a tablet/ smartphone (Table 4).

It was determined that 91.8% of the adolescents consumed less than 3 servings of vegetables, 76.9% of them consumed less than two servings of fruit, and 72.5% of them consumed less than five servings of F&V. It was determined 66.4% of them consumed less than 8 glasses of water and 80.5% of them consumed less than two glasses of milk (Table 5).

It was found that 31.7% of the adolescents had dinner with their families weekly, and breakfast frequency was less than seven days in 37.1%. It was determined that 86.1% ate out at least once a week. It was determined that 40.2% of them had a screen usage time of more than two hours, 47.6% of them had less than one hour of physical activity, and 30.2% of them had less than eight hours of sleep (Table 5).

Table 5 *Health habits of adolescents according to the limit value stated in the literature*

Variables	Limits	n	%
Daily consumption of vegetables	< 3 servings	293	91.8
	≥ 3 servings	27	8.2
Fruit consumption	< 2 servings	246	76.9
	≥ 2 servings	74	23.1
Fruit and vegetable consumption	<5 servings	232	72.5
	>5 servings	88	27.5
Daily water consumption	<8 glasses	193	66.4
	≥8 cups	127	33.6
Daily milk consumption	<2 cups	236	80.5
	≥ 2 glasses	84	19.5
Daily consumption of sugary drinks	=0 glass	90	28.4
	≥ 1 glass	230	71.6
Dinner with family in a week	<7 days	101	31.7
	=7 days	209	68.3
Number of days with breakfast in a week	<7 days	118	37.1
	=7 days	202	62.9
Food consumption in a week	< 1 time	44	13.9
	≥ 1 time	276	86.1
Daily screen time	<2 hours	198	59.8
	>2 hours	122	40.2
Daily physical activity	<1 hour	151	47.6
	>1 hour	169	52.4
Night time sleep duration	<8 hours	95	30.2
	>8 hours	225	69.8

4.DISCUSSION

This study, conducted with 320 adolescents to adapt the adolescent healthy habits questionnaire to the Turkish language and to test its validity and reliability, showed the Turkish version of the HHQ is a valid and reliable tool. It was found 91.8% of the adolescents' vegetables consumption, 76.9% of their fruit consumption daily, 66.4% their daily water amount, 30.2% of their sleep time, 47.6% of their physical activity time were insufficient. Also, 40.2% of the adolescents' daily screen time, 86.1% of their fast food consumption was above the recommended level.

Content validity is the main validity criterion that should be done first in data collection tool adaptations. It shows to what extent the questions define the concept to be evaluated (20).

In this study, the content validity index of the HHQ in adolescents was found to be 0.96. In the literature, it is stated that the content validity index value should be 0.80 and above (20). It was determined the content validity of the Turkish HHQ was at a high level.

In this study, the test-retest method was examined to show the invariance of the questionnaire items over time. In the literature, the interval between two tests is recommended to be two to four weeks. Test-retest correlation of 0.40 is stated as an acceptable level (21). In this study, the test-retest correlations of the questionnaire items made with a two-week interval were found to be within acceptable limits between 0.44 and 0.91.

Since the types of questions in the questionnaire are in different forms, internal consistency cannot be evaluated (eight questions are open-ended, one question is two-choice, and the other is multiple-choice).

In this study, it was determined that approximately two out of three adolescents did not consume the five servings of F&V recommended by the CDC, and the median F&V consumption was three servings (vegetable one, fruit two) (14). Similar to our results in a study conducted by Laska et al. in Minnesota, F&V consumption median of adolescents aged 11-19 was found to be three portions (22). In a study conducted by Gur et al. in Istanbul, the median of vegetable consumption was found to be 1.3 servings and fruit consumption as 1.1 servings (23). These results were shown the inadequacy of F&V consumption in adolescents is a significant level. Since vegetables and fruits are rich in vitamins, minerals, and fiber, they are especially important for health (24). In this study, daily F&V consumption of 72.5% of adolescents was found to be insufficient. Similarly, in a study conducted by Sidoti et al. 37% of the sample stated that they rarely consume F&V (25). In another study, it was reported that 24.96% of adolescents consume fruit once a day, 17.95% consume fruits twice a day, and vegetable consumption is at most once a day (26). These results support that F&V consumption is a problem for a large group of adolescents and emphasize the necessity of programs to increase F&V consumption. Programs and interventional studies are recommended to increase the consumption of F&V. In addition, public and institutional policies are needed to increase F&V consumption. In order to increase F&V consumption, it is recommended to sell F&V in the canteen, to include F&V in school meals, and to keep F&V at home as prepared.

The median adolescents' eating dinner with their families was seven days per week in this study, however, 68.3% of the adolescents reported that they did not eat dinner with their families seven days a week. In a study conducted by Wong et al. in Hong Kong, 55.6% of adolescents reported that they ate dinner with their families every day (27). In a study conducted by Berge et al. in Minnesota, when asked about eating dinner

with their families per week, 43% of adolescents stated that they ate five or more times a week (28). In a study conducted by Akman et alin Istanbul, 68.4% of adolescents stated that they ate together with their families (29). Study results show that adolescents mostly eat dinner with their families. Eating with the family is significant in terms of preventing obesity and protecting physical and mental health in adolescents (28). Therefore, school nurses can encourage adolescents and their families to have dinner together regularly.

It was determined that the median weekly consumption of breakfast of adolescents was seven day in this study, however, 37.1% of adolescents did not consume breakfast every day. In a similar study conducted by Olatona et al. in Nigeria, only 56.7% of adolescents stated they had daily breakfast (30). In a study conducted by Colak and Ergun in Istanbul, it was reported that 76.6% of adolescents had breakfast (31). Similarly, in a study conducted by Gökler et al. in Eskisehir, it was stated that only 37.6% of adolescents had breakfast (32). In our study, the rate of adolescents who had breakfast was found to be higher than the studies conducted in Nigeria and Eskişehir. However, our results are similar to the study conducted in Istanbul. It is thought that the regional and socioeconomic level differences may cause the frequency of having breakfast to be different. In this study, although the majority of adolescents eat breakfast, the rate of adolescents who do not have breakfast is high. These results show that there is a need for this topic of health education for adolescents who do not have regular breakfast habits.

In this study, 86.2% of the adolescents received outside food/ fast food at least once a week. In a study of adolescents living in 54 low – and middle-income countries conducted by Li et al. it was reported that 55.2% of them had food/ fast food taken out at least once a week (33). In a study conducted by Koca and Arkan in Izmir, it was reported that 88.2% of adolescents consume fast food (34). It is recommended to conduct education programs to reduce fast food consumption in adolescents.

The median daily screen time of adolescents was two hours in our study. In addition, 40.2% of the adolescents in our study had a daily screen time above the recommended limit of two hours. According to the data of the American Academy of Child and Adolescent Psychiatry, the time that adolescents spend in front of the screen can increase by up to nine hours (35). In a study conducted by Nagata et al. in the USA, the time spent by adolescents in front of the screen was found to be 2.42 hours (36). These results are similar to our study. These results show the need for educational programs for adolescents and their families with more than two hours of daily screen time.

In this study, it was determined that 73.4% of the students had an electronic media device in their bedroom. In a study conducted by Tezol in Mersin the adolescents living, 56.3% of adolescents stated that there is a television and computer in their bedroom (37). In a study conducted by Gilbert-Diamond et al. in the USA, it was stated that 64.5% of adolescents had a television in their bedroom (38). The presence of an electronic media device in the bedroom of adolescents makes it possible to use the screen before sleep. In the literature, there are studies on the negative effects of screen use time on sleep time (39). For this reason, it is important that electronic media devices are not used in bedrooms, and it is recommended to keep banners and posters in schools and implement educational programs.

In this study, it was determined that the median sleep duration was eight, and 30.2% of the adolescents slept less than the recommended sleep duration. Similarly, in a study conducted by Tezol in Mersin province, it was stated that the sleep duration of adolescents was eight hours (37). In a study conducted by Bay and Ergun in Istanbul, the median sleep duration was found to be seven hours (40). Similarly, in a study conducted on adolescents in Sweden, the median sleep duration was found to be 7.75 (41). According to the Centers for Disease Control and Prevention, the ideal sleep time for adolescents is 8-10 hours (18). These results indicate there is a significant number of adolescents with insufficient sleep duration. Programs to increase sleep duration are recommended for these groups.

The physical activity duration of 47.6% of the adolescents was found to be less than one hour which is the daily recommended limit in this study. In a study conducted by Kandola et al. in England, 79% of adolescents were reported to be inactive (42). In a study conducted by Yilmazel and Bozdogan in Çorum, it was stated that 72.9% of adolescents are inactive (43). Sedentary life causes various physical and mental problems such as obesity. Therefore, there is a need for programs that support adolescents to be physically active. In addition, there is a need for developing the health policies. The designed bike lanes and parks should be increased. For this, intersectoral cooperation is significant. Different sectors (health care, transportation/planning, parks/public spaces, and school) can create a physical activity policy that can impact physical activity.

In this study, 66.4% of the adolescents had insufficient water consumption to be less than eight glasses (1600 ml). In a similar study conducted in Çorum, 70.7% of adolescents were found to consume insufficient water (43). Similarly, in the HELENA study conducted in eight European cities, the daily amount of water consumed by adolescents was stated as 788 ml (44). In a study conducted by Franken et al. in the Netherlands, the median daily consumption of water was stated as three glasses (45). These results show that the water consumption of adolescents is below the recommended amount. Increasing the consumption of water by limiting the consumption of sugary drinks is extremely important for the protection of adolescents from chronic diseases such as Type 2 diabetes and hypertension (45). For this reason, it is recommended that education programs to increase water consumption be implemented in schools. At the same time, it is necessary to develop policies that allow adolescents to consume water free of charge at school. Also, in this study, the consumption of sugary drinks was found to be above the recommended limit. 77.1% of adolescents consumed at least

one glass of sugary beverage per day. Similarly, in a study conducted in the Netherlands, the median daily consumption of sugary beverages consumed by adolescents was stated as 1.1 glasses (45). In a study conducted by Gur et al. in Istanbul, it was reported that 76.6% of adolescents consumed instant fruit juice, and 77.71% consumed carbonated beverages (31). Consumption of sugary drinks causes dental caries, Type 2 diabetes, and obesity in adolescents (45). It is stated in the literature that water consumption has a positive effect on the weight management of adolescents (46). For these reasons, limiting the consumption of sugary drinks with low vitamin and mineral content and unnecessary calories and increasing water consumption is extremely significant for the protection of adolescents from chronic diseases such as obesity, Type 2 diabetes, and hypertension.

Milk consumption of 80.5% of the adolescents was found to be less than the recommended two glasses per day and the median milk consumption was one glass and insufficient in this study. Similarly, in a study conducted by Lee et al. in Korea, it was stated that the rates of milk consumption were low and only 14.7% of male adolescents and 8.1% of female adolescents consumed two glasses or more of milk (47). In a study conducted by Tucker et al.in the USA, the median milk consumption was stated as 0 cups (13). In a study conducted in Corum, it was stated that 78.8% of adolescents consumed less than two glasses of milk (43). Milk and dairy products are essential nutrients that must be consumed adequately at all ages. Therefore, education programs are needed to increase milk consumption in adolescents. Besides, it is recommended that the school milk program, which has been implemented since 2016 to support the milk consumption of children in kindergarten and primary schools, be extended to include adolescents.

When asked about the habits they want to change; 24.8% of the adolescents stated they sleep more, 20.8% are more active-exercise, and 16.4% spend less time watching TV or using a tablet/smartphone. Similar to our study results, in a different study conducted on adolescents by Aslan in Ankara, when asked about the behaviors they want to change, they answered eating too much junk food, drinking little water, doing insufficient physical activity, not being able to communicate adequately with friends, going to bed late, playing too many computer games (48). These results are an important finding showing that adolescents are aware of their unhealthy behaviors. For this reason, it is thought that programs that include helping to change behavior rather than knowledge and awareness educational programs will be more useful.

5. CONCLUSION

HHQ was found to be a valid and reliable measurement tool that can be used in adolescents over the age of 10, including diet, physical activity, screen time, and sleep habits. The questionnaire can be used by nurses and other health professionals working in primary care, pediatric clinics, and schools.

In addition, according to our study results evaluated with HHQ, approximately two-thirds of adolescents were found to have insufficient consumption of vegetables, fruits, water, and milk. In addition, nearly half of the adolescents had more than two hours of screen time and less than one hour of physical activity. Approximately one-third of adolescents had insufficient sleeping time. In line with these results, school-based behavior change programs are recommended for adolescents to gain healthy habits.

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Clinical and Experimental Health Sciences

CBCT Artifact Evaluation in a Single Device: Insights and Limitations

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ABSTRACT

Objective: To classify the types of artifacts in cone-beam computed tomography (CBCT) and to evaluate them according to age and gender.

Methods: CBCT images of 1500 patients (766 males and 734 females) aged 5-92 (mean age: 40.89 ± 18.82 years) were retrospectively evaluated and the patients were categorized into 4 age groups: under 20 years old, 20-39 ages, 40-59 and over 60 years old. The types of artifacts encountered in CBCT images were classified. The relationship between the artifact types with age and gender were investigated. Chi-square test was applied to analyze the relationships between variables and distribution of parameters.

Results: Of the cases, 284 (18.9%) were under the age of 20, 389 (25.9%) were between the ages of 20-39, 554 (36.9%) were between the ages of 40-59 and 273 (18.2%) were over the age of 60. Moire artifact was observed at the highest rate (100%), while motion artifact was determined at the lowest rate (19.5%), and no ring artifact was detected in the analyzed images. Metallic artifact, metallic artifact removal, streak artifact and presence of dark bands were found to be statistically significant in females ($p = .002$, $p = .001$, $p = .002$ and $p = .002$, respectively). There was no statistically significant correlation between cupping artifact, metallic artifact, metallic artifact removal, streak artifact, dark band and noise, and stitched artifact ($p > .05$).

Conclusion: Both device and patient-based artifacts in CBCT images should be known, as well as the ways to prevent them.

Keywords: Artifacts, metallic artifact, streak artifact, motion artifact, cone-beam computed tomography

1. INTRODUCTION

Cone-beam computed tomography (CBCT) has become one of the important diagnostic methods for dentists and researchers working in the rapidly changing field of digital dentistry with the innovations in computers and developments in scanning technology (1). Compared to medical computed tomography (CT) images used for similar purposes, the radiation dose required for CBCT is lower than for CT (2). Besides its many advantages, one of the main disadvantages of the CBCT system is the appearance of artifacts in reconstruction images due to various reasons. These artifacts cause image distortion and may lead to erroneous diagnosis or misdiagnoses (3, 4).

There are some studies on different artifact classifications in the literature (1, 5-10). Two studies were found that evaluated metallic and motion artifact according to age group (11, 12). Most of the previous work evaluates on algorithm and software developments to reduce and prevent metal and motion artifacts (5, 12-14). Efforts have been made to eliminate metal artifacts by applying this algorithm and software on images or phantom models of patients with materials such as dental implants, orthodontic materials, endodontic posts and metal-supported prostheses that cause metal artifacts (15-18). In studies regarding motion artifact, the effect on diagnostic accuracy was evaluated. In addition, the effect of patient anxiety on motion artifact was investigated (19, 20).

However, as far as we know, there is no study examining all types of artifacts according to age and gender. Therefore, the aim of this study is to classify the types of artifacts detected in CBCT images of patients, and to evaluate them retrospectively according to age and gender.

2. METHODS

Before commencing the study, the Clinical Research Ethics Committee of Gaziantep University granted ethical approval (Protocol No: 2020/403). The images used in this study were taken between 2017-2020 at the Department of Dentomaxillofacial Radiology, located in the Faculty of Dentistry at Gaziantep University, using the Planmeca

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Promax 3D (Helsinki, Oy, Finland) CBCT device. The images were retrieved from the tomography archive, asymptomatic patients who underwent CBCT exam for various indications were selected. Multiplanar images were obtained from 16×5, 16×9, 16×16 FOV (field of view) with 0.4 mm^3 voxel size and 1 mm slice thickness. Inclusion criteria for this study are CBCT scans acquired from patients aged 5-92 between 2017-2020. Exclusion criteria; include patients with syndromes or facial growth disorders, presence of distortion, magnification or foreign bodies in the study area on CBCT images, metabolic bone diseases, cysts, tumors, or fracture lines in the examination area, cysts affecting the maxillary sinuses, tumors, or trauma in the maxillofacial region, and odontogenic infections. CBCT images of 1500 patients (766 males and 734 females) aged 5-92 (mean age: 40.89 ± 18.82 years) were evaluated retrospectively. 1500 patients were classified into 4 age groups: under 20 years old, 20-39 ages, 40-59 ages and over 60 years old. More than one artifact can be found in the same CBCT scan.

2.1. Image Analysis

The images were analyzed using Romexis software (Helsinki, Oy, Finland). Common types of artifact encountered in CBCT images were analyzed and classified (Figure 1) as follows:

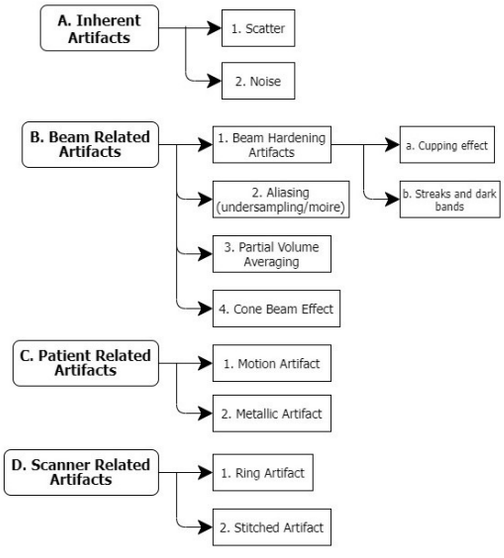

Figure 1. *Diagram showing the classification of artifacts considered.*

– Scatter is caused by X-ray photons being deflected from their original path as a result of interactions with matter. Since CBCT employs area detectors, scattered photons are captured, which contributes to an overall degradation of the image or "quantum noise" when compared to CT imaging (21). In projection images, noise can be identified by the presence of inconsistent gray values for attenuation, as well as larger standard deviations in areas where a constant attenuation is anticipated (1, 5, 8) (Figure 2).

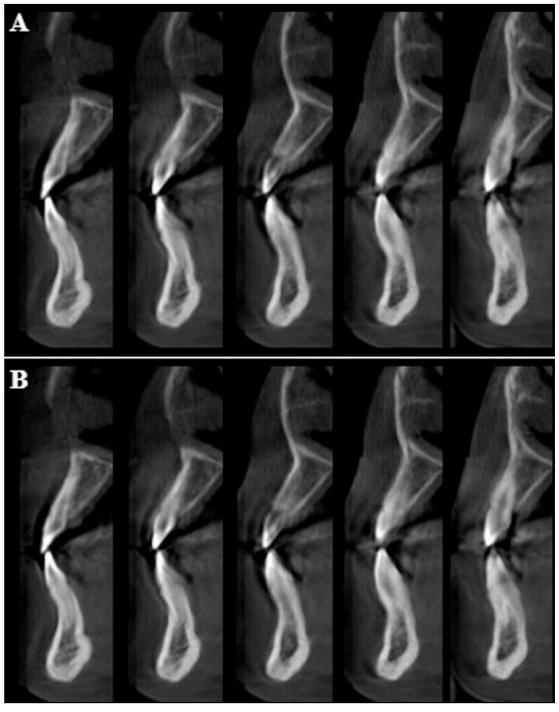

Figure 2. *It is known that the noise varies depending on the section thickness. Cross-sectional CBCT images show noise variation between images with slice thickness of 0.4 mm in A and slice thickness of 5.20 mm in B.*

-Low-energy rays emitted from the X-ray source are absorbed as they pass through objects, causing an increase in the remaining X-ray energy. Thus, beam hardening artifact occurs (22, 23). Beam hardening results in two phenomena: Cupping artifact is caused by the absorption of X-rays and results in the degradation of metallic structures (Figure 3A). It appears as dark streaks or bands between two dense objects and is more visible in axial planes and 3D reconstruction images (Figure 3B). The presence of this artifact can significantly reduce the quality of the image (23).

-White streaks called metallic artifact are observed in relation to metallic structures such as prosthetic and amalgam restorations, orthodontic brackets and wires, implants, surgical plates or screws (1, 24) (Figure 3).

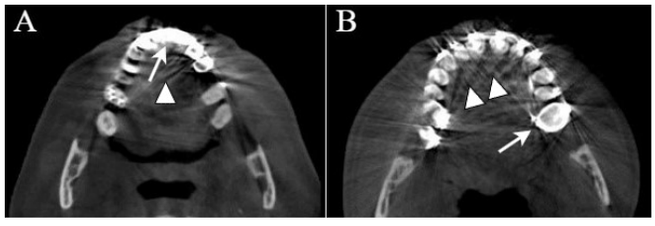

Figure 3. *Axial CBCT images (A and B): Cupping artifact (white arrows) and dark band (arrowheads) are shown.*

-Undersampling/aliasing artifacts, also known as the Moire pattern, can occur due to the undersampling of structures within the subject by the cone beam unit's detector, particularly when only a few basis projections are used for

the reconstruction. Slightly wavy lines that diverge towards the periphery of the image are observed (1, 5) (Figure 4).

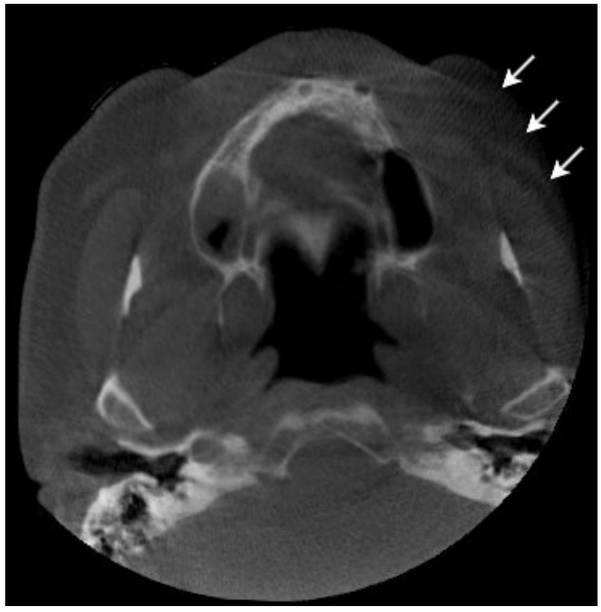

Figure 4. *Moire artifact (white arrows) is demonstrated in the axial CBCT image.*

-If the voxel size selected for a scan is larger than the size of the object being imaged, it can lead to partial volume averaging artifacts. Partial volume averaging artifacts are occured by regions with rapidly changing surfaces in the 'Z' direction, for example, in the temporal bone (1, 21).

– Cone-beam effect artifact occurs in the peripheral regions of the scan and is caused by the X-rays diverging in those areas. As a result of the cone beam effect, peripheral "V" artifact occurs, consisting of image distortion, lines, noise, and reduced contrast (1, 8, 21) (Figure 5).

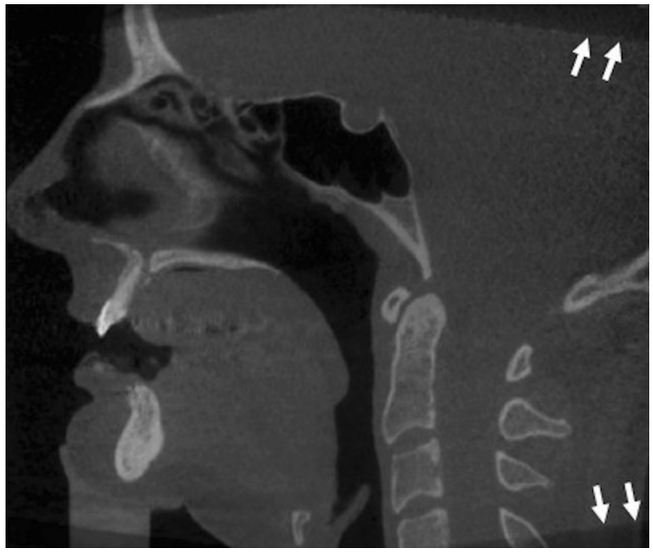

Figure 5. *Cone-beam effect (white arrows) is shown in the sagittal CBCT image.*

-Ring artifact is typically circular in shape and is caused by scanner detection defects or lack of calibration (21, 22).

-Patient motion artifact can cause erroneous recording of data, along with a lack of image acuity or a double image of bone contours (1, 21) (Figure 6).

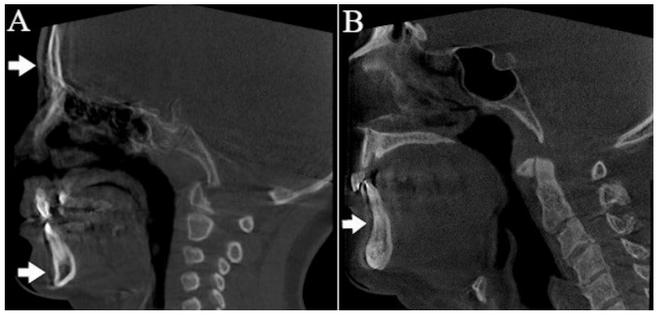

Figure 6. *Motion artifact is (white arrows) demonstrated in the sagittal CBCT images (A and B).*

-Stitched artifact formed during the reconstruction of the obtained data occurs in the 16×16 and 16×9 FOVs of the Planmeca Promax 3D device used in the present study (Figure 7).

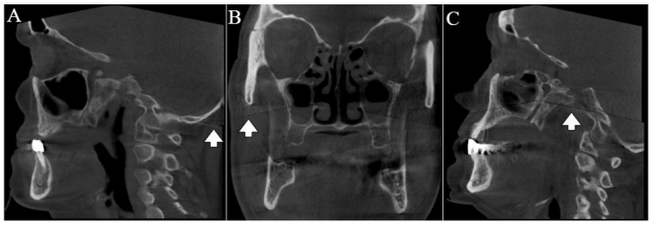

Figure 7. *Stitched artifact (white arrows) is indicated in the sagittal (A, C) and coronal (B) CBCT images.*

All evaluations were conducted by two dentomaxillofacial radiologists, one of whom was a four years experience specialist dentomaxillofacial radiologist (EMAO) and the other had nine years of experience dentomaxillofacial radiologist (EDY). In cases where there was disagreement between the observers, a consensus was reached through discussion. In order to ensure intra-examiner calibration and reliability of the evaluations, the same observers reviewed the images two weeks after the initial evaluation.

2.2. Statistical Analysis

The kappa statistics were utilized to determine inter-observer and intra-observer agreement. The relationships among categorical variables were analyzed using the Chi-square test. The data was analyzed using SPSS software version 22.0 (IBM Corp, Armonk, NY), and statistical significance was defined as $p < .05$.

3. RESULTS

The coefficient of intra-observer and inter-observer reliability for all assessments was determined to be excellent, with values of 0.93 and 0.88, respectively. The study analyzed a total of 1500 CBCT images, with 766 (51.1%) males and 744 (48.9%) females (with a mean age of 41.13 ± 18.55 and 40.64 ± 19.09, respectively) included in the analysis. Regarding the FOV of the exams evaluated, 45.1% were 16x16, 43.8% were 16x9 and 11.1% were 16x5. Cone beam effect 40.2%, moire artifact 100%, ring artifact 0% cupping artifact 40.5%, metallic artifact, streak artifact and dark band 67.6%, motion artifact 19.5%, stitched artifact 34.7% and noise at a rate of 76.6% were detected in the scans. In addition, metallic artifact removal rate was observed as 57.0%. The frequency of artifacts is shown in Table 1. The FOV with the most stitched artifact is 16×16 with 34.4%. It was detected 0.4% at 16×9 FOV and 0% at 16×5 FOV. A statistically significant correlation was observed between FOV and stitched artifact (p = .001).

Table 1. *The frequency of artifacts.*

Variables	Present N (%)	Absent N (%)
Cone-beam effect	603 (40.2)	897 (59.8)
Moire artifact	1500 (100)	0 (0)
Ring artifact	0 (0)	1500 (100)
Cupping artifact	608 (40.5)	892 (59.5)
Metallic artifact	1014 (67.6)	486 (32.4)
Metallic Artifact Removal	855 (57.0)	645 (43.0)
Streak Artifact	1014 (67.6)	486 (32.4)
Dark Band	1014 (67.6)	486 (32.4)
Motion Artifact	293 (19.5)	1207 (80.5)
Stitched Artifact	520 (34.7)	980 (65.3)
Noise	1151 (76.7)	349 (23.3)

Upon examining the relationship between parameters and gender, a statistically significant association was observed between cupping artifact, metallic artifact, streak and dark band artifacts, motion artifact, stitched artifact, and metallic artifact removal with gender. It was observed that the presence of metallic artifact, metallic artifact removal, streak artifact, and dark bands was significantly higher in females (p = .002, p = .001, p = .002 and p = .002, respectively). Table 2 displays the distribution of artifacts according to gender.

When the images were grouped according to age, 284 (18.9%) of the cases were under 20 years old, 389 (25.9%) were between 20-39 ages, 554 (36.9%) were between 40-59 ages and 273 (18.2%) were over 60 years old. Under the age of 20, the most frequently observed artifact was noise, with a rate of 72.5%. For the 20-39 age group, the most common artifacts were metallic artifact, streak artifact, and dark band artifact, with a rate of 72.7%. The most common artifact in the 40-59 age group was noise with 80.7%. It is followed by metallic artifact, streak artifact and dark band with 78.5% and cupping artifact with 61.6%. Age groups and the artifact frequencies are shown in Table 3.

After examining the relationship between the artifacts, a significant correlation was found between the cone-beam effect and all other artifacts (p < .05), except for stitched artifact (p = .737), motion artifact (p = .089), and noise (p = .337). No statistically significant correlation was determined between cupping artifact, metallic artifact, metallic artifact removal, streak artifact, dark band and noise and stitched artifact (p = .258, p = .182, p = 861, p = .182, p = .182 and p = 896, respectively). The relationship of the artifacts with each other is shown in Table 4. The distribution of artifact types with FOVs is shown in Table 5.

Table 2. *Distribution of artifacts by gender.*

	Male		Female		
	Present N (%)	Absent N (%)	Present N (%)	Absent N (%)	P
Cone-beam effect	325 (21.7)	441 (29.4)	278 (18.5)	456 (30.4)	.072
Moire artifact	766 (51.1)	0 (0.0)	734 (48.9)	0 (0.0)	****
Ring artifact	0 (0.0)	766 (51.1)	0 (0.0)	734 (48.9)	****
Cupping artifact	290 (19.3)	476 (31.7)	318 (21.2)	416 (27.7)	.031*
Metallic artifact	490 (32.7)	276 (18.4)	524 (34.9)	210 (14.0)	.002*
Metallic Artifact Removal	390 (26.0)	376 (25.1)	465 (31.0)	269 (17.9)	.001*
Streak Artifact	490 (32.7)	276 (18.4)	524 (34.9)	210 (14.0)	.002*
Dark Band	490 (32.7)	276 (18.4)	524 (34.9)	210 (14.0)	.002*
Motion Artifact	166 (11.1)	600 (40.0)	127 (8.5)	607 (40.5)	.033*
Stitched Artifact	290 (19.3)	476 (31.7)	230 (15.3)	504 (33.6)	.008*
Noise	587 (39.1)	179 (11.9)	564 (37.6)	170 (11.3)	.924

*Chi-square test; *p < .05*

Table 3. *Distribution of age groups and frequency of artifacts.*

	Cone-beam effect		Cupping artifact		Metallic artifact		Metallic Artifact Removal		Streak Artifact		Dark Band		Motion Artifact		Stitched Artifact		Noise	
Age Groups	Present N (%)	Absent N (%)	Present N (%)	Absent N (%)	Present N (%)	Absent N (%)	Present N (%)	Absent N (%)	Present N (%)	Absent N (%)	Present N (%)	Absent N (%)	Present N (%)	Absent N (%)	Present N (%)	Absent N (%)	Present N (%)	Absent N (%)
< 20 age	93 (32.7)	191 (67.3)	6 (2.1)	278 (97.9)	105 (37.0)	179 (63.0)	132 (46.5)	152 (53.5)	105 (37.0)	179 (63.0)	105 (37.0)	179 (63.0)	100 (35.2)	184 (64.8)	60 (21.1)	224 (78.9)	206 (72.5)	78 (27.5)
20-39 age	144 (37.0)	245 (63.0)	99 (25.4)	290 (74.6)	281 (72.7)	108 (27.8)	243 (62.5)	146 (37.5)	281 (72.7)	108 (27.8)	281 (72.7)	108 (27.8)	46 (11.8)	343 (88.2)	127 (32.6)	262 (67.4)	277 (71.2)	112 (28.8)
40-59 age	253 (45.7)	301 (54.3)	341 (61.6)	213 (38.4)	435 (78.5)	119 (21.5)	339 (61.2)	215 (38.8)	435 (78.5)	119 (21.5)	435 (78.5)	119 (21.5)	66 (11.9)	488 (88.1)	210 (37.9)	344 (62.1)	447 (80.7)	107 (19.3)
60+ age	113 (41.4)	160 (58.6)	162 (59.3)	111 (40.7)	193 (70.7)	80 (29.3)	141 (51.6)	132 (48.4)	193 (70.7)	80 (29.3)	193 (70.7)	80 (29.3)	81 (29.7)	192 (70.3)	123 (45.1)	150 (54.9)	221 (81.0)	52 (19.0)

Table 4. *Correlations between artifacts.*

	Cone-beam effect	Cupping artifact	Metallic artifact	Metallic Artifact Removal	Streak Artifact	Dark Band	Motion Artifact	Stitched Artifact	Noise
	p	p	p	p	p	p	p	p	p
Cone-beam effect	******	.001*	.001*	.040*	.001*	.001*	.089	.737	.337
Cupping artifact	.001*	******	.001*	.001*	.001*	.001*	.001*	.258	.001*
Metallic artifact	.001*	.001*	******	.001*	.001*	.001*	.001*	.182	.001*
Metallic Artifact Removal	.040*	.001*	.001*	******	.001*	.001*	.001*	.861	.001*
Streak Artifact	.001*	.001*	.001*	.001*	******	.001*	.001*	.182	.001*
Dark Band	.001*	.001*	.001*	.001*	.001*	******	.001*	.182	.001*
Motion Artifact	.089	.001*	.001*	.001*	.001*	.001*	******	.001*	.001*
Stitched Artifact	.737	.258	.182	.861	.182	.182	.001*	******	.896
Noise	.337	.001*	.001*	.001*	.001*	.001*	.001*	.896	******

*Chi-square test; *p < .05*

Table 5. *Distribution of artifact types with FOVs.*

	FOVs					
	16x5		16x9		16x16	
	Present N (%)	Absent N (%)	Present N (%)	Absent N (%)	Present N (%)	Absent N (%)
Cone-beam effect	0 (0.0)	166 (11.1)	291 (19.4)	367 (24.5)	312 (20.8)	364 (24.3)
Moire artifact	166 (11.1)	0 (0.0)	658 (43.9)	(0.0)	676 (45.1)	0 (0.0)
Ring artifact	0 (0.0)	166 (11.1)	0 (0.0)	658 (43.9)	1 (0.1)	675 (45.0)
Cupping artifact	32 (2.1)	134 (8.9)	285 (19.0)	373 (24.9)	291 (19.4)	385 (25.7)
Metallic artifact	76 (5.1)	90 (6.0)	480 (32.0)	178 (11.9)	459 (30.6)	217 (14.5)
Metallic Artifact Removal	72 (4.8)	94 (6.3)	383 (25.5)	275 (18.3)	400 (26.7)	276 (18.4)
Streak Artifact	75 (5.0)	91 (6.1)	480 (32.0)	178 (11.9)	459 (30.6)	217 (14.5)
Dark Band	75 (5.0)	91 (6.1)	480 (32.0)	178 (11.9)	459 (30.6)	217 (14.5)
Motion Artifact	44 (2.9)	122 (8.1)	107 (7.1)	551 (36.8)	142 (9.5)	534 (35.6)
Stitched Artifact	0 (0.0)	166 (11.1)	4 (0.3)	654 (43.6)	516 (34.4)	160 (10.7)
Noise	123 (8.2)	43 (2.9)	524 (34.9)	134 (8.9)	504 (33.6)	172 (11.5)

4. DISCUSSION

Artifacts present in medical images can significantly reduce the visibility of important details, and as a result, negatively impact the accuracy of diagnoses. When the quality of images is not sufficient for accurate reporting, re-imaging is often necessary. However, rescanning the patient with CBCT leads to an additional exposure to radiation, which does not comply with the ALARA (as low as reasonably achievable) principle.

Accurate recognition of artifacts is very important for clinically correct diagnosis and successful surgery. As far as we know, artifact studies in the literature are on algorithms to prevent artifacts (5, 12, 13, 25). There are different studies on the classification of artifacts in the literature (1, 6, 9, 10). Except for the study conducted by Nardi et al. (11) and Donaldson et al. (12), no study similar to the current study was found. Nardi et al. (11) only analyzed metal and motion artifact by age group. Donaldson et al. (12) only examined motion artifact according to age group.

In the study of Bhoosreddy et al. (1), artifacts were classified under the main headings of beam-related, patient-related, image noise, and poor soft tissue contrast. Subtypes of beam-related artifacts include beam hardening artifact, cone-shaped beam-related faults, scatter, exponential edge gradient effect, photon deprivation, and metallic artifact. The subtitles of patient-related are unsharpness, double image, scanner-related artifacts and foreign bodies. In the study of Jaju et al. (6), artifacts were classified into four categories: physics-based, patient-based, scanner-based, and motion artifact. Physics-based artifacts are caused by the physical processes that take place during the acquisition of CBCT data. Physics-based artifacts subgroup: noise, beam hardening, filtration, antiscatter grids, calibration, software corrections, partial volume artifacts. Patient-based artifacts arise due to factors related to the patient's form or function. Metallic artifact is in the subgroup of patient-based artifact. Scanner-based artifacts are resulted from by imperfections in the function of the scanner itself. Ring artefact is in the subgroup of scanner-based artifacts. In the research of Nagarajappa et al. (7), artifacts were categorized as x-ray beam artifacts, patient-related artifacts, scanner-related artifacts, and image noise main groups. In the present study, artifacts were classified according to their causes. Unlike other studies, the inherent artifact group was created in the classification and scatter and noise were included in this group. The beam-related artifact group comprises beam hardening artifacts, aliasing, partial volume averaging, cone-beam effect.

In the study conducted by Donaldson et al. (12), 200 CBCT images were examined and repetitive CBCT images were evaluated for motion artifact formation for under 16 and over 65 years old. 0.5% of the images required repeating the exam because of double bone contours and motion artifact that prevented the diagnosis. In the study performed by Nardi et al. (11), 416 CBCT images were examined, the analyzed images were divided into groups as 6-10 years old, 11-18 years old, 19-60 years old and over 60 years old, and the metal artifact percentages were examined in these age groups. Metallic artifact was found to be 12.2% in the 6-10 age group, 14.7% in the 11-18 age group, 41.4% in the 19-60 age group, and 27.2% over the age of 60. Motion artefact was detected as 10.8% in the 6-10 age group, 14.2% in the 11-18 age group, 42.4% in the 19-60 age group, and 32.6% over the age of 60. In this study, metallic artifact, streak artifackt and dark band were observed as 37.0% in the under-20 age, 72.7% in the 20-39 age group, 78.5% in the 40-59 age group, and 70.7% in over 60 age group, and mostly in females. The difference between the study of Nardi et al. (11) and this study may be due to the difference in the number of images examined, different classification of age groups and different ethnic origins. In the present study, it is thought that the reason for the high number of metal artifacts in the 40-59 age group is the increase in the need for prosthetic restoration in this age group. In addition, the reason for the metallic, line and dark bands are more in the group under 20 years of age compared to the other groups may be the

increase in the need for orthodontic treatment in this age group. The difference in motion artifact between the study of Nardi et al. (11) and this study may be the use of devices with different technical characteristics (e.g. patient position during acquisition (sitting or standing), head stabilization features of each equipment, and scanning time of both equipments, factors affecting image quality; spatial resolution, contrast, density, sharpness, tube current, tube voltage, FOV, number of projections, detector type, etc.), the creation of different age groups, increased dental anxiety and claustrophobia under the age of 20.

A smaller FOV also means a shorter scan time. The shorter scan time allows the amount of artifacts to decrease by increasing the detector frame rate, reducing patient motion, and reducing the number of projections (26). In the present study, we observed an increase in almost all artifact types with increasing FOV. The results of our study support the information in the literature.

As a result of the cone-beam effect seen in the peripheral parts of the scanning area and caused by the separation of X-rays in these areas, the image distortion, lines, and peripheral noise occur. The present investigation showed the cone-beam effect was observed at a rate of 40.2% and there was a statistically significant relationship between other artifacts except noise, stitched and motion artifacts ($p < .05$). In this study, a result compatible with the general information in the literature was found (1, 8, 10), but sufficient comparison could not be made because there were no similar studies. For more comprehensive results, it is recommended to increase the studies on this subject.

Ring artifact caused by insufficient calibration was never encountered in the current study. To evaluate this comprehensively, it is suggested to conduct comparative studies with different brand devices.

No study was found in the literature regarding the stitched artifact that occurred during the reconstruction of the obtained data. It is suggested to develop software and algorithms to prevent this by examining different brands of devices.

Moire artifact, which was observed at a rate of 100% in this study, could not be compared with the literature since there was no similar study.

The limitation of this study is the use of a single brand device and the evaluation of artifacts only in the "Planmeca Promax 3D" brand device. In future studies, it is recommended to produce devices to minimize artifacts in terms of technology, according to the results obtained by using different branded devices and making comparisons. In addition, multicenter studies by increasing the number of samples and age groups are important in terms of guiding technological developments.

5. CONCLUSION

CBCT images have artifacts that can arise from both the patient and the device. These artifacts should be well known so that the physician does not misdiagnose and avoid re-imaging. The formation of some artifacts can be prevented by taking the necessary precautions (such as informing the patient before the shooting and fixing the head, removing metal-containing objects and performing periodic maintenance of devices: preventive technical maintenance of equipment, control of all physical and irradiation parameters and software). With the developing technology, these artifacts can be prevented. The devices should be calibrated on time and care should be taken to ensure that the software required for reconstruction is up-to-date.

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Clinical and Experimental Health Sciences

The Association Between Obesity, Mediterranean Diet Adherence, Zinc, Depression and COVID-19 Susceptibility: An Observational Study

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ABSTRACT

Objective: Unhealthy lifestyle factors have been associated with COVID-19 susceptibility, but data for diet and related lifestyle factors are conflicting. The objective of this study was to identify whether obesity, Mediterranean diet, Zn or depression could be associated to the risk of COVID-19 occurrence.

Methods: This observational case-control study was conducted in Türkiye (between December 2020 – September 2021) with face-to-face interview. A total of 100 former COVID-19 subjects as case group and 100 healthy control group, aged 20-54 were included in the study. By semi-structured questionnaire; demographic characteristics and anthropometric measurements was collected. Adherence to the Mediterranean diet was assessed using the Mediterranean Diet Adherence Screener and Mediterranean Diet Score. Daily dietary zinc intake was calculated using a 25-item food frequency questionnaire and blood samples for zinc levels was obtained from each participant. The level of depression was evaluated by Center for Epidemiologic Studies Depression Scale.

Results: No differences were found between the anthropometric characteristics of two groups (p>.05). Average adherence to the Mediterranean diet were lower in the case groups compared to controls (p<.05). While the total zinc intake showed a significant difference between the groups (12.6±13.0 vs 12.8±7.2 mg, respectively, p=.003), no difference was observed in the food sources related to zinc intake (9.4±5.71 vs 10.1±9.45 mg, p=.052). Case group had significantly lower zinc levels (64.7±17.6 µg/dL vs 76.1±16.7 µg/dL, p<0001) in both genders (for male p=.009 and female p<.001, respectively). The majority of case group subjects (76.1 vs 23.8%) had a serum zinc concentration below the reference ranges (p<.001).

Conclusion: Our findings suggest a negative relationship between Mediterranean diet adherence or serum zinc levels, and COVID-19 occurrence, however further studies are required to examine whether Mediterranean diet consumption or serum zinc status reduces the risk of COVID-19 causally.

Keywords: Mediterranean Diet, zinc, obesity, depression; COVID-19

1. INTRODUCTION

The worldwide coronavirus disease 2019 (COVID-19) pandemic has become the important global health emergency. Accordingly, World Health Organization (WHO) defined it as a global epidemic (pandemic) on 11 March 2020 (1).

The COVID-19 pandemic involved movement restrictions, also known as 'lockdown' or 'mass quarantine', to stop or limit the spread of the virus by restricting individual mobility and face-to-face interaction (2). These restrictions involved travel restrictions, stay-at-home orders, curfew enforcements, working-from-home advisories, self-quarantine, and also nationwide closure of schools, non-essential businesses and territorial borders (3). These measures have led to critical changes in lifestyle, resulting in high levels of stress, anxiety, and depression in addition to the risk of obesity (4).

Globally, virus-associated restrictions and lifestyle changes have led to adverse health consequences. It was highlighted the importance of maintaining a healthy lifestyle in fighting against COVID-19 pandemic (5). COVID-19 susceptibility may have been impacted by the eating, physical activity and other weight-related lifestyle behaviors. In this context, it was found that high body mass index (BMI) and insufficient physical activity associated with the risk of COVID-19 severe illness, with an implication that maintaining a healthy lifestyle could protect from COVID-19 susceptibility (6). It has also been identified that individuals with overweight and obesity increased as a result of virus-associated lifestyle

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changes. Body weight might increase as a result of unhealthy eating behaviors to cope with the pandemic (7). In addition to these, stress, depressive symptoms, financial strain, and loneliness exacerbated during the COVID-19 pandemic that might influenced the emotional state of individuals and lead to unhealthy eating behaviors like overeating (8-10).

Adherence to healthful dietary patterns may also protect from COVID-19 susceptibility. Data from 592 571 participants of the smartphone-based COVID-19 Symptom Study provide evidence that a healthy diet, characterized by healthy plant-based foods, was associated with lower risk and severity of COVID-19 (11).

Unhealthy lifestyle risk factors which might led to low grade inflammation have been associated with COVID-19 susceptibility (5). Obesity and other unhealthy lifestyle risk factors interactively impair immune function and increase the risk of severe infectious disease. It is currently not known whether patients with obesity are also more likely to have greater COVID-19 severity of illness (12).

Maintain a balanced function of the immune system and the redox system, zinc deficiency can probably be added to the factors predisposing individuals to infection and detrimental progression of COVID-19 (13). Numerous studies have demonstrated that zinc modulates immune response. Therefore, this possibility show the importance that zinc deficiency may have been associated with COVID-19 susceptibility (14-16).

Unhealthy lifestyle factors have been associated with COVID-19 susceptibility, but data for diet and associated lifestyle factors are conflicting (17). The present study aimed at examining the effects of the COVID-19 susceptibility on obesity, Mediterranean Diet adherence, zinc, and depression in a sample of adults.

2. METHODS

2.1. Study population and design

This observational (comparative) case-control study was conducted in Sarayköy District in Denizli, Türkiye, between December 2020 and September 2021. The sample size was calculated with a tolerable error of 2% and a confidence level of 95%, resulting in a minimum sample of 200. All individuals were selected through the simple random sampling process. A total of 200 individuals aged 20-54, 100 in the former COVID-19 case group and 100 in the control group (individuals with no COVID-19 occurrence) were included in the study. Individuals with any chronic disease, regular medication users, BMI<19 kg/m^2, elite athletes, pregnant and lactating women were excluded from the study.

This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects/patients were approved by the EMU Scientific Research and Publication Ethics Committee with the decision dated 07.04.2021 and numbered 2021/0068. Written informed consent was obtained from all subjects. This study funded and supported by the C-Type Scientific Research Project (date: 08.04.2021, decision: BAPC-OD-21-02). In order to carry out the study and to evaluate the serum zinc level, an application was made to the Private Denipol Hospital in Denizli and written permission was obtained. In this study, it was planned to evaluate the impact of obesity, adherence to the Mediterranean Diet, zinc and depression on COVID-19 susceptibility.

2.2. Demographic characteristics

A semi-structured questionnaire containing questions for collecting data on socioeconomic and demographic characteristics (gender, age, alcohol and cigarette consumption, cigarette and alcohol consumption, meal skipping) was used. Questions including whether or not to have COVID-19, the number of cases of contracting COVID-19, and the status of being vaccinated against COVID-19 were asked. All questions were filled in by the researcher dietician in a face-to-face interview according to the pandemic rules, by following the social distance, mask and hygiene rules.

2.3. Anthropometric measurements

Anthropometric measurements, including weight, height, and waist circumference (WC), were questioned according to individual declaration. Body mass index **(**BMI) was calculated by dividing the weight in kilograms by the square of the height in meters.

2.4. Zinc intake

Daily dietary zinc intake was calculated using a 25-item food frequency questionnaire, which includes foods rich in zinc. Questioned foods were developed based on the data from a short food frequency questionnaire including 18 items (18). The daily zinc intake (mg) of the participants was calculated by using the Nutrition Information Systems Package Program (BEBIS) version 8.2.

2.5. Serum zinc level

Serum zinc levels of all individuals were evaluated. 1 mL of blood was taken from the participants with a injector and transferred to a sodium EDTA tube. After the blood taken into the tube was kept for 30-60 minutes, it was centrifuged and the serum zinc level was determined in the Spectrophotometer device. According to the Private Denipol Hospital, the reference range for serum zinc was accepted as 68-115 µg/dL for women and 70-125 µg/dL for men, according to gender.

2.6. Adherence to the Mediterranean diet

Adherence to the Mediterranean diet were assessed using the Mediterranean Diet Adherence Screener (MEDAS) and Mediterranean Diet Score (MedDietScore).

2.7. Mediterranean Diet Adherence Screener (MEDAS)

The 14-item MEDAS questionnaire was indicated to be a moderate and reasonably valid tool for the rapid estimation of MD adherence (19). Turkish Validation and Reliability of Mediterranean Diet Adherence Screener conducted by Pehlivanoglu et al (20).A total score of 7 and above indicates that the individual has an acceptable degree of compliance with the Mediterranean diet (moderate), and a score of 9 or above indicates that the individual has a strict adherence to the Mediterranean diet (high) (19).

2.8. Mediterranean Diet Score (MedDietScore)

MedDietScore questionnaire has been validated for assessing Mediterranean diet adherence and providing dietary modification advice for primary prevention purposes. According to the rationale of the Mediterranean dietary pattern, the weekly consumption of the following 9 food groups: non-refined cereals (whole grain bread and pasta, brown rice, etc), fruit, vegetables, legumes, potatoes, fish, meat and meat products, poultry, full fat dairy products (like cheese, yoghurt, milk), as well as olive oil and alcohol intake have included in the diet score. A total score ranging from 0 to 55 was calculate/ed that higher values of this diet score indicate greater adherence to the Mediterranean diet (21). Accordingly, 0-20 points of adherence to the Mediterranean diet are considered low, 21-35 points of adherence to the Mediterranean diet are considered high, and 36-55 points of adherence to the Mediterranean diet are considered high (22).

2.9. CES Depression Scale (CES-D)

The Center for Epidemiologic Studies Depression Scale (CES-Depression Scale) was developed by The American National Mental Health Institute (23). It is a short self-report scale that has been found sensitive in measuring depressive symptoms in general population. The Adaptation of the CES-Depression Scale into Turkish was performed by Tatar and Saltukoglu (24).There are 20 items in the scale, and these items include questions to determine one-week feelings and thoughts. The items in the questionnaire are evaluated according to a 4-point Likert scale. (0: Never-Rarely, 1: A little-A few times, 2: Sometimes-Sometimes, 3: A lot-Most of the time). Among the questions, items 4, 8, 12 and 16 are scored in reverse. Participants receive points between 0 and 60 in line with their answers. CES-D Scale classified as <15 points not depression, 16-20 points as mild depression, 21-30 points as moderate depression, and >31 points as severe depression (23, 24).

2.10. Statistical analyses

All statistical analyses were performed using the statistical software package Statistical Package for the Social Sciences (SPSS) Statistics for Windows version 24.0 and p values were considered to be statistically significant if less than .05. Data are expressed as means ± standard deviation (SD) for continuous variables, and as number (percentage) for categorical variables. Variables were tested for normality using the Kolmogorov-Smirnov test. It was determined that the data set were not follow a normal distribution. For this reason, non-parametric hypothesis tests were used in the study. Comparisons of the basic and anthropometric measurements of the participants were performed. Mann Whitney U-test was used to determine the differences between former COVID-19 case group and control group. Differences between categorical variables were tested by Chi-square test. Binary logistic regression was applied to get the factors potentially related to COVID-19 occurrence.

3. RESULTS

3.1. Baseline characteristics

In this study, 100 patients with COVID-19 history were confirmed as a case group, and 100 healthy individuals with no COVID-19 occurrence were included as the control group. There was no chronic disease (non-included in the study) in any of the participants. The average age of the participants were 37.7±10.2 and 68% of the participants were women (Table 1).

Table 1. *Baseline characteristic of participants based on COVID-19 occurrence.*

Characteristics	All	Former COVID-19 case group	Control group	*p* value
Participants n (%)	200 (100)	100 (100)	100 (100)	
Gender (male) n (%)	64 (32)	38 (59.4)	26 (40.6)	
Gender (female) n (%)	136 (68)	62 (45.6)	74 (54.4)	
Age (years), X±SD [a]	37.7±10.2	37.7±9.9	37.7±10.4	.927
Age (years), X±SD (male) [a]	38.1±10.1	38.2±10.3	38.1±10.0	.978
Age (years), X±SD (female) [a]	37.5±10.2	37.5±9.9	37.6±10.6	.962
Vaccinated against COVID-19, n (%)[b]	124 (62.0)	63 (63.0)	61 (61.0)	.771
Current smoker n (%)[b]	57 (28.5)	30 (30.0)	27 (27.0)	.638
≤10 cigarettes/day n (%)[b]	25 (44.6)	13 (46.4)	12 (42.9)	.788
>10 cigarettes/day n (%)[b]	31 (55.4)	15 (53.6)	16 (57.1)	
Alcohol abstainers n (%)[b]	149 (74.5)	78 (78.0)	71 (71.0)	.256
2 drinks or more for men/1 drink or more in a day for women n (%)[b]	23 (11.5)	11 (47.8)	12 (52.2)	.98
Skipping meals n (%)[b]	69 (34.5)	34 (34.0)	35 (35.0)	.893

[a] *Data are presented as mean (standard deviation), X±SD. P: Mann Whitney U test ; p <.05*

[b] *n, number of participants; % , percentage of participants. P: Chi-squared test ; p<.05*

The comparative analysis of former COVID-19 patients and healthy controls showed no significant difference between

lifestyle modifications in terms of age, vaccination, alcohol consumption, smoking status and meal skipping rate (p>.05) (Table 1). Regarding lifestyles, similar conclusions were obtained after stratifying by gender categories between case and control groups (p>.05) (Table 1)

3.2. Assessment of anthropometric measures

Mean BMI upon admission to the study was 26.4±4.4 kg/m^2. Distribution of mean values of weight (kg), height (cm), WC (cm) and BMI (kg/m^2) did not differ between case and control groups. In other words, there was no statistically significant difference between the anthropometric variables specified according to the state of infection in either gender (p>.05) (Table 2).

Table 2. *Comparison of mean (X±SD) anthropometric variables in former COVID-19 patients with control group.*

	All	Former COVID-19 case group	Control group	*p* value
Anthropometric measurements				
Weight (kg)	74.6±14.9	73.7±13.7	75.5±16.1	.532
Weight (kg) male	85.9±14.7	83.8±11.6 (38)	88.9±18.1 (26)	.356
Weight (kg) female	69.3±11.8	67.5±11.0 (62)	70.7±12.3 (74)	.112
Height (cm)	167.5±9.2	167.7±8.6	167.4±9.8	.675
Height (cm) male	175.4±7.4	174.3±6.2 (38)	177.1±8.8 (26)	1.00
Height (cm) female	163.8±7.5	163.7±7.4 (62)	163.9±7.6 (74)	.715
WC (cm) male	99.9±12.8	2.4±0.9 (38)	2.4±0.9 (26)	1.00
WC (cm) female	86.6±12.8	1.8±0.9 (62)	1.9±0.9 (72)	.302
BMI (kg/m^2)	26.4±4.4	1.8±0.7 (100)	1.8±0.8 (100)	.732
BMI (kg/m^2) male	27.7±3.7	2.1±0.7 (38)	2.0±0.8 (26)	.595
BMI (kg/m^2) female	25.8±4.6	1.6±0.7 (62)	1.8±0.8 (72)	.185

X, mean values; SD, standard deviation. P: Mann Whitney U test ; p <.05 BMI, body mass index; WC, waist circumference.

We classified our study participants based on different adiposity risk categories, adopting WCas an indicator of abdominal fat distribution and BMI as an indicator of body fatness. The clinical characteristics of patients with low, moderate, high risk abdominal obesity or with normal weight, with overweight and with BMI-based obesity are shown in Table 3. No patients was underweight and 39.5% (n=79) had a BMI of between 25 and 29.9 kg/m^2, while 20.5% (n=41) had a BMI over 30 and above kg/m^2 (Table 3). There were no significant differences between participants with normal weight, with overweight or with BMI-based obesity and COVID-19 occurrence (p>.05). By way of explanation, there were no differences in between case-control groups and BMI classes (p=.321) (Table 3). Likewise, no significant COVID-19 occurrence differences were found between participants with or without abdominal obesity risk for men or women (p=.431, p=.169; respectively), although the rate of the male participants is higher in high risk waist category, overweight or who suffered from BMI-based obesity than those with normal weight.

Table 3. *Difference in BMI and WC risk categories between groups.*

	All	Former COVID-19 case group	Control group	*p* value
BMI classes				
BMI category, n (%)	200 (100)	100 (50)	100 (50)	
Normal weight*	80 (40)	39 (48.8)	41 (51.2)	.321
Overweight**	79 (39.5)	44 (55.7)	35 (44.3)	
Obesity***	41 (20.5)	17 (41.5)	24 (58.5)	
BMI category for genders				
BMI category male, n (%)				
Normal weight*	15 (23.4)	7 (18.4)	8 (30.8)	.431
Overweight**	30 (46.9)	20 (52.6)	10 (38.5)	
Obesity***	11 (28.9)	8 (30.8)	19 (29.7)	
BMI category female, n (%)				
Normal weigh*	65 (23.4)	32 (51.6)	33 (44.6)	.169
Overweight**	49 (36.0)	24 (38.7)	25 (33.8)	
Obesity***	22 (16.2)	6 (9.7)	16 (21.6)	
WC classes for genders				
WC category for male, n (%)				
Low risk*	19 (29.7)	11 (28.9)	8 (30.8)	.704
Moderate risk**	1 (1.6)	1 (2.6)	-	
High risk***	44 (68.8)	26 (68.4)	18 (69.2)	
WC category for female, n (%)				
Low risk*	68 (50)	35 (56.5)	33 (44.6)	.205
Moderate risk**	12 (8.8)	3 (4.8)	9 (12.2)	
High Risk***	56 (41.2)	24 (38.7)	32 (43.2)	

*n, number of participants; %, percentage of participants. P: Chi-squared test; p <.05; *18.5-24.9 kg/m²; **25-29.9 kg/m²; ***≥30 kg/m²; *male ≤ 94 cm, female ≤ 80 cm; **male 95-102 cm, female 81-82 cm; ***male ≥ 102cm, female > 88 cm; BMI, body mass index; WC, waist circumference.*

3.3. Adherence to the Mediterranean diet and CES-D scale

Table 4 shows the levels of the adherence to the Mediterranean diet; in the total sample the mean±SD of the MedDiet and MEDAS scores among the study participants are 28.2±7.3 and 7.6±2.5, respectively. Average adherence to the Mediterranean diet scores are lower in the case groups compared to controls, even stratified by in both genders (Table 4). However only the cases of total and female MedDiet scores (p<.001) , total and MEDAS male scores (p <.05) showed a significantly lower mean±SD values compared to controls (Table 4).

In Table 5, the statistical relationship between the groups according to the gender and scale scores of MEDAS, MedDiet and CES-D of the participants, whether they had COVID-19 or not, has been examined. The vast majority (81.6%) of men who have had COVID-19 have adapted moderately to the Mediterranean diet (MEDAS adherence). But there is no such clear distinction for women. Of women who had COVID-19, 61.3% had good (moderate) adherence to the diet, while 38.7% had high compliance. In addition, the difference between the scale scores of the women, only MedDiet scores showed a difference with the cases of having COVID-19 (p <.05) while It was determined that MedDiet and MEDAS scores showed a difference in men who had COVID-19 (p <.05) (Table 5).

There was no difference between the scale groups according to gender of people who did not have COVID-19. Being female or male who did not have COVID-19, did not affect the scale scores (p>.05) (Table 5).

3.4. Serum zinc and COVID-19 occurrence

While the total zinc intake (food sources and supplements) showed a significant difference between the former case and control groups (12.6±13.0 vs 12.8±7.2 mg, respectively, p=.003), no statistically significant difference was observed in food sources related zinc intake mean values between groups (9.4±5.71 vs 10.1±9.45 mg, p=.022) (Table 6).

Table 4. *Comparison of mean (X±SD) CES-D scale, MedDietScore and MEDAS score values among groups.*

Score values	All	Former COVID-19 case group	Control group	*p* value
CES-D scale	18.6±10.9	19.4±10.6	17.9±11.2	.241
CES-D scale male	17.9±10.8	17.5±9.7	18.6±12.5	.945
CES-D scale female	18.9±10.9	20.5±11.0	17.6±10.8	.119
MedDietScore	28.2±7.3	26.3±6.1	30.1±7.9	**<.001***
MedDietScore male	27.5±7.9	26.2±6.1	29.2±9.8	.092
MedDietScore female	28.6±7.0	26.4±6.1	30.4±7.2	**.001***
MEDAS score	7.6±2.5	7.2±2.4	7.9±2.5	**.035***
MEDAS score male	7.5±2.4	6.9±1.9	8.2±2.8	**.032***
MEDAS score female	7.6±2.5	7.3±2.6	7.9±2.4	.316

*X, mean values; SD, standard deviation. P: Mann Whitney U Test ; *Denotes significant difference (p<.05) cases vs .control MEDAS, Mediterranean Diet Adherence Screener; MedDietScore, Mediterranean Diet Score; CES-D scale, The Center for Epidemiologic Studies Depression Scale.*

Table 5. *Adherence to the MEDAS , MedDietScore and CES-D scale of the participants.*

Characteristics	Former COVID-19 case group Male n (%)	Control group Male n (%)	*P* value male	Former COVID-19 case group Female n (%)	Control group Female n (%)	*p* value female	Difference in COVID-19 occurrence between genders
MEDAS Adherence							
Moderate	31 (81.6)	11 (42.3)	**0.001***	38 (61.3)	38 (51.4)	.245	0.427
High	7 (18.4)	15 (57.7)		24 (38.7)	36 (48.6)		
MedDietScore Adherence							
Low	9 (23.7)	6 (23.1)	**0.003***	12 (19.4)	6 (8.1)	**<.001ŧ**	0.066
Average	26 (68.4)	9 (34.6)		47 (75.8)	41 (55.4)		
High	3 (7.9)	11 (42.3)		3 (4.8)	27 (36.5)		
CES-D scale							
No depression	14 (36.8)	14 (53.8)	0.067	17 (27.4)	26 (48.6)	.060	0.733
Possibility of mild depression	8 (21.1)	2 (7.7)		16 (25.8)	10 (13.5)		
Possibility of moderate depression	14 (36.8)	5 (19.2)		17 (27.4)	18 (24.3)		
Possibility of severe depression	2 (5.3)	5 (19.2)		12 (19.4)	10 (13.5)		

*%, percent of participants. P – value: Pearson's Chi-square test. *Denotes significant difference (p< .05) between male cases vs .control. ŧDenotes significant difference (p<.05) between female cases vs. control. MEDAS, Mediterranean Diet Adherence Screener; MedDietScore, Mediterranean Diet Score; CES-D scale, The Center for Epidemiologic Studies Depression Scale.*

In addition, women in former COVID-19 case group had an average of 11.3±11.2 mg/day total zinc intake, while women who were in control group had 12.6±6.8 mg/day total zinc intake. While the serum zinc level of women who had COVID-19 was 60.6±15.7 mg/dL, it was 74.4±14.2 mg/dL for those who did not. A statistically significant difference was found between total zinc and serum zinc levels according to the COVID-19 transmission status of the women included in the study (p<.05). However, according to COVID-19 infection status, there was no statistically significant difference found between the men's average zinc intakes (p>.05) (Table 6).

Meanwhile, average serum Zn levels for all participants are as follow, 70.4±18.0 µg/dL. Accordingly, former COVID-19 patients had significantly lower zinc levels in comparison to the healthy controls (64.7±17.6 µg/dL vs 76.1±16.7µg/dL, p<.001). Consistently, in both genders, in case groups significantly lower serum zinc levels were observed (for male p=.009 and female p<.001, respectively) (Table 6).

Additionally, serum zinc level and MedDiet score were found to be significant variables explaining the status of COVID-19 occurrence (p<.05). When the serum zinc level and MedDiet score increase, the occurrence of COVID-19 decreases (Table 7).

Table 8 shows the distribution of the serum zinc level based on the reference value of the individuals, according to whether they had COVID-19 or not. The serum zinc level of 52.5% (n=105) of the total participants, 76.1% (n=80) of those who had COVID-19 and 23.8% (n=25) of those who did not have COVID-19, was below the reference value.

A significant difference was found between the cases of COVID-19 occurrence and the distribution of serum zinc levels. While the serum zinc value of the majority of those who had COVID-19 is below the reference value, it is between and/or above the reference value of the majority of those who did not have COVID-19. (Table 8)

Tablo 6. *Difference in mean (X±SD) serum zinc levels (µg/dL) and zinc intake (mg) values in former COVID-19 patients and healthy controls.*

	All	Former COVID-19 case group	Control group	*p* value
Total Zinc intake (mg) (food sources and supplements)	12.7±10.5	12.6±13.0	12.8±7.2	**.003***
Total Zinc intake (mg) (min.-max) male	14.05±11.85	14.6±15.4 (1.6-75.0)	13.5±8.3 (2.4-34.3)	.187
Total Zinc intake (mg) (min.-max) female	11.95±9.0	11.3±11.2 (2.4-62.2)	12.6±6.8 (3.6-35.9)	**.002***
Zinc intake (food sources) (mg)	9.8±5.38	9.4±5.71	10.1±9.45	.052
Serum Zinc levels (µg /dL)	70.4±18.0	64.7±17.6	76.1±16.7	**<.001***
Serum Zinc levels (µg /dL) (min.-max) male	16.15±29.55	71.3±18.6 (35.0-120.0)	81.0±21.9 (60.0-177.0)	**.009***
Serum Zinc levels (µg /dL) (min.-max) female	97.8±14.95	60.6±15.7 (36.0-142.0)	74.4±14.2 (41.0-126.0)	**<.001***

*X, mean values; SD, standard deviation. P: Mann Whitney U test ; *significant results (p<.05)*

Table 7: *Binary logistic regression analysis for the factors that are associated with COVID-19 occurrence.*

Variables	Regression coefficient (β)	Standard error (SE)	Wald	df	Sig	Odds ratio (Exp(β))	95% CI for Odds ratio Lower	Upper
Age (years)	-0.002	0.017	0.008	1	0.927	0.998	0.966	1.032
Weight (kg)	-0.008	0.015	0.259	1	0.611	0.992	0.964	1.022
Serum Zinc levels (µg /dL)	**0.043****	0.011	15.520	1	0.000	1.044	1.022	1.067
Total Zinc intake (mg)	0.006	0.015	0.130	1	0.718	1.006	0.976	1.036
CES-D scale	-0.004	0.015	0.064	1	0.402	0.996	0.967	1.026
MEDAS score	0.061	0.073	0.703	1	0.015	1.063	0.922	1.225
MedDiet Score	**0.062****	0.026	5.879	1	0.024	1.064	1.012	1.118

*Cox & Snell R^2= 0.171; Nagelkerke R^2= 0.227; – 2 Log Likelihood = 239.861; Chi Square= 37.398**; CI: Confidence interval, bold values were statistically significant at *: p≤.05; **: p<.01*

Table 8. *Difference in Serum Zinc Intake and Zinc status based on reference values.*

	All	Former COVID-19 case group	Control group	*p value*
Zinc intake below reference n (%) (male < 9.4 mg , female < 7.5 mg)	84 (42.0)	55 (65.5)	29 (34.5)	**.001***
Serum Zinc levels below reference n (%) (male < 70 µg /dL, female < 68 µg /dL)	105 (52.5)	80 (76.1)	25 (23.8)	**<.001***

*n, number of participants; %, percentage of participants. P: Chi-squared test; *significant results (p <.05)*

4. DISCUSSION

Although it was emphasized that there is a limited number of evidence to determine whether obesity increases the susceptibility of virus infection, several reports including meta-analysis and systematic reviews have confirmed the correlation between increased BMI and worse clinical outcome (ICU admission, use of mechanical ventilation, hospital admission and mortality) of COVID-19 in adult patients (25-28). Moreover, it was stressed that obesity defined by BMI could be an independent risk factor for COVID-19 (29). However, as opposed to BMI which poorly describes actual body fat excess, WC is shown to be more associated to chronic low-grade inflammation (30-32). Besides that, patients with high visceral fat measurements observed to have elevated inflammatory cytokine levels which linked to elevated obesity-associated morbidity in infections (30). Indeed, it recently stressed that visceral fat and upper abdominal circumference specifically increased the likelihood of severity of COVID-19 (32, 33).

In the current study no significant association was observed between COVID-19 occurrence, BMI and the mean WC values of participants. The subgroup analyses of participants with normal weight, overweight and those with BMI-based obesity also showed no significant difference in the COVID-19 occurrence. In addition, no significant effect of WC on COVID-19 cases in any (low, medium and high risk) subgroups found. This results is compatible with the finding of a recent study where it was proposed that apart from BMI, body fat distribution, in particular visceral adiposity, plays no direct causal role regarding COVID-19 severity and susceptibility (29). It is proposed that because of the single centered observational study design, including residual confounding and reverse causality, correlations of BMI and visceral fat accumulation with COVID-19 can be subjected to biases (29).

It is noteworthy that we could slightly underestimate the BMI and weight lost in COVID-19 patients. First of all, large cohort studies (34, 35) showed that self-reported weight upon admission cause underestimation of weight. Due to the given COVID-19 pandemic restrictions to prevent viral spread self-reported weights used in this study. Moreover, neither body weight at hospital discharge nor body composition at the base line were recorded and were missing for the analysis. It has been proposed that COVID-19 might negatively impact body weight and nutritional status. In many COVID-19 patients, independent of hospitalization, a weight loss of >5% which defined as the threshold used to diagnose cancer cachexia was observed (36). Additionally, it was emphasized that in COVID-19 patients weight loss even in patients with obesity in the hospital setting should be taken carefully because of the malnutrition risk which is known not only by low body mass but also unhealthy body composition and loss in the skeletal muscle mass (37).

Taken together, a cohort study presented that in COVID-patients weight loss may occur in a relatively short time period (32 days) and the patients could not return at the initial body weight at the follow up visits easily (a median of 23 days since discharge). This highlights the importance of questioning the disease duration and the length of hospitalization where both of the factors found as significant independent variables of weight loss by reflecting the disease severity and inflammation (34). With regards, another possible underestimation reason for the study could be that the evaluated patients was COVID-19 survivors whose prevalence of weight loss and the risk of malnutrition among seriously ill patients were lower.

Furthermore, taste/smell disturbances and other emotional factors such as fear, sadness, frustration, anxiety and anger may reduce the desire to eat or limit the access to food and/or the variety of food choices which having direct implications for nutrition and weight status of COVID-19 patients (38). Above all, the sample size was statistically representative of the population. However, trials with an increased number of participants should be evaluated (39).

The increased Mediterranean Diet score values were associated with a lower risk of COVID-19 occurrence (40). Furthermore, two recent observational studies showed an inverse correlation between the adherence to the Mediterranean diet and COVID-19 cases in selected European countries (41, 42). Similar to referred studies when adjusted for factors of well-being and physical activity, there was a negative association examined between the status of having COVID-19 and adherence to Mediterranean diet through two different tools in this study. However, the preventive role of Mediterranean diet in COVID-19 solely could not be completely approvable due to the limitations of the study. First of all, the observational study design does not lead to causative conclusion to be reached based on results. Secondly, although the adherence to the Mediterranean diet showed a significant negative association with depression score in adjusted models. Other life satisfaction factors which could have influence on viral infection occurrence and Mediterranean diet adherence such as income, education, age, gender, genetics and other sociodemographic characteristics should also be considered in the assessment models (40, 41). Additionally, not collecting data on medications and other nonpharmacological preventive

measures (masks, hand washing etc.) could be the possible confounding factors of the study (42).

A number of review articles (43, 44) have examined Zn deficiency could be associated to the risk of infection or severe complication of COVID-19. Zn adequacy is needed to support anti-oxidant, anti-inflammatory, immune-boosting and other protective effects in COVID-19 patients (45). Notably, Zn is referred as a common subject in both prophylactic and curative for COVID-19 (43).

A recent case control study showed that serum zinc levels in COVID-19 patients are lower than control groups (39). Moreover, a prospective study data clearly demonstrated that a significant number of COVID-19 patients were zinc deficient compared to healthy controls (46). On the similar lines, in the current study former COVID-19 patients had a significantly lower zinc levels. Moreover, amongst them significant number were found zinc deficient in both genders. However, it is unclear whether low status zinc levels is simple causation or an epiphenomenon of COVID-19. We should be cautious about the interpretation of the results in this scenario.

In other words, serum zinc level is highlighted as one of the most important recommended method to estimate dietary zinc status in individuals (47). So far, it was observed that Zn absorption could be influenced by some foods. Particularly animal proteins result a greater Zn absorption, while phytates reduce its absorption (43). Moreover, according to the existing evidence, Zn deficiency could be highly prevalent in Türkiye secondary to high phytate consumption (48). Given that, only questioning the zinc-rich foods could be a possible limitation of the study. Dietary constituents (content) and physiological conditions that affect the bioavailability of Zn should also be evaluated. Furthermore, clinical studies have revealed that cellular Zn intake can be improved by ionophores including chloroquine and some of its derivatives such as hydroxychloroquine (43). Interestingly, hydroxychloroquine, a drug used initially in the management of COVID-19, is an ionophore that transports zinc across the hydrophobic cell membrane. Additionally, study results generally recommends that zinc supplements with antiviral drugs containing zinc ionophores precisely target and bind to SARS-CoV-2 preventing its replication within the infected host cells. Intracellularly, zinc binds with RNAdependent RNA polymerase causing elongation inhibition and decreased template binding of the viral mRNA (28, 46). On the other hand, a concern had arisen that long term (over 6 week) large doses (300 mg/day) Zn treatment can cause suppression of the immune system (43).

In the present study we failed to follow up and assess patients Zn levels and Zn supplementation in different stages of disease as we only collect the serum Zn levels and question Zn intake once after recovery. On this basis, before concluding we should have data clarifying which patients were zinc deficient and which were not before COVID-19. Furthermore, the baseline clinical and treatment characteristics as well as supplementation should be taken into consideration at initial assessment, throughout the course of disease, and after clinical remission. This could have been probably yield a better explanation for future studies.

5. CONCLUSION

In conclusion, even though our findings suggest a negative relationship between Mediterranean diet adherence or serum zinc levels, and COVID-19 occurrence, further studies are required to examine whether Mediterranean diet consumption or serum zinc status reduces the risk of COVID-19 causally. Also, future research is necessary to investigate the underlying mechanisms linking obesity and zinc status with COVID-19. In particular, as an integral part within the treatment and management of COVID-19 patients; nutritional and anthropometric evaluation, nutrition counselling and nutritional treatment should be applied at initial assessment, during the course of the disease, and after clinical remission.

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Clinical and Experimental Health Sciences

The Effect of Sexual Education and Counseling Based on the Ex-PLISSIT Model on the Sexual Life in Primigravidas

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ABSTRACT

Objective: This study was conducted to determine the effect of sexual education and counseling based on the Ex-PLISSIT model on sexual function, attitudes towards sexuality during pregnancy, sexual distress, and quality of sexual life in primigravidas.

Methods: This study was carried out on 62 primigravidas in a pretest posttest, a quasi-experimental control group. Primigravidas meeting the inclusion criteria were assigned to the groups by simple random sampling method. The intervention group received training and counseling sessions twice. The control group received routine care during the follow-up period. The results were compared with the evaluation forms 10 weeks after each training and counseling session. The forms were administered to the control group at the same time as the intervention group.

Results: The mean scores of the intervention and control groups' attitude towards sexuality during pregnancy in the second test (95%CI=2.33-19.01; d=0.65, p=.013) and in the posttest (95%CI=2.28-19.34; d=0.64, p=.015) were statistically significantly different. Sexual life quality scale mean scores of the intervention and control groups were statistically significant in the second test (95%CI=8.27-18.54; d=1.33, p=.000) and the posttest (95%CI=12.14-22.01; d=1.76, p=.000) level was different. In addition, the mean scores of the intervention group were higher after sexual education and counseling based on the Ex-PLISSIT model.

Conclusion: In this study, sexual education and counseling based on the Ex-PLISSIT model positively affected primigravida's attitudes towards sexuality and their quality of sexual life. Therefore, this model can be used as a cost-effective and simple counseling method to improve the sexual life.

Keywords: Ex-PLISSIT model, Primigravida, sexual counseling, sexual education, sexual life

1. INTRODUCTION

Pregnancy, which is one of the physiological changes that affect sexual health, is a process that plays an important role in women's sexual functions and behaviors, and sexual problems are experienced intensely (1). In this process, sexual life is affected by anatomical, physiological and mental changes and differences occur in the sexual response cycle (2). Pregnancy symptoms and negative beliefs and feelings of couples about sexuality during pregnancy can cause long-term reductions in sexual activity. However, sexuality is important in maintaining a positive relationship of couples (3,4). In a study, it was determined that 40% of pregnant women had a negative attitude towards sexuality during pregnancy (5). In some studies, it has been reported that sexual function generally decreases during pregnancy, especially in the first and third trimesters (6,7).

To maintain a healthy pregnancy and a healthy family relationship, it is very momentous to maintain harmony and communication between spouses, to enhance the quality of life of women, to prevent sexual problems that may occur in the postpartum period, and to raise awareness by correcting false information. Although it is a factor affecting general health and quality of life, sexual problems during pregnancy can be caused by privacy, lack of communication, lack of guidance, etc. It is known that it is still a neglected condition for various reasons (8). The World Health Organization reports that, in addition to interventions for sexual dysfunctions, identifying and addressing sexual anxiety and problems are important basic factors in the improvement of sexual health services. But sexual health is often overlooked by healthcare providers. Those who have sexual health problems usually do not seek help for it (9,10). Therefore, it is important to evaluate sexual health during pregnancy. There are some models (ALARM, ALLOW, BETTER, Ex-PLISSIT and PLISSIT models) that can be used by health professionals to evaluate sexual wellness and provide a solution-oriented approach to sexual problems. Among these models, PLISSIT and Ex-PLISSIT models, which are widely used, easy to apply and effective, are accepted as safe tools (11,12).

The absence of a guideline in the literature based on a systematic model for the sexual health of pregnant women has also been identified as an important deficiency. With model-based sexual education and counseling, it is possible to contribute to the prevention of sexual problems in

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pregnancy by eliminating the concerns of primigravidas and their partners, creating reliable information, correcting false information and beliefs.

The aim of this study is to determine the effect of sexual education and counseling based on the Ex-PLISSIT model on sexual function, attitudes towards sexuality during pregnancy, sexual distress, and quality of sexual life in primigravidas.

The following hypotheses were tested:

H1: The sexual education and counseling for primigravidas developed according to the Ex-PLISSIT model increases women's sexual function during pregnancy.
H2: The sexual education and counseling for primigravidas developed according to the Ex-PLISSIT model increases women's positive attitudes toward sexuality during pregnancy.
H3: The sexual education and counseling for primigravidas developed according to the Ex-PLISSIT model reduces pregnant women's sexual distress.
H4: The sexual education and counseling for primigravidas developed according to the Ex-PLISSIT model increases the quality of sexual life of pregnant women.

2. METHODS

2.1. Design and Sample

This study is in the type of pretest and posttest, a quasi-experimental research with a control group.

The study was conducted in the obstetrics and gynecology outpatient clinics of a hospital.

Sample size was calculated by power analysis. Power analysis showed that a minimum number of individuals should be included in the sample (Power-G 3.1). In order to keep the sample size at the maximum level, the p-rate was set as 0.50. The sample consisted of 70 primigravida (35 in the intervention group and 35 in the control group). For each group, uptake of 35 primigravida provided a margin of error of 5%, an effect size of 30%, and a population representation (power) of 80%. The population of the study consisted of 357 pregnant women who applied to the outpatient clinic for pregnancy control.

The inclusion criteria of the study; being primigravida, 8-16th to be between gestational weeks, not to have a risky pregnancy situation, not to have a sexual dysfunction diagnosis in her partner or herself, and not to be pregnant with infertility treatment. These criteria were chosen to ensure sample homogeneity and to minimize the impact on sexuality during pregnancy. Of the primigravidas who made up the universe and met the limitation criteria, 119 refused to participate in the study because they found the subject confidential or did not have time. Using simple random sampling from primigravidas meeting these criteria, 35 primigravida were initially included in the intervention group, and after this group was completed, 35 primigravida were included in the control group. Abortion occurred in two of the pregnant women in the intervention group, and one pregnant did not continue the study. Abortion occurred in three of the pregnant women in the control group, and two of the pregnant women did not continue the research. In order to evaluate the study data, pregnant women had to participate in all steps of the application. Therefore, at the end of the study, the data of 32 pregnant women in the intervention group and 30 pregnant women in the control group were evaluated (Figure 1).

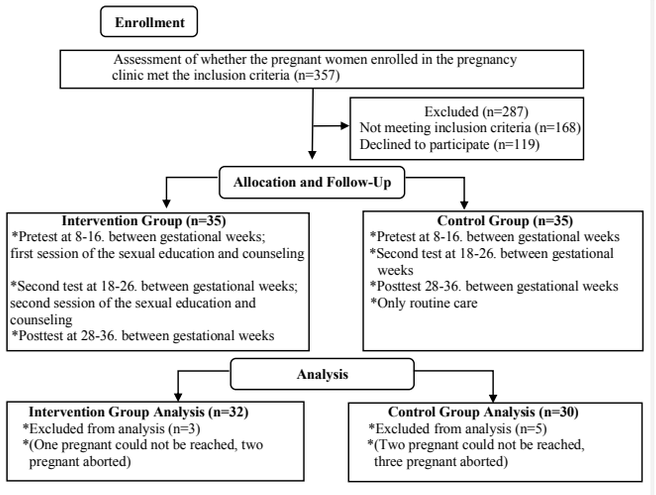

Figure 1. *Flow diagram*

2.2. Intervention

The aim of the intervention in the study is to determine the effect of sexual education and counseling given to primigravidas based on the ex-PLISSIT model on the sexual lives of pregnant women. The total duration of the intervention is nine months.

The researcher, who will provide sexual education and counseling to pregnant women, attended "Sexual Health for Health Professionals" and "Sexual Therapy" courses before starting the practice. These courses were given by expert sexual therapists and covered topics such as sexuality, the physiology of sexuality, factors affecting sexuality, sexual approaches in special situations, and models used in the evaluation of sexuality. Subsequently, a comprehensive literature review on sexual life during pregnancy was carried out, and a comprehensive guide to sexual life during pregnancy was prepared by compiling the information obtained. Opinions were obtained from two experts in gynecology and obstetrics and one in psychiatric nursing about the guide.

Evaluation forms were applied to the pregnant women in the intervention and control groups three times (pretest, second test, and posttest). Pregnant women in the intervention group received two times education and counseling interventions according to the Ex-PLISSIT model (between 8-16 weeks of gestation, between 18-26 weeks of gestation). There was a 10-week period between the sessions of the sexual education and counseling given to pregnant women. Interviews with the pregnant women were conducted individually. Individual Information Form, Female Sexual Function Index (FSFI), Attitude Scale Toward Sexuality During Pregnancy (ASTSP), Female Sexual Distress Scale – Revised (FSDS-R) and Sexual Life Quality Scale-Female (SQLS-F) were applied in the pretest (between 8-16 weeks of gestation). FSFI, ASTSP, FSDS-R

and SQLS-F were applied in the second test (between 18-26 weeks of gestation) and in the posttest (between 28-36 weeks of gestation). The reason for sampling pregnant women between these weeks is that the pregnancy is finalized and the interventions and evaluations are ensured to coincide with every trimester of pregnancy. Thus, the pregnant woman was kept under observation throughout her pregnancy. In the literature, it was stated that sexual education and counseling given according to the Ex-PLISSIT model can be 70%-%90 effective in solving sexual problems even in the first education (13). In the application of the intervention twice, reinforcing the information given to the pregnant woman and solving individual sexual problems were effective.

All steps of the Ex-PLISSIT model were applied sequentially in education and counseling in the first intervention (between 8-16 weeks of gestation). Sexuality of each pregnant woman was evaluated at this stage, the pregnant woman was allowed to express herself and her needs were determined. The implementation of the steps of the model was shaped according to individual requirements. Allow step was applied before each step. At the end of the session, the pregnant women were given homework to do at home, including sharing sexual feelings and thoughts with your spouse, sharing problems and concerns about sexuality during pregnancy, taking care of your physical appearance (clothes, makeup, etc.), using alternatives to sexual intercourse, sexual life information booklet, and were asked to read the information in the booklet and share it with their spouses. In the Intensive Therapy phase of the model, two pregnant women were referred to a sex therapist because they did not find relief from their sexual problems. However, the pregnant women preferred not to see the therapist and to continue with the education and counseling. In the second intervention (between 18-26 weeks of gestation) the step of giving permission was centered and education and counseling were provided in line with the needs of the pregnant woman (Chart 1). A few examples can be given to the information given to pregnant women in the steps of the Ex-PLISSIT model, according to the guide to sexual life in pregnancy we have prepared. Step 1: Permission (How did pregnancy affect your sexual life?, What kind of concerns did you have about your sexual life during pregnancy?); Step 2: Limited Information (physiology of sexuality, sex life during pregnancy); Step 3: Special Suggestions (sexual intercourse positions during pregnancy, sexual intimacy other than sexual intercourse during pregnancy); Step 4: Intensive Therapy (referral of the pregnant woman to a specialist who has received additional training to give intensive therapy).

Chart 1. *Implementation steps of sexual education and counseling intervention based on ex-plissit model*

Tests and Sessions	Time	Techniques and Tasks
Pretest and first session of the sexual education and counseling 8-16. between gestational weeks	60 minute	*Obtaining written consent from pregnant women *Application of data collection forms (pretest) • Individual Information Form • The Female Sexual Function Index • Attitude Scale Toward Sexuality During Pregnancy • Female Sexual Distress Scale-Revised • Sexual Quality of Life Scale-Female *10 minute break *Sexual education and counseling based on the Ex-PLISSIT model • Permission • Limited Information • Permission-Special Suggestions • Permission-Intensive Therapy if needed *Giving homework *Giving the brochure "Sexual Life in Pregnancy" *Making an appointment for the second session after 10 weeks
Second session of the sexual education and counseling 18-26. between gestational weeks	40 minute	* Application of data collection forms (second test) • The Female Sexual Function Index • Attitude Scale Toward Sexuality During Pregnancy • Female Sexual Distress Scale-Revised • Sexual Quality of Life Scale-Female *10 minute break *Discussing homework * Sexual education and counseling based on the Ex-PLISSIT model • Permission • Limited Information • Permission-Special Suggestions • Permission-Intensive Therapy if needed * Appointment for posttest at 28-36. between weeks of pregnancy
Posttest 28-36. between gestational weeks	20 minute	* Application of data collection forms (posttest) • The Female Sexual Function Index • Attitude Scale Toward Sexuality During Pregnancy • Female Sexual Distress Scale-Revised • Sexual Quality of Life Scale-Female

The control group received routine care only. Routine care consisted of prenatal care such as blood pressure monitoring, weight monitoring, measurement of fundal height, auscultation of fetal heart rate, and laboratory testing. After completion of the application, the results of the scale were communicated to the women who requested it.

2.3. Data Collection Process

Data were collected between September 2019 and May 2020. The data of the study were collected using Individual Information Form, Female Sexual Function Index (FSFI), Attitude Scale Toward Sexuality During Pregnancy (ASTSP), Female Sexual Distress Scale-Revised (FSDS-R), and Sexual Quality of Life Scale-Female (SQLS-F).

Individual Information Form: This form contains 18 questions to evaluate the pregnant woman's age, education level, obstetric characteristics and thoughts about sexuality during pregnancy (14-17).

Female Sexual Function Index: The scale was developed to evaluate sexual function in women. The scale consists of 19 items and six subscales. The cut-off point of the scale is ≤26.55 points, which is considered to indicate a negative change in sexual function (18). The reliability coefficient of the scale is 0.98 (19). In this study, the reliability coefficient of the scale is 0.96.

Attitude Scale Toward Sexuality During Pregnancy: It evaluates the attitudes of the pregnant women and partners towards sexuality during pregnancy. The scale consists of 34 items and three subscales. The lowest score that can be obtained from the entire scale is 34, and the highest score is 170. It is accepted that as the scale score increases, the positive attitude towards sexuality increases during pregnancy. The reliability coefficient of the scale is 0.90 (20). In this study, the reliability coefficient of the scale is 0.93.

Female Sexual Distress Scale-Revised: It is a scale to measure personal distress related to sexuality in women with sexual dysfunction. The scale consists of 13 items. The lowest score that can be obtained with the FSDS-R is zero, and the highest score is 52. A higher score indicates a higher level of sexual distress (21). The cut-off point of the scale was ≥11.5 points to determine the presence of gender-related personal distress in Turkish women. The reliability coefficient of the scale is 0.98 (2). In this study, the reliability coefficient of the scale is 0.92.

Sexual Life Quality Scale-Female: The scale consists of 18 items. Each item is answered by considering the sexual life in the last four weeks. The scale score ranges from 18 to 108 and the scale score is converted to 100 using the formula (scale raw score-18) x 100/90. A high score on the scale means that the quality of sexual life is good (23). The reliability coefficient of the scale is 0.83 (24). In this study, the reliability coefficient of the scale is 0.82.

2.4. Data Analysis

Statistical analysis, IBM SPSS Statistics Implemented using for Windows, version 27.0. Continuous data were defined using the mean and 95% Confidence Interval (95% CI). Individual variables in each group were defined as frequency and percentage. It was annotated for comparison applied an independent sample t-test for continuous variables. The effect size of the difference between both conditions on individual variables was determined by calculating Cohen's d. The significance level was accepted as p<.05.

2.5. Ethical Considerations

The study was confirmed by a university in Non-Invasive Clinical Research Ethics Commission of Sivas Cumhuriyet University (2019-6/54-27/02/2019). Primigravidas were informed about the purpose and procedure of the study, and their consent was obtained. The study was performed according to the Helsinki rules.

3. RESULTS

A total of 62 primigravidas participated in this study (Intervention group: 32, Control group: 30). The mean age of the intervention group was 27.50 (SD=3.75; range 17-34), and the mean age of the control group was 27.00 (SD=4.28; range 22-38). The individual characteristics, sexual characteristics, thoughts and knowledge levels of the primigravidas participating in the study were similar, and no statistically significant difference was found between the groups (p>.05) (Table 1).

There was no statistically significant difference between the FSFI total and subscale mean scores in the second and posttests of primigravidas in the intervention and control groups (p>.05). However, in the second and third tests, the sexual function score of the intervention group increased compared to the control group (Table 2).

In the second test, mean total ASTSP scores of the intervention group (95%CI=2.33-19.01; d=0.65, p<.05), mean scores of beliefs and values regarding sexuality during pregnancy (95%CI=0.02-5.17; d=0.51, p<.05) and mean scores of confirming sexuality during pregnancy (95%CI=0.96-9.95; d=0.61, p<.05) were statistically significant compared to the control group. Total ASTSP score averages at the posttest (95%CI=2.28-19.34; d=0.64, p<.05), sexual beliefs and values during pregnancy (95%CI=-0.40-4.69; d=0 .43, p<.05) and confirmation of sexuality during pregnancy (95%CI=2.01-10.39; d=0.75, p<.01) subscales, there was a statistically significant difference in favor of the intervention group (Table 2).

There was no statistically significant difference between the mean FSDS-R values of the primigravidas in the intervention and control groups in the second and last test (p>.05). However, in the second and third tests, the FSDS-R score of the intervention group decreased compared to the control group (Table 2).

Intervention group between the second-test mean SQLS-F score (95%CI=8.27-18.54; d=1.33, p<.001) and the post-test mean SQLS-F score (95%CI=12.14-22.01; d=1.76, p<.001) a statistically significant difference was found in favor of (Table 2).

Table 1. *The comparison of the individual characteristics of the pregnant*

Variables	Intervention group (n=32)	Control group (n=30)	χ^2; p
	n (%)	n (%)	
Age			
17-25 ages	3 (9.4)	8 (26.7)	3.325; .190[a]
26-34 ages	21 (65.6)	17 (56.7)	
≥35 ages	8 (25.0)	5 (16.6)	
Educational level			
Secondary school	1 (3.1)	2 (6.7)	0.720; .698[a]
High school	3 (9.4)	4 (13.3)	
University and above	28 (87.5)	24 (80.0)	
Occupational status			
Yes	22 (68.8)	19 (63.3)	0.203; .652[a]
No	10 (31.2)	11 (36.7)	
Partner's age			
23-27 ages	5 (15.6)	8 (26.7)	1.730; .421[a]
28-32 ages	21 (65.6)	19 (63.3)	
≥33 ages	6 (18.8)	3 (10.0)	
Partners' educational school			
Secondary school	3 (9.2)	1 (3.3)	1.049; .592[a]
High school	6 (18.8)	5 (16.7)	
University and above	23 (71.9)	24 (80.0)	
Income status			
More than expenses	9 (28.1)	7 (23.3)	1.704; .427[a]
Equal to expenses	21 (65.9)	18 (60.0)	
Less than expenses	2 (6.0)	5 (16.7)	
Married ages			
17-25 ages	8 (25.0)	12 (40.0)	1.594; .451[a]
26-34 ages	20 (62.5)	15 (50.0)	
≥35 ages	4 (12.5)	3 (10.0)	
Assessment of marriage			
Middle	4 (12.5)	0 (0.0)	4.930; .085[a]
Good	7 (21.6)	11 (36.7)	
Very good	11 (65.9)	19 (63.3)	
Pregnancy planning status			
Planned	27 (84.4)	26 (86.7)	0.066; .798[a]
Unplanned	5 (15.6)	4 (13.3)	
Can coitus be continued during pregnancy?			
Yes	24 (75.0)	19 (63.3)	0.992; .319[a]
No	8 (25.0)	11 (36.7)	
Do you find it safe to maintain coitus during pregnancy?			
Yes	13 (40.6)	14 (46.6)	3.440; .179[a]
No	4 (12.5)	8 (26.7)	
No idea	15 (46.9)	8 (26.7)	
Sex during your partner's pregnancy what is his thought about it?			
Positive	17 (53.1)	19 (63.3)	4.513; .105[a]
Negative	5 (15.6)	8 (26.7)	
No idea	10 (31.3)	3 (10.0)	
Having sex during pregnancy does it harm the fetus?			
Yes	1 (3.1)	3 (10.0)	1.664; .435[a]
No	18 (56.3)	18 (60.0)	
No idea	13 (40.6)	9 (30.0)	
Do you know the coitus positions that can be used during pregnancy?			
Yes	12 (37.5)	14 (46.7)	5.226; .054[a]
No	6 (18.7)	0 (0.0)	
No idea	14 (43.8)	16 (53.3)	

Is sex just coitus?			0.217; .642[a]
Yes	4 (12.5)	5 (16.7)	
No	28 (87.5)	25 (83.3)	
Have you a sex-related problem is there?			4.737; .054[a]
Yes	7 (21.9)	1 (3.3)	
No	25 (78.1)	29 (96.7)	
You did get it information on sexual life in pregnancy?			0.203; .652[a]
Yes	10 (31.2)	11 (36.7)	
No	22 (68.8)	19 (63.3)	
You did get it information from whom about sexual life in pregnancy?			4.052; .542[a]
Physician	5 (15.6)	8 (26.7)	
Nurse-Midwife	1 (3.1)	1 (3.3)	
Media	1 (3.1)	0 (0)	
Internet	3 (9.4)	2 (6.7)	
	x̄±SD / (min-max)	**x̄±SD / (min-max)**	
Age mean	27.50±3.75 (17-34)	27.00±4.28 (22-38)	
Age of partner mean	29.25±2.27 (23-34)	29.37±3.56 (24-41)	
Marriage age	26.06±3.70 (17-33)	25.50±4.29 (21-38)	

a: chi-square test

Table 2. *The comparison of the pretest-posttest scale scores of the intervention and control groups*

	Pretest		**Second test**		**Posttest**	
	x̄±SD / t; p (95%CI)		**x̄±SD / t; p (95%CI) / d**		**x̄±SD / t; p (95%CI) / d**	
	Intervention group (n=32)	**Control group (n=30)**	**Intervention group (n=32)**	**Control group (n=30)**	**Intervention group (n=32)**	**Control group (n=30)**
The Female Sexual Function Index and sub-dimensions						
Sexual desire	3.00±0.76	3.18±0.92	3.48±0.90	3.32±0.87	3.16±1.15	2.98±0.77
	t=-0.740; p=.462[a] (-0.61-0.25)		t=-0.841; p=.404[a] (-0.29-0.69)		t=-0.750; p=.456[a] (-0.32-0.69)	
Sexual arousal	2.44±1.91	3.17±1.59	3.34±1.53	3.29±1.37	3.22±1.45	2.70±1.61
	t=-1.610; p=.113[a] (-1.62-0.18)		t=0.153; p=.879[a] (-0.69-0.80)		t=1.314; p=.184[a] (-0.26-1.31)	
Lubrication	3.01±2.20	3.72±2.08	3.99±1.53	3.84±1.70	3.59±1.72	2.89±2.04
	t=-1.284; p=.204[a] (-1.79-0.39)		t=0.374; p=.710[a] (-0.67-0.98)		t=1.462; p=.149[a] (-0.26-1.66)	
Orgasm	2.72±2.03	3.41±1.93	3.86±1.58	3.53±1.61	3.33±1.79	2.85±2.08
	t=-1.364; p=.178[a] (-1.70-0.39)		t=0.811; p=.421[a] (-0.48-1.14)		t=0.982; p=.330[a] (-0.50-1.48)	
Satisfaction	3.58±1.66	4.13±1.75	4.65±1.09	4.02±1.42	4.11±1.47	3.61±1.53
	t=-1.255; p=.214[a] (-1.42-0.32)		t=1.937; p=.057[a] (-0.02-1.27)		t=1.308; p=.196[a] (-0.26-1.26)	
Pain	2.92±2.36	3.38±2.11	4.12±2.04	3.36±2.09	3.73±2.08	2.61±2.13
	t=0.808; p=.422[a] (-1.60-0.68)		t=1.455; p=.151[a] (-0.29-1.82)		t=2.098; p=.060[a] (0.05-2.20)	
Total score	17.70±9.96	21.00±9.09	23.46±7.30	21.37±7.64	21.17±8.31	17.65±9.22
	t=-1.369; p=.179[a] (-0.34-0.11)		t=1.104; p=.274[a] (-0.11-0.39)		t=1.580; p=.119[a] (-0.06-0.36)	
Attitude Scale Toward Sexuality During Pregnancy and sub-dimensions						
Anxiety about Sexual Intercourse during Pregnancy	30.81±6.21	33.36±7.50	36.18±5.80	33.56±6.60	35.53±6.02	33.06±6.20
	t=-1.463; p=.149[a] (-6.05-0.94)		t=-1.663; p=.102[a] (-0.53-5.77)		t=-1.587; p=.118[a] (-0.64-5.57)	
Dysfunctional Beliefs and Values about Sexuality during Pregnancy	41.25±5.25	42.83±5.23	45.06±4.48	42.46±5.60	45.78±4.52	42.63±5.47
	t=-1.188; p=.240[a] (-4.25-1.08)		**t=2.019; p=.048[a] (0.02-5.17) / d= 0.51**		**t=2.017; p=.049[a] (-0.40-4.69) / d=0.43**	
Approving Sexuality during Pregnancy	47.87±7.32	48.63±8.43	54.71±7.56	49.26±10.03	55.03±7.84	48.83±8.65
	t=-0.379; p=.706[a] (-4.77-3.25)		**t=2.426; p=.018[a] (0.96, 9.95) / d=0.61**		**t=2.958; p=.004[a] (2.01-10.39) / d=0.75**	
Total score	119.93±6.91	124.83±19.09	135.96±14.89	125.30±17.87	136.34±15.14	124.53±18.36
	t=-1.065; p=.291[a] (-14.09-4.30)		**t=2.559; p=.013[a] (2.33-19.01) / d=0.65**		**t=2.519; p=.015[a] (2.28-19.34) / d=0.64**	
Female Sexual Distress Scale-Revised						
Total score	11.53±10.34	4.43±6.92	5.28±3.92	6.93±8.10	6.56±7.14	8.66±9.12
	t=3.193; p=.003[a] (-0.53—0.08)		t=-1.010; p=.307[a] (-0.10-0.25)		t=-1.015; p=.314[a] (-0.25-0.22)	
Sexual Quality of Life Scale-Female						
Total score	68.85±11.15	69.40±8.20	81.73±8.20	68.33±11.79	85.97±9.36	68.89±10.08
	t=-.228; p=.820[a] (-5.40-4.29)		**t=5.223; p=.000[a] (8.27-18.54) / d=1.33**		**t=6.915; p=.000[a] (12.14-22.01) / d=1.76**	

a: Independent sample t test

4. DISCUSSION

The aim of this study was to determine the effect of sexual education and counseling based on the Ex-PLISSIT model on the sexual life of primigravidas. First, the individual characteristics of primigravidas and some thoughts and knowledge levels about sexuality during pregnancy were evaluated. It was examined whether the characteristics of primigravidas in the intervention and control groups were similar. Both groups were homogeneous in terms of these variables.

The sexual functions of primigravidas, their attitudes towards sexuality during pregnancy, their sexual distress and sexual life quality and their subscales were evaluated with the obtained data.

First, sexual function scores of primigravidas were measured. The mean score obtained was below the cut-off point of FSFI of 26.55 (Intervention group: 17.70±9.96; Control group: 21.00±9.09). The aim was to improve sexual function after counseling. FSFI scores did not rise above the cut-off point after counseling, but increased after the first (Intervention group: 23.46±7.30; Control group: 21.37±7.64) and second session (Intervention group: 21.17±8.31; Control group: 17.65±9.22) of counseling in the intervention group. The score after the first session was slightly higher than the second session. It can be thought that the coinciding of the second evaluation with the second trimester may be effective in this. In some studies, it has been determined that sexual function during pregnancy is higher in the second trimester. In addition, it is stated that some pregnancy-related symptoms, lack of knowledge and myths about sexuality during pregnancy can negatively affect sexual function (2-4,6,7,25). This information supports our results. However, in our study, it was determined that education and counseling based on the Ex-PLISSIT model were not effective on sexual function in primigravidas. Therefore, our first hypothesis was rejected. Considering the studies conducted in the world, the studies in which counseling based on the Ex-PLISSIT model were given to primigravidas were very limited. Generally, the PLISSIT model was used. According to Ziaei et al. (26) reported that the sexual function level of pregnant women increased with sexual education and counseling given using the Ex-PLISSIT model. In this study, pregnant women were evaluated four weeks after the counseling and their sexual function scores were better than our study in the first measurement. There was a statistically significant difference between the FSFI mean scores of the intervention group and the control group. In our study, no significant difference was found between the mean scores. One of the reasons for this may be that the FSFI scores of primigravidas were quite low in the first measurement in our study. Another reason is Ziaei et al.(26). It may be that they made an evaluation in their studies four weeks after the counseling. In our study, the evaluation was made 10 weeks after the counseling. Therefore, the period in our study may be a long time interval for the evaluation of sexual function during pregnancy. Since there are not enough studies investigating the effect of counseling based on the Ex-PLISSIT model on primigravidas, it may be recommended to increase studies on this subject. Counseling based on the PLISSIT model, which is a similar model in pregnant women, was effective in improving sexual function (27,28). It was seen in the literature that other studies based on the Ex-PLISSIT model were conducted in different sample groups such as Multiple Sclerosis, Systemic Lupus Erythematosus and postpartum women. In these studies, it was determined that counseling was effective in improving sexual function (29-33). It is thought that the randomized controlled studies and the shorter measurement intervals compared to our study may have an effect on the results. In addition, since sexual function in pregnant women is affected by many factors and is below the cut-off point, it is recommended to develop a scale for the evaluation of sexual function specific to pregnant women. Thus, sexual function during pregnancy can be evaluated more effectively.

One of the factors affecting sexual life during pregnancy is the attitude towards sexuality during pregnancy. During the prenatal period, health professionals are recommended to help pregnant women maintain their positive attitudes towards sexuality (25). In our study, it was determined that the level of positive attitudes towards sexuality in primigravidas increased with sexual education and counseling in both measurements, and the intervention was effective in attitudes towards sexuality in pregnant women. Therefore, our second hypothesis was accepted. However, counseling had no effect on anxiety about sexual intercourse during pregnancy. In a study, it was reported that 88.8% of pregnant women had negative attitudes towards sexuality during pregnancy, and these primigravidas experienced stress and anxiety due to their limited knowledge on the subject (4). In addition, the low education level of the spouses negatively affected the sexual attitudes of the pregnant women (5). In support of this, lack of knowledge is emphasized as the most important factor in the emergence of sexual problems (33). For this reason, there is a need for training and counseling programs to create positive attitudes and raise awareness about sexuality during pregnancy. The results of the study also support our study and revealed that sexual education and counseling during pregnancy increase the knowledge and positive attitudes of pregnant women about sexuality (20).

Research suggests that couples should be provided with sexual counseling by a health professional and their sexual distress should be evaluated using effective measurement tools (21,22). In this study, it was determined that the sexual distress scores of primigravidas decreased after sexual education and counseling, but there was no significant change compared to the control group. Therefore, our third hypothesis was rejected. Similar to our study, in the study of Topatan and Koç (34) it was determined that the sexual information given to pregnant women did not change the level of sexual distress during pregnancy. According to these results, it is thought that physiological factors, cultural structure of the society, lack of knowledge and myths may be more effective in sexual distress experienced during

pregnancy. It has been reported that counseling given to non-pregnant women based on the PLISSIT model is effective in reducing sexual distress (35). In our study, sexual problems could have been solved more effectively if we had included pregnant women and their spouses for counseling sessions. However, in our society, individuals are hesitant to talk about sexual problems and they think that these problems are private. Therefore, we could not include spouses in our study. We recommend researchers to counsel primigravidas with their spouses.

It is known that the quality of sexual life changes negatively as the gestational week increases (36). In this study, it was determined that the quality of sexual life in primigravidas increased significantly compared to the control group as the pregnancy progressed with the sexual education and counseling provided. Therefore, our last hypothesis was accepted. In a study, it was reported that the quality of sexual life of women increased after the training program, as in this study (15). It was determined that sexual education and counseling based on the Ex-PLISSIT model in women with Multiple Sclerosis increased the sex quality of life scores, but there was no significant increase. Some diseases can prevent the increase in the quality of sexual life (14). In a study, it was determined that the health education given to pregnant women was effective in increasing the sexual quality of life scores from the first trimester to the last trimester of pregnancy (37). Another study reveals that the educational initiative is effective in increasing the sexual satisfaction of pregnant women (38).

Although our findings were compared with other studies, we think that the studies on the effect of counseling based on the Ex-PLISSIT model on the sexual life of primigravidas are insufficient. Therefore, it was very difficult for us to compare the superiority of the methods against each other. In addition, we suggest conducting a quasi-experimental studies to evaluate attitudes towards sexuality, sexual distress and sexual quality of life in primigravidas during pregnancy.

Study design, follow-up and intervention times, sample size and type, cultural and geographical differences may be effective in the differences in some results from the literature. Despite these limitations, our study was established with a solid foundation for the use of standard questionnaires with proven reliability, since the Ex-PLISSIT model is one of the most effective models that is easy to implement, given its design. This study is especially valuable in terms of the new information that primigravidas have brought to the literature in terms of their attitudes towards sexuality during pregnancy and their sexual life quality.

Limitations of the study

This study has several limitations. Randomization could not be done while determining the sample group, and pregnant women who met the inclusion criteria by simple random sampling according to the sample number determined according to the power analysis in both groups were included. In addition, the fact that the spouses of pregnant women were not included in the counseling sessions is another limitation. The participation of the spouses can help pregnant women to solve their sexual problems.

5. CONCLUSION

According to the findings, sexual education and counseling based on the EX-PLISSIT model has a positive effect on the sexual life of primigravidas. It is effective in supporting women's positive attitudes towards sexuality during pregnancy and increasing their sexual life quality. It is also an effective method in improving the relationships of primigravidas with their spouses, and in providing useful solutions for sexual dysfunction and sexual distress experienced during pregnancy.

For this reason, the Ex-PLISSIT model is recommended for primigravidas as a cost-effective and simple counseling method to improve sexual life.

In addition, considering the decrease in mean sexual function score and increase in sexual distress in the control group, it is recommended to include spouses in future studies.

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Clinical and Experimental Health Sciences

Nutrition Education via A Mobile Application on Weight Loss and Quality of Life: A Randomized Controlled Trial

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ABSTRACT

Objective: To evaluate the effect of nutrition education supported by MOtiVE mobile application on weight loss and quality of life (QoL).

Methods: In this pilot randomized-controlled study, 79 overweight/obese patients who presented to University Hospital between March-September 2018 to consult a dietitian, were included. All the participants were provided with a weight-loss diet program. Then, participants were randomized to experimental and control groups. During the first interview, all participants completed the questionnaire and anthropometric measurements were done. BMI, the scores obtained from different QoL scales, and Healthy Eating Index (HEI) were the dependent variables. Daily messages were sent to cases for 3 months via MOtiVE mobile application. All the participants were asked to be present three months later for a follow-up appointment. Using SPSS 25.0, change in BMI, QoL scores, and other variables within both groups were assessed via Wilcoxon signed-rank test and McNemar chi-square test.

Results: 20 cases and 18 controls completed the study. The mean BMI decreased significantly in both groups being more predominant in cases (p=.001 for cases and p=.006 for controls). Waist circumference decreased (p=.029), self-esteem (p=.035) and healthy eating scores (p=.007) increased only in cases. Hence there were significant improvements in cases, in the final evaluation, there were no significant differences between the groups (p>.05)

Conclusion: Nutrition education supported by MOtiVE mobile application improved anthropometric measurements, self-esteem, quality of life, and healthy eating habits of the overweight/obese participants. Free mobile applications can be used in increasing motivation to adopt new behaviors to tackle obesity.

Keywords: Obesity, mobile applications, nutrition, education, quality of life,

1. INTRODUCTION

Obesity is a multifactorial and chronic disease characterized by excessive accumulation of fat in the body resulting from the interaction between genetic and environmental factors. According to World Health Organization in 2016, more than 1.9 billion adults were overweight and over 650 million were obese (1). Several studies showed that obese persons report a lower health-related quality of life (HRQoL) than their non-obese counterparts (2). In weight loss programs, the most important factor is lifestyle changes. Technology-based interventions are considered a valid tool for weight loss and seem to increase program adherence (3). Studies conducted on this issue have shown that such programs, although short-term, lead to positive changes in diet, physical activity level, glycemic control, anthropometric measurements, and biochemical parameters (4-7). In a systematic review of adults, Alnuaimi et al. (8) found that mobile applications are more effective than standard interventions in weight management and maintenance of weight loss. In a study by Nikolaou and Lean (9), it was found that mobile health apps are the fastest growing area in the e-health sector, among 100000 health apps in app stores, about 29000 of them are related to obesity (physical activity, diet, energy intake and expenditure etc.) but only 17 of them were developed by professionals. As a result, even though these applications are very common, they lack professional content expertise. MOtiVE is an application developed exclusively for this study by expert researchers. It is aimed to increase compliance with the diet by both informing the participants and increasing their motivation by sending compact information messages to the participants. The app can also track and report which participant opened which message to see if the messages reached the target. In several studies, low self-esteem and poor body image were determined to be associated with obesity (10, 11). Providing motivational support to achieve lifestyle changes can play a significant role in maintaining weight loss by increasing self-esteem. As far as we can see, when the study was conducted, this was the first study that aimed to provide nutrition education and motivation through a mobile application, thus aiming to improve

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anthropometric indices and quality of life measures. In parallel, the application was the first application developed in the field with these features in Turkish. The main purpose of the present study was to compare the effect of standard education on weight loss, quality of life (QoL), and healthy eating behavior with that of nutrition education supported by mobile applications.

2. METHODS

2.1. Trial Design

The present study was conducted with patients who presented to the Ege University Hospital Endocrine and Metabolic Diseases Outpatient Clinic in March-September 2018 to consult a dietitian and to receive routine nutrition therapy, aged between 18-64 years and with Body Mass Index (BMI) ≥ 25 kg/m^2. The participants were first given the standard nutrition training by the dietitian in the outpatient clinic, and then a person-specific diet program. After this, the patients who gave their consent to participate in the study were randomly assigned to the case and control groups. Then, anthropometric measurements of all the participants were performed and the data collection forms were filled in. The "MOtiVE" mobile application for smartphones designed specifically for this study was installed on the smartphones of the cases, and text, visual, or video messages were sent to them via this application for three months. The participants in the control group underwent the routine procedure. In line with the routine practice of the outpatient clinic, all the participants were told to be present three months later for control appointments. At the end of December 2018, at the end of the three-month follow-up period, the data collection process ended. In a systematic review conducted by Aguilar-Martınez et al (12), showed that most of the trials last for 2-4 months.

2.2. Participants and Recruitment

Of the patients, those who did not have a smartphone with an internet connection, underwent bariatric surgery, took medication, or practice a special diet for thyroid problems, diabetes, celiac, gout, and kidney diseases, and whose body fat analysis was not fulfilled due to the presence of a pacemaker, prosthesis, etc. were not included in the study.

When we consider 80% power with a %5 error and medium effect size (dz:.5) we had to have 27 participants in each group calculated via G-power. According to the results of a study conducted by Allen et al, 25 participants in each group was enough to reach 80% power (13). When we evaluate admissions to the outpatient clinic, we saw that each week we can have 4 or 5 new participants. Thus, considering this data, we decided to take 30 participants in each group. Considering the loss in follow-up we included 40 participants in each group. The flowchart of the study is given in Figure 1. At the end of the study, the loss in follow-up was more than expected. Thus, the study was concluded with 20 cases and 18 controls. In G-power post hoc power calculations yield 98% power for BMI difference in cases (two dependent means, dz:.89, alpha:.05, n=20).

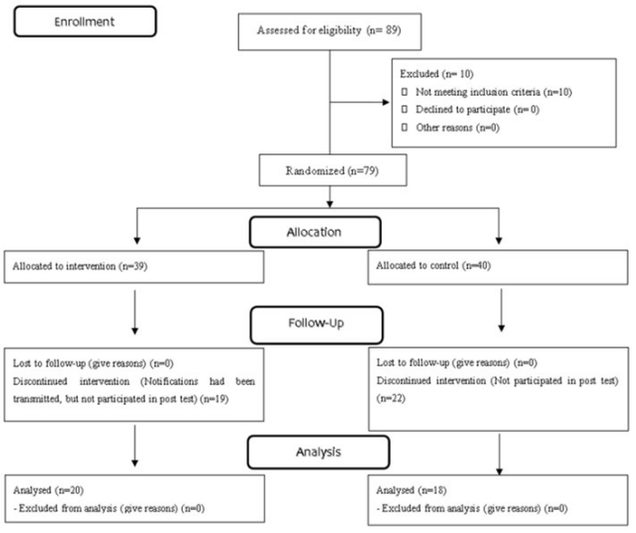

Figure 1. *The flowchart of the study*

In the present study, randomization was carried out in the following block allocation sequence: AABB, ABAB, BABA, BBAA, ABBA, BAAB.

Change in the BMI and other anthropometric indices and the scores obtained from the Weight Efficacy Lifestyle (WEL) test, Rosenberg Self-Esteem Scale (RSES), Obesity and Weight-Loss Quality of Life Instrument (OWLQoL), Healthy Lifestyle Behavior-II Scale (HLSB-II) and Healthy Eating Index (HEI) were the dependent variables.

2.3. Measurements

Anthropometric Measurements

The body height was measured with a stadiometer whereas the body weight, body fat percentage, and BMI were measured with the Tanita BC-418 Segmental Body Composition Analyzer. Waist circumference was measured with a non-elastic standard measuring tape, with the individual wearing light clothes, standing still, in an upright position, and with arms open sideward. WC was measured from the midpoint between the distal border of the lowest rib and the superior border of the iliac crest (14). Neck circumference was measured by placing the inelastic measuring tape around the neck from the point just below the laryngeal prominence.

WEL Test: It consisted of 20 questions about negative feelings, food accessibility, social pressure, physical disturbance, and positive activity. The higher the score is the better the weight efficacy lifestyle is (15, 16).

HEI: It was developed by the United States Department of Agriculture to investigate the quality of diet in Americans (17). The new HEI-2015, which was published in 2018, consists of 13 components related to diet. The increase in scores indicates a positive development (18). Nutritional values for individuals based on their 24-hour food consumption records were calculated using the BEBIS, Turkish dietary data system.

RSES: The first 10 items of the scale are used to measure self-esteem. Self-esteem increases as the score obtained from the RSES decreases (19, 20).

HLSB-II: The scale includes health responsibility, exercise, nutrition, self-actualization, interpersonal support, and stress management subscales (21, 22).

OWLQOL: The higher the score obtained from the scale, the higher the quality of life of the person is (23, 24).

All of the scales were validated in Turkish and found to be reliable to use in Turkish. Among the scales, WEL test, HLSB-II, HEI could be accessed freely through internet sources, thus no permission was obtained for these scales. Turkish version of the Rosenberg Self-esteem Scale is under the license of Turkish Children and Adolescent Psychiatry Association and the fee for the license was paid by the authors. Written permission was obtained from the authors of the OWLQoL-Turkish version.

2.4. MOtiVE Application for Mobile Phones

The program was developed by researchers with technical support from a software engineer, specifically for this study and it is compatible with both Android and IOS. The messages were developed in Turkish by the researchers using the latest guidelines by Ministry of Health and other authorities (25-27). The messages were sent to the participants in the case group once a day for three months. Messages sent to the phone screen every morning at 9:00 AM were either text, visual, or video messages. The visual messages designed to draw the attention of the reader included both a text and an illustration (Figure 2). There were 90 messages disseminated, 26 of them visual, 10 of them were videos, and the rest were text messages containing compact information.

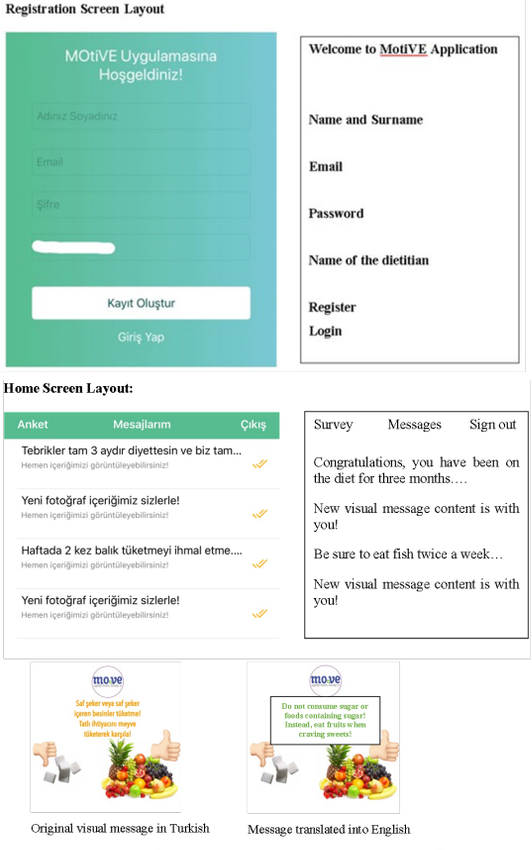

Figure 2. *Screenshots from MOtiVE and examples of messages*

By sending text messages like "*Sunday is the only day when most of us do not work. You will be more comfortable during the weekdays if you do your shopping and prepare meals on the weekend. Prepare your meatballs this Sunday and freeze them….*", practical solutions were offered to the participants. By sending text messages like "*Lifestyle changes improve your quality of life and enable you to lead a better quality of life. Improve your quality of life with diet and regular exercise. Thus, do not give up...*" the participants were motivated.

Video messages were uploaded to YouTube and a link address (e.g., What is an antioxidant? https://www.youtube.com/watch?v=TIaQq1CbNBM) was sent to the cases, which enabled them to access. The videos were also subtitled so that the watcher could get the message in noisy environments. The registration screen of the MOtiVE, home screen layout, and samples of messages sent for 90 days are given in additional files.

One of the features of the MOtiVE is to calculate how many days the participant was following the program and to send the message specific to that day to the person's phone. Thanks to this feature of the MOtiVE, it was possible to send a message like *"Congratulations, you have been on a diet for a full month…"* to the person who participated in the program for a month. Thus, the program was made person-specific. Another feature of the MOtiVE is that only one-way communication was possible to avoid bias. The application enabled only the researchers to send messages to the participants but did not allow the participants to communicate with the researchers. In this way, standardization was established between the participants in terms of determining their knowledge and interest. Another feature of the MOtiVE is to report whether the participants see the message of that day, which enabled the researchers to find out whether the participant has read the messages.

2.5. Statistical Analysis

In the analysis of the study data, IBM SPSS Statistics (version 25; IBM, New York, NY, USA) was used. Continuous variables were presented with mean ± standard deviation. At the baseline, the homogenous randomization of the participants to the case and control groups was investigated using the independent samples t-test (Mann Whitney-U test if the parametric condition could not be met) and chi-square tests. The efficacy of the intervention was assessed using Wilcoxon signed rank test separately in the case and control groups. $p<.05$ considered significant.

2.6. Ethical Issues

The study was conducted following the Declaration of Helsinki and ethical approval was obtained from Ege University Medical Research Ethics Committee (no:17-7.1/14, 08.08.2017) and the written consent was obtained from the patients. The study was recorded in clinical trials (no: ClinicalTrials.gov NCT04026971). This study adheres to CONSORT guidelines.

3. RESULTS

Of the participants (cases:20, controls:18), 90% in the case group and 83.4% in the control group were women. There was no significant age difference between cases and controls (34.7±14.0 years vs. 38.7±13.5 years; p=.349). The analysis performed to test whether the participants who took the final test had been homogenously randomized to the case and control groups at the baseline revealed that there were no significant differences between the groups in terms of their sociodemographic characteristics, anthropometric measurements, or the scores they obtained from the RSES, OWLQOL, HLSB-II scale, WEL test, and HEI and their sub-dimensions except for the nutrition subscale of the HLSB-II scale (p>.05). The participants in the control group obtained significantly higher scores from the nutrition subscale of the HLSB-II scale (Case group: 20.9±3.6, Control group: 23.6±4.2, p=.043). Therefore, it can be said that the participants were homogenously distributed to the case and control groups. According to the feedback provided by MOtiVE, all participants read all the messages.

Although weight, BMI and neck circumference measurements of the participants significantly decreased in both groups at the end of the 3 months, these differences were more dominant in the cases. Waist circumference decreased significantly only in cases. But in the final evaluation there were no significant differences between the groups. The changes in the anthropometric measurements of the participants at the end of the three-month follow-up were given in Table 1.

RSES scores significantly decreased in the cases which showed an increase in self-esteem. There were no significant changes in the WEL test scores of the participants in both groups. HLSB-II scale scores significantly increased only in the cases. As for the subscales of the HLSB-II scale, there were significant increases in the nutrition and stress management subscale scores in the cases. While the mean HEI scores of the controls did not change significantly at the end of the three months, those of the cases increased significantly. But in the final evaluation there were no significant differences between the groups. The comparison of all the data collection tools used in the present study is presented in Table 2.

Table 1. *Comparison of Anthropometric Measurements*

Measurements	Groups	n		Mean	SD	Median	Min.	Max.	p* within groups	p** First	p** Final
Weight, kg	Case	20	First test	91.4	18.0	89.3	67.7	129.0	**.001**	.682	.726
			Final test	88.5	17.1	87.2	66.3	120.9			
	Control	18	First test	89.4	17.9	86.2	67.1	128.9	**.006**		
			Final test	86.4	16.9	83.7	61.8	125.4			
BMI, kg/m^2	Case	20	First test	33.8	6.0	33.2	25.7	45.7	**.001**	.907	.953
			Final test	32.8	5.8	31.9	23.8	43.0			
	Control	18	First test	33.3	5.0	32.6	26.0	41.6	**.006**		
			Final test	32.2	4.7	32.5	23.8	40.4			
Body Fat Percentage, %	Case	20	First test	39.5	5.2	26.6	46.7	40.3	.489	.511	.569
			Final test	39.3	6.0	24.9	47.7	39.5			
	Control	18	First test	39.1	4.6	30.5	48.3	38.3	.556		
			Final test	38.7	4.9	29.3	46.0	39.1			
Waist circumference (cm)	Case	20	First test	108.2	15.1	86.0	141.0	109.5	**.029**	.770	.895
			Final test	106.1	15.1	82.0	135.5	107.0			
	Control	18	First test	108.3	12.7	87.0	130.0	108.5	.060		
			Final test	105.5	12.4	86.0	126.0	105.0			
Neck circumference (cm)	Case	20	First test	37.3	3.5	33.0	45.0	36.2	**.001**	.428	.371
			Final test	36.4	3.3	33.0	44.5	35.7			
	Control	18	First test	38.3	4.1	32.5	48.0	38.0	**.028**		
			Final test	37.5	4.3	32.0	48.0	36.5			

(*): Wilcoxon signed rank test, (**) Mann Whitney-U test. P values printed in bold indicate a significant difference.

Table 2: *Comparison of all the data collection tools used in the present study*

Scales	Groups	n		Mean	SD	Median	Min.	Max.	p*	p** First	p** Final
RSES	Case	20	First test	1.1	0.7	1.0	0.25	2.9	**.035**	.489	.407
			Final test	0.7	0.5	0.6	0.25	2.0			
	Control	18	First test	0.9	0.5	0.8	0.25	2.3	.723		
			Final test	0.9	0.6	0.7	0	2.3			
OWLQOL	Case	20	First test	55.2	16.6	58.0	28.0	87.0	.064	.568	.219
			Final test	45.3	21.5	38.5	20.0	95.0			
	Control	18	First test	51.3	21.0	50.0	11.0	95.0	.331		
			Final test	49.7	22.4	52.5	2.0	93.0			
HLSB-II	Case	20	First test	131.0	15.4	126.5	11.0	173.0	**.021**	.404	.396
			Final test	141.0	19.5	144.0	111.0	188.0			
	Control	18	First test	135.0	20.2	135.0	99.0	177.0	.981		
			Final test	135.0	19.3	129.5	109.0	167.0			
HLSB-II Health Responsibility	Case	20	First test	21.7	4.7	21.0	12.0	31.0	.294	.445	.977
			Final test	23.0	5.2	23.0	16.0	33.0			
	Control	18	First test	22.8	5.4	23.0	11.0	33.0	.896		
			Final test	22.8	5.3	22.0	14.0	33.0			
HLSB-II Exercise	Case	20	First test	15.7	4.2	15.5	8.0	24.0	.245	.481	.660
			Final test	17.0	5.6	17.5	8.0	29.0			
	Control	18	First test	14.8	5.4	14.0	8.0	25.0	.325		
			Final test	16.1	4.6	16.5	9.0	24.0			
HLSB-II Nutrition	Case	20	First test	20.9	3.6	21.0	15.0	31.0	**.001**	**.043**	.362
			Final test	25.0	4.2	25.5	17.0	36.0			
	Control	18	First test	23.6	4.2	23.5	18.0	33.0	.950		
			Final test	23.9	4.1	23.0	18.0	32.0			
HLSB-II Self-actualization	Case	20	First test	26.7	2.9	26.5	21.0	33.0	.124	.965	.363
			Final test	27.7	3.1	28.0	20.0	34.0			
	Control	18	First test	26.8	3.8	26.0	21.0	34.0	.457		
			Final test	26.6	4.2	26.0	19.0	34.0			
HLSB-II Interpersonal Support	Case	20	First test	26.9	3.5	27.5	21.0	33.0	.323	.713	.325
			Final test	27.5	4.1	29.0	21.0	35.0			
	Control	18	First test	27.7	4.1	28.0	21.0	36.0	.175		
			Final test	26.3	4.6	24.5	18.0	35.0			
HLSB-II Stress Management	Case	20	First test	18.9	2.9	18.5	15.0	25.0	**.036**	.791	.116
			Final test	20.7	3.2	21.0	14.0	28.0			
	Control	18	First test	19.0	3.3	19.5	13.0	26.0	.876		
			Final test	19.1	3.1	18.0	14.0	26.0			
WEL Test	Case	20	First test	51.0	13.7	48.0	24.0	89.0	.256	.492	.306
			Final test	47.7	16.9	46.5	20.0	91.0			
	Control	18	First test	47.2	16.8	48	20.0	83.0	.177		
			Final test	42.0	12.7	39.0	26.0	70.0			
HEI Score	Case	20	First test	49.2	9.8	52.0	30.0	65.0	**.007**	.230	.849
			Final test	56.1	11.4	56.5	34.0	72.0			
	Control	18	First test	53.7	8.2	54.0	43.0	68.5	.486		
			Final test	55.7	10.3	55.7	37.0	72.5			

(*) Wilcoxon signed rank test. (**) Mann Whitney-U test.
P values printed in bold indicate a significant difference.
HEI: Healthy Eating Index; HLSB-II: Healthy Lifestyle Behavior-II Scale; OWLQOL: Obesity and Weight-Loss Quality of Life; RSES: Rosenberg Self-Esteem Scale; WEL: Weight Efficacy Lifestyle

4. DISCUSSION

The use of internet-based applications to develop a healthy lifestyle is increasing. However, most of these programs have not been evaluated with appropriate and standardized methods and have different exposure times. Safran Naimark et al. (28) investigated the effect of an internet-based application on improving a healthy lifestyle in a randomized controlled trial. They collected data on nutrition knowledge, diet quality, and physical activity periods using online data collection forms. The cases used the Internet-based application designed based on healthy lifestyle

recommendations of the US Department of Agriculture and the Israeli Ministry of Health for 14 weeks. Of the 99 participants, 86% of them (n=85, 56 in the case group, 29 in the control group) completed the study. Besides significant weight loss (p=.03), knowledge score, diet quality score, and success score indicating success in maintaining a healthy life increased significantly in the cases. There was a significant correlation between the frequency of using the application and a high success score (p<.01). Similarly, in their randomized controlled study aimed at weight loss, Patrick et al. (29) sent SMSs and/or multimedia messages to the participants in the case group 2-5 times a day for 16 weeks. At the end of the 16th week, the weight loss in the cases was significantly higher (p=.02). It was concluded that SMSs and multimedia messages could promote behaviors supporting weight loss in obese adults. In the present study, all participants took nutritional training first with their diets and then randomized to cases and controls. That could be the reason for controls to lose weight significantly as well as cases, cases being more predominant. Besides weight loss, the present study showed significant improvements regarding self-esteem; healthy lifestyle behaviors, quality of life, and healthy eating habits were observed only in cases. Therefore, in line with Patrick et al.'s study, mobile applications can improve behaviors that support weight loss. Moreover, a recently published meta-analysis concluded that even though there are concerns about the study designs, mobile apps and wearables can be effective self-regulating tools for weight loss (30).

A meta-analysis including 14 randomized controlled trials to investigate whether internet-based interventions were effective in empowering patients concluded that these interventions yield positive improvements. On the other hand, in 3 studies using general self-efficacy scales and in 1 study using the RSES conducted to assess self-esteem, no changes were observed. The comparison of face-to-face interviews and internet-based interventions demonstrated that no significant differences were observed in self-esteem (31). In the present study, cases received messages in addition to routine nutritional therapy. Unlike the meta-analysis, a significant decrease in RSES scores thus, a significant increase in self-esteem was observed in cases in the present study. However, according to the analysis of the self-efficacy scores obtained from the WEL test, we also could not show significant changes in both groups. It is thought that internet-based interventions can be used to improve self-esteem, lifestyle behaviors, and quality of life of patients but not self-efficacy.

Although the use of a mobile application led to changes in health behaviors, the mechanisms by which these applications facilitate behavior change are generally not known. West et al. (32) conducted a cross-sectional study including 217 participants. The participants gave their feedback about their diet and nutrition applications in the last 6 months and most of the participants agreed that the application increased their dietary motivation, improved their self-efficacy, and increased their willingness to set dietary goals and to reach the target. Therefore, it was concluded that diet and nutrition-related practices focusing on the improvement of motivation, willingness, self-efficacy, attitude, knowledge, and goal setting might be particularly useful. Jacobs et al. (33) demonstrated the importance of adherence to intervention on weight loss in a large sample using a smartphone application *Noom* tracking individual self-monitoring and showed that after three months, a significant reduction in BMI was accomplished. They concluded that smartphone application use can induce weight loss associated with adherence. The present study was also aimed at improving healthy lifestyle behaviors by inducing motivation. At the end of the present study, there was a significant improvement in the mean scores of BMI as well as from the overall HLSB-II scale and only its nutrition and stress management subscales.

Limitations and Strengths

The mobile application MOtiVE and its unique features solely designed for this study can be considered the main strength of this study. Personal feedback about messages, personal timing, and sending messages according to enrollment time makes this program tailored to participants. All the participants read the messages, so the messages were considered to be attractive and not boring. Moreover, one-way communication ensured the standardization of knowledge and motivation. In the final evaluation, there were no significant differences between the groups in terms of anthropometric measurements. This can be expected since both groups received nutritional therapy and counseling. On the other hand, some of the quality measures significantly increased in cases, but not in controls, even though it did not reach a significant difference in the final evaluation. This can be due to loss in follow-up and relatively small sample. Even though there were significant losses during the follow-up period, the study reached 98% power to detect significant differences in BMI within cases.

5. CONCLUSIONS

In conclusion, it is thought that provision of mobile application-based nutrition education to overweight and obese individuals in addition to routine nutritional therapy may lead to improvements in anthropometric measurements, self-esteem, healthy lifestyle behaviors, quality of life, and healthy eating habits of the participants, and it might help to achieve the targeted weight loss. As the number of dietary and nutritional practices continues to increase, to ensure healthy lifestyle behavior changes, application developers together with health professionals should consider integrating the appropriate theoretical structures into the newly developed mobile applications because these types of mobile applications are easily accessible. Such applications should be created and made available to the public free of charge for the protection of public health, and the prevention and reduction of obesity.

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Author Contributions:

Research idea:DP, RM
Design of the study: DP, SS, RM
Acquisition of data for the study: DP, SS
Analysis of data for the study: DP, RM
Interpretation of data for the study: DP, SS, RM
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Clinical and Experimental Health Sciences

Care Burden and Compassion in Caregivers of Stroke Survivors

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ABSTRACT

Objective: This study was conducted to evaluate the factors related to care burden and compassion levels of caregivers of family members who had a stroke and the relationship between the two variables.

Method: The study was designed as a descriptive, cross-sectional, and correlational one. The sample included 280 stroke survivors and family caregivers. The data were collected using a Family Caregiver Information Form, the Stroke Survivors Information Form, the Zarit Burden Interview (ZBI), the Compassion Scale (CS), and the Modified Barthel Index (MBI).

Results: In the study, it was determined that the compassion scores (3.71±0.85), and caregiving burden scores (34.30±17.42) of the family caregivers of stroke survivors were moderate. There was a negative, strong and significant relationship between caregiving burden and compassion ($p<.001$). It was determined that as the caregiving burden of the family caregiver's increased, their levels of compassion decreased.

Conclusion: Determining the care burden and compassion levels of family caregivers of stroke survivors might contribute to improvement of the health of both family caregivers and stroke survivors.
Keywords: Family caregivers, care burden, compassion, stroke survivors.

1. INTRODUCTION

Stroke survivors may experience many problems in bodily functions such as muscle strength, movement, energy, sleep and memory and in activities of daily living such as speaking, mobility, dressing, self-care, eating and doing daily chores. These problems cause the stroke survivor to be dependent and increase their care needs (1-3). This need for care is usually met by family members. "Informal caregivers" are family members, close relatives, or friends who provide at-home care for a stroke survivor without regard to time constraints or reimbursement and who are not medically trained (4). Family caregivers take important responsibilities in the care of stroke survivors. These responsibilities may be listed as the daily activities of the survivor such as nutrition, dressing, bathing and moving or some other activities such as preparing food, shopping, household chores, home care, transportation, medication management, communication with the medical team, coordination and meeting the needs of other family members. These responsibilities may cause care burden (4-6). Care burden refers to negative objective and subjective results such as psychological, physical, economic and social problems, the deterioration of family relationships and feelings of failure to maintain control (5-7). Care burden causes the family caregiver to postpone their own needs and problems such as a decrease in interpersonal relationships, impairment of own health, stress, depression, behavioral and cognitive problems and parental conflicts (2,4,6-8). These problems may cause a decrease in the quality of their life (2,4) and feelings of compassion (9,10). Compassion is frequently defined as sensitivity to the pain or suffering of another, coupled with a deep desire to alleviate that suffering (11-13). Caregivers define compassion as a feeling that emerges from witnessing another's suffering, which then motivates the desire to help others (14). Compassion plays an important role in a caregiving context involving strong individual connections and shared worldviews between the caregiver and the survivor (14,15). The sense of compassion affecting the behaviors of family caregivers towards survivors is therefore important.

In recent years, compassion has become a subject of increasing interest in the field of health. This is because compassion increases the quality of care and ensures that care is given individually, appropriately, correctly and with positive results (16-18). In studies on the effects of compassion

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in health professionals on patients, it has been reported that compassion relieves patients, supports their treatment process and has positive effects on their physical health (9,19). Furthermore, in a study conducted with family caregivers of Alzheimer patients, it was found that family caregivers experienced less care burden by means of compassion, and compassion was found to provide psychological benefits such as decreased levels of depressive symptoms (15). Because of these important effects, compassion is recognized as one of the cornerstones of high-quality care by stroke survivors, clinicians and family caregivers (16-18). Compassion may play an important role in reducing negative symptoms and depressive symptoms experienced by family caregivers who help stroke survivors overcome many problems. It may also help them feel better, communicate with the patient who is receiving care better and provide better care for the patient (14,15,17,20). It is observed that the number of studies on compassion has increased in recent years and that the topic has been investigated among healthcare professionals in general (9,18,21-23). However, studies on compassion among the family members of family caregivers are almost non – existent (14,15). It was stated that caring for a family member is similar to professional care, and both types of care are based on compassion (24). In the literature, it is seen that there are many studies on care burden in family caregivers of stroke survivors (7,8,20,25,26). However, in the accessible literature, compassion in family caregivers of stroke survivors and the relationship between compassion and care burden have not been examined. Therefore, this study was conducted as a descriptive study to determine the relationship between care burden and level of compassion in family caregivers of stroke survivors.

2. METHODS

2.1. Ethical Considerations

The study was carried out in accordance with the Declaration of Helsinki's principles, and it received clearance from the university's Clinical Research Ethics Committee (approval number: 2018-01/08). The aim and method of the study were explained to the participants by the researcher, and verbal and written consent was obtained.

2.2. Design and Sample

This study was carried out with a descriptive, cross-sectional and correlational design.

The population of the study consisted of stroke survivors who visited the neurology service of a city hospital and received treatment and their family caregivers. A neurology department is the place where not only patients with neurological diseases-related problems (e.g., cerebrovascular disease, epilepsy, multiple sclerosis) are treated and given healthcare but also stroke survivors are given routine stroke treatment (antiaggregant, anticoagulant therapy). The number of stroke survivors visiting the neurology services for a whole year was found to be 750 without repetition. The study was conducted between 01 March 2018 and 30 December 2018, and the minimum required sample size of the study was determined by power analysis. The sample size for the study was calculated as: α=.05, β=.20 and 1-β=.80. As a result, 280 stroke survivors and their family caregivers were included in the sample (p=.80046). The study was completed with 280 stroke survivors and family caregivers as there were no dropouts among the participants.

Among the people constituting the population, regarding family caregivers, the following criteria were defined as the inclusion criteria: caregiver being a close family member (such as mother, father, spouse and child), being 18 years old or older, not having hearing loss and being able to communicate, being responsible for the care of the survivor for at least 3 months, being able to speak and understand the Turkish language. In addition to these, the stroke survivor's and family caregiver's willingness to participate in the study was another inclusion criterion. Family caregivers of stroke survivors with transient ischemic attacks, professional caregivers, caregivers who provided care with charge and non-permanent family caregivers who provided short-term care to stroke survivors were excluded from the study. Stroke survivors diagnosed at least 3 months ago and those who agreed to participate in the study were included in the sample. Stroke survivors who had a transient ischemic attack were excluded from the sample. In the results of the study, while the word "literate" refers to a person who is able to read and write but has no formal education degree, the word "illiterate" refers to a person who is not able to read and write.

2.3. Data Collection Process

The data were collected using the following tools:

2.3.1. Family Caregiver Information Form: This form, developed by the researcher based on the literature, consisted of questions to determine the sociodemographic characteristics (e.g., age, marital status) and caregiving-related characteristics (e.g., caregiving time, the situation of receiving help from another individual while providing care) of the family caregivers.

2.3.2. Stroke Survivors Information Form: The form, which was developed by the researcher after the literature review and evaluated the sociodemographic characteristics (e.g., age, gender, marital status, educational status) and medical characteristics (e.g., stroke diagnose duration, number of strokes and type of stroke) of the stroke survivors, consisted of 12 questions.

2.3.3. The Zarit Burden Interview (ZBI): This form, developed by Zarit, Reever and Bach – Peterson , is a scale used to evaluate the care burden of caregivers for the person in need of care (27). The validity and reliability of the scale in Turkey were tested by İnci and Erdem , and its reliability coefficient was found as .99 (28). There are 22 items on the scale. The scale's items are scored on a 5-point Likert scale with a range

of 0 to 4. The scale yields results between a minimum score of 0 and a maximum score of 88. The results from the scale were analyzed in this study and classified into four categories of burden: none/mild (0–20 points), moderate (21–40 points), severe (41–60 points), and severely severe (61–88 points) (5,27,28). The scale's Cronbach's alpha value in this investigation was determined to be .92.

2.3.4. Compassion Scale (CS): The scale was developed by Pommier , and the validity and reliability of the scale were tested by Akdeniz and Deniz in Turkey (11,12). It consists of 24 items and six subscales: kindness, indifference, common humanity, separation, mindfulness and disengagement. The negligence, indifference and disengagement subscales of the scale are scored inversely. The average of the total scores is taken after this computation. The scale's items are scored on a 5-point Likert-type scale, with 1 representing "never" and 5 representing "always". The combined average is used to determine the overall scale's final score. In other words, the average score for the entire scale is calculated by adding the mean scores for each item, dividing the result by the number of items, and then averaging the results. Consequently, the overall scores. The scale's stated Cronbach's alpha value was .85 (12). The scale's Cronbach's Alpha score in this study came out to be .94. By taking into account the lowest and highest scores on the scale, the mean values for the scale were employed in the evaluation.

2.3.5. Modified Barthel Index (MBI): The Modified Barthel Index (MBI) was developed to determine the level of independence of individuals in activities of daily living. Kucukdeveci et al. conducted a validity and reliability study on neurology patients using the Turkish translation of the index created by Mahoney and Barthel in 1965 (29,30). The index's Cronbach's alpha value in the study conducted by Kucukdeveci et al. was calculated to be .93. A score of 0 to 20 on the index denotes "complete" dependence, a score of 21 to 60 denotes "severe" dependence, a score of 61 to 90 denotes "moderate" dependence, a score of 91 to 99 denotes "slight" dependence, and a score of 100 denotes independence (5,30). The scale's Cronbach's alpha coefficient in this study was found to be .90.

2.4. Procedure

The institution where the study will be conducted granted written consent prior to the study's start. The individuals who accepted to take part in the study gave their verbal and written consent after being informed about the research process. The researcher conducted face-to – face interviews with the family caregivers in the hospital setting by giving them a Family Caregiver Information Form, ZBI, and CS in a separate room. MBI for Activities of Daily Living, the Stroke Survivors Information Form, face-to-face interviews with the survivors' relatives, and a review of medical records were all used to gather data on the stroke survivors. The forms and scales were applied by the researchers. The application of the forms took approximately 30 minutes for each participant.

2.5. Data Assessment

Utilizing the computer program SPSS 22.0, the data were examined. Frequencies, percentages, and mean values were used in the statistical analysis of the data relating to the family caregivers and stroke survivors. In the quantitative data satisfying normal distribution assumptions, independent-samples t-test was applied, while Mann Whitney U test was applied in the groups that did not show normal distribution. In cases where there were more than two groups, analysis of variance (ANOVA) was used for the groups conforming with normal distribution, LSD Post Hoc test was used to find out the source of the difference in the groups. Kruskal-Wallis test was applied for the groups that did not show normal distribution, and Dunnett T3 Post Hoc test was used for determining the source of the difference between the groups. Pearson's correlation analysis was used to determine the relationships between the care burden, compassion and dependency level scales. Besides, multiple linear regression analysis was used for the variables predicting the family caregiver's compassion. The level of significance was determined as p<.001.

3. RESULTS

Among the family caregivers with a mean age of 42.60±11.19, it was determined that 37.5% of them were in the age group of 40-50, 57.5% were women, 48.9% had an educational background of high school or above, 82.5% had social security, 67.5% were in a nuclear family, 71.1% lived with the stroke survivors, and 48.2% provided care for a period between 3 and 9 months. Among the stroke survivors, it was seen that 63.6% of them were in the age group of 62 or older, 52.1% were male, 70% were married, 41.1% were illiterate, 83.6% were not employed, 71.4% were diagnosed with stroke between the periods of 3 months and 4 years, 57.1% had one stroke attack, 53.2% of them experienced ischemic stroke, and 53.9% had a severe level of dependency.

Table 1. *ZBI, CS subscale and total mean scores of the family caregivers, and distribution of score ranges according to ZBI (n=280)*

Scales	X±SD	Min	Max
ZBI	34.30±17.42	8.00	80.00
		n	**%**
None (0-20 points)		69	24.6
Moderate care burden (21-40 points)		127	45.4
Severe care burden (41-60 points)		54	19.3
Highly severe care burden (61-88 points)		30	10.7
CS	3.71±0.85	1.21	4.83
Kindness	3.94±0.86	1.00	5.00
Indifference*	3.56±1.18	1.00	5.00
Common humanity	3.58±0.90	1.00	5.00
Separation*	3.76±1.06	1.00	5.00
Mindfulness	3.68±0.91	1.00	5.00
Disengagement*	3.76±1.10	1.00	5.00

ZBI: Zarit Burden Interview CS: Compassion Scale, SD: Standard Deviation.
** Scoring for the total points of compassion was calculated in reverse order.*

As presented in Table 1, the mean ZBI and CS scores of the participants (34.30±17.42 and 3.71±0.85 respectively) were close to average. The analysis of the mean scores for the subscales of the CS revealed that the participants' mean scores for kindness (3.94±0.86), indifference (3.56±1.18), common humanity (3.58±0.90), separation (3.76±1.06), mindfulness (3.68±0.91) and disengagement (3.76±1.10) were close to average (Table 1). According to the ZBI score ranges of the family caregivers, it was determined that 45.4% of them had a moderate level of care burden.

The results of the comparisons of some variables of the family caregivers to their ZBI and CS mean scores are shown in Table 2. Accordingly, it was found that the mean care burden score of those with the following factors was significantly higher than the other groups: those in the age ranges of 40-50 years and 51 years or older, those who were literate with no formal education degree or illiterate, those without any social security, those without any chronic disease, those living with the stroke survivor, and those providing care for a period of 31 months or longer (p<.001). The compassion mean scores of the following groups were significantly higher than the other groups: those in the age range of 18-28 years, those with an education level of high school or higher education, those with social security, those with a nuclear family, those who had a chronic disease, those who were not living with the stroke survivors, and those providing care for a period of 3-9 months (p<.001) (Table 2).

The results of the comparisons of some variables of the stroke survivors to their mean ZBI and mean scores are shown in Table 3. Accordingly, the mean care burden score of those with the following factors was significantly higher than the other groups: those in the age range of 62 years or older, those who were single, those who were literate, those who were employed, those with a stroke diagnosis for 10 years or longer, those with two or more stroke attacks, those who had experienced ischemic stroke, and those who were completely dependent (p<.001). Additionally, the mean compassion scores of the following groups were significantly higher than the other groups: those in the age range of 40-61 years, those who were married, those with an education level of high school or higher education, those who were not employed, those with a stroke diagnosis for 3 months to 4 years, those who had experienced one stroke attack, those who had experienced a hemorrhagic stroke, and those who were slightly dependent (p<.001). The mean total MBI score of the stroke survivors was found to be 47.14±22.68, and they were found to be severely dependent in general (Table 3).

Table 2. *The comparison of some variables of the family caregivers with ZBI and CS mean scores*

				ZBI		CS	
Variables		**N**	**%**	**X± SD**	Test	**X± SD**	Test
Mean of age	42.60±11.19						
Age	18-28 29-39 40-50 51 <	46 68 105 61	16.4 24.3 37.5 21.8	27.07±12.24 28.21±14.20 35.58±17.24 44.36±19.21	**F=14.147** **p=.000** ***3-4>1-2**	4.01±0.51 3.92±0.79 3.63±0.81 3.40±1.05	**x^2_{kw}=16.821** **p=.001** ****1-2>3-4**
Education level	Illeterate Literate but not graduate of any school Primary school Middle school and higher	55 15 73 137	19.6 5.4 26.1 48.9	40.82±19.17 41.67±21.20 37.22±18.03 29.33±14.32	**x^2_{kw}=20.903** **p=.000** ****1-2-3>4**	3.29±1.08 3.25±1.04 3.63±0.79 3.97±0.64	**x^2_{kw}=27.834** **p=.000** ****4>1-2-3**
Social security	There is No	231 49	82.5 17.5	32.30±15.61 43.76±22.02	t=-3.462 **p=.001**	3.87±0.70 2.95±1.08	**U=2902.000** **p=.000**
Family Type	Nuclear family Extended family	189 91	67.5 32.5	33.33±16.54 36.33±19.04	t=1.353 p=.177	3.83±0.76 3.46±0.96	**U=6510.000** **p=.001**
Chronic disease presence	There is No	61 219	21.8 78.2	30.60±14.30 47.59±20.91	**t=7.349** **p=.000**	3.91±0.64 2.99±1.09	**U=32.076** **p=.000**
Living with the patient	Living Together Non-Living	199 81	71.1 28.9	36.35±17.79 29.28±15.45	**t=3.316** **p=.001**	3.59±0.89 4.02±0.67	**U=5500.000** **p=.000**
Duration of caregiving	3-9 mo 10-16 mo 17-23 mo 24-30 mo 31 – 40 mo	135 51 27 18 49	48.2 18.2 9.6 6.4 17.5	27.23±12.08 28.88±12.69 34.19±16.10 37.50±17.33 58.33±13.28	**F=52.678** **p=.000** ***5>1-2-3-4**	4.06±0.47 3.96±0.55 3.94±0.71 3.52±0.66 2.44±0.87	**x^2_{kw}=88.319** **p=.000** ****1-2-3-4>5**

*p<.001, ZBI: Zarit Burden Interview, CS: Compassion Scale, SD: Standard Deviation, F: One Way Anova, x^2_{kw}: Kruskal Wallis Test, t: Independent Samples t Test, U: Mann-Whitney UTest, *LSD Post Hoc Test, ** Dunnet T3 Post Hoc Test*

Table 3. *The comparison of some variables of the stroke survivors with the family caregivers'ZBI and CS mean scores*

				ZBI		CS	
Variables		**n**	**%**	**X± SD**	Test	**X± SD**	Test
Age	40-61 62 <	102 178	36.4 63.6	30.71±16.07 36.37±17.86	**t=2.644** **p=.009**	3.92±0.71 3.59±0.91	**U=7061.000** **p=.002**
Marital status	Married Single	196 84	70.0 30.0	29.56±14.63 45.37±18.43	**t=-6.976** **p=.000**	3.92±0.71 3.23±0.96	**U=4798.000** **p=.000**
Education level	Illeterate Literate but not graduate of any school Primary school Middle school and higher	115 50 67 48	41.1 17.9 23.9 17.1	36.09±18.28 40.42±17.82 33.01±16.61 25.46±11.92	**F=7.150** **p=.000** ***1-2-3>4**	3.54±0.95 3.51±0.85 3.80±0.70 4.20±0.55	**x^2_{kw}=31.485** **p=.000** *** *4>1-2-3**
Employment status	Employed Unemployed	46 234	16.4 83.6	35.35±17.54 28.98±15.93	**t=2.286** **p=.023**	3.66±0.88 3.99±0.64	**U=4207.000** **p=.019**
Duration of stroke diagnosis	3 mo-4 year 5-9 year 10 year <	200 46 34	71.4 16.4 12.1	12.98±0.92 17.43±2.57 22.83±3.92	F=43.444 **p=0.000** ***2-3>1**	1.09±0.18 0.87±0.12 0.59±0.04	**x^2_{kw}=53.967** **p=.000** *** *1>2-3**
Number of stroke event	1 2 ≤	160 120	57.1 42.9	30.93±12.81 38.81±21.36	**t=4.032** **p=.000**	3.94±0.64 3.40±0.99	**U=6156.500** **p=.000**
Stroke type	Ischemic Hemorrhagic	149 131	53.2 46.8	38.05±19.71 30.05±13.20	**t=4.032** **p=.000**	3.46±0.93 4.00±0.64	**U=6156.500** **p=.000**
Dependency level	Total dependent Severe dependent Moderate dependent Slight dependent	47 151 81 1	16.8 53.9 28.9 0.4	52.74±18.64 34.17±15.81 23.93±8.94 28.00±0.00	**F=38.021** **p=.000** ***1>2-3-4**	2.86±1.01 3.67±0.76 4.27±0.36 4.54±0.00	**x^2_{kw}=77.560** **p=.000** *** *2-3-4>1**

*p<.001, ZBI: Zarit Burden Interview, CS: Compassion Scale, SD: Standard Deviation, F: One Way Anova, x^2_{kw}: Kruskal-Wallis Test, t: Independent Samples t Test, U: Mann-Whitney UTest, *LSD Post Hoc Test, ** Dunnet T3 Post Hoc Test*

The analyses that were conducted in this study revealed a significant negative correlation between the family caregivers' care burden and compassion total scores (r=-.786), a significant positive correlation between the patients' MBI independence levels and the family caregivers' total compassion scores (r =.607), and a significant moderate negative correlation (p<.001) between the family caregivers' care burden and the patients' MBI independence level (r=-.607) (Table 4).

Table 4. *The correlation between CS, ZBI and MBI mean scores*

Variables	**1**	**2**	**3**
1.CS	1		
2. ZBI	-0.786**	1	
3. MBI (patient)	0.607**	-0.623*	1

*CS: Compassion Scale, ZBI: Zarit Burden Interview, MBI: Modified Barthel Index. *Correlation is significant at the .05 level **Correlation is significant at the .01 level.*

Table 5 shows the results of the regression analysis for the prediction of CS scores by ZBI and MBI. The variables of care burden and dependence level showed a significant relationship with compassion (R=.800, R^2=.641, p<.001). The care burden and dependence level variables explained 64% of the total variance in compassion. According to the standardized regression coefficient (β), the care burden and dependence variables were found to be significant predictors of compassion. According to the results of the t-test on the significance of the regression coefficients, the care burden of the family caregivers and the dependency level of the stroke survivors were significant predictors of compassion. As a result, it was determined that having higher care burden levels predicted lower compassion scores, and having a high level of dependency in the stroke survivors predicted higher levels of compassion in the caregivers (Table 5).

Table 5. *Regression analysis of the prediction of CS scores according to ZBI and MBI*

Variables	**B** (95% CI)	**SE**	**β**	**t**	**p**
Constant	4.491	.146		30.679	.000
ZBI	-0.033	0.002	-0.667	-14.480	.000
MBI	0.017	0.002	0.449	8.136	.000

R=0.800 Adj.R2=0.641 $F_{(2,277)}$=247.007 p<.001. Adj.R^2: Adjusted R square, ZBI: Zarit Burden Interview, MBI: Modified Barthel Index, t: Independent Samples t Test, B: Partial Regression coefficient, SE: Standart Error, β: Standard partial regression coefficient; 95% CI: 95% confidence interval.

4. DISCUSSION

In this study, the mean ZBI scores of the family caregivers of stroke survivors were found to be on a moderate level. This result was similar to studies conducted with family caregivers of stroke survivors both outside Turkey (7,20,26) and in Turkey (5,6,31). However, there are also studies in the literature that have provided results which differed from the results of this study. In their study on the family caregivers of stroke survivors, Tosun and Temel found that family caregivers

suffered a severe burden of care (47.42±11.91) (31). The difference between the results of this study and those of Tosun and Temel may have stemmed from the difference between the durations of the caregiving processes (31). In Tosun et al.'s study, the duration of the caregiving process (60 months or more) was longer than that in this study (36-40 months), which was considered to affect the outcome (31). In the literature, it was stated that the duration of the caregiving process affects the burden of care, and the burden of care increases as this duration increases (1).

In this study, which is the first study examining compassion in family caregivers of stroke survivors, the compassion scores of the family caregivers were found to be moderate. In their studies on the compassion levels of parents of children with special care needs, Avşaroğlu and Güleş found the level of compassion to be high (32). The difference between the results of the study of Avşaroğlu and Güleş and this study may be due to the fact that the samples were different (32). While Avşaroğlu and Güleş were working with parents of children with special care needs, this study was conducted with family caregivers who were providing care for stroke survivors (32). Being a mother or father requires caring for one's children in a dedicated way. However, it was reported that parents of children with disabilities can consider their children's birth as divine grace because they are very good and compassionate (33). Additionally, the care provided for elderly individuals is perceived as more difficult for family caregivers, and this leads to heavy responsibilities, stress, frustration, anger and exhaustion in family caregivers (34,35). Moreover, the finding of the reduced compassion scores of the family caregivers who were included in this study as the age of the stroke survivors increased could be shown as another finding supporting this idea.

When some demographic characteristics of the family caregivers and the relationships between these characteristics and their care burden and compassion levels were examined, care burden and compassion were found to be significantly related to age. In this study, the family caregivers who were 51 years old or older had higher care burden levels than those in other age groups, and their compassion scores were lower. This result was consistent with studies in the literature on the relationship between care burden and age (5,25,36). However, in studies conducted in different samples, unlike the result of this study, it was seen that as age increases, compassion scores also increase (9,21). There are also some other studies showing no significant relationship between age and compassion (23,37). Education level is a factor that affects the care burden (36,38) and compassion of caregivers (21). In this study, the education levels of the family caregivers were found affect their care burden and compassion scores, and as the education levels of the family caregivers increased, their care burden decreased, and their compassion increased. This finding was consistent with those of some studies in the literature that have investigated care burden in caregivers of stroke survivors (1,39) and one study on compassion in nurses (21). This result is important in terms of showing that by means of increasing the level of education, compassion may be increased, and care burden may be decreased. Additionally, the higher the education level of family caregivers is, the greater the financial means of theirs and the lesser their burden of care may be. While no significant relationship was found between family type and care burden, a significant relationship was found between family type and compassion. It was determined that the family caregivers with extended families had lower levels of compassion. While this result was similar to a study showing that family caregivers of stroke survivors are not affected by family type (34), it was different from studies in which compassion was examined in nursing students (22) and classroom teachers (37) and showed that there was no relationship between compassion and family type.

In this study, the family caregivers without any chronic diseases had higher care burden levels. This result differed from the results of some previous studies (36,38). This result may be interpreted as that those who have chronic diseases may be strengthened while adapting to their disease, and those who do not have any chronic disease experience more care burden during the caregiving period by feeling under more pressure. Additionally, in this study, like some other studies (34,38), it was determined that the care burden levels of the family caregivers increased as their caregiving durations increased. On the other hand, long-term care may lead to more physical, emotional, social and financial problems as family caregivers are exposed to various stressors for prolonged durations (34,40). It is thought that this may lead to a decrease in the compassion levels of family caregivers. Therefore, by evaluating the caregiver's burden of care and compassion levels and by implementing supportive interventions to reduce the duration of the caregiving process, the caregiver's burden of care may be reduced, and their compassion levels may be improved or at least prevented from increasing.

In this study, it was determined that some characteristics of the stroke survivors such as their age, marital status, level of education, working status, duration of stroke diagnosis, number of strokes, stroke type and level of dependency affected care burden and compassion. In the comparison of the results of this study to the results of other studies in the literature where care burden has been examined, it was seen that this study produced similar results with regard to the survivor's age (5,34), education level (34,38) and level of dependency (5) and dissimilar results with regard to marital status (8,34).

The care burden experienced during the caregiving process may cause family caregivers to experience negative emotions, and they may neglect their own needs and feel less compassion in the process of time (9,10). In this study, it was determined that as the care burden levels increased, the compassion levels decreased. Additionally, it was found that the compassion levels of the family caregivers significantly predicted their care burden levels. Similar to the results of this study, in a study which investigated compassion in spouses who cared for Alzheimer's patients, it was found that compassion reduced care burden (15). Compassion enhances the quality of care by providing individual, appropriate and accurate care, it allows both better clinical outcomes

for stroke survivors and improved quality of life for family caregivers, and it causes a decrease in the rates of negative consequences such as burnout and feelings of care burden (16,41). Studies have shown that compassion increases the happiness, positive thinking and positive mood of family caregivers (42), and it has been proven that compassion is a significant predictor of subjective well-being (43). Given that caregivers of stroke survivors are mostly family members, and they experience care burden, both for the health of the family caregiver and the stroke survivor, it may be stated that the examination of compassion and the development of methods to increase compassion or reduce care burden are important issues.

Another finding obtained in this study was that as the dependency level of the stroke survivors increased, the compassion scores of the family caregivers decreased. It was stated that an increase in the dependency level of survivors may cause physical and mental exhaustion in the family caregiver by increasing the time the family caregiver devotes to care, reducing the time devoted to their own life and responsibilities and limiting their social life (34,40). This situation may also be effective in lowering the compassion scores of family caregivers of stroke survivors. Thus, it was stated that compassion may change over time due to stress, lack of support and learned behaviors devoid of compassion (13).

5. CONCLUSIONS

In this study, it was found that the care burden and compassion levels the family caregivers of stroke survivors were moderate. However, there was a negative, strong and significant relationship between the family caregivers' scores of care burden and compassion. According to the results of the study, the care burden levels of the family caregivers of stroke survivors were effective on their compassion levels. Therefore, in order for survivors to receive better quality care, it is recommended to determine care burden and compassion levels, develop studies and methods for reducing care burden and raising compassion and investigate the matter with a larger population for a better understanding of the issue. Determining the care burden and compassion levels of family caregivers of stroke survivors will contribute to improving the health of both the family caregiver and the stroke survivor.

This research had several restrictions. First off, the results cannot be applied to people outside of the tiny sample size used and one health center. Second, because the study was conducted in a clinical setting, it cannot be used to judge the care provided at home. This restriction could be removed by conducting comparable research in the home setting and reevaluating the results.

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Analysis of data for the study: HEY, TKF
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Clinical and Experimental Health Sciences

The Relationship Between Achilles Tendon Thickness and Instent Restenosis in Patients with Carotid Stents

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ABSTRACT

Objective: Considering that atherosclerosis and Achilles tendon thickening share common mechanisms, the aim of this study to reveal the relationship between Achilles tendon thickness (ATT) and carotid in-stent restenosis (ISR).

Methods: In this study, 89 patients who had carotid stenting for carotid artery disease at our institute between 2016 and 2020 were included. Subjects were divided into two groups as restenosis (+) and restenosis (-) based on the ultrasonographic and/or angiographic findings. The development of 50% or more restenosis in the carotid stent was defined as ISR. Bilateral ATT was measured for all patients who satisfied the inclusion criteria.

Results: In our study, 16 (17.9%) patients constituted the restenosis group and 73 (82%) the no-restenosis group. ATT values were similar between groups (4.9±0.8 vs 4.7±0.6, p=.27). However, in the marginal effect graphic, it has been demonstrated that the probability of carotid stent restenosis increases with the increase in the mean ATT. The probability of restenosis was 14% when the mean ATT value was 4.16 mm (mean – 1 SD) and the probability of restenosis was 22% when the mean ATT value was 5.36 mm (mean +1 SD).

Conclusion: No significant difference was found in ATT between the restenosis and no-restenosis group, however, the probability of restenosis increased with increasing ATT. In addition, ultrasonographic measurement of ATT is an easy, inexpensive and safe method that can be used to identify patients at high risk for carotid stent restenosis.

Keywords: Carotid artery disease, carotid stent restenosis, Achilles tendon thickness, atherosclerosis, cerebrovascular disease

1. INTRODUCTION

Advances in catheter-based techniques have allowed symptomatic carotid artery patients and asymptomatic patients at increased risk of stroke to be treated with results similar to conventional carotid endarterectomy. Carotid artery stenting (CAS) is an alternative to carotid endarterectomy (CEA). Despite advances in stent technology, carotid in-stent restenosis (ISR) continues to occur at a rate of approximately 10% (1–3).

Restenosis can be defined as measuring the degree of recurrent stenosis ≥50% (moderate) or ≥70% (severe) in the treated artery (4). While half of the cases are seen in the first 6 months, data on stent restenosis are limited in long-term follow-ups (5). Female gender, advanced age, diabetes mellitus (DM), hyperlipidemia, smoking, history of radiation to the neck, type of stent used, and residual stenosis after CAS are the most important risk factors (6).

Two important mechanisms have explained the development of carotid restenosis. Early carotid restenosis that develops within 12 months after the procedure is associated with neo-intimal hyperplasia (7,8). As a result of the force applied by the stent to the vessel wall, there is smooth muscle and fibroblast migration from the media layer to the intima in response to the damage to the intima layer. Activation of platelets and lymphocytes causes the release of cellular mediators. These mediators result in smooth muscle cell and collagen matrix proliferation, resulting in neointimal hyperplasia. Damage from stenting causes a more severe reaction compared to angioplasty (9,10). Carotid restenosis

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that developed after 12 months after stenting was associated with progressive neo-atherosclerosis (11).

Achilles tendon thickness (ATT) has been identified as an independent predictor of coronary artery disease (CAD) and atherosclerosis (12–16). Especially in patients who are genetically predisposed to hypercholesterolemia, lipid and connective tissue accumulation occurs in the extracellular matrix of tendons. Lipid deposition in tendons appears clinically as a tendon xanthoma and is often seen in the Achilles tendon. The initial form of xanthomas is the thickening of the tendon. This is not usually reflected in the clinic, but can be viewed via ultrasonography or magnetic resonance imaging (MRI). In fact, xanthomas are accumulations of lipid-laden macrophages (foam cells) in the tendon matrix. Xanthomas and cardiovascular diseases, beyond being related to each other, share common pathophysiological mechanisms (17). The purpose of this study was to examine the relationship between ATT and carotid stent restenosis.

2. METHODS

In this study, 89 patients who had carotid stenting for carotid artery disease at our institute between 2016 and 2020 were included. The study was constituted in accordance with the Declaration of Helsinki and protocol of study was approved by own ethics committees of our institution**.** The patients who signed the informed consent form were included in our study population**.** The patients with severe stroke sequelae that cannot be mobilized, Achilles tendon rupture, foot deformity that may affect image quality, a history of amputation owing to peripheral artery disease, rheumatoid arthritis, ankylosing spondylitis, Achilles tendinitis or tenosynovitis, bursitis, tuberculum attriticum, and Achilles patients with conditions that will affect the thickness of the Achilles tendon, such as tendon surgery were excluded.

2.1. Measurement of Achilles Tendon Thickness

Bilateral ATT was measured for all patients who satisfied the inclusion criteria. The measurement of thickness and width of the Achilles tendons of the patients was performed blindly by an experienced radiologist. A high-resolution B-mode ultrasonography device (Hitachi Medical Systems – HiVision Preirus) with a 7 MHz linear probe was used in measurement**.** The Achilles tendon was imaged with the patient lying prone (prone position) and ankles extended from the examination stretcher(Fig. 1(A)). The ankle was slightly bent by 90 degrees to increase the contact between the probe and tendon (Fig. 1(B)). The thickness and width of a tendon; they measured bilaterally by taking the mean of three measurements at the medial malleolus level in transverse scans above the tuber calcaneus insertion site, and the mean values were taken (Fig. 1(C),1(D)).Ultrasonographically measured ATT and Achilles tendon width (ATW) were measured as right and left for both extremities, mean values were obtained by summing the right and left measurements and dividing them into two. Average measurements were divided by body surface area (BSA) and average Achilles tendon thickness and width were indexed. BSA was calculated using the formula $BSA = 0.007184 \times Height^{0.725} \times Weight^{0.425}$.

2.2. Carotid Duplex Ultrasonography (DUS)

The primary outcome of our study was carotid stent restenosis. The development of 50% or more stenosis in the stent is defined as stent restenosis (18). In the Radiology and Imaging Center of our hospital, Peak Systolic Velocity (PSV) and B Mode imaging are used in the grading of carotid stent restenosis. As described by Setacci et al., gradients above 3 m/s were defined as significant restenosis (18). Carotid Doppler USG was performed on the patients at the 1st and 6th months, 1st year and then annually after CAS, and it was evaluated whether there was an ISR. Patients diagnosed with restenosis ultrasonographically were confirmed by angiography.

2.3. Statistical analysis

The distribution of numerical data was evaluated with a histogram and Shapiro-Wilks test. Numerical baseline characteristics were evaluated by the Mann-Whitney-u test and independent t-test, and according to the distribution of variables, categorical ones were evaluated by the chi-square test. Normally distributed numerical variables were expressed as mean±standard deviation, and categorical variables were expressed as absolute numbers and percentages. A p value of <.05 was identified as significant. For carotid stent restenosis, which is the main outcome, the Achilles tendon thickness parameter, which is the parameter we are interested in, was added to the regression model, except for the parameters taken in previous studies (variables such as age, gender, creatinine). The predicted probability model was checked for the best explanatory model. In addition, the explanatory feature of Achilles tendon thickness was shown with the marginal mean.

3. RESULTS

89 patients who underwent CAS due to carotid artery disease were enrolled in our study. In the study population, restenosis was found in 16 (17.9%) patients. The mean age of the study population was 65.9±7.2 years. Comparisons of clinical, demographic and laboratory features according to the presence of restenosis are shown in Table 1. Variables such as age, DM, hypertension (HT), CAD, lower extremity peripheral arterial disease, smoking, statin use, low or high dose statin use were similar between groups. The association between thickness and width of the Achilles tendon and carotid stent restenosis, is shown in Table 2. When the right ATT measurements were 5±0.9 mm in the restenosis group, it was 4.8±0.6 mm in the non-restenosis group (p=.21). When the left ATT was 4.8±0.7 mm in patients with restenosis, it was 4.7±0.6 mm in patients without restenosis (p=.41). The mean ATT values were 4.9±0.8 mm and 4.7±0.6 mm in patients with restenosis and without restenosis, respectively. There was no remarkable difference between the groups

(*p*=.27). In addition, ATT was indexed to BSA but, results were similar between restenosis and no-restenosis groups (2.6±0.5 mm/m2 vs 2.5±0.4 mm/m2, *p*=.37, respectively). Additionally, measurements of Achilles tendon width did not differ between groups.

Table 1. *Comparison of clinical and laboratory characteristics according to the presence of restenosis.*

Variables	Restenosis (-) (N:73)	Restenosis (+) (N:16)	All patients (N:89)	*p* value
Age, year	65.7±7.2	66.6±7.7	65.9±7.2	.64
BMI, kg/m^2	27.8±4.06	28.2±4.40	27.8±4.1	.80
BSA, m^2	1.9±0.2	1.9±0.2	1.9±0.2	.97
DM, n (%)	31.0 (42.5)	7.0 (43.8)	38.0 (42.7)	.92
HT, n (%)	63.0 (86.3)	11.0 (68.8)	74.0 (83.1)	.089
HL, n (%)	52.0 (71.2)	14.0 (87.5)	66.0 (74.2)	.178
CAD n (%)	55.0 (75.3)	12.0 (75)	67.0 (75.3)	.97
Smoking, n (%)	42 (57.5)	8 (50)	50 (56.2)	.58
Peripheral artery disease, n (%)	16 (21.9)	4 (25)	20 (22.5)	.079
Statin therapy, n (%) Not using Low dose High dose	 25 (34.2) 27 (37) 21 (28.8)	 6 (37.5) 6 (37.5) 4 (25)	 31 (34.8) 33 (37.1) 25 (28.1)	.95
Total cholesterol, mg/dl	168 ±44	182±57	171±46	.26
HDL cholesterol mg/dl	44±11	46±11	45±11	.57
LDL cholesterol, mg/dl	91±38	103±47	93±40	.25
Triglyceride, mg/dl	164±87	164±61	164±82	.99
Creatinin, mg/dl	1.2±1.1	1.1±0.4	1.2±1	.75
CRP, mg/L	8.2±25	7.5±8.9	8.1±23	.90
Albumin, g/dl	6±8.1	4.2±0.5	5.6±7.4	.38
WBC, 10^3/µL	8.6±2.6	7.4±1.1	8.4±2.4	.08
Neutrophil, 10^3/µL	5.2±2.3	4.7±0.8	5.1±2.2	.38
Lymphocyte, 10^3/µL	2.3±0.8	1.8±0.6	2.2±0.7	**.02**
Hemoglobin, g/dl	13.5±2.1	12.8±2.3	13.4±2.2	.25
Platelet, 10^3/µL	267±75	244±63	263±73	.26

BSA, body surface area; BMI, body mass index; DM, Diabetes mellitus; HT, Hypertension; HL, hyperlipidemia; CAD, Coronary artery disease; HDL, High Density lipoprotein; LDL, low density lipoprotein; CRP, C-reactive protein; WBC, white blood cell.

Table 2. *Comparison of Achilles tendon thickness and Achilles tendon width according to the presence of restenosis.*

Variables	Restenosis (-) (N:73)	Restenosis (+) (N:16)	All patients (N:89)	*p* value
Right ATT, mm	4.8±0.6	5±0.9	4.8±0.7	.21
Left ATT, mm	4.7±0.6	4.8±0.7	4.7±0.6	.41
Mean ATT, mm	4.7±0.6	4.9±0.8	4.8±0.6	.27
Mean ATT index, mm/m^2	2.5±0.4	2.6±0.5	2.5±0.4	.37
Right ATW, mm	16.3±2.1	17±2.5	16.5±2.2	.26
Left ATW, mm	16±1.8	16.1±2.7	16±2	.74
Mean ATW, mm	16.1±1.9	16.6±2.5	16.2±2	.44
Mean ATW index, mm/m^2	8.5±1.3	8.7±1.4	8.5±1.3	.61

ATT, Achilles tendon thickness; ATW, Achilles tendon width.

The distribution of ATT values in subjects with and without restenosis is shown in Figure 2. After correcting for age, the probability of restenosis was 14% when the mean ATT value was 4.16 mm (Mean-1 SD), while the probability of restenosis was 22% when the mean ATT value was 5.36 mm (mean + 1SD) (Table 3). This value suggests that ATT may be associated with the possibility of restenosis. The marginal effect graph showing the relationship between ATT and the probability of restenosis is shown in Figure 3. In this graph, when the ATT value is 5.7 mm, the probability of restenosis is 25%, when the ATT value is 6.8 mm, the probability reaches 37.5%.

Table 3. *Mean Achilles tendon thickness and probability of restenosis.*

	ATT	Probability of restenosis	Standard error
Mean – 1 SD	4.16	0.14	0.05
Mean	4.76	0.18	0.04
Mean +1 SD	5.36	0.22	0.06

SD, standard deviation; ATT, Achilles tendon thickness.

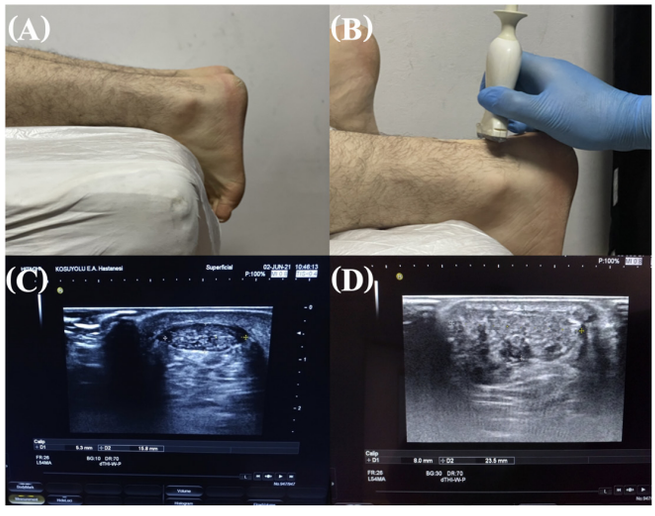

Figure 1. *Position of patient and probe in Achilles tendon thickness measurement. (A). Achilles tendon imaging was performed with the patient prone position and ankles extended from the examination stretcher. (B). To facilitate contact between the probe and the tendon, the probe was positioned by bending the ankle slightly by 90 degrees. (C). Measurement of Achilles tendon thickness (5.3 mm) and width (15.8 mm) in transverse section. (D). Achilles tendon ultrasonography of a patient with familial hypercholesterolemia and stent restenosis on carotid DUS. The thickness (8 mm) and width (23.5 mm) of the Achilles tendon have increased, the fibrillar structure of the tendon has deteriorated, and a type 3 sonographic pattern with nodular xanthomatous appearance is observed.*

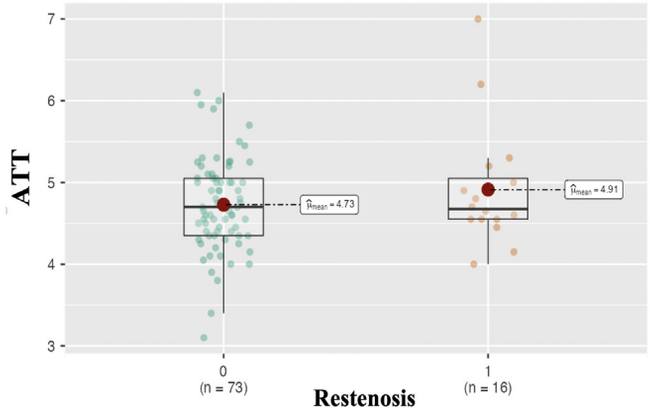

Figure 2. *Bar graph showing the distribution of mean ATT values with standard error in patients with and without restenosis.*

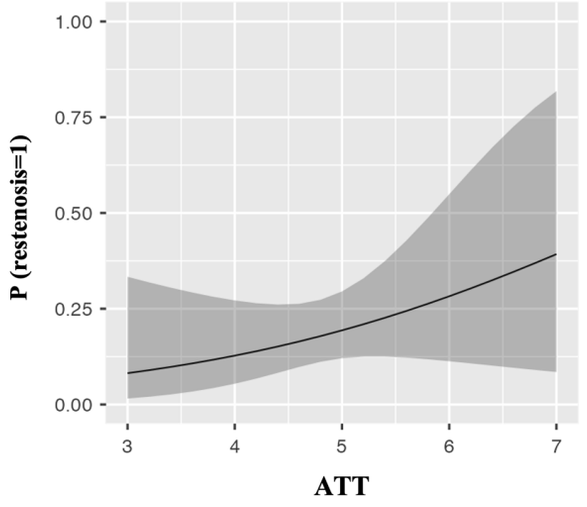

Figure 3. *Marginal effect plot showing the relationship between the mean Achilles tendon thickness (ATT) and the probability of restenosis (P).*

4. DISCUSSION

ATT, which shares common mechanisms with atherosclerosis, has been identified as an independent predictor of CAD and atherosclerosis (12,13,15,17). In present study, we revealed that mean ATT values were similar in subjects with and without restenosis. However, according to marginal effect graphic (Figure 3), the probability of carotid stent restenosis may tend to be increased when the ATT level increased.

Identification of predictors of ISR is clinically important. However, there is no study in the literature determining the relationship between carotid ISR and ATT, hence our study is the first. The association of ATT with restenosis has been attributed to atherosclerosis, but the relationship of ATT to neo-intimal hyperplasia is unclear.Perhaps this uncertainty can be investigated in more comprehensive and larger studies. Our findings showed that the right, left, mean ATT and mean indexed ATT were not different in patients with and without restenosis. However, the probability of carotid stent restenosis increased with the increase in mean ATT (Figure 3). It might suggest that ATT value may be associated with carotid stent restenosis. Currently, several studies have evaluated the association between ATT and carotid artery disease. A previous study revealed that a remarkable correlation occurred between ATT and carotid intima media thickness in patients diagnosed with familyal hyperlipidemia (19). In another study, ATT was identified as an independent predictor of atherosclerosis in carotid artery (20). ATT, which was discovered that it has a relationship with carotid plaque burden, was found significantly increased in patients with carotid stenosis. It has been discovered that when ATT reaches to ³5.07 mm, predicting the presence of atherosclerotic plaques in the carotid arteries might be possible (sensitivity: 68.3% and specificity: 62.5%) (20). Similarly, the mean ATT value of 89 patients who underwent CAS in our study was measured as 4.76 mm. At this value, the probability of carotid stent restenosis was 18% with a standard error of 0.04. With an increase in this value, the probability of restenosis increases. Although a cut-off value for ATT required for restenosis is not clear in the literature, these results suggest that the Achilles tendon must reach a certain thickness for restenosis to occur.

Several studies evaluated the relationship between ATT and CAD (12–15,21,22) in literature. In our study, 67 (75.3%) patients were diagnosed with CAD and 12 (75%) patients with carotid stent restenosis had CAD. CAD was more widespread in patients with increased ATT, and also they have more left main coronary artery (LMCA) disease and more advanced and/or vulnerable plaque structure (14). Thus, it has been claimed that ATT may be a marker to identify high-risk patients. Hirobe et al. reported that ATT was thicker in patients diagnosed with familial hyperlipidemia with CAD than without CAD (22). ATT was found to be higher in diabetic patients and patients with a Syntax score >23, in a study included patients who underwent percutaneous coronary procedure (12). It has been also discovered a linear relationship between tendon xanthomas and coronary calcium score (15). These studies demonstrated that ATT is an independent predictor of CAD and atherosclerosis.

ATT is a clinical marker to identify patients who have a high risk for cardiovascular diseases and elevated LDL cholesterol levels (23). A few studies showed that tendon xanthomas regress after drugs such as statin, ezetimibe, and PCSK-9 inhibitors are used in the treatment of hyperlipidemia (24,25). As we showed in our study, while the increase in the mean ATT value increases the probability of restenosis, when we think in the opposite direction, it seems possible to decrease the probability of restenosis with the decrease in the mean ATT value. Reducing Achilles tendon thickness with lipid-lowering therapy might be an affective method to reduce the possibility of restenosis. Nonetheless, the relationship between the use of lipid-lowering therapy and the possibility of restenosis could not be demonstrated in our study. The small size of patient population and the low persistence on statin therapy might make the clinical impact of statin therapy uncertain.

Recently, also a relationship between ATT and, diabetes, hyperuricemia,obesity has been revealed (26). In particular, ATT has been associated with diabetes in many studies (26,27). It is well known that DM is also a risk factor for carotid stent restenosis. This association can be attributed to diabetes being a strong predictor of atherosclerosis. Interestingly, significant difference was not occured between DM and restenosis in our study.

In diabetic patients, ATT has increased in male patients with retinopathy or neuropathy compared to the group without these complications (26). When the relationship between DM, HT or obesity and ATT was evaluated, no significant difference was found in our study. This results can be explained by the fact that our patient group consists of very high-risk patients. However, the examination of the ATT is important in determining the high-risk group that will be exposed to the complications of diabetes (26).

Consequently, ultrasonographic measurement of ATT is an easily accessible, inexpensive and applicable method to determine the high-risk group for carotid stent restenosis. Our study has some limitations: 1) In our small sample group with very high cardiovascular risk, DM and multiple drug use may have affected ATT; 2) The single-center design of our study may prevent the generalization of the study results; 3) Evaluation with Doppler USG and B mode requires user experience, in our study, the measurements were made blindly by a radiologist. If the measurements were made blindly by two different users, more reliable results could be obtained.

5. CONCLUSION

To our best knowledge, our study is the first single institutional study evaluated the association of ATT with carotid stent restenosis. No significant difference was found in ATT between the restenosis and the no-restenosis group. However, the probability of restenosis increased with increasing ATT. In addition, ultrasonographic measurement of ATT is an easy, inexpensive and safe method to identify patients at increased risk for carotid stent restenosis. Further large-scale multicenter studies are required to accept ATT, which is considered a marker of atherosclerosis, as a predictor of carotid stent restenosis.

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Design of the study: CY, RZ.
Acquisition of data for the study: RZ, AFK, BGŞ, MHÖ.
Analysis of data for the study: AK, NH, LÖ.
Interpretation of data for the study: LO, AK, RZ.
Drafting the manuscript: RZ, CY, BGŞ.
Revising it critically for important intellectual content: RZ, MVY, LO.
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Clinical and Experimental Health Sciences

Female Adolescents' Experiences with Contraceptive Method Decision-Making, Access, and Continuation: Qualitative Research

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ABSTRACT

Objective: Because of early sexual activity that starts in adolescence, critical problems such as unwanted pregnancies arise. The aim of this study was to examine the experiences of female adolescents in decision making, accessing, and maintaining contraception.

Methods: The study was conducted as a case study with a phenomenological design and a qualitative approach. The study included adolescents who had contraception experience and were present in the gynecology clinic(s) of the hospital to receive any care service. Participants (n=22) was selected through purposive sampling. Semi-structured in-depth interviews were conducted, transcribed, and analyzed using the approach, content, and descriptive analysis.

Results: Through the analysis of in-depth interview data with female adolescents examining their experiences related to contraceptive method use, four themes were identified: "reasons for using and deciding on pregnancy prevention methods, accessibility to pregnancy prevention methods, experiences during the use of pregnancy prevention methods, and continuity of pregnancy prevention method use."

Conclusion: In this study, it was determined that female adolescents faced some difficulties in deciding on, accessing, and maintaining contraceptive methods. In this context, units such as youth centers where adolescents can apply can be established. Unwanted pregnancies can be prevented by regulations regarding the provision of counseling and contraceptive services to adolescents.
Keywords: Adolescents; contraception; reproductive health; safe sex

1. INTRODUCTION

Adolescence is a period when the normal growth and development of both sexes accelerate, and reproductive and sexual health needs emerge (1). It is defined by the World Health Organization (WHO) (2021) as the period between the ages of 10 and 19 years. Serious problems related to reproductive health arise (i.e., unwanted pregnancies) because of early sexual activity linked to the development of sexual identity during adolescence (3,4). In this context, it is critical to examine the experiences of adolescents regarding the contraceptive methods (CM) they use to prevent unwanted pregnancies.

One of the United Nations' sustainable development goals related to health is to ensure universal access for individuals around the world to sexual and reproductive health (SRH) services, including family planning, information, education, and integration of RH, by 2030(1). The WHO (2021) reported that every year 21 million girls aged 15-19 and 2 million girls under the age of 15 experience pregnancy and approximately 16 million adolescents aged 15-19 and 2.5 million adolescents under 16 years of age give birth in developing regions (2). It is estimated that most of these pregnancies and births are not planned or desired by these adolescents. According to 2018 data, the fertility rate among adolescents in Turkey tends to decrease, but the birth rate in the 15-19 age group is 17 per thousand, whereas the overall birth rate is 14.3 (5). These results indicate that there is a significant unmet need among adolescents regarding their access to CM and necessitate knowing more about their experiences about contraceptive use.

In addition to social barriers related to the use of CM, adolescents experience problems related to access and finances. Studies indicated that most adolescents (52%) found contraceptive methods too expensive (6), CM and free methods offer reduced unwanted pregnancy rates (7), and the cost was an obstacle for contraceptive use for a significant portion of adolescents (23%) (8). Adolescents are exposed to maternal deaths and permanent health problems because of the increased rate of miscarriages and abortions in unsafe conditions linked to inadequate contraceptive use (1,2). Moreover, adolescent pregnancy has many negative

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social and economic effects on girls and their families. These girls and their families may be subject to social stigma or rejection. Serious problems such as dropping out of school, lack of opportunities for work and economic independence, and exposure to violence are especially common among young girls who become pregnant (2,9).

Several studies have addressed the experiences of adolescents related to the use of CM. These studies reported that adolescents had concerns about the side effects because of the lack of proper knowledge about CM (10), had difficulties in accessing contraception methods (11), obtained contraceptives from pharmacies (12), and used condoms primarily to prevent pregnancy rather than to prevent sexually transmitted infections (STI) (13). It was found in some studies that unmarried young people were not able to plan and were not ready to use modern CM before each sexual intercourse (14) and encountered several systemic and interpersonal barriers in accessing SRH services (15).

Studies on the use of CM in Turkey were mostly conducted with married women aged 15-49, focused on the method, and were quantitative (16,17). There is a need for qualitative studies that can reflect Turkish cultural characteristics and provide more comprehensive information about the experiences of adolescents regarding the use of CM. It is expected that the information obtained from such studies will reveal the cultural and structural characteristics related to the experiences of adolescents regarding contraceptive use, contribute to the quality of health services, and improve the physical and mental health of the youth and their socioeconomic well-being. Thus, the aim of this study was to examine the experiences of female adolescents in decision-making, accessing, and maintaining contraception. The research questions were as follows:

(1) What are the reasons for adolescents to use contraception and make decisions?

(2) How do adolescents access contraceptive methods?

(3) What are the experiences of adolescents with the use of contraceptive methods?

(4) What is the continuity of adolescents on contraceptive methods?

2. METHODS

2.1. Research Design

The study was conducted as a case study with a phenomenological design and a qualitative approach. Phenomenology is a perspective that form the basis of qualitative research. Qualitative phenomenological studies allow for a detailed understanding of human experiences by examining the underlying hidden realities and causes in the natural environment (18). The study was conducted as a qualitative case study using a phenomenological design in accordance with the COREQ guidelines.

2.2. Participants and Setting

There is no set rule for sample size in qualitative research. The sample size may vary according to the qualitative research approach, the diversity of the selected sample, and the participant's ability to provide sufficient information. When the answers received from the participants begin to repeat, the interviews can be terminated assuming that the data has reached sufficient saturation (18).

Data collection was carried out in gynecology and obstetrics clinics of any public hospital in Izmir between May 2020 and December 2020. Twenty-seven female adolescents, who were determined by the purposive sampling method, were invited to the study, but five of them did not agree to be interviewed. Data collection continued until the responses started to repeat and was completed with the participation of 22 female adolescents. Voluntary female adolescents who were 19 years of age or younger and had experience with contraceptive methods were included in the study sample. The reasons for the presence of the female adolescents included in the sample group in the hospital were not questioned, and the participants were interviewed about their contraception experience in accordance with the purpose of the study. However, adolescents who stated that they were diagnosed with any physical or mental illness and did not speak Turkish were excluded from the study. Adolescents who agreed to participate in the study were interviewed in a quiet and comfortable room at the hospital for approximately 30/40 min. All interviews were completed in a single session, and there were no repeated interviews. Written and verbal consent was obtained from all participants.

2.3. Data collection tools

The research data were collected using a semi-structured form consisting of open-ended questions prepared by the researchers based on the literature on the subject (3,4,6,10,14,17).

The form included the following: Section 1 (descriptive characteristics): age, marital status, education, and number of pregnancies. Section 2: Adolescents' experiences with contraceptive method use. To ensure the content validity of the interview form, the opinions of experts (five people) experienced in qualitative research were taken and rearrangements were made in line with the suggestions made. To improve the comprehensibility and applicability of this form and to ensure the standardization of the interview, a pre-application was conducted with five people and the interview form was finalized. The data collected through the pre-application were excluded from the analysis.

2.4. Ethical Considerations

Ethics approval was obtained from Aydın Adnan Menderes University Health Science Faculty Non-Interventional Research Ethics Committee (Date: 26.08.2019; Number of approval: 2019/051). The research was conducted in accordance with the principles of the Declaration of Helsinki.

2.5. Procedure

In the research group of this study, there were two female researchers, a professor and a doctoral lecturer who were experienced in the field of midwifery. Both researchers have taken qualitative research courses and have experience in this field. The first researcher has previous experience as a counselor in long-term family planning. The second researcher has conducted a qualitative research course and has published numerous studies.

First, they were informed about the research, and oral and written consent was obtained from the adolescents who agreed to participate in the research. Participants were assured that they could stop the interview at any time and skip any questions they did not want to answer. Participants were assured that their identity information would not be disclosed in the study results. The privacy and confidentiality of the meeting room has been meticulously ensured. Individual interviews were conducted in a quiet and convenient room in the hospital. Documentation of the data was carried out in a special room. The documentation was shared only with qualitative researchers. Research data were stored on the personal computer of the first researcher and protected with a password. Written data were kept in a secured cabinet in a safe room with no access to others. Interview records will be deleted after they have been documented.

2.6. Statistical Analysis

Descriptive data obtained from the questionnaire forms were reported numerically. In the analysis of the data, the voice recordings of the women were converted into a text word by word; a raw data document was created in Microsoft Word. The data obtained from the interviews were analyzed through content analysis. In this analysis, women's responses were coded in line with the research objectives. Categories were created by considering the similarities, differences, and relationships of the codes and were placed in the determined categories. The adolescents were asked to evaluate whether these codes, categories, and themes corresponded to their own views (AD, ZK). Sentences were used as the unit of analysis. Qualitative data were then quantified by determining how often each category was repeated (frequency). No statistical program was used for the analysis of qualitative data. Support was received from a competent faculty member in the field of qualitative research in coding and analyzing the data and preparing the research report. None of the participants gave any negative feedback, and the results were confirmed. For reliability, the coefficient between coders (2 people) was calculated and obtained as 0.80 (18).

3. RESULTS

The female adolescents included in this study were between the ages of 16 and 19 years. Ten participants had eight years or more of education, 14 of them had less income than their expenses, four were students, nine were in an official marriage, and four were in an unofficial (religious) marriage. Twelve participants had children, four had spontaneous abortions, and two had induced abortions. For contraception, seven participants used condoms; four had intrauterine devices (IUD); four used combined oral contraceptives (COC); three used the coitus interruptus method; and four used injections (Table 1).

We identified four main categories based on the qualitative analysis: 1) reasons for female adolescents to use the contraceptive method and to decide; 2) female adolescents' access to CM; 3) experiences of female adolescents during the use of CM; 4) continued use of female adolescents to CM. The data analysis yielded several developed meanings and subthemes (Table 2).

Table 1. *Participants characteristics*

Characteristic	Number of Participants (N=22)
Age range	
16-17	10
18-19	12
Education	
≤5 years	5
6-12 years	16
≥ 13 years	1
Income expense status	
Income less than expenses (bad)	14
Income equal to expenses (medium)	7
Income more than expenses (good)	1
Marital status	
Official Marriage	9
Religious Marriage	4
Single	9
Family planning method	
Modern method	19*
Coitus Interruptus	3

**(Condom (n=7), IUD(n=4), COCP (n=4), Injection(n=4)*

3.1. Theme. 1: reasons for female adolescents to use the contraceptive method and to decide

It has been determined that the advice of health workers and friends is important during decision-making and use of the contraceptive method by adolescents participating in the research. Some statements on this subject were as follows:

"*I got married when I was 15. We met when we were at school; he was older than me, and he always came [to us] after school. We in 2-3 months. I was a child when I …*" (A12)

"*…When I took the baby to the family physician for a checkup, they told me, 'The breastmilk does not protect you [from pregnancy], let's get you an injection.'. I had the injection because I afraid of getting pregnant. My mother young like me and gave birth to us one after the other. I'm afraid I'll end up like her.*" (A15)

Table 2. *Main and sub-theme*

Main theme	Sub-theme
Reasons for female adolescents to use the contraceptive method and to decide	Contraception Being underage The decision-making process
Female adolescents' access to contraceptive methods	Accessing the contraceptive methods Not being able to access the contraceptive methods Social life
Experiences of female adolescents during the use of contraceptive methods	Personal experience Feeling uncomfortable Peer experience
Continued use of female adolescents to contraceptive methods	Continuity to contraceptive methods Lack of information/ incorrect information Social status

3.2. Theme 2: Female adolescents' access to contraceptive methods

In this study, it was determined that some adolescents could not easily obtain CM, and they had important economic and social problems. It has been determined that some of them are purchased from public or private institutions and pharmacies. However, it has been determined that those who use oral contraceptives for medical reasons can easily obtain them.

"*…I went to a private physician and had it fitted. I bought my coil [IUD] from the pharmacy myself. I pay for the coil [IUD], and I pay the physician to get it fitted. Then I went to the physician to get it checked, so everything costs money…*" (A2)

"*When I stay with my family, I am afraid that I will be caught. Because I also saw that my mother had the pills. I take [the pills] in secret; other than that, there is no problem; I am quite comfortable.*" (A20)

3.3. Theme 3: experiences of female adolescents during the use of contraceptive methods

In this study, it was determined that some adolescents encountered some side effects specific to the contraceptive method they used, but they were still satisfied. Some adolescents also declared that spousal/partner harmony is important. In addition, some adolescents explained that it is difficult to take pills every day because they live with their families. Some statements on this subject were as follows:

"*…it hurt with the condom. I used to ask [my partner] to [ejaculate] outside, but that wasn't always the case either. Of course, I was afraid that I would get pregnant. My baby is still young; there is no financial security, and we live with my mother-in-law…*" (A10)

"*…My husband first told me not to get the IUD, he said he heard that it would hurt him, but I didn't listen to him. He's enjoying himself; I'm suffering. Those at the health center said it would not hurt him. I am well satisfied.*" (A13)

3.4. Theme 4: continued use of female adolescents to contraceptive methods

In the study, a significant portion of the adolescents stated that they wanted to continue using the method they used. Some adolescents also stated that they received information from various sources or health workers through their own efforts. Some statements on this subject were as follows:

"I'm *going to change [the method I use], I'm going to have an IUD installed to be sure. Even if he doesn't come to the health center, I wait a little bit; I will even go to the private... "Because I don't want to have children anymore.*" (A3)

"*… the physician told me to use it for a year. They said I could have children after that if I want, but I say I will take them for at least two years. At least I should be 20. Of course, we must have children; I will stop using then [when I am ready to have children].*" (A14)

4. DISCUSSION

In this study, in-depth interviews were conducted with 22 female adolescents in a phenomenological design with a qualitative research approach to examine their experiences regarding the use of CM. Significant data were obtained regarding the descriptive characteristics of these adolescents and their experiences with the use of CM. These data may reflect some basic characteristics of adolescents and contribute to the development and delivery of health services that can be offered to those who need to use CM.

Some participants stated that they did not want to get pregnant, were single, and used CM because they were afraid of becoming pregnant and ending up like their mother. WHO reported that the rate of contraception use among adolescents varies between 42% and 68% in Latin American, European, and Asian countries and between 3% and 49% in African countries (19). In addition, it has been reported that 19% of young women in developing countries become pregnant before reaching the age of 18, more than 15 million girls between the ages of 15 and 19 give birth each year, and

40% of unsafe abortions worldwide involve adolescents (20). Other research has reported that women use modern CM to prevent unwanted and unplanned pregnancies (9,21-23). Accordingly, it can be said that there is a need for CM because of the prevalence of sexual activity among adolescents.

Some of the participants in this study stated that midwife/doctor's advice during pregnancy/birth influenced their decision to use CM, whereas the recommendation of their friends in social environments was influential for others. Studies have indicated that friends and relatives (23,24), healthcare personnel (13,21), education given in schools, and radio-television broadcasts are influential in SRH issues (23,24). A study examining the use of modern contraceptives after giving birth and related factors in Ethiopia reported that postpartum counseling was influential in deciding on the use of CM (22). These findings indicate that providing detailed information about contraception methods is an important factor in the continued use of contraception, and it is necessary to expand the availability of safe, effective, and continuously accessible services and to create relevant training programs, especially for healthcare personnel.

In this study, only some adolescents indicated that they could buy CM from public health institutions. Many of them had difficulty in obtaining CM because they had to obtain it from private health institutions, pharmacies, or markets for a fee; some had difficulty because they were embarrassed. Those who used oral CM for medical reasons stated that they could easily obtain them. The Turkish Demographic and Health Survey, which is the most comprehensive study conducted in Turkey on this subject, reported that a significant portion of women (36%) obtained contraception methods through purchasing (25). Goodman et al. reported that free access to contraceptive counseling and methods reduced unwanted pregnancy rates (7). Other studies on the subject reported that participants found contraceptives too expensive and that the cost prevented the use of contraceptives (6). A study conducted by Bloomberg School of Public Health through the examination of demographic and health data from more than 40 countries reported that RH services for adolescents might be limited due to the feeling of shame and fear of stigmatization and violence if the relatives/acquaintances determine about receiving such services (26). It has also been reported that there are serious problems among the young who experience pregnancy, such as dropping out of school, lack of work opportunities and economic independence, and experiencing more violence in the future (2,9). A study conducted in Ghana also reported that social stigma is an important obstacle to the use of CM (27). A study conducted in Kisenyi reported that most of the adolescents were not satisfied with the fact that RH services were based on a health institution (28). These findings indicate that the provision of services related to CM in a nonjudgmental, free, and accessible manner to adolescents may contribute to the prevention of adolescent, maternal, and neonatal health problems.

In this study, some adolescents expressed experiencing discomfort specific to the contraceptive method they used but were still satisfied, while others emphasized the importance of the cooperation of spouse/partner. Other studies examining the same issue in Turkey have also reported that women experience various problems related to the CM they use and that their spouse/partner's opinion is important in the use and preference of a method (17,29). Similarly, previous studies reported that the side effects were "very important" in the participants' preference of CM and that they were worried about side effects such as weight gain, acne, and migraine (4,10). In another study, it was reported that women who were given more comprehensive information about the method more frequently continued to use the method without quitting (30). These findings indicate that the side effects that may develop due to the method and the spouse/partner's opinion are important factors in choosing a contraceptive method. During the delivery of reproductive services to adolescents, healthcare professionals' consideration of both sexes and informing them about the side effects that may develop due to CM may help increase the acceptability and continuity of the method used.

In this study, most of the adolescents stated that they wanted to continue with the method they used and that they learned about the contraceptive method on their own, while some stated that they received help from healthcare professionals. In a cohort study examining the relationship between the quality of contraceptive counseling and continuity of contraceptive use in Pakistan and Uganda, it was reported that the rate of continuing the method (64% in Pakistan and 80% in Uganda) was higher in women who were given more comprehensive information than in the control group (30). Another study reported that individuals who received comprehensive information about CM were less likely to change to a modern contraceptive method (8). It has also been reported that relatives and friends (19,20) or school, healthcare personnel, family, radio-television, and magazines were the most frequently cited sources of information (19,20). UNESCO reports (2015) declared that a person has the right to receive education about their body, relationships, sexual behavior, and sexual health and that accessing the correct information on time is the most important basic principle (1). Karaçalı and Özdemir reported that a significant portion of women wanted to receive information and counseling about family planning (15). Jonas et al. reported that most women received information about family planning from nurses, midwives, doctors, and their social environment (11). These findings indicate that providing detailed information about the method is an important factor for the continuous use of CM and that it is essential to provide relevant training to healthcare workers and make safe, effective, and continuously accessible services widely available. In addition, healthcare professionals' explanations during the provision of services about the side effects that may be encountered may increase the continuity of the use of these methods.

Study limitations

Because the women were determined by the purposeful sampling method, the results obtained cannot be generalized and can only represent the women participating in this study. In addition, the research results may vary depending on cultural reasons.

5. CONCLUSION

In this study, it was obtained that some adolescents have an active sex life and used CM due to having a pregnancy at an early age, not wanting children recently, and still being a student for reasons such as. However, it was determined that adolescents faced some difficulties in deciding on, accessing, and maintaining contraceptive methods. Based on these results, it is important for midwives and other healthcare professionals to question adolescents' contraceptive needs and use, provide counseling, and facilitate the provision of CM. Administrators may establish youth centers where adolescents can easily share their SRH problems and find solutions, and provide legal arrangements for the provision of counseling and services related to CM in places such as schools and universities.

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Design of the study: ZK, AD
Acquisition of data for the study: AD
Analysis of data for the study: AD and, ZK
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Clinical and Experimental Health Sciences

Effect of Skin Antiseptics Used in Peripheral Intravenous Catheter Application on Phlebitis Development: A Double-Blind Randomized Controlled Trial

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ABSTRACT

Objective: This study aimed to examine the effect of antiseptics used in peripheral intravenous catheter (PIVC) application on phlebitis development.

Methods: This is a double-blind randomized controlled trial that is suitable for the Consolidated Standards of Reporting Trials (CONSORT) statement. The study was carried out at a University Hospital in Turkey. The study participants were 60 patients (experiments 30 and control 30). 2% chlorhexidine was used as a skin antiseptic in the intervention group and 70% alcohol was used in the control group. In both groups, the catheter insertion site was observed every 8 hours for 72 hour. Data were obtained using the "Personal Information Form" and "Phlebit Scale". Independent samples t-test (t-table value), Mann–Whitney U test (Z-table value) and Fisher's exact test, continuity correction, or Pearson's $\chi 2$ cross tables were used for data analysis.

Results: No statistically significant difference was found between 2% chlorhexidine and 70% alcohol in preventing the development of phlebitis after PIVC application ($p > 0.05$).

No statistically significant relationship was found between the intervention and control groups in terms of sex, BMI, substance abuse, alcohol use, smoking, chronic disease, or PIVC application area ($p>0.05$). The highest degree of phlebitis that developed in the intervention and control groups was 1st degree. There was also no statistically significant difference between the groups in terms of degree of phlebitis and phlebitis development time ($p > 0.05$).

Conclusions: In line with the findings obtained from this study, it is thought that both skin antiseptics used when inserting a peripheral intravenous catheter are effective in preventing the development of phlebitis and will guide healthcare personnel in the selection of antiseptics.

Keywords: Nursing, phlebitis, antiseptic, peripheral intravenous catheter

1. INTRODUCTION

Peripheral intravenous catheter (PIVC) application and care are very important for the maintenance and success of treatment in individuals receiving healthcare (1). PIVC insertion is one of the most common and highly invasive nursing interventions (2). It has been reported that 58%–87% of patients receive treatment via PIVC during hospitalization (3). Although PIVC is of great benefit to patients and is lifesaving, problems such as infiltration, extravasation, and phlebitis may arise owing to damage to the endothelial layer as a result of inadequate care or erroneous applications. Studies have shown that the rate of complications necessitating premature removal of PIVC has reached 50% in Turkey (4,5). Such complications increase the risk of infection in individuals, prolong hospitalization, threaten patient safety, and cause unnecessary labor and material expenditures in healthcare institutions, thereby increasing the healthcare cost (6,7).

Phlebitis is a state of inflammation in the intima layer of the vein and develops in response to tissue damage. This condition is characterized by pain, erythema, redness, edema, and vascular hardening (8). In the literature, phlebitis has been reported to be one of the most common complications, with incidence rates ranging from 1.25% to 80% in patients with PIVC (9). PIVC-related phlebitis development is recognized as a major problem in clinical practice. A study including data from 51 countries shows that the development of phlebitis remains a global threat (10).

The incidence of phlebitis can increase because of patient-related factors such as age, sex, and certain chronic diseases

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as well as other causes such as dose and osmolarity of the drugs administered, the technique used for establishing vascular access, and the knowledge level of the nurses (11). One of the many factors that can cause PIVC-related phlebitis is microorganisms in the skin that are transported first to the catheter surface and then to the bloodstream with the catheter, thereby leading to infection in the intima layer of the vein and also systemic infection. Therefore, to ensure antisepsis in the area of application, the INS and the healthcare infection control practices advisory committee (HICPAC) recommend that the area of PIVC application be cleaned with 70% alcohol, povidone–iodine, or 2% chlorhexidine. Moreover, the antiseptic must fully contact the skin and then be allowed to dry for at least 2 minutes (5,8,12).

The guidelines prepared by The Centers for Disease Control and Prevention (CDC) and Infection Prevention Society emphasize that the principle of surgical asepsis should be considered during and after PIVC application and that symptoms of phlebitis should be monitored at 8-hour intervals (13). In accordance with INS and HICPAC, the National Vascular Access Management Guideline recommends the use of 70% alcohol solution, povidone–iodine, or 70% alcohol containing >0.5% chlorhexidine as antiseptic in the PIVC application area (4,5).

When scientific studies are examined, no study examining the effect of skin antiseptics used in PIVC applications in preventing phlebitis has been found in Turkey. When studies in the world on this subject are examined, some studies reported that disinfection method of chlorhexidine in isopropyl alcohol before the implementation is the more effective than alcohol (14-17). However some studies reported there is no significant difference between the two antiseptics(18-20). Therefore due to controversy in this area, comprehensive study is required. Therefore, this study aimed to investigate the ability of 2% chlorhexidine and 70% alcohol, which are antiseptics commonly used in PIVC applications, to prevent phlebitis development.

The following hypotheses are proposed:

H0: The use of 2% chlorhexidine or 70% alcohol while inserting PIVC has no superiority over each other in reducing the development of phlebitis.

H1: Using 2% chlorhexidine or 70% alcohol when placing PIVC is superior to each other in reducing the development of phlebitis.

2. METHODS

2.1. Ethical Considerations

The study was reviewed and approved by the ethics committee of university faculty of medicine in November 2019 (14/ 01.11.2019). Ethical standards founded on informed and voluntary consent were adhered to. Written consent was obtained from the participants prior to their inclusion in the study (Declaration of Helsinki, 2013). Participation was voluntary. Participant withdrawal from the study was respected without any disadvantage to or repercussions for the participant. The randomization of participants ensured that all participants had an equitable chance of being allocated to either the intervention or control arm. The study protocol was reviewed and approved by on Clinical Trials.gov (NCT04817020).

2.2. Trial Design

This study was a double-blind randomized controlled trial. The study was carried out between January 2020 and September 2020 at a University Medical Faculty Hospital General Surgery Clinics. The hospital is a university hospital located within the provincial border of Adana. Based on similar studies in the literature, the sample size was calculated using G Power 3.0.10 program based on 95% confidence range, 0.81 power level, 0.05 margin of error, and 0.75 effect size. Sixty patients are determined to be sufficient to represent the research population (18,19).

Considering that patients may withdraw from the study, their treatments may change, and they may be discharged before 72 hours, the maximum number of patients that can be included within the period and meet the criteria has been reached.

From the population determined within the scope of the research, 79 patients who accepted the research were reached. Among 85 patients, 79 patients met the study criteria. Nineteen patients were not included in the study due to being discharged before 72 hours, being admitted to the intensive care unit after surgery, and changing antibiotic treatments. Phlebitis development status was evaluated using the phlebitis scale every 8 hours after the application. The scale was applied for 72 hours.

2.3. Participants

2.3.1. Inclusion Criteria: The participants consisted of patients who met the following inclusion criteria: (a) 18 years or older; (b) newly admitted to the clinic; (c) vascular access established in the clinic; (d) upper extremity available for PIVC; (e) administration of antibiotics and/or analgesics containing the same active substance as part of intravenous (IV As drug therapy only (antibiotics with ceftriaxone and ornidazole as active ingredients and analgesics with paracetamol as active ingredients); (f) no problems in terms of state of consciousness or sensory organs.(g) Not being discharged or transferred to a different unit before 72 hours.

2.3.2. Exclusion Criteria: The exclusion criteria were as follows: (a) PIVC inserted by someone other than the researcher; (b) having hematological, oncological, or allergic disease (c) having peripheral vascular disease; (d) having any incision or scar tissue in the IV region.

2.4. Interventions

All patients in the intervention and control groups were treated with 20G intravenous catheters made from the same material (Vialon), and transparent, non-allergic, 6 cm × 7 cm transparent covers (Tegaderm; 3M, St Paul, MN, USA) were used to stabilize and secure the PIVC. The upper extremity was selected in all patients for PIVC application.

After applying skin antiseptic from top to bottom along the vein in the PIVC application area, it was dried for 2 minutes according to INS (2016) and HICPAC (2011) recommendations, and PIVC insertion was performed. All PIVCs were washed with a 5-mL ready-to-use injectable saline solution (BD PosiFlush™) before, during, and after different types of treatment and were secured with PIVC cover. A special label was attached to the arm of the patient receiving the PIVC, which indicated that the PIVC application was for research purposes, and all nurses at the clinic were informed about it.

To prevent or minimize the formation of treatment-induced chemical phlebitis, antibiotics with only ceftriaxone or ornidazole as the active substance and analgesics with only paracetamol as the active substance were infused through the PIVC. If the patient was about to receive a different treatment, consent was obtained and a second catheter was inserted to the other arm.

Phlebitis development was evaluated 8 hours after the application with the "Phlebitis Scale" recommended by INS (2016). Within the scope of this evaluation, millimetric measurement was performed with a transparent ruler and the degree of phlebitis was determined and recorded. The scale was applied every 8 hours for 72 hours. According to many studies, it has been observed that prolonged stay of PIVC's in the vein increases the risk of phlebitis, and as a result of these studies, it has been decided to routinely change PIVC's every 72-96 hours worlwide (8,13). When the researcher was not present, the measurement was carried out by nurses who were informed about the research. PIVC was completely removed in case of phlebitis development before 72 hours. Forms filled out before PIVC removal were included in the research.

2.5.Outcomes

The primary outcome measure is the rate of phlebitis development according to the antisepsis used. In addition, the degree of phlebitis according to the antisepsis used in the primary results and the time it develops are included.

As secondary results, it was aimed to examine the development of phlebitis among some variables. These variables are; age, gender, body mass index, alcohol smoking habits, chronic disease status and the region where the catheter is inserted, and the development of phlebitis was examined according to these variables.

2.6. Randomization

The randomization of patients into 2% chlorhexidine and 70% alcohol groups was made by a statistician other than the researcher. Block randomization method was used for randomization of the patients. Within the research population, 79 patients were included in the study. The patients were evaluated according to the inclusion and exclusion criteria. In the first stage, 79 people we randomly selected (n=39) formed the intervention group and (n=40) the control group. This randomized trial adhered to the Consolidated Standards of Reporting Trials (CONSORT) 2010 guidelines. A flow diagram of the study is shown in Figure 1.

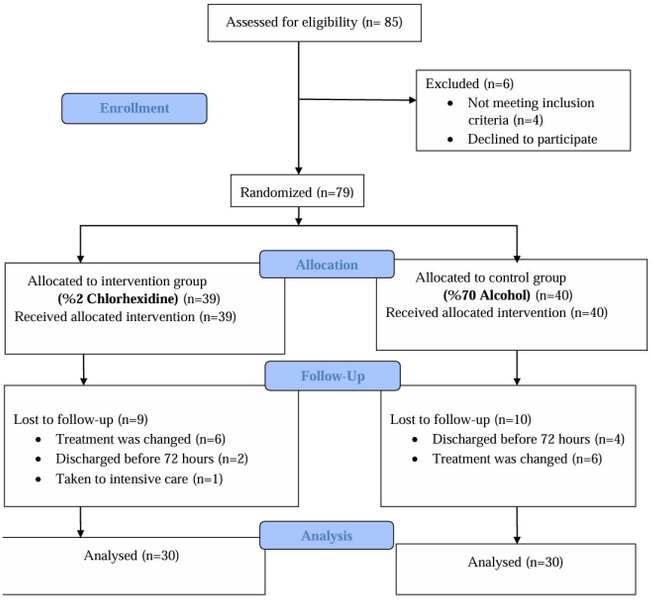

Figure 1. *Consort 2010 Flow Diagram fort the data collection procedure*

2.7. Validity and Reliability

Since the researcher was working in the same institution as a nurse, all PIVC initiatives and antiseptic applications were performed by the researcher to minimize errors that may result from differences in application. Another nurse who was on the infection control committee volunteered to observe the researcher's compliance with the PIVC implementation directive and determined the accuracy of the intervention. PIVC applications and data collection were performed at time points suitable to the participants, and all data were maintained in a confidential manner. In addition, the data were checked by both researchers to ensure accuracy before performing statistical analyses.

2.8. Instruments

2.8.1. Personal Information Form

Data on age, sex, body mass index (BMI), chronic disease, current medical diagnosis, substance or alcohol use, smoking, and PIVC application area were collected for both groups.

2.8.2. The INS Phlebitis Scale

The scale was developed by Gallant and Schultz and published by the INS (21). Psychometric properties of the scale were evaluated by Groll et al. (2010). The Phlebitis Scale has been recognized as a valid, clinically applicable, and reliable scale to determine when intravenous catheters should be removed (22).

2.9. Blinding of Research

Participants were randomly assigned to the intervention and control groups by a statistician other than the researcher. The groups were named Group I and Group II instead of the intervention and control groups. The researcher was blinded to the groups. To eliminate possible differences, the solutions were prepared only by the nurse responsible for the General Surgery-1 Clinic. Both solutions were placed in light-proof bottles of the same color and the same size. Group I (intervention group) was treated with 2% chlorhexidine (solution in blue-labeled bottle), and Group II (control group) was treated with 70% alcohol (solution in pink-labeled bottle). The solutions were indistinguishable in terms of color and smell. Thus, both researchers and participants were blinded to the solutions used.

After statistical analysis was performed, the researcher was informed by the clinical nurse that Group I was the intervention group treated with 2% chlorhexidine (blue-labeled solution), while Group II was the control group treated with 70% alcohol (pink-labeled solution).

2.10. Data Analysis

Statistical analysis was performed using SPSS package program (IBM SPSS Statistics 24). Independent samples t-test (t-table value) was used to compare the measurements between the two independent groups when parametric test conditions were met, and Mann–Whitney U test (Z-table value) was used otherwise. Fisher's exact test, continuity correction, or Pearson's χ^2 cross tables were used to examine the relationship between two qualitative variables. The significance level was accepted as $p<0.05$.

3. RESULTS

3.1. Patients and PIVC Characteristics

Thirtynine patients were assigned to the experimental group (2% chlorhexidine) and 40 patients to the control group (70% alcohol). 9 of 39 patients included in the control group were excluded from the application. Among the reasons for exclusion: Treatment changes were made in 6 patients, 2 patients were discharged before 72 hours, and 1 patient was transferred to a different clinic. In the control group; Among the 40 patients who accepted the application, a total of 10 patients were excluded from the application because 4 patients were discharged before 72 hours and the treatment of 6 patients was changed. A total of 30 patients in both groups were included in the application.

The mean age of the participants in the intervention group was 50.07 ± 10.82, and the mean age of the participants in the control group was 46.53 ± 12.57 years. It was noted that 56.7% of the participants were men and that the mean BMI was 25.03 ± 5.74 kg/m^2 (overweight). Substance abuse was observed in only one participant. Furthermore, 11.7% of the participants used alcohol and 30% were smokers. Moreover, 26.7% of the participants included in the study had a chronic disease. Of these participants, 37.5% had diabetes mellitus (DM) and hypertension simultaneously.

The most commonly used area for PIVC insertion was the forearm (61.7%). No statistically significant relationship was found between the intervention and control groups in terms of sex, BMI, substance abuse, alcohol use, smoking, chronic disease, or PIVC application area ($p > .05$). The groups were found to be independent and homogeneous in terms of the specified characteristics (Table 1).

3.2. Outcome Measures

After PIVC application, five patients (16.7%) in the intervention group that was treated with 2% chlorhexidine developed phlebitis, whereas eight patients (26.7%) in the control group that was treated with 70% alcohol developed phlebitis. No statistically significant difference was found between 2% chlorhexidine and 70% alcohol in preventing the development of phlebitis after PIVC application ($p > 0.05$). In line with this result, the H0 hypothesis was approved. The highest degree of phlebitis that developed in the intervention and control groups was 1st degree. Although phlebitis development occurred at all time intervals, it was most frequent between 32 and 40 hours (37.5%). There was also no statistically significant difference between the groups in terms of degree of phlebitis and phlebitis development time ($p >.05$) (Table 2).

Table 1. *Patient Characteristics*

Variable	Intervention group (2% chlorhexidine) (n = 30) n %		Control group (70% alcohol) (n = 30) n %		Total (N = 60) N %		Statistical analysis* Significance level
Mean Age	50.07±10.82		46.53±12.57		49.5±11.6		t=1,167 p=0.248
Sex							
Male	15	50.0	19	63.3	34	56.7	χ^2=0.611 p=0.434
Female	15	50.0	11	36.7	26	43,3	
BMI class (kg/m²)					2		χ^2=1.714 p=0.634
Underweight (<18.5)	1	3.3	1	3.3	28	3.3	
Normal (18.5-24.9)	12	40.0	16	53.3	16	46.7	
Overweight (25.0-29.9)	8	26.7	8	26.7	14	26.7	
Obese (≥30.0)	9	30.0	5	16.7		23.3	
Average BMI	26.80±5.32kg/m²		26.40±6.22kg/m²		25.03±5.74kg/m²		Z=-0,591 p=0.554
Substance abuse							
Yes	-	-	1	3.3	1	1.7	p=1.000
No	30	100.0	29	96.7	59	98.3	
Alcohol use							
Yes	3	10.0	4	13.3	7	11.7	p=1.000
No	27	90.0	26	86.7	53	88.3	
Smoking							
Yes	8	26.7	10	33.3	18	30.0	χ^2=0.079
No	22	73.3	20	66.7	42	70.0	p=0.778
Chronic disease							
Yes	9	30.0	7	23.3	16	26.7	χ^2=0.000
No	21	70.0	23	76.7	44	73.3	p=1.000
Name of the disease							
Diabetes mellitus	3	33.3	2	28.58	5	31.25	χ^2=5.623
Hypertension	-	-	2	28.58	2	12.5	p=0.229
DM+hypertension	5	55.6	1	14.28	6	37.5	
Hepatitis B	1	11.1	1	14.28	2	12.5	
Epilepsy	-	-	1	14.28	1	6.25	
Catheter area							
Back of the hand	5	16.7	7	23.3	12	20.0	χ^2=3.577
Wrist	6	20.0	3	10.0	9	15.0	p=0.311
Forearm	17	56.6	20	66.7	37	61.7	
Inside the elbow	2	6.7	-	-	2	3.3	

** "Pearson – χ2"; "Independent Samples t-test" (t-table value); "Mann–Whitney U test" (Z-table value)*

Table 2. *Phlebitis Development, Degree of Phlebitis, and Phlebitis Development Time in the Intervention and Control Groups*

Variable	Intervention group (2% chlorhexidine) (n = 30)		Control group (70% alcohol) (n = 30)		Statistical analysis* Significance level
	n	%	n	%	
Phlebitis development status					
Positive	5	16.7	8	26.7	χ^2=0.393
Negative	25	83.3	22	73.3	p=0.531
Degree of Phlebitis					
1	3	60.0	5	62.5	χ2=0.008
2	2	40.0	3	37.5	p=0.928
Phlebitis development time					
8–16 hours	1	20.0	-	-	χ^2=2.790
32–40 hours	1	20.0	3	37.5	p=0.732
40–48 hours	1	20.0	1	12.5	
48–56 hours	-	-	1	12.5	
56–64 hours	1	20.0	1	12.5	
64–72 hours	1	20.0	2	25.0	

** "Pearson-χ2"*

4. DISCUSSION

This study was conducted to highlight the importance of skin antisepsis in preventing phlebitis, which causes tissue damage as a result of inflammation in the vein's intima layer. It was done to compare the effectiveness of two different solutions. The findings were discussed in line with the relevant literature. In the present study, both antiseptics used in the groups were similarly effective in preventing the development of phlebitis. Similar to our results, there are studies in the literature reporting no difference between the use of 2% chlorhexidine and 70% alcohol (18,23), among 70% alcohol, 2% chlorhexidine, and povidone-iodine (19), between 2.5% chlorhexidine containing 70% alcohol and 70% alcohol (20), and between 2% chlorhexidine and 2% nitroglycerin (24) as antiseptics for PIVC application in terms of preventing the development of phlebitis.

When other studies in the literatüre similar to our study were examined, phlebitis development was observed in 36.7% of the patients in the chlorhexidine group and 53.3% of the patients in the alcohol group, and no significant difference was found in terms of phlebitis development between the groups (18). In another study investigating the effects of alcohol, chlorhexidine and povidone–iodine on preventing phlebitis, no significiant difference was found between the groups (19). Kaur et al. (2012) examined the effect of chlorhexidine and alcohol on phlebitis. Although fewer patients developed in the chlorhexidine group, no statistically significant difference was found between the groups in line with our study (20).

In order to use 2% chlorhexidine containing 70% alcohol solution more reliably, there are studies on whether solutions with lower concentrations are reliable enough (25,26). In a study, the effectiveness of 2% chlorhexidine containing 70% alcohol and 0,5% chlorhexidine containing 70% alcohol on staphylococcus epidermis biofilm formation was investigated in witro, and it was reported that the 2% chlorhexidine solution had a higher efficiency (27).

There is also study reporting less phlebitis development in patients treated with 2% chlorhexidine when compared with those treated with 70% alcohol (14). Furthermore, 2% chlorhexidine solution containing 70% alcohol has been reported in a study to be more effective in preventing phlebitis compared with 70% alcohol and chlorhexidine only (15).

Another study has observed less phlebitis development in patients treated with 70% alcohol–5% chlorhexidine solution compared with patients treated with 70% alcohol solution (16).

In the study in which Maki et al. (2014) looked at its effectiveness in antisepsis, they reported that a 2% aqueous chlorhexidine solution was statistically superior to 70% isopropyl alcohol or 10% povidone-iodine for the prevention of catheter-related bloodstream infections with catheters (28).

In the present study, phlebitis development was most frequent in the time period of 32–40 hours. In the study conducted, phlebitis development was most frequently observed between 40 and 49 hours (6). In another study, it was reported that the incidence of phlebitis was highest within the first 48 hours; this rate decreased between 48 and 96 hours and was the lowest between 96 and 120 hours (29,30). In the present study, only 1st degree and 2nd degree phlebitis development was observed in the patients. These results emphasize the importance of monitoring phlebitis development every 8 hours in line with the CDC report. Furthermore, the findings highlight that chemical and mechanical variables that can cause phlebitis in the early hours as well as patient groups with comorbid diseases should be examined separately for phlebitis development.

This study was conducted during the COVID-19 pandemic. Therefore, hospitalization of urgent and complicated cases was prioritized in clinics, which posed difficulties in reaching a greater number of participants meeting the inclusion criteria. At the same time, the study will be particularly relevant for low – and middle-income countries where chlorhexidine skin preparation may not always be available. The results of the study are limited to the patients who were given antibiotics and/or analgesics containing the same active substance in drug therapy (antibiotics whose active substance is ceftriaxone and ornidazole and analgesics whose active ingredient is paracetamol).

5. CONCLUSIONS

According to the results of this study, It was found that 2% chlorhexidine and 70% alcohol used for skin antisepsis before PIVC application were not superior to each other in terms of preventing the development of phlebitis. The results of this study will help reduce the dilemma of nurses and other healthcare professionals in choosing the type of antiseptic to avoid phlebitis development and also assist healthcare institutions in selecting antiseptics from a cost-effectiveness perspective. Future studies should be conducted with different antiseptics and larger samples, evaluating different time intervals with more groups. In addition, further studies that examine patient groups with different diagnoses and receiving different treatments, together with individual variables, should be planned.

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Author Contributions:
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Design of the study: AK, ZE
Acquisition of data for the study: AK
Analysis of data for the study: AK, ZE
Interpretation of data for the study: AK, ZE
Drafting the manuscript: AK, ZE
Revising it critically for important intellectual content: ZE
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Clinical and Experimental Health Sciences

Evaluation of Food Security Status and Mediterranean Diet Adherence of Air Services Employees in İstanbul, Türkiye

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ABSTRACT

Objective: Since airports operate in foreign currency and are far from cities, employees could be limited to purchase food and beverages from more expensive sales points. Additionally, air services employees may be at risk for poor nutritional status due to job-specific unhealthy dietary habits and lifestyle behaviors. Yet, air services as workplaces have been understudied in terms of nutritional environment. Hence, in this study the aim is to assess food security (FS) and Mediterranean diet adherence (MDA) among Atatürk and Istanbul airports' employees.

Method: The face-to-face cross-sectional study was conducted with a non-probability sample of 381 employees that were recruited between February and May 2022. FS was assessed with Food Insecurity Experience Scale and MDA with Mediterranean Diet Adherence Screener (MEDAS). Regression analyses were conducted to analyze the independent variables affecting FS.

Results: MDA was found to be 7.6±2.07 and most of the participants were within a moderate adherence range. While 14.6% (n: 55) of the participants have moderate or severe food insecurity, 2.9% (n: 11) have severe food insecurity. The regression analyses revealed the factors affecting food insecurity as education, income, smoking, use of medication, and dieting status ($p<.05$). On the other hand, MDA was not found to be an effective factor of food security status of air services employees.

Conclusion: Ultimately, addressing food insecurity among airport staff will require systemic changes to ensure that all workers are paid fair wages and have access to essential benefits like affordable food and supportive food environment that encourages healthy eating.
Keywords: Food security, Mediterranean diet, workplace nutrition, airport employees, Türkiye

1. INTRODUCTION

Proper workplace nutrition is necessary for maintaining good health and productivity (1). Air services as workplaces has been understudied in terms of nutritional environment. Hence, there is limited research that is specifically focused on the nutritional status of airport and aircrew employees. Considerable sections of the airports operate in duty-free areas that sell foods and beverages significantly more expensive than the average national prices (2). Also, since the airports are mainly located in the outskirts of the cities, airport employees need to purchase these more expensive foods and beverages. Additionally, one of the sectors most affected by COVID-19 has been the airlines and their employees due to the restricted number of flights between the years of 2020 and 2022 (3).

Even before the onset of COVID-19 pandemic, many airport workers, such as baggage handlers, janitors, and food service workers were shown to earn wages that were near or below the poverty line (4). Furthermore, some airport workers may not have access to affordable healthcare or other benefits, which can contribute to more financial strain. Even in the European Union region, aircrew employment and work conditions, including pilots and cabin crew, were reported to deteriorate because of intense competition and the pressure to reduce costs. Thus, it is possible for some airport employees to experience food insecurity problems at varying degrees (5). Food insecurity, uncertain access to adequate food owing to resource limitations, found to be linked with adverse health outcomes including poor nutrition, chronic diseases, worsened depressive symptoms, and increased healthcare expenditure (6).

In addition to the aforementioned adverse employment conditions, people who work at the airports have an increased exposure to noise, vibration in addition to possible disruptions with their circadian rhythm due to the nature of shift-based work (7). Moreover, aircrew personnel have increased electromagnetic field and cosmic radiation exposure in flights, which has been increasingly shown to be associated with elevated rates of cancer (8, 9). Therefore, consuming a nutrient-dense diet as part of healthier lifestyle patterns could help to mitigate these adverse conditions.

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Particularly a diet that is rich in fruits and vegetables, whole grains, seafood; yet low in simple carbohydrates, processed meats, and fried foods has been recommended by food-based dietary guidelines (10). One widely acknowledged such dietary pattern is the Mediterranean Diet (MedDiet) that encompass both nutritional and cultural features that even UNESCO considers as an intangible cultural heritage (11, 12). However, longitudinal and cross-sectional studies recorded a shift toward the adoption of diets higher in fat and refined carbohydrates around the Mediterranean Sea countries including Türkiye, Lebanon, Albania, Spain, Portugal long before the economic crises (13). Since adhering to MedDiet has been shown to be expensive, the abrupt rise in food insecurity and financial constraints may put additional constraints among populations around the Mediterranean basin. Coexistence of economic downturn and shift from the traditional healthy dietary patterns has been instrumental in increased obesogenic food environment. Food insecure households and individuals were shown to sacrifice food quality and dietary variety in favor of quantity and unhealthful satiation practices (14). For MedDiet in particular, socioeconomic inequalities have been reported unanimously in the literature as a reason for poorer adherence (15-17).

Air services employees may also be at risk for poor nutritional status due to unhealthy dietary habits and lifestyle behaviors that are related to their job tenure (7). Hence, more research is needed to better understand the nutritional status of this population and develop interventions to promote healthy eating and lifestyle habits among airport employees. To address this gap in the literature, using data collected from Atatürk and Istanbul airports' employees, the occupational dietary and lifestyle determinants of food insecurity were aimed to be examined among airport workers in Istanbul, Türkiye. The study further explored the relationship between adherence to Mediterranean diet and food security with the scope of ameliorating workplace nutrition.

2. METHODS

2.1. Research Design and Setting

The current cross-sectional study is conducted face to face with air service employees that are working in Istanbul and Atatürk airports, both located in Istanbul, Türkiye. The inclusion criteria for the consenting respondents were to be over the age of 18 and being actively employed in either ground operations or as part of an aircrew. The estimated sample size of this study was determined by taking 40,000 people working at Istanbul Airport and 2,000 employees working at Atatürk Airport into account. The information on the total number of employees was received from the airport civil administration of each airport separately. Thus, the cumulative number of employees was determined to be 42,000 people. The sample was calculated as 381 participants with a 95% confidence level.

The present study was first approved by the Istanbul Aydin University Non-Interventional Clinical Research Ethics Committee (Decision Number: B.30.2. AYD.0.00.00-050.06.04/30). Then administrative permits were obtained from both airports' civil administration departments. The data collection took place between February and May 2022.

2.2. Characteristics of the Survey

The first set of questions were related to socio-demographic characteristics of the participants including date of birth to precisely calculate age, then sex, educational status in terms of the last degree obtained and the total number of years studied, marital status, employment characteristics, income level as it was inquired in Turkey Nutrition and Health Survey (TNHS) 2017 (18), presence of chronic disease were inquired. The participants self-reported their weight (kg) and height (cm), and the researchers calculated the body mass index (BMI) by dividing body weight by height squared (kg/m^2) (19). Then, whether participants were being on a diet was inquired with the questions from TNHS 2017. Smoking, exercise, and alcohol use were inquired with the questions that were used in Turkey Demographic and Health Survey 2013 with following response options: i. No, ii. Regularly, iii. Irregularly (20). Following, participants' adherence to Mediterranean Diet (MedDiet) is assessed with Mediterranean Diet Adherence Screener (MEDAS). MEDAS was developed by Martínez-González et al. (21) and has been shown to be a reliable and valid tool for assessing MedDiet adherence in various populations including Türkiye. MEDAS was adapted to Turkish by Özkan Pehlivanoğlu et al. (22) and it consists of fourteen questions that assess the consumption of selected foods with Yes and No response options. The scores obtained from these questions are used to determine the level of adherence to the Mediterranean diet. Finally, food security status was evaluated by using Food Insecurity Experience Scale (FIES) that was developed by the Food and Agriculture Organization (FAO) in 2013 and also used in TNHS 2017. FIES is a self-administered questionnaire that consists of eight questions. It is used to evaluate an individual's or household's experience and behavior regarding food accessibility. FIES uses an experience-based metric that captures the access dimension of food security. FIES metric is calculated from participants' direct responses to questions regarding their access to adequate quality and quantity of food (23).

2.3. Statistical Analysis

Continuous data were presented as means ± SD, mean (min, max) and categorical variables were shown as percentages. Normality was assessed using Shapiro-Wilk and Kolmogorov tests. The chi-square test was used to compare categorical data between groups, and the Bonferroni-corrected z-test was used to examine multiple comparisons of proportions. The Mann-Whitney U test was used to compare binary data for non-normally distributed variables, and the independent two-sample t-test was used to compare normally distributed variables.

Table 1. Descriptive statistics of the study participants

	Frequency (n)	Percentage (%)
Sex		
Man	168	44.1
Woman	213	55.9
Education status		
Primary school	2	0.5
Middle-school	2	0.5
High-school	11	2.9
University degree	317	83.2
Graduate degree	49	12.9
Marital status		
Married	217	57
Unmarried	164	43
Employment category		
Government official	96	25.2
Medical staff	8	2.1
Flying staff	73	19.2
Field staff	52	13.6
Ground operations personnel	48	12.6
Internal preparation personnel	6	1.6
Director	18	4.7
Office staff	62	16.3
Running their own business	16	4.2
Carrying personnel	2	0.5
Monthly Income status		
Comfortably sufficient income for the monthly expenses	137	36
Sufficient income for the monthly expenses	158	41.5
Barely sufficient income for the monthly expenses	68	17.8
Insufficient income for the monthly expenses	18	4.7
Presence of chronic disease		
Yes	18	4.8
No	359	95.2
Use of medications		
Yes	25	6.6
No	355	93.4
Smoking status		
No	201	52.8
Regular	119	31.2
Irregular	61	16
Use of alcohol		
No	125	33.2
Regular	31	8.2
Irregular	221	58.6
Dieting status for weight loss		
No, I am on an ideal weight	204	53.7
No, but I need to lose weight	131	34.5
No, but I need to gain weight	18	4.7
Yes	27	7.1
Vegetarianism		
Yes	8	2.1
No	373	97.9

The psychometric assessment of FIES data is done with the item response theory measurement model, which is the single-parameter logistic measurement model commonly known as the Rasch Model. For the Rasch reliability test, reliability scores ≥ 0.7 was accepted as reasonably good overall model fit. Conditional independence and dimensionality were assessed via the items' residual correlations. Correlations ≥ 0.4 between pair of items indicated that the responses to the items were not independent of each other (24). Regression analyses were performed for logistic and linear regression for assessing the determinants of food security. Linear regression analysis was used to analyze independent variables affecting food security scores. Binary logistic regression analysis was used to examine risk factors for moderate or severe food insecurity. Statistical significance was evaluated as $p < .05$ for all the tests. The data were analyzed using IBM SPSS 20.0 for Windows (SPSS, Chicago, IL, USA) and through the use of R (version 3.2.3; R Foundation, Vienna, Austria).

Table 2. Participants' MD[1] adherence comparison by sex

	Total (n=381)	Women (n=213)	Men (n=168)	p-value
MEDAS[2] (mean ± SD)	7.6 ± 2.07	7.6 ± 1.97	7.6 ± 2.19	.994
MEDAS (median (min, max))	7.0 (2,13)	7.0 (2,13)	8.0 (2,13)	
MEDAS Categories (%, N)				
Low (<7)	24.7, 94	21.1, 45	29.2, 49	
Moderate (7-9)	44.1, 168	50.7, 108	35.7, 60	**.013**
High (≥9)	31.2, 119	28.2, 60	35.1, 59	

[1]MD: Mediterranean Diet, [2]MEDAS: Mediterranean Diet Adherence Screener

3. RESULTS

Out of 381 participants, there were 213 (55.9%) women. The mean age of the participants was 34.64±8.78, where the minimum participant age was 20.0, and the maximum value was 64.00 years. 83.2% of the participants have at least a university degree. The average education period was 15.67±2.86 years with a range of 2 to 22 years. 57% of the participants were found to be married. With respect to occupational status, most of the participants (68.8%) were working for private entities and 80.8% were working in the ground operations including administrative workers, food service operators, field personnel, medical staff, cleaning personnel, etc. Regarding income status as categorized according to the TNHS-2017, 36% of the participants were found to live comfortably with their income, 41.5% of the participants were categorized as having incomes that were deemed sufficient to not experience any serious problems, and the rest of the 22.5% of the participants had either barely sufficient income or insufficient income to spend the rest of the month. An overwhelming majority of the participants did not report presence of disease (95.2%) and the mean BMI was found to be 25.09±5.11 (Table 1).

Table 3. Questions of the Food Insecurity Experience Scale (FIES) along with item fit statistics

Questions	Label	Affirmative Responses (n, %)	Severity±SE[1]	Infit[2]	Outfit[3]
Q1. You were worried you would not have enough food to eat because of a lack of money or other resources?	WORRIED	58 (15.2%)	-0.77±0.25	1.11	1.17
Q2. You were unable to eat healthy and nutritious food because of a lack of money or other resources?	HEALTHY	62 (16.3%)	-0.99±0.25	1.03	1.09
Q3. You ate only a few kinds of foods because of a lack of money or other resources?	FEWFOOD	60 (15.8%)	-0.88±0.25	1.03	1.03
Q4. You had to skip a meal because there was not enough money or other resources to get food?	SKIPPED	50 (13.1%)	-0.35±0.25	0.88	0.77
Q5. You ate less than you thought you should because of a lack of money or other resources?	ATELESS	53 (13.9%)	-0.51±0.25	0.67	0.52
Q6. Your household ran out of food because of a lack of money or other resources?	RANOUT	30 (7.9%)	0.75±0.26	0.87	0.95
Q7. You were hungry but did not eat because there was not enough money or other resources for food?	HUNGRY	31 (8.1%)	0.69±0.26	0.96	1.13
Q8. You went without eating for a whole day because of a lack of money or other resources?	WHLDAY	12 (3.2%)	2.05±0.34	1.27	4.19

Rasch reliability was 0.71. [1:] Severity parameter of the FIES items indicates the severity of food insecurity associated with each raw score. The calibrations were estimated on a logit scale (with equal discrimination = 1), mean set to 0, and SD of 1. [2:] Infit, item-infit mean square statistic. [3:] Outfit, item-outfit mean square statistic.

Table 4. *Food insecurity items and item fit statistics after the WHLDAY item is removed*

Label	Affirmative Responses (n, %)	Severity±SE[1]	Infit[2]	Outfit[3]	Probability of moderate or severe FI by raw score	Probability of severe FI by raw score
WORRIED	53	-1.96±1.10	1.15	1.30	0.033	0.000
HEALTHY	57	-1.03±0.87	1.03	1.08	0.102	0.000
FEWFOOD	55	-0.34±0.81	1.06	1.11	0.303	0.001
SKIPPED	45	0.30±0.81	0.94	0.89	0.612	0.007
ATELESS	48	1.02±0.89	0.69	0.57	0.856	0.075
RANOUT	25	1.98±1.12	0.94	1.09	0.956	0.391
HUNGRY	24	3.09±1.48	1.12	1.35	0.979	0.703
Threshold Value[4]					0.07	2.29

Rasch reliability was 0.65. [1:] Severity parameter of the FIES items indicates the severity of food insecurity associated with each raw score. The calibrations were estimated on a logit scale (with equal discrimination = 1), mean set to 0, and SD of 1.[2:] Infit, item-infit mean square statistic.[3:] Outfit, item-outfit mean square statistic.[4:] Adjusted thresholds of food insecurity on the latent trait

MedDiet adherence is presented in Table 2. The mean adherence to MedDiet was found to be 7.6 ± 2.07 and a median score of 7.0 (2, 13) as assessed by MEDAS. Although no significant difference was found between the scores of women and men, a significant difference in the distribution of men and women among the MEDAS categories was observed, with men showing a slightly higher adherence to MedDiet (p =.013). A relatively low percentage of the study participants (31.2%) were classified as having a high adherence to MedDiet, with most of the participants falling within a moderate adherence (44.1%) and 24.7% having a low adherence.

The infit statistics of FIES items were within the acceptable range of 0.67 to 1.27 (Table 3). This condition indicates that the items met the equal discrimination assumption of the model. The highest infit was found for “whlday” as an item that may need to be improved. Consistently, the outfit value of “whlday” was also ≥2.0. The outfit statistics of FIES items were within 0.52 to 4.19. Other than the outfit value of “whlday”, all the other values were in the acceptable range. For the eight-item FIES scale, a Rasch reliability value of is found as 0.71 and since it was higher than 0.7, this value was considered acceptable (25). Despite the suitable coefficient values of the residual correlations of each item, which must be <0.4, the whole Rasch analysis was repeated after removing “whlday” after consulting with an expert statistician. Finally, the overall model fit using the Rasch reliability assessment was found to be 0.65. This value was deemed acceptable as it needed to be >0.6 for a 7-item instrument (25). Thus, the necessary compliance criteria were met after the “whlday” item was removed. Also, there was no significant residual correlation between the items. Table 4 provides the infit and outfit statistics of the 7-item FIES along with the probabilities of moderate and severe food insecurity by raw scores. As a

result of the Rasch model, the adjusted thresholds of food insecurity on the latent trait were found to be 0.07 for moderate or severe and 2.29 for severe food insecurity. While 14.6% (n: 55) of the participants have moderate or severe food insecurity, 2.9% (n: 11) have severe food insecurity.

Considering individual-level food insecurity score as the dependent variable, a multiple linear regression model was developed to explain its relationship between Mediterranean Diet adherence after adjusting for age, sex, education duration, marital status, income level, presence of chronic disease, BMI, smoking, alcohol intake, vegetarianism status, use of medication, and dieting status (Table 5). The regression model was found to be statistically significant (F=7.360; p<.001) and it explained 24.8% of the food insecurity with the independent variables. Analysis of the food insecurity determinants showed total duration of education as a statistically significant determinant (p=.007). As the years of education increased, the food insecurity score decreased by 0.073 points.

Table 5. *Multiple linear regression analysis results determining the food insecurity score*

	β_0 (%95 CI)	SE	β_1	t	p	r^1	r^2	VIF[a]
(Constant)	0.087 (-1.516 – 1.691)	0.815		0.107	.915			
Sex (Reference: Woman)	0.116 (-0.163 – 0.394)	0.142	0.038	0.816	.415	0.079	0.044	1.046
Age	-0.01 (-0.029 – 0.008)	0.009	-0.059	-1.092	.276	-0.039	-0.058	1.407
Total duration of education	-0.073 (-0.125 – -0.02)	0.027	-0.135	-2.736	**.007**	-0.234	-0.145	1.185
Marital status (Reference: Married)	0.089 (-0.221 – 0.4)	0.158	0.029	0.566	.572	0.142	0.030	1.289
Income status (Reference: Insufficient income for the monthly expenses)								
Comfortably sufficient income for the monthly expenses	-1.527 (-2.3 – -0.755)	0.393	-0.483	-3.888	**<.001**	-0.060	-0.204	7.524
Sufficient income for the monthly expenses	-1.713 (-2.482 – -0.944)	0.391	-0.556	-4.380	**<.001**	-0.249	-0.229	7.862
Barely sufficient income for the monthly expenses	-0.605 (-1.402 – 0.192)	0.405	-0.154	-1.493	.136	0.317	-0.080	5.170
Presence of chronic disease (Reference:No)	-0.324 (-1.22 – 0.572)	0.455	-0.045	-0.712	.477	0.107	-0.038	1.931
BMI (kg/m^2)	-0.023 (-0.053 – 0.007)	0.015	-0.078	-1.527	.128	-0.050	-0.082	1.265
Smoking (Reference: No)								
Regular	0.371 (0.031 – 0.71)	0.173	0.113	2.149	**.032**	0.112	0.114	1.352
Irregular	0.617 (0.195 – 1.04)	0.215	0.150	2.874	**.004**	0.158	0.152	1.330
Alcohol (Reference: No)								
Regular	0.055 (-0.566 – 0.676)	0.316	0.009	0.175	.861	0.006	0.009	1.432
Irregular	0.397 (0.072 – 0.722)	0.165	0.128	2.403	**.017**	0.042	0.128	1.392
Vegetarianism (Reference: No)	0.498 (-0.511 – 1.506)	0.513	0.048	0.970	.333	0.040	0.052	1.183
Use of medications (Reference: No)	1.314 (0.524 – 2.105)	0.402	0.205	3.270	**.001**	0.185	0.173	1.919
Dieting status for weight loss (Reference: No, I am on an ideal weight)								
No, but I need to lose weight	0.102 (-0.237 – 0.442)	0.173	0.032	0.591	.555	-0.014	0.032	1.423
No, but I need to gain weight	0.664 (-0.044 – 1.372)	0.360	0.092	1.845	.066	0.125	0.098	1.207
Yes	0.798 (0.2 – 1.397)	0.304	0.137	2.623	**.009**	0.220	0.139	1.331
MEDAS[b] score	0.039 (-0.032 – 0.11)	0.036	0.053	1.072	.284	0.095	0.057	1.213

$F=7,360$; $p<.001$; $R^2=\%28,7$, Adjusted $R^2=\%24,8$, β^0: Non-standard beta coefficient; SE: Standard Error; β^1: Standard beta coefficient; r^1: Zero-order correlation; r^2: Partial correlation, [a]VIF: Variance Inflation Factor, [b]MEDAS: Mediterranean Diet Adherence Screener

When participants with insufficient income for the monthly expenses are taken as reference, both sufficient income category participants have a significantly lower food insecurity status (p<.001). The food insecurity score of regular smokers was 0.371 points higher than non-smokers (p=.032) and the food insecurity score of irregular smokers was 0.617 points higher than non-smokers (p=.004). The food insecurity score of irregular alcohol users was 0.397 points higher than non-drinkers (p=.017). The scores of those who use medication are 1.314 units higher than those who do not use them (p=.001). Current dieters for weight loss have 0.798 units higher food insecurity scores compared to non-dieters who are on their ideal weight significantly higher (p=.009). Other variables were not found to be statistically significant.

The factors affecting moderate or severe food insecurity were analyzed with binary logistic regression analysis as univariate and multivariate models (Table 6). In the univariate model, the risk of moderate or severe food insecurity decreases as the total education duration increases (OR=0.815; p<.001). In multivariate analysis, this ratio was 0.854 units (p=.008). In the univariate model, the risk of moderate or severe food insecurity was 2.039 times higher in singles than in married people (p=.016). Yet, the model was not found to be significant in the multivariate model (p=.796). When participants with

insufficient income for the monthly expenses are taken as reference, those that have a "comfortably sufficient income" and "sufficient income" for the monthly expenses have lower risk of moderate or severe food insecurity (OR=0.114, OR=0.054; p<.001, p<.001). In the multivariate model, these ratios were obtained as 0.048 and 0.031 (p<.001). Such an association was significant in the univariate model for smoking status. When non-smokers are taken as a reference, the risk of moderate or severe food insecurity was 2.247 times higher for regular smokers and 3.615 times higher for irregular smokers (p values .019 and .001, respectively). In the multivariate model, irregular smokers were 3.23 times more likely to have the risk of moderate or severe food insecurity. The risk of moderate or severe food insecurity was 3.571 times higher among participants who take regular medication (p=.006) while this value was 10,326 the in multivariate analysis (p=.010). Compared to non-dieters who are on their ideal weight as a reference, the risk of food insecurity is 3.704 times higher in those who need to gain weight and 7,705 times higher in those who need to lose weight. In the multivariate model, the risk of food insecurity is 5,316 times higher in those who need to lose weight. No statistically significant effect of other variables was observed (p>.050).

Table 6. *Logistic regression analysis results determining the factors affecting moderate or severe food insecurity*

	Univariate		Multivariate	
	OR[1] (%95 CI[2])	p	OR (%95 CI)	p
Sex (Reference: Woman)	1.157 (0.652 – 2.053)	.618	1.07 (0.517 – 2.216)	.855
Age	0.995 (0.962 – 1.029)	.772	0.975 (0.93 – 1.023)	.305
Total duration of education	0.815 (0.75 – 0.885)	**<.001**	0.854 (0.76 – 0.96)	**.008**
Marital status (Reference: Married)	2.039 (1.142 – 3.642)	**.016**	0.899 (0.4 – 2.019)	.796
Income status (Reference: Insufficient income for the monthly expenses)				
Comfortably sufficient income for the monthly expenses	0.114 (0.037 – 0.351)	**<.001**	0.048 (0.01 – 0.234)	**<.001**
Sufficient income for the monthly expenses	0.054 (0.016 – 0.18)	**<.001**	0.031 (0.006 – 0.16)	**<.001**
Barely sufficient income for the monthly expenses	0.581 (0.194 – 1.741)	.333	0.25 (0.058 – 1.083)	.064
Presence of chronic disease (Reference:No)	2.484 (0.848 – 7.28)	.097	0.331 (0.041 – 2.681)	.300
BMI[3] (kg/m^2)	0.977 (0.921 – 1.036)	.439	0.936 (0.86 – 1.018)	.122
Smoking (Reference: No)				
Regular	2.247 (1.142 – 4.421)	**.019**	1.486 (0.574 – 3.849)	.415
Irregular	3.615 (1.711 – 7.639)	**.001**	3.231 (1.147 – 9.1)	**.026**
Alcohol (Reference: No)				
Regular	0.771 (0.209 – 2.852)	.697	1.397 (0.283 – 6.898)	.682
Irregular	1.362 (0.711 – 2.609)	.351	2.283 (0.882 – 5.913)	.089
Vegetarianism (Reference: No)	1.994 (0.392 – 10.14)	.406	4.134 (0.561 – 30.487)	.164
Use of medications (Reference: No)	3.571 (1.434 – 8.892)	**.006**	10.326 (1.736 – 61.438)	**.010**
Dieting status for weight loss (Reference: No, I am on an ideal weight)				
No, but I need to lose weight	1.649 (0.837 – 3.248)	.149	1.399 (0.569 – 3.442)	.465
No, but I need to gain weight	3.704 (1.191 – 11.519)	**.024**	2.393 (0.552 – 10.364)	.243
Yes	7.705 (3.151 – 18.84)	**<.001**	5.316 (1.444 – 19.576)	**.012**
MEDAS[4] score	1.121 (0.976 – 1.288)	.105	1.033 (0.875 – 1.221)	.701

[1]OR: Odds ratio; [2]CI: Confidence interval; [3]BMI: Body mass index; [4]MEDAS: Mediterranean Diet Adherence Screener

4. DISCUSSION

The limited availability of data regarding food security status among air services employees, as well as the lack of information on adherence to the Mediterranean diet assessment, underscores the relevance of this study. Since adherence to healthy dietary patterns has gained significant attention in the field of public health nutrition, this study assessed food security and MedDiet adherence among employees of Ataturk and Istanbul airports, recognizing the unique challenges faced by this occupational group in maintaining healthy eating habits. Findings obtained from the FIES assessment indicated that the majority of the employees demonstrated average food security levels. MedDiet adherence assessed with MEDAS in the sample of 381 individuals showed a mean score of 7.6±2.07. Apart from this, the mean BMI was found to be 25.09±5.11 and more than half of the participants deem themselves to be on their ideal weight.

In the most recent Turkey Nutrition and Health Survey (TNHS-2017), despite use of FIES, the data were not analyzed according to the FAO guidance and solely the descriptive results of each item were reported. Thus, the precise

country-specific food insecurity prevalence is not known, however the frequency of individuals who were worried that they would not be able to find enough food was 23.4%, and 22.7% of the participants were not able to consume healthy and nutritious food in TNHS. Moreover, while 13.1% of the individuals had to skip meals due to insufficient funds, 8.4% of the population had the experience of not being able to eat despite being hungry. Although an accurate comparison could not be made between TNHS sample and the current study participants due to methodological shortcomings, food security status appears to be above the national levels for the air services employees (18). Nonetheless, MEDAS scores of the present study participants appear to be similar and even slightly higher than similar studies that were conducted among Turkish adults. In a study that was conducted with 1053 healthy young adults from Türkiye (mean age of 28.77±11.62 years), the mean MEDAS score was found as 7.27 (26). Secondly, in a study that investigated the eating habits of 3294 adults in Türkiye during the COVID-19 quarantine, the mean MEDAS score of the participants were found as 7.0±2.37 (27). However, the mean MEDAS value was lower in another study of 256 people with 6.15±2.16 points (28). Similarly, in another study that investigated the validation of MEDAS among a sample of young Turkish adults (mean age of 31.7±10.97 years), mean total MEDAS score was found as 6.05±2.11 points (29). As is seen from multiple recent studies that were conducted in Türkiye, MedDiet adherence ranges from low to moderate. Although our study participants are employed in a workplace with expensive foods and beverages, free provision of at least one meal a day for the employees might have resulted in a moderate to high MedDiet adherence for more than 75% of the participants. The availability of free meals to the employees could explain the higher MEDAS scores of some participants with poorer food security levels. Since the structured meal provision to air services employees are mostly arranged by institutional dietitians, the menu planning is likely to be achieved with meals that are in line with dietary guidelines (30). However, despite the provision of lunches by dietitians, less than one third of the participants were found to have high MedDiet adherence. This indicates that there is room for improvement in promoting better adherence to the Mediterranean diet among air services employees. Nevertheless, individual preferences, cultural factors, lack of awareness about MedDiet's health benefits or challenges in implementing dietary changes in the workplace could be other possible reasons for the relatively low overall MEDAS scores. However, it is also crucial to note that a reduced level of adherence to the MedDiet does not inherently imply a diminished quality of the overall dietary pattern. To give an example, in a study conducted among young adults, compared to Lebanese peers, German students had lower MEDAS scores, yet their diet quality scores were significantly higher irrespective of their food security levels (31).

The relationship between food insecurity and MedDiet adherence at workplace could be complex and multifaceted and is influenced by various individual, environmental, and contextual factors (13). While there may not be a direct cause-and-effect relationship between food insecurity and MedDiet adherence, several factors can influence how food insecurity affects an individual's ability to adhere to the MedDiet at workplace (32). Food insecurity limits an individual's access to a variety of fresh, nutritious, and culturally appropriate foods that are essential for MedDiet (14). Secondly, food insecurity-associated stress could influence people's food choices and dietary behaviors. These conditions may lead to a greater reliance on cheaper, energy dense ultra-processed food choices that are not in line with MedDiet principles (33). The availability of supportive workplace resources and initiatives can play a role in mitigating the impact of food insecurity on MedDiet adherence. Workplace interventions, such as free or subsidized healthy meal options, educational programs, on budget-friendly MedDiet alternatives, or employee assistance programs can provide valuable support to employees facing food insecurity and help them make healthier food choices (34). While food insecurity poses challenges to adhering MedDiet, supportive workplace strategies and interventions can help mitigate some of these challenges and promote healthier eating habits among employees, regardless of their food security status.

The potential implications of the findings for the health and wellbeing of airport employees could be manyfold since inadequate food security and suboptimal adherence to MedDiet could hinder their physical and mental health, as well as their work performance. Further strategies and interventions could include targeted educational programs, workplace initiatives, or collaborations with air services companies and dietitians to enhance the availability and accessibility of nutritious and Mediterranean-style meals as part of a sustainable dietary pattern.

Although the cross-sectional design of the study precludes establishing a cause-and-effect relationship along with self-reported anthropometric variables, the study has several strengths. Firstly, the research novelty is present in assessing food insecurity and its relationship with MedDiet adherence among air services employees. Secondly, carrying out FIES data analysis in line with FAO recommendations would hopefully take food insecurity research further in Türkiye. Last, but not least assessing MedDiet adherence with MEDAS, which has repeatedly been demonstrated as an internationally valid and reliable scale enhances the robustness of the findings. Future research should investigate the specific barriers and facilitators of MedDiet adherence among airport employees, as well as the long-term effects of improved food security and dietary habits on their health outcomes.

5. CONCLUSION

By examining the interplay between food security and adherence to Mediterranean diet, this study provided insights into the nutritional status and potential areas for improvement among airport employees. Despite its cross-sectional design, by employing robust tools we have evaluated the food insecurity status and its relationship

with Mediterranean diet among an understudied group of workers. Understanding the food security and dietary habits of this population can inform the development of targeted interventions and workplace initiatives to promote better health outcomes and quality of life among airport personnel. Ultimately, addressing food insecurity among airport staff will require systemic changes to ensure that all workers are paid fair wages and have access to essential benefits like affordable food and supportive food environment that encourages healthy eating.

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Clinical and Experimental Health Sciences

Assessing Oral Cancer Awareness Among Dental Students in Van Province, Turkey

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ABSTRACT

Objective: The aim of this study was to assess and compare oral cancer awareness among 3rd year and 5th year undergraduate dental students in Faculty of Dentistry, Van Yüzüncü Yıl University.

Method: A validated questionnaire testing oral cancer awareness was distributed to third and fifth year dental students at Faculty of Dentistry, Van Yüzüncü Yıl University. A total of 140 students participated in this survey. Knowledge of oral cancer risk factors and diagnostic procedures, dental students' attitudes towards oral cancers, management practices related to oral cancer and sources of oral cancer information were evaluated using 25 questions. The level of significance was set at $p<.05$.

Results: Among the 140 participating dental students, there were 70 (50.%) third-year and 70 (50%) fifth-year students. The responses of 3rd grade students were significantly lower than 5th grade students in evaluating tobacco use, chewing maras grass and primary oral lesions as risk factors. The rate of 3rd grade students identifying leukoplakia and erythroplakia as the two lesions with the highest susceptibility to carcinogenesis (54.5%) was statistically significantly lower than that of 5th grade students (87.0%). The rate of 3rd grade students (35.9%) identifying squamous cell carcinoma as the most common form of oral cancer was statistically significantly lower than that of 5th grade students (84.1%).

Conclusion: This study emphasized the importance of improved training methods for dentistry in oral cancer detection and prevention. As the oral cancer expertise of graduating dental students advances, so does the number of dentists who are informed and competent in delivering proper oral cancer therapy to their patients. In this study, 5th-grade students were shown to be more aware of oral cancer than 3rd-grade students.
Keywords: Awareness, dentistry students, oral cancer, dental education

1. INTRODUCTION

Oral cancer is an important health problem. Cases of oral cancer represent the 16th most common malignant neoplasm worldwide, with approximately 355,000 new cases annually (1). In Turkey, oral cancers are the second most common cancers of the head and neck region after laryngeal carcinoma (2). It is more common in men over the age of 40 (2-4).

Oral cancer includes a subgroup of neoplasms arising from the lips, anterior two-thirds of the tongue, gums, hard and soft palate, oral mucosal surfaces and floor of the mouth. More than 90% of these oral cancers are oral squamous cell carcinomas (OSCC) (2-6). Various factors such as tobacco, alcohol, betel chewing, poor oral hygiene, malnutrition, ultraviolet light, immunosuppression, viruses, chronic trauma play a role in its etiology (7). Among more than 4000 organic compounds in tobacco smoke, especially N-nitrosamines and polycyclic hydrocarbons are known to cause dysplastic changes in cells. Studies have reported that the risk of developing oral cancer is related to the duration and amount of smoking (8). Betel chewing is associated with oral submucous fibrosis (OSF) and co-use with tobacco can lead to leukoplakia formation, both of which are potentially precancerous in the oral cavity. Therefore, measures to stop betel use are recommended to control conditions such as OSF and oral cancer. (9) Given that most risk factors can be eliminated, oral cancer can be considered a largely preventable disease. However, it is still possible for it to occur in patients who do not belong to risk categories (8,10). It might show up as uneven and patchy lesions on the epithelium that are red,

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white, or reddish-white in appearance. Over time, ulceration, hard border with malignant cell invasion, palpable infiltration into adjacent muscle and bone may occur (11).

In general, the prognosis deteriorates as the disease advances and the tumor's site becomes more difficult to access. The most important factor impacting prognosis is the clinical and pathologic stage at diagnosis (12). Given the high death rate, early identification of oral cancer and prediction of diagnosis resulted in a better prognosis and survival rate, as well as less morbidity (13).

There are various number of studies reporting oral cancer awareness of dental students (14-17). In a survey study conducted in the United Kingdom, oral cancer awareness of medical and dental students was evaluated and the need for better education of undergraduate students on oral cancer was emphasized (14). Despite increasing knowledge in recent years, oral cancer mortality and morbidity rates have not improved significantly (18,19). In a research of 541 patients with oral squamous cell carcinoma presented to the North West England Regional Maxillofacial Unit between 1992 and 2002, 5-year survival rates were reported to be around 50%, with patients often diagnosed at an advanced stage (20). Therefore, more invasive treatments may lead to a lower quality of life and disfigurement for these patients (21,22). Deficiencies in education prevent the recognition of lesions and may lead to late diagnosis (23). Therefore, it is important for health professionals, especially dentists, to perform oral cancer examinations and be aware of the pathogenesis of the disease as well as the first clinical signs (24). The aim of this study was to evaluate oral cancer awareness among undergraduate dental students at the Faculty of Dentistry, Van Yüzüncü Yıl University.

2. METHODS

Ethical approvals were obtained from the Non-Interventional Ethics Committee of Van Yüzüncü Yıl University (2023/03-08). A validated questionnaire testing oral cancer awareness was given to third and fifth year students at the Faculty of Dentistry, Van Yüzüncü Yıl University. A total of 140 students (70 third-year and 70 fifth-year) participated in this survey. Knowledge of oral cancer risk factors and diagnostic procedures, dental students' attitudes towards oral cancers, oral cancer management practices and oral cancer information sources were assessed using 25 questions. The questionnaire was distributed in paper format. Participation was voluntary and all participants were clearly informed that participation was anonymous.

Table 1. *Knowledge about oral cancer risk factors of respondents*

Risk factors		Third grade	Fifth grade	Total	P
		n (%)	n (%)	n (%)	
Do you consider use of tobacco as a risk factor?	**Yes**	54 (77.1)	68 (97.1)	122 (87.1)	**.001[1]***
	No	15 (21.4)	2 (2.9)	17 (12.1)	
	I don't know	1 (1.4)	0 (0)	1 (0.7)	
Do you consider low consumption of fruit and vegetable as a risk factor?	**Yes**	56 (80.0)	60 (85.7)	116 (82.9)	**.612[1]**
	No	12 (17.1)	9 (12.9)	21 (15.0)	
	I don't know	2 (2.9)	1 (1.4)	3 (2.1)	
Do you consider betel quid chewing as a risk factor?	**Yes**	41 (61.2)	55 (78.6)	96 (70.1)	**.020[1]***
	No	3 (4.5)	5 (7.1)	8 (5.8)	
	I don't know	23 (34.3)	10 (14.3)	33 (24.1)	
Do you consider ultraviolet exposure as a risk factor?	**Yes**	60 (85.7)	62 (88.6)	122 (87.1)	**.925[1]**
	No	7 (10.0)	5 (7.1)	12 (8.6)	
	I don't know	3 (4.3)	3 (4.3)	6 (4.3)	
Do you consider viral infection (eg. HPV…) as a risk factor?	**Yes**	65 (92.9)	67 (97.1)	132 (95.0)	**.560[1]**
	No	4 (5.7)	2 (2.9)	6 (4.3)	
	I don't know	1 (1.4)	0 (0)	1(0.7)	
Do you consider alcohol use as a risk factor?	**Yes**	55 (78.6)	57(81.4)	112(80)	**.870[1]**
	No	13(18.6)	12(17.1)	25(17.9)	
	I don't know	2(2.9)	1(1.4)	3(2.1)	
Do you consider prior oral cancer lesion as a risk factor?	**Yes**	61(87.1)	66(94.3)	127(90.7)	**.049[1]***
	No	3(4.3)	4(5.7)	7(5.0)	
	I don't know	6(8.5)	0(0)	6(4.3)	
Do you consider older age as a risk factor?	**Yes**	52(75.4)	51(72.9)	103(74.1)	**.953[1]**
	No	13(18.8)	15(21.4)	28(20.1)	
	I don't know	4(5.8)	4(5.7)	8(5.8)	

[1]*Fisher-Freeman-Halton test* [2]*Chi-square test* *$p < .05$

Table 2. *Knowledge about oral cancer diagnostic procedures of third and fifth grades*

Diagnostic procedures		Third grade	Fifth grade	Total	P
		n (%)	n (%)	n (%)	
The most common sited for oral cancer	**All sites equally**	10 (14.7)	1(1.6)	11(8.3)	**.047[1]***
	Floor of mouth and under the tongue	16(23.5)	24(37.5)	40(30.3)	
	Mucous membrane cheek/lip/gums and back of the tongue	26(38.2)	23(35.9)	49(37.1)	
	Hard and soft palate and floor of mouth	5(7.4)	4(6.3)	9(6.8)	
	Back of the tongue and mucous membrane cheek/ lip/gums	11(16.2)	12(18.8)	23(17.4)	
	Under the tongue and hard and soft palate	0 (0)	0 (0)	0 (0)	
Two lesions most likely to be precancerous	**Eryhtroplakia and Morbus Bowen**	12(18.2)	6(8.7)	18(13.3)	**.00008[1]***
	Leukoplakia and eryhtroplakia	36(54.5)	60(87.0)	96(71.1)	
	Blue nevus and leukoplakia	10(15.2)	3(4.3)	13(9.6)	
	Morbus Bowen and blue nevus	8(12.1)	0(0)	8(5.9)	
The most common form of oral cancer	**Squamous cell carcinoma**	23(35.9)	58(84.1)	81(60.9)	**.000001[2]***
	Large cell carcinoma	14(21.9)	1(1.4)	15(11.3)	
	Small cell carcinoma	16(25.0)	8(11.6)	24(18.0)	
	Adenosquamous cell carcinoma	11(17.2)	2(2.9)	13(9.8)	
Age group more likely to be diagnosed with oral cancer	**10-20**	4(5.7)	1(1.4)	5(3.6)	**.087[1]**
	20-40	23(32.9)	14(20.0)	37(26.4)	
	40-60	32(45.7)	46(65.7)	78(55.7)	
	60-80	11(15.7)	9(12.9)	20(14.3)	
Clinical properties of a prior oral cancer lesion	**Small, painful, white area**	13(20.3)	10(14.5)	23(17.3)	**.177[2]**
	Small, painless, white area	26(40.6)	37(53.6)	63(47.4)	
	Small, painful, red area	15(23.4)	8(11.6)	23(17.3)	
	Small, painless, red area	10(15.6)	14(20.3)	24(18.0)	

[1]*Chi-square test* [2]*Fisher-Freeman-Halton test* *$p < .05$

2.1. Statistical analysis

IBM SPSS Statistics 22.0 (IBM SPSS, Turkey) program was used for statistical analysis. The normal distribution of the parameters was evaluated by Shapiro Wilks test. Chi-square test and Fisher-Freeman-Halton test were used to compare descriptive statistics (mean, standard deviation, frequency) and qualitative data. The significance level was determined as $p < .05$.

3. RESULTS

A total of 140 participants, 70 (50%) 3rd grade students and 70 (50%) 5th grade students, aged between 19-25 years, studying at Faculty of Dentistry, Van Yüzüncü Yıl University participated in the study. 84 (60%) of the participants were male and 56 (40%) were female with a mean age of 22.58 ± 1.47 years.

To test their knowledge about oral cancer, questions about risk factors and diagnostic procedures were asked to 3rd and 5th year dental students who participated in the survey. The distribution of the respondents is presented in Table 1 and Table 2.

There was no statistically significant difference between 3rd and 5th grade students in the rate of low consumption of vegetables and fruits, exposure to ultraviolet rays, viral infections, alcohol use and high age as risk factors ($p>.05$). The rate of 3rd grade students perceiving tobacco use as a risk factor (77.1%) was found to be statistically significantly lower compared to 5th grade students (97.1%) ($p= .001$). The 3rd grade students' perception of chewing maras grass as a risk factor (61.2%) was statistically significantly lower compared to the 5th grade students (78.6%) ($p=.02$). The rate of 3rd grade students perceiving primary oral cancer lesion as a risk factor (87.1%) was statistically significantly lower compared to 5th grade students (94.3%) ($p=.925$).

3rd grade students were significantly more likely to identify cheek/lip/gingival mucous membrane and dorsum of tongue as the most common sites of oral cancer (38.2%) than 5th grade students (35.9%) ($p=.047$). The 3rd grade students were statistically significantly less likely to identify leukoplakia and erythroplakia as the two lesions with the

Table 3. *Attitude towards oral cancer of the respondents*

		Third grade	Fifth grade	Total	P
		n (%)	n (%)	n (%)	
I advise my patients with suspicious oral lesions.	**Strongly agree**	29(42.6)	40(57.1)	69(50.0)	**.047[1]***
	Agree	18(26.5)	20(28.6)	38(27.5)	
	Uncertain	16(23.5)	5(7.1)	21(15.2)	
	Disagree	4(5.9)	5(7.1)	9(6.5)	
	Strongly disagree	1(1.5)	0(0)	1(0.7)	
My patients are sufficiently informed on risk factors for oral cancer.	**Strongly agree**	24(35.8)	15(21.4)	39(28.5)	**.021[1]***
	Agree	12(17.9)	28(40)	40(29.2)	
	Uncertain	24(35.8)	20(28.6)	44(32.1)	
	Disagree	5(7.5)	7(10)	12(8,8)	
	Strongly disagree	2(3.0)	0(0)	2(1.5)	
My patients sufficiently know signs and symptoms of oral cancer.	**Strongly agree**	4(5.8)	5(7.1)	9(6.5)	**.861[1]**
	Agree	12(17.4)	8(11.4)	20(14.4)	
	Uncertain	18(26.1)	17(24.3)	35(25.2)	
	Disagree	27(39.1)	32(45.7)	59(42.4)	
	Strongly disagree	8(11.6)	8(11.4)	16(11.5)	
I am adequately trained to perform an oral cancer examination.	**Strongly agree**	9(12.9)	2(2.9)	11(7.9)	**.0001[2]***
	Agree	11(15.7)	19(27.1)	30(21.4)	
	Uncertain	17(24.3)	33(47.1)	50(35.7)	
	Disagree	21(30)	15(21.4)	36(25.7)	
	Strongly disagree	12(17.1)	1(1.4)	13(9.3)	
I am adequately trained to perform patient's lymph nodes palpation.	**Strongly agree**	8(11.6)	12(17.1)	20(14.4)	**.0001[1]***
	Agree	12(17.4)	29(41.6)	41(29.5)	
	Uncertain	17(24.6)	23(32.9)	40(28.8)	
	Disagree	24(34.8)	5(7.1)	29(20.9)	
	Strongly disagree	8(11.6)	1(1.4)	9(6.5)	

[1]*Fisher Freeman Halton Test* [2]*Chi-square test* **p<.05*

highest susceptibility to carcinogenesis (54.5%) than the 5th grade students (87%) (p=.000). The rate of 3rd grade students identifying squamous cell carcinoma as the most common form of oral cancer (35.9%) was statistically significantly lower than that of 5th grade students (84.1%) (p=.000). There was no statistically significant difference between 3rd and 5th year dental students in terms of the distribution of the age range in which oral cancer was most commonly diagnosed and in terms of identifying the clinical features of the primary oral cancer lesion (p>.05).

Table 3 shows the distribution of the participants' responses to questions concerning their opinions toward oral cancer. The participation rate of 3rd grade students in informing patients with suspicious oral lesions (42.6%) was significantly lower than that of 5th grade students (57.1%) (p=.047). The participation rate of 3rd grade students in adequately informing patients about oral cancer risk factors (42.6%) was statistically significantly lower than that of 5th grade students (57.1%) (p=.02). The rate of 3rd grade students being undecided about having sufficient training to perform oral cancer examination (24.3%) was statistically significantly lower than 5th grade students (47.1%) (p=.000). The rate of 3rd grade students agreeing that they have sufficient training to perform lymph node palpation (17.4%) was statistically significantly lower than that of 5th grade students (41.6%). There was no statistically significant difference between 3rd and 5th grade dental students in the rate of agreement that their patients were sufficiently aware of the signs and symptoms of oral cancer (p>.05).

The distribution of the participants' answers to the questions regarding the evaluation of the patient's medical history is presented in Table 4. There was no statistically significant difference between 3rd and 5th grade students in terms of evaluating the patient's current tobacco use, current alcohol use, patient's cancer history, patient's past tobacco use and patient's family history of cancer (p>.05). The rate of 3rd grade students evaluating the patient's past alcohol use (75.8%) was statistically significantly higher than that of 5th grade students (58.8%).

The distribution of participants' oral cancer information sources is presented in Table 5. The 3rd grade students' rate of evaluating textbooks as a source of oral cancer information (47.1%) was statistically significantly lower than that of the 5th grade students (82.9%) (p=.000). There was no statistically significant difference between 3rd and 5th grade students in terms of evaluating training courses, scientific journals and dental congresses as oral cancer information sources (p>.05).

Table 4. *Patient's health history assessment by the respondents*

		Third grade	Fifth grade	Total	p
		n (%)	n (%)	n (%)	
Patient's current use of tobacco.	Yes	61(89.7)	61(89.7)	122(88.4)	.638
	No	7(10.3)	9(12.9)	16(11.6)	
Patient's current use of alcohol.	Yes	50(76.9)	51(73.9)	101(75.4)	.686
	No	15(23.1)	18(26.1)	33(24.6)	
Patient's history of cancer.	Yes	58(86.6)	63(91.3)	121(89)	.378
	No	9(13.4)	6(8.7)	15(11)	
Patient's previous use of tobacco.	Yes	53(79.1)	51(72.9)	104(75.9)	.393
	No	14(20.9)	19(27.1)	33(24.1)	
Patient's previous use of alcohol.	Yes	50(75.8)	40(58.8)	90(67.2)	.037*
	No	16(24.2)	28(41.2)	44(32.8)	
Patient's family history of cancer.	Yes	50(73.5)	56(81.2)	106(77.4)	.286
	No	18(26.5)	13(18.8)	31(22.6)	

Chi-square test **p<.05*

Table 5. *Information sources of the respondents*

Oral cancer information source/ sources	Third grade	Fifth grade	Total	p
	n (%)	n (%)	n (%)	
Educational courses	28 (40.0)	26 (37.1)	54 (38.6)	**.728**
Scientific journals	23 (32.9)	22 (31.4)	45 (32.1)	**.856**
Textbooks	33 (47.1)	58 (82.9)	91 (65.0)	**.000009***
Dental congresses	17 (24.3)	18 (25.7)	35 (25.0)	**.845**

Chi-square test **p<.05*

4. DISCUSSION

Early diagnosis of oral cancers is very important for the prognosis, treatment and quality of life of the patient (25, 26). The education and knowledge of dental students on this subject may be insufficient (27-29). Therefore, the aim of this study was to examine the knowledge of dental students about oral cancer risk factors, clinical aspects and attitudes.

Awan et al. (30) conducted a questionnaire study evaluating the oral cancer knowledge of dental and medical students. Dental students were able to identify risk factors better than medical students. Keser and Pekiner (26) evaluated the level of awareness of third and fifth year dental school students about oral cancer risk factors in their questionnaire study. The majority of students reported tobacco use as a risk factor for oral cancers and the researchers reported that there was no significant difference between the groups. Although tobacco use was considered as a risk factor in present study, 3rd grade students were statistically significantly lower than 5th grade students.

Camélo et al. (27) conducted a questionnaire study to evaluate the oral cancer awareness of second and fifth year dental students. The researchers reported that factors such as smoking, alcohol consumption, and UV exposure were known by the students as predisposing factors for oral cancers. As a result of the study, it was reported that there was no significant difference between the groups Öztaş et al. (31) asked 32 questions in an oral cancer awareness questionnaire to 53 intern students, 51 research assistants and 36 faculty members. Candida albicans, ultraviolet rays, thermal irritation, chronic oral traumas, viral infections such as HPV, white and red mouth lesions were evaluated as risk factors by more than 90% of the participants. In present study, there was no statistically significant difference between 3rd and 5th grade students in the rate of evaluating low consumption of vegetables and fruits, exposure to ultraviolet rays, viral infections, alcohol use and high age as risk factors for oral cancers.

The knowledge of future dentists in the United Arab Emirates on risk and non-risk factors for oral malignancies was assessed in a different research carried out in the country. A questionnaire regarding oral cancer risk factors was completed by first – and fifth-year dentistry students involved in the study. 83% of the students correctly recognized cigarette use as a risk factor for oral cancer, followed by 52% who properly identified high age, 45.6% who correctly identified poor consumption of fruits and vegetables, and 74.4% who correctly identified alcohol use. The knowledge of risk factors and academic year did not have the predicted statistical significance (32). Camelo et al. (27) evaluated the knowledge and attitudes of second and fifth year dental students about oral cancer. Smoking (92.4%) and alcohol (84.2%) were found to be the most frequently cited oral cancer risk factors. When the risk factors were analyzed according to grades, it was observed that only sunlight exposure and tobacco use were more clearly identified by advanced graduate students and there was no significant difference between the student groups in terms of other factors. In present study, 3rd grade students were found to be statistically significantly lower than 5th grade students in evaluating maras grass chewing, primary oral lesion and tobacco use as risk factors.

Silva et al. (33) they evaluated the oral cancer awareness of newly graduated dentists and dental students. In the study, the majority of both newly graduated dentists and dental students stated that the most common type of oral cancer is squamous cell carcinoma. The authors also reported a significant disparity between dental students and freshly graduated dentists in the prevalence of leukoplakia as a precursor lesion of oral cancer. This effect was ascribed by the researchers to the continuance of theoretical instruction as well as practical application. The researchers attributed this result to the continuation of theoretical education along with practical application. Koca and Yenidünya (34) conducted a 13-question questionnaire study on 55 students who did and did not complete surgical internship. There was a statistically significant correlation between completing the internship and correctly answering the question "Which is the precursor lesion of oral cancer?". In the study by Camelo et al. (27) squamous cell carcinoma was identified as the most common type of oral cancer by 48.1% of the students. In present study, the rate of 3rd year students identifying squamous cell carcinoma as the most common form of oral cancer (35.9%) was statistically significantly lower than that of 5th year students (84.1%). There was no statistically significant difference between 3rd and 5th year dental students in the

distribution of the age range in which the most common oral cancer was diagnosed and in terms of identifying the clinical features of the primary oral cancer lesion. Keser and Pekiner (26) reported that the majority of students stated that the most common site of oral cancers was the floor of the mouth and the sublingual region and that there was no statistically significant difference between the groups. In addition, it was mentioned that the number of third-year students who knew that erythroplakia and leukoplakia were precancerous lesions was statistically significantly higher. The researchers attributed these results to the fact that practical training was given to third-year students supported by seminars and presentations.

In the study conducted by Keser and Pekiner (26), the participation rate of 3rd grade students in informing patients with suspicious oral lesions was found to be statistically significantly lower than that of 5th grade students. In terms of awareness of the signs and symptoms of oral cancer patients, third grade students (23.2%) were significantly higher than fifth grade students (10.1%). In this study, the participation rate of 3rd grade students in informing patients with suspicious oral lesions (42.6%) was found to be statistically significantly lower than that of 5th grade students (57.1%). The participation rate of 3rd grade students in adequately informing patients about oral cancer risk factors (42.6%) was statistically significantly lower than that of 5th grade students (57.1%).

In the study by Camelo et al. (27), most students reported that they regularly performed a comprehensive oral examination (81.9%); 81.2% of the students stated that they informed their patients about the harms of alcohol and tobacco; 73.6% said that they could at least partially detect precancerous lesions; 69.1% stated that they would refer the patient to someone else. No statistically significant difference was found between the groups.

The goal of Shamala et al's (35) study was to evaluate senior dentistry students in Yemen's knowledge, attitudes, and practices about OC. Data were gathered online using a pre-validated questionnaire. It was formed up of many closed-ended questions about OC-related knowledge, attitudes, and practices. The survey was sent to nine dental schools located in four main cities, and it was intended for Yemeni dental students enrolled in clinical levels (4th and 5th years).927 students in all completed the survey, representing a 43% response rate. While most people (93.8%) and smokeless tobacco users (92.1%) recognized smoking and sun exposure as potential risk factors for oral cancer (OC), only 76.2% and 50.0%, respectively, recognized sun exposure and lip cancer as potential risk factors. Merely two thirds of the participants acknowledged that OC can manifest as a lesion that is either white or red in color. Regarding practices, only 78% of participants said they routinely do a soft tissue examination. The fifth-year students demonstrated noticeably superior practices and knowledge compared to the fourth-year students ($p < .05$). In our study there was no statistically significant difference between 3rd and 5th year dental students in terms of identifying the clinical features of the primary oral cancer lesion.

A profound limitation of the present study was that Likert Scale questions produce ordinal data, which means that even if a response is given, some opinions may fall between two scale points in reality. Careful construction of the scale and question is needed to ensure accurate results. The results could be influenced by a tendency toward results clustering.

Present study compared the 3rd graders' first visits to the clinic and their first examinations with patients and the 5th graders' knowledge accumulated during their graduation. 5th grade students' awareness of oral cancer was found to be higher than 3rd grade students. The beneficial correlation between dentistry students' activities to evaluate oral cancer risk factors and their increased awareness about oral cancer was also demonstrated by our data. As a result, it is critical that dentistry students get instruction that emphasizes the prevention and detection of oral cancer. Raising the level of oral cancer awareness among dental students who graduate also raises the number of dentists who are qualified and experienced in treating patients with the oral cancer. This study emphasized the value of practical and theoretical training for dental collages. We suggest devoting additional instructional time to teaching about disease identification. Incorporating a focus on the early identification of oral cancer is vital in order to provide dental students with accurate knowledge regarding the risk factors associated with malignant diseases. Present study supports the importance of dental education on oral cancer.

5. CONCLUSION

Oral cancer awareness and knowledge among dentistry students were investigated in this study. Several methodological limitations should be addressed when evaluating our study's findings; all data was self-reported and subjective. As a result, the generalizability of our findings may be restricted, however our study underscored the necessity of improved training techniques and programs for dentists and dental students in terms of early detection and prevention of oral cancer. Furthermore, medical history questionnaires at dentistry schools should be reviewed and revised to provide a thorough list of available and new risk factors for oral cancer.

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Author Contributions:

Research idea: FNP.
Design of the study: FNP, AGÖT.
Acquisition of data for the study: AGÖT, NF.
Analysis of data for the study: AGÖT, NF.
Interpretation of data for the study: AGÖT.
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Clinical and Experimental Health Sciences

The Used Masks of The Food Industry Employees During the Covid-19 Pandemic: Did the Mask Promote Other Diseases While Protecting from the Coronavirus? A Survey Study Supported by Microbiological Data

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ABSTRACT

Objective: Use of disposable face masks helps prevent infection by airborne pathogens. The effectiveness of such masks in excluding diseases and contaminants depends on many factors. As a result of misuse, mask loses its protective role and becomes a source of microbial contamination. It is aimed to investigate the attitudes of food workers towards use of masks in proportion to the bacterial load and microorganism species in the masks they wear.

Method: Total aerobic mesophilic, Yeast-mould and Coliform counts were determined as log colony forming units per mL. Phenotypically different colonies grown on three different agar plates were purified and fresh cultures were classified using matrix assisted laser desorption/ ionization time-of-flight mass spectrometry (MALDI-TOF MS).

Results: In this study, bacterial contamination was found at different levels on all tested (103) disposable face masks. The screened bacterial pathogens by MALDI-TOF MS Bacillus cereus was detected at the highest level 17.86%, Enterobacter cloacae, Klebsiella oxytoca, Kurthia gibsonii and Pseudomonas aeruginosa with rates 14.29%, 10.72%, 7.14%, and 7.14%, respectively. Long-term working, inappropriate mask usage, poor hygiene attitudes of employees, and the fact that staff wore the mask out of need rather than for its protective advantage all signal that the investigated masks had low microbiological quality.

Conclusion: The findings show that because most masks used by food industry employees to protect themselves from COVID-19 and avoid infecting others contain bacteria of intestinal origin, serious health problems may occur in both employees who use contaminated masks and consumers who consume food contaminated by mask contamination.
Keywords: COVID-19; face masks; MALDI-TOF MS; food industry employees; microbiology

1. INTRODUCTION

The novel coronavirus, which started as a localized zoonotic disease outbreak in China – December 2019 – spread rapidly to many other countries, causing it to be declared a pandemic by the World Health Organization (WHO) on March 11, 2020 (1). The pandemic has greatly affected social life in every sense. For this reason, various guidelines and resources have been developed at local, national, and international levels, both in the private and public sectors. Almost all countries, especially those most affected by the epidemic, have taken various precautions to reduce the epidemic. Full or partial quarantine practices, travel restrictions, and mandatory use of masks in public areas are among the foremost of these measures (2).

Food industry is one of the most important sections of a country's critical infrastructure along with health, energy, and communication (2). Nutrition, which is the most basic need of people during the pandemic, should continue to be maintained at a normal life level (3). Therefore, during pandemic, the food industry continues to struggle with some challenges such as preventing disruptions from the supply chain (4), meeting consumer demand, protecting the workforce while ensuring food safety, and not disrupting consumer confidence (5). Personal hygiene and health are essential to maintaining a hygienic food processing environment. The implementation of Food Safety Management Systems (FSMS) based on Hazard Analysis and Critical Control Point (HACCP) principles provides control of

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food safety (6). Studies indicate that the factors potentially affecting the risk of infection include difficulties with physical distance, hygiene, crowded living, and transportation conditions in the workplace (7, 8). As stated by the European Food Safety Authority (9) there is no evidence that food poses a risk to public health in Europe. Infection occurs through respiratory droplets formed by sneezing, coughing, or breathing in infected persons (10). Respiratory droplets do not stay suspended in the air, but quickly fall on floors and surfaces. Although it has greatly affected social life, there seems to be no report that COVID-19 is transmitted through food consumption. For these reasons, the risk of COVID-19 transmission increases in the form of touching a contaminated surface or touching the mouth, nose, and eyes (11).

The most common way to avoid the risk of contamination is the use of Personal Protective Equipment (PPE) (12). When used correctly, PPE, together with proper hygiene and hand washing practices, help reduce the spread of cross-infection (COVID-19) and cross-contamination (food safety) (1, 11). PPE commonly used in the food industry may include face masks, face shields, gloves, aprons, bonnets, and work shoes. Since SARS-CoV-2 is a respiratory virus, the mask has been put in the first place in terms of precautions among other PPE. WHO has stated that the most effective ways to prevent an epidemic and minimise its spread are the use of surgical masks, along with hand hygiene and other preventive measures (13).

Face masks have previously been used by paramedics and infected people, especially in epidemics such as the 2009 influenza pandemic, avian flu, Middle East respiratory syndrome coronavirus (MERS-coronavirus) and Ebola virüs (14). The most used disposable surgical and N95 type masks are produced to filter droplets containing microorganisms expelled from the mouth and nose. However, to prevent the spread of airborne pathogens, some factors should be considered, especially the selection of masks with different pore sizes and the types (15). The generally accepted assumption is that use of masks, both medical and non-medical, is safe, but this should be closely monitored and studied in detail. Studies on mask effectiveness are available in the literature (16, 17). Masks, in continuous and correct use, reduces the rate of virus infection (18) but it is less effective in protecting the user from being infected (19). The fact that microorganisms in human saliva and exhaled breath can pose a biosafety problem is often overlooked, especially when worn for too long, not stored properly, or reused without proper disinfection. Human saliva contains around 100 million bacterial cells per milliliter. These bacteria include pathogens such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Candida albicans*, *Klebsiella pneumoniae*, *Neisseria*, *Prevotella* and *Veillonella* spp. (16). Cotton contained in the masks a moisture-retaining property, making cotton masks suitable for microbial contamination. For this reason, reused cotton masks increase the risk of respiratory transmission (20). Studies show that surgical masks may not be sufficient to protect people from airborne pathogens, and moreover, these masks may be a source of infection (21). Surgical face masks worn for more than 4 hours show higher contamination than those worn less often. On the other hand, the use of face masks affects individual and social life for reasons such as discomfort, respiratory distress, headache, skin acne and difficulty in communication (16).

We hypothesized that masks used to minimise bacterial contamination from the mouth, nose, and face can be a source of contamination when worn for a long time, reused several times, and when used masks come into contact anywhere. The present study examines the role of the masks in personal and community hygiene and their role as a potential source of bacterial contamination. For this reason, it is aimed to investigate the hygiene status of food workers, their attitudes towards mask use, the bacterial load proportional to the duration of use in the masks they wear, the microorganism types in the contaminated masks and their correlation with survey and microbiological results.

2. METHODS

2.1. Sampling

The research was carried out between 01-31 December 2021 in the city center of Burdur, Türkiye (37.7183° N, 30.2823° E). The study aimed to determine the status and microbiological quality of the masks used by food industry employees. Prior to sampling, the permission of the owner of the enterprise was obtained, and then the participation of food industry employees was determined on a voluntary basis. Before starting the research, the employees were informed about the study and questionnaire were applied to the employees who read and accepted the voluntary consent form. Attention was paid to the fact that participants were between 18-65 ages without any psychological problems, and a total of 103 participants were subject of the research. The protocol (GO 2021/422) was approved by the Non-Interventional Clinical Research Ethics Committee of Burdur Mehmet Akif Ersoy University.

2.2. Collection of Research Data

The survey data were prepared by the researchers after the relevant literature was searched and the data were collected by face-to-face interview method. The questionnaire form consists of 3 sections in total, including the socio-demographic information of the food industry employees, the questions about the workplace and working conditions, and the questions prepared to determine the use of masks. Individuals between the ages of 18-65 who participated in the research were given general information about the research, and the volunteer consent form was read to the participants who wanted to participate in the study and their approval was obtained.

2.3. Microbiological Evaluation

2.3.1. Bacterial isolation

The masks collected throughout the survey were sent to the laboratory in closed sterile stomacher bags. Initially, all the samples were enriched onto buffered peptone water for 24 hours at 37 °C. Appropriate serial dilutions were prepared in 0.1% peptone water solution, and bacterial populations were counted. Total aerobic mesophilic counts were enumerated on plate count agar (*Oxoid CM325*) incubated at 30±1 ºC for 48 h. Yeast and mould counts were enumerated on potato dextrose agar (*Difco B 13*). Plates were incubated at 22±1 ºC and evaluated after 5 days. Coliform counts were enumerated on violet, red bile agar (*Difco B12*). Plates were incubated at 30±1 ºC and evaluated after 24 h. Inoculated plates in duplicate were incubated and at the end of incubation only plates containing 30-300 colonies were evaluated. Microorganism counts of samples were expressed as log colony forming units per mL (log_{10} CFU/mL).

2.3.2. MALDI-TOF MS identification of colonies

Phenotypically different colonies grown on three different agar plates were purified by spreading on Tryptic Soy Agar (TSA) (*Merck 1.05458*). Pure and fresh cultures were classified using matrix assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS; Microflex® LT/SH, Bruker Daltonik, Bremen, Germany) and the flexControl and MALDI Biotyper® Compass software packages (Bruker Daltonik). After the pure cultures were obtained, formic acid + ethanol extraction processes were started for MALDI TOF MS analysis. Picked single colonies were transferred to a MALDI-plate and covered with 1 µL of 70% formic acid and allowed to dry at room temperature. The plates were overlayed with 2 µL of α-Cyano-4-hydroxycinnamic acid (HCCA) matrix solution and placed into the MALDI-TOF MS. Samples were analysed using the Bruker Daltonics flexControl-MicroFlex LT system. The spectra captured with the Flex control software program were compared with the MALDI Biotyper Real-Time Classification (RTC) software and the diagnosis process was completed. Measurements were continued until the bacterium was clearly identified. The meaning of the resulting scores was evaluated according to the manufacturer's instructions. In this context, a score between 2.300-3.000 (green color) indicates highly probable species identification; a score between 2.000-2.299 (green color) indicates secure genus identification, probable species identification; a score between 1.700-1.999 (yellow color) indicates probable species identification; and a score between 0.000-1.699 (red color) indicates not reliable identification (22).

2.4. Statistical Analysis

The data obtained in the study were evaluated using Statistical Package for The Social Sciences (SPSS Inc., Chicago, IL, USA) 26.0 package program. Number (n) and percentage (%) rates are given for categorical data and mean (X) standard deviation (SD) values are given for numerical data. Mean (X) standard error (SE) is given as the mean of the number of diseases that participants had in the last year. In statistical analysis, frequency in socio-demographic information, independent sample t-test according to the characteristics of the variable in numerical data, and one-way analysis of variance (ANOVA) were used. In one-way analysis of variance, differences between groups were calculated using Tukey's multiple comparison test. $p < .05$ was considered statistically significant.

3. RESULTS

3.1. Socio-demographic Data of the Participants

The results of the survey conducted with the food industry employees and the microbiological analysis of the masks used by the employees were examined. Accordingly, when the age group distributions of the individuals participating in the study are examined, it is seen that 37.9% (n=39) are between the ages of 18-24 and 21.4% (n=22) are between the ages of 25-34. It was determined that the mean of individuals in the 55-65 age range was 5.8% (n=6), the group with the lowest distribution among all age groups. The distribution of age groups showed similarity in both genders; it is seen that the number of individuals in the 25-34 age range is low only in the female gender (Table 1).

It was determined that the participants were sick on average 1.9±0.2 times in the past year, and 55.3% (n=57) of the diseases were respiratory system diseases and 16.5% (n=17) were COVID-19. 25 people (24.3%) stated that they had not had any disease in the last year. It was found that 60.2% (n=62) of the people working in food businesses are permanent employees, 25.2% (n=26) are business owners or partners and 14.6% (n=15) are part-time employees. It was determined that 33.0% (n=34) of the individuals participating in the study kept the masks they used in their pockets, and 16.5% (n=17) kept their chins. It was observed that only 18.4% (n=19) of the individuals did not store the mask they used or used a new mask. 41.7% (n=43) of the individuals participating in this study stated that they used a mask for 9-24 hours, and 23.3% (n=24) used it for more than 1 day. When the individuals participating in the study were questioned whether they used masks or other protective equipment used by others, it was seen that 93.2% (n=96) answered no. Although the rate of individuals who say yes is low, it is estimated that they are from individuals with family ties. When asked whether masks protect the person from diseases, 51.5% (n=53) of the individuals participating in the study answered no; It was observed that 47.5% (n=49) answered yes and 1 person did not express an opinion.

Table 1. *Socio-demographic data of the participants*

	Female		Male		Total	
	X	SE	X	SE	X	SE
Number of diseases in the last year						
	2.5	0.3	1.5	0.2	1.9	0.2
	n	%	n	%	n	%
Age distributions of participants						
18-24	21	47.7	18	30.5	39	37.9
25-34	5	11.4	17	28.8	22	21.4
35-44	8	18.2	13	22.0	21	20.4
45-54	8	18.2	7	11.9	15	14.6
55-65	2	4.5	4	6.8	6	5.8
Hygienic attitude of after their needs such as toilet, cigarette, lunch break*						
Hand washing	44	100.0	54	91.5	98	95.1
Cologne	6	13.6	11	18.6	17	16.5
Disinfectant	1	2.3	12	20.3	13	12.6
Diseases in the past year						
Absent	8	18.2	17	28.8	25	24.3
Respiratory system diseases	26	59.1	31	52.5	57	55.3
COVID-19	7	15.9	10	16.9	17	16.5
Other	3	2.3	1	1.7	4	4.0
Position at work						
Business owner-partner	9	20.5	17	28.8	26	25.2
Permanent employee	28	63.6	34	57.6	62	60.2
Part time employee	7	15.9	8	13.6	15	14.6
Storage place of participant's masks						
Do not store	8	18.2	11	18.6	19	18.4
Chin	11	25.0	6	10.2	17	16.5
Arm	2	4.5	6	10.2	8	7.8
Pocket	13	29.5	21	35.6	34	33.0
Bag	6	13.6	2	3.4	8	7.8
Hanger	2	4.5	4	6.8	6	5.8
Countertop	2	4.5	9	15.3	11	10.7
Mask usage duration						
< 4 h	5	11.4	12	20.3	17	16.5
4-8 h	5	11.4	14	23.7	19	18.4
9-24 h	21	47.7	22	37.3	43	41.7
> 24 h	13	29.5	11	18.7	24	23.3
Usage of mask or other protective equipment used by others						
Yes	2	4.5	5	8.5	7	6.8
No	42	9.5	54	91.5	96	93.2
Whether people think that the use of masks protects them from diseases						
Yes	27	61.4	22	37.3	49	47.5
No	16	36.4	37	62.7	53	51.5
Indecisive	1	2.3	-	-	1	1.0
TOTAL	**44**	**100.0**	**59**	**100.0**	**103**	**100.0**

** More than one option has been ticked. X: mean; SE: standard error*

3.2. Microbiological Results

Bacterial contamination was found at different levels on all tested (103) face masks. Total aerobic mesophilic counts were found to be around 9 $\log_{10}$ CFU/mL, while yeast and mould counts were approximately 8 $\log_{10}$ CFU/mL in each age group as indicated in Figure 1. There was no statistically significant relationship between bacterial growth and gender. Coliform findings indicate a bacterial growth range between 8.41-10.56 $\log_{10}$ CFU/mL.

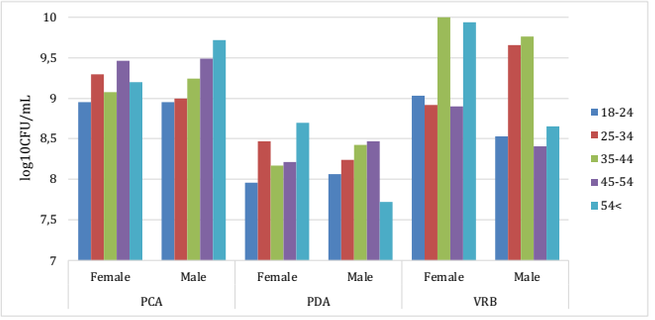

Figure 1. *Total bacterial counts (log10 CFU/mL) within indicated age groups*

Figure 2 shows that the highest coliform counts (10.56 $\log_{10}$ CFU/mL) were seen in women aged 35 – 44 (p <.05). It is notable that the mean counts isolated from part-time employees was found to be lower than that of permanent employees and owners (p > .05). This might be due to the mask being changed more frequently.

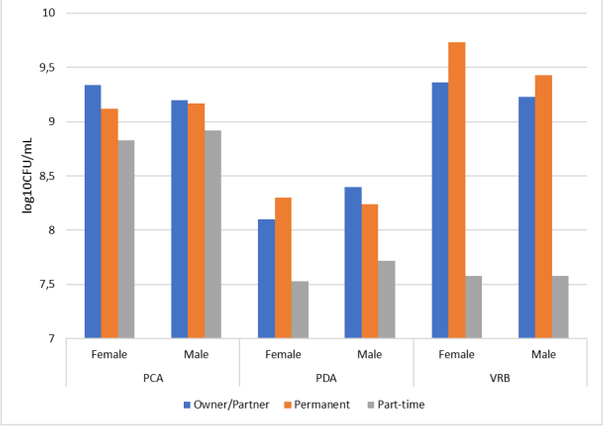

Figure 2. *Total bacterial counts ($\log_{10}$ CFU/mL) within indicated work position*

3.2.1. Identification Results by MALDI-TOF MS

In current study, the number, ratio and habitats of microorganisms classified by MALDI-TOF MS are presented in Table 2.

Bacillus cereus was detected at the highest level with 17.86%. This was followed by *Enterobacter cloacae*, *Klebsiella oxytoca*, *Kurthia gibsonii* and *Pseudomonas aeruginosa* with rates of 14.29%, 10.72%, 7.14%, and 7.14%, respectively. All of the other classified microorganisms were detected at the level of 3.57%. When the detected species were classified according to their families, it was seen that the species belonging to *Enterobacteriaceae*, which also included pathogens, were dominant, followed by the *Bacillaceae* family, which is generally composed of ubiquitous environmental bacteria, with 25%.

Table 2. *Number and percentage of prevalence of microorganisms detected via MALDI-TOF MS in surgical mask samples of food industry employees.*

Organism identified	Number	%	Typical habitat
Firmicutes			
Bacillaceae			
Bacillus cereus	8	17.86	ubiquitous, environment, soil
Bacillus subtilis	1	3.57	ubiquitous, environment, soil, water
Lysinibacillus pakistanensis	1	3.57	ubiquitous, environment, soil, water
Planococcaceae			
Kurthia gibsonii	2	7.14	ubiquitous, intestinal tract
Enterococcaceae			
Enterococcus faecalis	1	3.57	gut, feces
Proteobacteria			
Enterobacteriaceae			
Enterobacter cloacae	4	14.29	ubiquitous, environment, soil, water, intestinal tract
Enterobacter xiangfangensis	1	3.57	ubiquitous, environment, soil, water, intestinal tract
Klebsiella oxytoca	3	10.71	ubiquitous, environment, colon, nasopharynx, skin
Klebsiella pneumoniae	1	3.57	ubiquitous, environment, mouth, skin, intestinal tract
Pseudomonadaceae			
Pseudomonas aeruginosa	2	7.14	ubiquitous, environment, soil, water, plant, nasopharynx, skin, intestinal tract
Moraxellaceae			
Acinetobacter pittii	1	3.57	environment, sewage, soil, plant, water, skin, foods
Acinetobacter radioresistens	1	3.57	environment, soil, water, skin, cotton
Erwiniaceae			
Mixta calida	1	3.57	plant, food products
Ascomycota			
Saccharomycetaceae			
Candida lusitaniae	1	3.57	skin, mouth, vaginal mucous membranes, feces
Total	28	100.0	

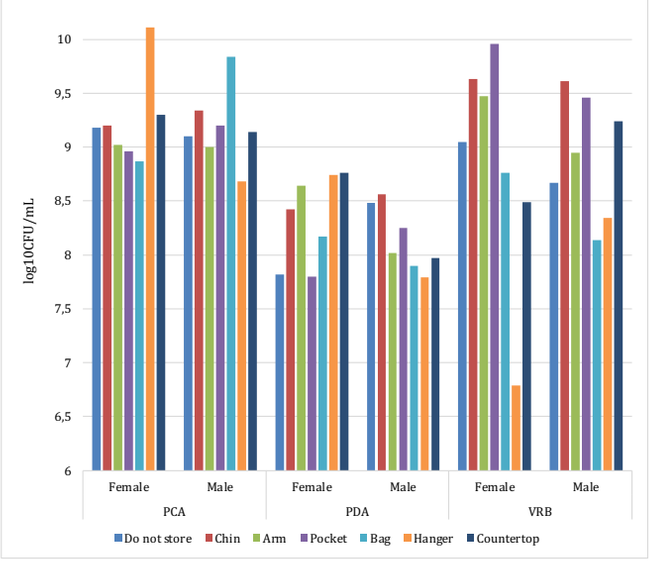

Figure 3. *Total bacterial counts (log_{10} CFU/mL) within indicated storage places*

4. DISCUSSION

When the hygienic attitudes of the people were questioned after their needs such as toilet, cigarette, and meal breaks, it was determined that all the women (n=44) and 95.1% of the men washed their hands after the specified needs (Table 1). Hand washing is extremely important for the protection and improvement of public health (23). Like this study, the Turkey hand washing research report stated that hand washing rates were high (24). It was observed that only 18.4% (n=19) of the individuals did not store the mask they used or used a new mask. The WHO has published a report as a mask use guide in the COVID-19 pandemic. Suggestions such as 'perform hand hygiene before putting on the mask, inspect the mask for tears or holes, and do not use a damaged mask, replace the mask as soon as it becomes damp with a new clean, dry mask, either discard the mask or place it in a clean plastic resalable bag where it is kept until it can be washed and cleaned, do not store the mask around the arm or wrist or pull it down to rest around the chin or neck, do not share your mask with others, discard single-use masks after each use and properly dispose of them immediately upon removal' have been developed in this report (25). 41.7% (n=43) of the

individuals participating in this study stated that they used a mask for 9-24 hours, and 23.3% (n=24) used it for more than 1 day. Based on these findings, it is believed that the masks are being handled improperly, and that personnel should be trained in this regard.

Figure 2 shows that the highest coliform counts (10.56 $\log_{10}$ CFU/mL) were seen in women aged 35 – 44 ($p< .05$). It is notable that the mean counts isolated from part-time employees was found to be lower than that of permanent employees and owners ($p > .05$). This might be due to the mask being changed more frequently. WHO suggested that the masks do not store the mask around the arm or wrist or pull it down to rest around the chin or neck (25).

The storing places of the masks used by the participants in this study are shown in Table 1. The bacterial load of the masks used by the participants according to the hiding places of the masks is shown in Figure 3. Sachdev et al. (26) found similar results in their study. Accordingly, the microbiological counts of the participants in the current study on the mask storage areas have again proven the importance of WHO's recommendations.

Masks have traditionally been recognized to serve a significant role in ensuring workplace hygiene. However, it is worth debating whether the measures in place to prevent germs or viruses shed by employees can become a source of bacterial contamination. In this study, we investigated the bacterial contamination of face masks used by food industry employees. The mean bacterial counts rose with increased wearing time, although this was not statistically significant. Similar to this study, Zhiqing et al.(27) reported that the bacterial count on the surface of surgical masks increased with extended operating times. Several bacterial pathogens including *Enterobacter cloacae*, *Klebsiella pneumoniae*, *Bacillus cereus*, *Pseudomonas aeruginosa*, *Kurthia gibsonii* etc. were detected. According to research, germs may be produced and spread via exhaled breath, offering an elevated risk of infectious illness transmission (28). Several investigations have indicated airborne and/or droplet transmission as the primary pathways for transmitting human and animal germs such as *E. coli*, *S. aureus*, *K. pneumonia*, and *P. Aeruginosa* (29, 32).

When the detected species were classified according to their families, it was seen that the species belonging to *Enterobacteriaceae*, which also included pathogens, were dominant, followed by the *Bacillaceae* family, which is generally composed of ubiquitous environmental bacteria, with 25%. Gund et al. (33), who applied a method similar to our study methodologically, reported that 79% of the masks of dental clinic workers were contaminated, and *Staphylococcus epidermidis*, the natural habitat of which was skin, was the most common. Similarly, in a study conducted with 130 dental clinic participants, the count and genus of microorganisms in used masks were determined by classical cultural techniques, and it was stated that bacterial species was predominated by *Staphylococci* species 26.35% followed by *Pseudomonas* 17.82% and *Streptococci* 15.50%. It is seen that the natural habitats of the bacteria detected in this study are the skin and nasopharynx (26).

One of the most striking results of this research was the distribution of the species identified from the masks according to their natural habitats. It was determined that 53.16% of the species were of intestinal origin. This shows that the hygiene attitudes of the employees are quite bad, and they gave misleading answers to the questions about hygiene in the questionnaire part of the research.

5. CONCLUSION

The current study was performed to determine mask usage habits and to assess the presence of bacteria and fungi among food industry employees. Participants of this study's cross-sectional questionnaire lacked sufficient information about how to use masks properly in the workplace. Although the majority reported to replace the mask on a regular basis, the results show that the main source of contamination in the mask is the users' hands and their tendency of putting them in locations like the mouth, arm, and pocket, creating external contamination. Similarly, although the majority (95.1%) of the food industry employees participating in the study claim that they have hand washing habits, it is thought that the microbial contamination in the masks they use is due to insufficient hygiene. The fact that 71.8% of the participants suffered from infections in the last year also supports this situation. The use of masks containing high levels of germs in food facilities owing to poor cleanliness and storage conditions is regarded as a cause of aerosol formation. In the present study, long-term working conditions, especially inaccuracies in the use of masks, poor hygiene attitudes of the employees, and the fact that the employees wear the mask out of necessity rather than its protective effect suggest that is why the poor microbiological quality of the analyzed masks. The results show that since most of the masks to protect yourself from COVID-19 and prevent infecting others used by food industry employees contain bacteria of intestinal origin, serious health problems may occur both in employees using contaminated masks and in consumers because of contamination of food by masks. Finally, the results could have been affected by the fact that the data was not collected at the beginning of the pandemic and, consequently, the tendency of people to pay fewer attention to masks or protective equipment with time. Therefore, this situation may be regarded as a limited aspect of the study.

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Author Contributions:
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Design of the study: AHD, MÖ
Acquisition of data for the study: AHD, EBÖ, ZÇ, JR
Analysis of data for the study: MÖ
Interpretation of data for the study: AHD, JR
Drafting the manuscript: MÖ, EBÖ, ZÇ, JR
Revising it critically for important intellectual content: AHD, MÖ
Final approval of the version to be published: AHD

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Clinical and Experimental Health Sciences

Experiences and Attitudes of Dentists with Different Demographic Characteristics Towards Dental Photography

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ABSTRACT

Objective: Dental photography is used for such purposes as the evaluation of treatment process, providing patients with information and motivating them, education/academic activities, and medico-legal recording. To investigate the prevalence of dental photography use and the experiences and attitudes of dentists with different demographic characteristics towards dental photography.

Methods: An online survey consisting of three parts as, which are demographic characteristics (age, sex, professional experience, institution of employment, and professional qualification), experiences, and attitudes of dentists towards dental photography, was prepared. A link of the survey was randomly sent to dentists by researchers. Descriptive statistical analysis and Chi-square tests were used.

Results: A total of 444 volunteer participants (female:56.8%; male:43.2%) were included in the study. Dental photography was seen to be used by 66.7% of the participants. The prevalence of dental photography use was higher in specialist dentists than in general dental practitioners. A statistically significant difference was found between the demographic characteristics and purpose of using dental photography and the considered receiving training on dental photography.

Conclusion: The prevalence of dental photography use was 66.7%. The experiences and attitudes of dentists towards dental photography can be diverse according to demographic characteristics.

Keywords: Dentist, dental photography, survey

1. INTRODUCTION

Photography is a combination of the word's 'photo' meaning light and 'graph' meaning drawing and refers to the processing of light. The first medical photography was used in the 19th century (1). Nowadays, photographic images as well as traditional patient records and radiography images are important for a patient's clinical record, in medical field. Photographs are commonly used in almost every clinical branch (2). Patient photographs used in dental clinics are called as dental photography.

Dental photography is the recording of healthy or pathological conditions in the soft and hard tissues of patients, extraoral and/or intraorally (3). Dental photography is commonly used in all areas of dentistry, especially in orthodontics and esthetic dental treatment. Dental photography is used for many purposes such as the clinical evaluation of case, evaluation of the treatment process (pre/post-operation), providing education/information/motivation to patients about treatment, to communicate between the dentist and the laboratory, to show the success of the restoration, education/academic activities, to consult with dentist or physician, teledentistry and forensic dentistry (4,5). Another important use of dental photography is for clinical marketing/advertising purpose in especially private clinics. The marketing tools can be considered such as practice brochures and newsletters, newspapers, journals, or web pages. It is also significant to follow local guidelines or ethical standard in this regard (4). In, addition, dental photography makes a positive contribution to the medico-legal process for both the dentist and the patient (2).

Although dental photography was previously applied with traditional methods, today it is generally practiced with digital methods. The developing digital world provides many advantages to dentists and patients, especially in taking and storing photographs. The photography is obtained in a short time. Improvements such as lighting, darkening, cropping, and rotating can be made to the photographs (6). Additionally, the photographs can be stored and retrieved at any time (7). Thus, digital dental photography is simple, fast, and very useful (5).

The standardization of dental photography procedures is critical for presentation. The procedure includes appropriate lighting, exposure, patient positioning, perspective, depth of

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field, and background (7). Therefore, a high-quality resolution reflecting both soft tissue and hard tissue details is desired, as well as a suitable color representation that includes the correct exposure in an ideal dental photograph image (8). For an appropriate color representation, the extraoral photo should be obtained in front of a dark background while the intraoral photo should be obtained using accessories such as cheek retractor, intraoral mirror, and black background/ contrasters (5,9).

The use of dental photography is easy and helpful. In many countries such as Turkey and India, dental photography is taught to students as a course in dentistry faculties (10). In addition, there are many review and research articles about technical details, purposes, and uses of dental photography, in the literature (11-16). However, there has been a limited survey research about dental photography (12-16). The aim of the present study is to investigate the prevalence of dental photography use and the experiences and attitudes of dentists with different demographic characteristics towards dental photography.

2. METHODS

Ethical approval was obtained from the Gazi University Ethical Committee for the present study (2020–661). The survey form was prepared for the present study based on the surveys were used in previous studies (12-16). The form consisted of fifteen questions grouped in three main parts.

Part-I: Demographic characteristic of the participants: Five questions about age, sex, professional experience, institution of employment, and professional qualification.

Part-II: The experiences of dentists towards dental photography: Seven questions on whether they use it or not, reasons for using/not using it, in which situation, type of consent, type of device and storing.

Part-III: The attitudes of dentists towards dental photography: Three questions on whether dental photography is necessary, whether they had previous training, and whether they consider receiving training.

The questions of the survey form were transferred to the online platform, and a survey link was created via Google Forms (Google Inc., Mountain View, California, USA) (www.google.doc). The link was sent randomly to the dentist via e-mail or WhatsApp© (WhatsApp Inc., Mountain View, California, USA) message by researchers. Participation was voluntary. The identities of the participants were not recorded. Since the answers given by the volunteer participants to the survey questions constituted the study data, they were recorded as Microsoft Excel (.xlsx) tables via Google Forms.

Statistical analysis

The Statistical Package for Social Sciences (SPSS), Windows version 23.0 (SPSS Inc. Chicago, USA) program was used for statistical analysis. Descriptive statistical analysis, which included frequencies and percentages, and cross tabulation was used to report the data. Association with the factors was tested for significance using Pearson chi-square tests. $P < .05$ was accepted to be statistically significant.

Sample size calculation were accepted at the 5% precision, and 36% prevalence (the prevalence of dental photography use in the previous study), and 95% confidence interval (6). The number of samples was calculated using Epi info Statcalc (CDC, Atlanta, USA) program as 354.

3. RESULTS

A total of 444 dentists (minimum age 24, maximum age 65) were included in the present study. The original survey form and the distributions of the responses, in terms of numbers and percentages, are shown in Table 1-3.

Table 1. *Distribution of demographic characteristics in Part-I, according to their responses on original survey, presented as N (%) with N=444*

Part-I: Demographic characteristics of the participants			N (%)
Age			35.1±10.5#
Sex	Female		252 (56.8 %)
	Male		192 (43.2 %)
Professional experience	0–5 years		171 (38.5 %)
	6–10 years		100 (22.5 %)
	≥ 11 years		173 (39 %)
Institution of employment	Private clinic		218 (49.1 %)
	Public clinic		100 (22.5 %)
	University		126 (28. 4%)
Professional of qualification	General dental practitioner		225 (51.7 %)
	Specialist dentist		219 (49.3 %)
		Oral and Maxillofacial Surgery	26 (5.9 %)
		Oral and Maxillofacial Radiology	47 (10.6 %)
		Endodontic	16 (3.6 %)
		Oral Pathology	1 (0.2 %)
		Orthodontic	35 (7.9 %)
		Pediatric Dentistry	20 (4.5 %)
		Periodontology	25 (5.6 %)
		Prosthodontics	43 (9.7 %)
		Restorative Dentistry	6 (1.4 %)

 # : mean±standart deviation

A statistically significant difference was found between the prevalence of dental photography use and the institution of employment and professional qualification ($p < .05$). The prevalence of dental photography use by dentists working in private clinics and universities was higher than that of

dentists working in the public clinics. Specialist dentists' prevalence of dental photography use was higher than that of the general dental practitioner (Table 4).

A statistically significant difference was found between the purpose of using dental photography and the institution of employment, and professional qualification ($p < .05$). The communicating with the laboratory and education/academic activities showed statistically significant differences according to institution of employment and professional qualifications (Table 5).

A statistically significant difference was found between those who received training in dental photography and the institutions they worked in ($p < .05$). The rate of those who were trained about dental photography was the lowest in the public clinic (Table 6).

A statistically significant difference was observed between those who were considered receiving training on dental photography and their sex, professional experience, and institution of employment ($p < .05$) (Table 6).

Table 2. *Distribution of experiences of dentists towards dental photography in Part-II, according to their responses on original survey, presented as N (%) with N=444*

Part-II: Experience of dentists towards dental photography		N (%)
Do you use dental photography for cases in the clinic?	Yes	296 (66.7 %)
	No	148 (33.3 %)
What is your purpose(s) of using dental photography? *	For the clinical evaluation of case	140 (47.3 %)
	For the evaluation of treatment process	247 (83.4 %)
	For patient information/motivation	175 (59.1 %)
	Communicating with the laboratory	141 (47.6 %)
	For consultation among physicians/dentists	121 (40.9 %)
	For education/academic activities	143 (48.3 %)
	For clinical promotion	75 (25.3 %)
	For medico-legal condition	59 (19.9 %)
	For interest	87 (29.4 %)
What is your reason for not using dental photography? *	No interest/need	53 (35.8)
	Due to lack of time	107 (72.3%)
	Cost burden	15 (10.1%)
	Difficult infection control	10 (6.8%)
In which cases do you use of dental photography?	All cases	52 (17.5 %)
	Only in special cases	244 (82.5 %)
What type of consent do you take from the patient before taking the photography?	Written consent	40 (13.5%)
	Verbal consent	150 (50.7%)
	Written and verbal consent	66 (22.3%)
	No consent	40 (13.5%)
Which device do you use to take the photo?*	DSLR camera	85 (28.7%)
	DSLR with ring/twin flash camera	79 (26.7%)
	Video camera	15 (5.1%)
	Smartphone camera	202 (68.2 %)
How do you store the photography? *	Personal or clinic computers	181 (61.1%)
	Portable storage (disc, usb flash)	118 (39.9%)
	On the internet (Google drive, icloud, e-mail)	58 (19.6%)
	In the memory of the used device	140 (47.3%)

**:Participants can select more than one answer.*

Table 3. *Distribution of attitudes of dentists towards dental photography in Part-III, according to their responses on original survey, presented as N (%) with N=444*

Part-III: Attitudes of dentists towards dental photography N (%)		
Is dental photography necessary in the clinic?	Yes	326 (73.4 %)
	No	3 (0.7 %)
	Sometimes	115 (25.9 %)
Have you received any training (lecture, course, etc.) about dental photography?	Yes	129 (29.1 %)
	No	315 (70.9 %)
Would you consider receiving training for dental photography?	Yes	327 (73.6 %)
	No	117 (26.4 %)

Table 4. *Distribution of the prevalence of dental photography use according to demographic characteristics, N (%) and results of the statistical analysis*

Demographic characteristics	Do you use dental photography for cases in the clinic?			
		Yes N (%)	**No N (%)**	*p-value*
Sex	Female	177 (59.8%)	75 (50.7%)	*.067*
	Male	119 (40.2 %)	73 (49.3%)	
Professional experience	0–5 years	109 (36.8%)	62 (41.9%)	*.200*
	6–10 years	63 (21.3%)	37 (25%)	
	≥ 11 years	124 (41.9%)	49 (33.1%)	
Institution of employment	Private clinic	158 (53.4%)	60 (40.5%)	*.000**
	Public clinic	27 (9.1%)	73 (49.3%)	
	University	111 (37.5%)	15 (10.1%)	
Professional qualification	General dental practitioner	140 (47.3%)	85 (57.4%)	*.044**
	Specialist dentist	156 (52.7%)	63 (42.6%)	

**: statistically significant at the level p<.05*

Table 5. *Distribution of the purpose of using dental photography according to the institute of employment and professional qualification, N (%) and results of the statistical analysis*

Purpose of using dental photography	Demographic characteristics						
	Institute of employment			*p*-value	Professional qualification		*p*-value
	Private clinic N=158 N (%)	**Public clinic N=27 N (%)**	**University N=111 N (%)**		**General dental practitioner N=140 N (%)**	**Specialist dentist N=156 N (%)**	
For the clinical evaluation of case	87 (55.1%)	14 (51.9%)	39 (35.1%)	.000*	74 (52.9%)	66 (42.3%)	.000*
For the evaluation of treatment process[&]	145 (91.8%)	24 (88.9%)	78 (70.3%)		126 (90%)	121 (77.6%)	
For patient information/ motivation[&]	113 (71.5%)	16 (59.3%)	46 (41.4%)		91 (65%)	84 (53.8%)	
Communicating with the laboratory[&#]	105 (66.5%)	10 (37%)	26 (23.4%)		80 (57.1%)	61 (39.7%)	
For consultation among physicians/dentists	65 (41.1%)	12 (44.4%)	44 (39.6%)		59 (42.1%)	62 (39.7%)	
For educational/academic activities[&#]	36 (22.8%)	21 (12.4%)	96 (86.5%)		49 (35%)	94 (60.3%)	
For clinical promotions[&]	58 (36.7%)	0 (0%)	17 (15.3%)		34 (24.3%)	41 (26.3%)	
For medico-legal condition	41 (25.9%)	5 (18.5%)	13 (11.7%)		26 (18.6%)	33 (21.2%)	
For interest	52 (32.9%)	10 (37%)	25 (22.5%)		40 (28.6%)	47 (30.1%)	

**: statistically significant at the level p<.05, &: statistically significant the according to institution of employment, #: statistically significant the according to professional qualification*

Table 6. *Distribution of the attitudes of dentists towards dental photography according to the demographic characteristics, N (%) and results of the statistical analysis*

Demographic characteristics		Have you received any training about dental photography?			Would you consider receiving training for dental photography?		
		Yes N (%)	No N (%)	*p-value*	Yes N (%)	No N (%)	*p-value*
Sex	Female	74 (57.4%)	178 (56.5%)	*.869*	197 (60.2%)	55 (47%)	*.013**
	Male	55 (42.6%)	137 (43.5%)		130 (39.8%)	62 (53%)	
Professional experience	0–5 years	47 (36.4%)	124 (39.4%)	*.454*	143 (43.7%)	28 (23.9%)	*.001**
	6–10 years	26 (20.2%)	74 (23.5%)		70 (21.4%)	30 (25.6%)	
	≥ 11 years	56 (43.4%)	117 (37.1%)		114 (34.9%)	59 (50.4%)	
Institution of employment	Private clinic	76 (58.9%)	142 (45.1%)	*.008**	155 (47.4%)	63 (53.8%)	*.011**
	Public clinic	18 (14%)	82 (26%)		67 (20.5%)	33 (28.2%)	
	University	35 (27.1%)	91 (28.9%)		105 (32.1%)	21 (17.9%)	
Professional qualification	General dental practitioner	64 (49.6%)	161 (51.1%)	*.774*	160 (48.9%)	65 (55.6%)	*.219*
	Specialist dentist	65 (50.4%)	154 (48.9%)		167 (51.1%)	52 (44.4%)	

**: statistically significant at the level p<.05*

4. DISCUSSION

The use of dental photography offers numerous benefits to dentists. It has many different uses. The characteristics such as the institution of employment, professional qualification, and professional experience of dentist can affect the behavior towards dental photography. In the present study, the experiences, and attitudes of dentists with different demographic characteristics towards dental photography were evaluated.

Dental photography is used more and more every day. The prevalence of dental photography use was 36% in a study conducted in 2004, was 48% in a study conducted in 2010, and was 71% in a study conducted in 2018 (12-14). In the present study, the prevalence of dental photography use was found 66.7%. The increase in the interests of dentists towards dental photography, as well as the development of technological opportunities, may have contributed to the increase in the dental photography use over time.

The purpose of using dental photography is diverse. Previous studies reported that the purpose of using dental photography is generally for treatment planning, evaluation of treatment process, patient information, and medico-legal conditions (12-16). In addition to these survey studies for dentists, survey studies were also conducted on dental students. In studies conducted among dentistry students, the main reasons for the use of dental photography were reported as medico-legal conditions, patient education, and treatment planning (10-17). In the studies conducted, the purpose of using dental photography among both dentists and dentistry students are generally similar. In the present study, the purpose of using dental photography was found as the evaluation of treatment process, patient information/motivation, and communication with the laboratory. These findings are consistent with previous findings (12,13).

The dentist should obtain both verbal and written consent from the patient for the photograph. In the previous studies, it was stated that the rate of verbal consent obtained from the patient before a dental photograph taken was higher than written consent (14,17). In the present study, this rate was consistent with the previous studies. The dentist should be explained detailly to the patients the purpose, type, and location of use, storage location and duration, and estimated results of the photograph taken to the patient (2,18). In addition, written consent can be useful important especially in any medico-legal condition. Therefore, written consent should be obtained, and these consents should be stored under appropriate conditions.

Various devices are used for dental photography. DSLR cameras are the most recommended devices. The DSLR camera offers users the convenience of portability, auto exposure, and the use of special synchronized flashes (8). In previous studies, the use of DSLR cameras has ranged from 17% to 64% (10,12,13). In the present study, the rate was 38%. In previous studies, it was reported that the most used device is a smartphone camera for dental photography (10,11,13,15). In the present study, in accordance with previous studies, it was determined that the most used device was the smartphone camera. Mobile phones are frequently used in our daily life, are easily accessible, simple to use, and do not take much time.

Different characteristics of dentists can affect the experience and attitude towards dental photography. In a previous

study, it was reported that the prevalence of dental photography use has higher in males than females, in private clinics than in public clinics, and in specialist dentists than in general dental practitioners (12). Additionally, in previous studies, it has been reported that the prevalence of dental photography use was higher in dentists with 10 or more years of experience than other participants (16). In the present study, dental photography was found to be used statistically more by dentists working in private clinics and universities than dentists working in the public clinics and by specialty dentists than general dental practitioners. A wide variety of factors such as clinical conditions, interest, qualification and experience of dentist, patient consent, and device adequacy can affect the experiences and attitudes of dentist towards dental photography.

This study has some limitations. The first limitation, as in other observational studies, is the internal limitation that associations can only be made with certain variables. Additionally, the findings belong to a certain time-period. Another limitation is that we kept the number of questions relatively short in order not to lengthen the survey and not to bore the participants, so that the objectionable aspects of the photographs were not addressed in the survey questions.

5. CONCLUSION

In the present study, the prevalence of dental photography use was 66.7% in dental clinics. The experiences and attitudes of dentists towards dental photography can differ according to demographic characteristics. The awareness of dentists should be increased in terms use of dental photography which has numerous benefits. Dentists should be supported with courses and training on this subject.

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Author Contributions:

Research idea: N.B.

Design of the study: N.B., İ.P.

Acquisition of data for the study: N.B.

Analysis of data for the study: N.B.

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Drafting the manuscript: N.B.

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Clinical and Experimental Health Sciences

Evaluation of Bone Microstructure Parameters by Using Tomographic Methods and Compressive Strength Estimation

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ABSTRACT

Objective: The aim of this study was to evaluate the microstructure of the mandible by micro computed tomography (µCT), cone beam computed tomography (CBCT) and computed tomography (CT) and to estimate the compressive strength of the bone based on the values obtained by these methods.

Methods: Thirty specimens obtained from ex-vivo sheep mandible were scanned by µCT cone beam computed tomography and computed tomography. These specimens were also subjected to compression testing and compression strength values were calculated. Morphometric parameters were evaluated using ImageJ software Bland-Altman lower upper bound agreement and ICC coefficient were used to evaluate the agreement between the tomography methods used and the gold standard. Linear and multivariate stepwise regression analysis was performed to calculate the compression strength value based on the radiomorphometric parameters. Statistical significance level was accepted as .05.

Results: Bone Surface/Total Volume, Bone Volume/Total Volume and Degree of Anistoropy parameters evaluated by CBCT and Fractal Dimension parameter evaluated by CT showed a statistically significant agreement with the gold standard method µCT. Bone Volume/Total Volume and Degree of Anistoropy parameters obtained with µCT (R^2:0.75), Bone Volume/Total Volume, Degree of Anistoropy , Connectivity Density parameters (R^2:0.62), and the Structure Model Index parameter (R2:0.13) obtained by CT can be used to predict the compression strength value.

Conclusion: Bone compression strength can be estimated by CBCT and µCT methods in a desired level. Bone Volume/Total Volume and Degree of Anistoropy parameters are significant determinants of bone mechanical property in not only µCT but also CBCT method.

Keywords: computed tomography, cone beam computed tomography, micro computed tomography, compression strength.

1. INTRODUCTION

In dental practice, predicting the quality and quantity of the alveolar bone increases the success of treatments. The ability to make these predictions can greatly increase the success of periodontal, orthodontic, and surgical treatments, particularly implant applications (1). In the past years, bone structure analyses have mainly been based on bone mineral density measurements (2). However, studies have shown that measuring only bone mineral density is insufficient for these analyses and that trabecular bone microstructure analysis should also be performed (3). The bone structure histologically consists of trabecular and cortical structures. The trabecular structure is more active in the metabolic metabolic procedure and more effective in bone remodeling than the cortical bone because it has a larger surface area (4). This causes the trabecular bone to be more affected during the bone resorption mechanism. However, the standard morphologic parameters used in bone microstructure evaluations are as follows: the ratio of the bone volume (BV) to the total bone volume (TV) i.e., bone volume to total volume the relative volume of calcified tissue in the selected volume of interest (BV/TV), trabecular thickness; mean thickness of trabeculae, assessed using direct 3D method (Tb/Th), bone surface (BS), trabecular separation; mean distance between trabeculae, assessed using direct 3D methods (Tb/Sp), trabecular number; number of trabecules that crosses a particular one pe runit of length across the VOI; (Tb/N), cortical thickness; mean thickness of cortical bone assessed using direct 3D method (Ct/Th), connectivity density; examination of thinning and thickening of trabecular bone in each volume set (Con. Dens), degree of anisotropy; which is the presence or absence of aligned trabecules in a particulardirection (1 is considered isotropic,>1 is considered anisotropic (DA), fractal dimension; which indicates the complexity ofthe specimen surface (FD), and structural model index; which gives information aboutpreponderance of trabecular morphology (0 is an ideal plate, whereas 3 is an ideal cylinder) (SMI) (5). The gold standard for the two-dimensional evaluation of bone microstructures is histologic

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or histomorphometric evaluation, and the gold standard for three-dimensional (3D) evaluation is microcomputed tomography (µCT) (6,7).

The computed tomography (CT), method, particularly multislice computed tomography (MSCT), has a larger radiation dose than other bone quality assessment methods (8,9). Kulah et al. compared the images they obtained using two different cone-beam computed tomography devices with voxel sizes in the range of 0.075 to 0.2 mm and field of view (FOV) of 40×40 to 80×80 with the images obtained by the micro computed tomography device, which is considered the gold standard among tomography methods, for bone microstructure parameters. As a result of their research, they demonstrated that the bone microstructure parameters, specifically BV/TV and DA, obtained from the images acquired with the low voxel size cone-beam computed tomography device exhibited the highest compatibility with micro computed tomography and could be used as an alternative (10). Ibrahim et al. evaluated bone structure in human cadavers using a cone-beam computed tomography device with a voxel size of 0.08 mm. They investigated which parameter exhibited better compatibility with the gold standard. It was observed that trabecular number showed the best agreement with micro computed tomography results, followed by trabecular thickness and trabecular separation parameters (11).

2. METHODS

All procedures performed in studies involving animal researches local ethic committee rules. The study was approved by Van Yüzüncü Yıl University Animal Researches Local Ethic Committee (06.02.2020-approval number: 2020/01).

2.1. Sample Preparation

Thirty samples were obtained ex vivo from sheep mandibles. The samples were obtained by inserting a 10*3 mm trepan burr parallel to the axial plane. To prevent moisture loss, the samples were wrapped in cotton wool impregnated with saline solution and stored at – 20 °C (Figure 1).

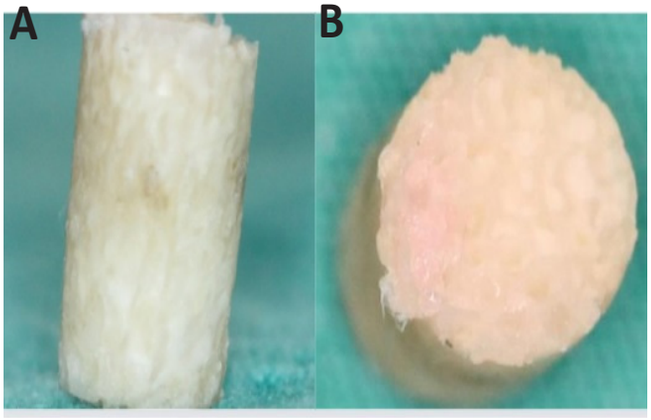

Figure 1. *Image of samples.*

2.2. Acquisition of µCT Images

Each sample were placed perpendicular to the ground plane and scanned (SkyScan, Kontich, Belgium) at 80 kVp, 125 µA, a scan time of 50 ms, and a section thickness of 10.00 µm.

2.3. Acquisition of CBCT Images

Each sample was placed perpendicular to the ground plane and scanned using a KaVo 3D eXam cone beam tomography device (KaVo Dental, Biberach, Germany) at 120 kVp, 5 mAs, a scan time of 7 s, a voxel size of 0.125 mm, and 16*4 cm FOV (Field of View).

2.4. Acquisition of CT Images

The samples were placed parallel to the ground plane in accordance with the scanning axis and scanned at a voxel size of 0.625 mm using a 2011 GE brand Brightspeed Model 16-section CT scanner (Healthcare, Milwaukee, WI) with a 12226 Exam X-ray tube at 120 kVp, 69 mA, and a scan time of 0.6 s.

2.5. Radiologic Analysis

To equalize the different slice thicknesses of the devices, all the CT and CBCT images were analyzed using five slice intervals, while the µCT images were analyzed using sixty-two slice intervals. All the consecutive slice images were analyzed and aligned using the study protocol of Panmekiate et al.. The bone microstructure parameters were evaluated using ImageJ-Bone J software (National Institutes of Health, USA) (Figure 2) (12). All transferred images were converted into a single file containing consecutive images (Image-Stacks-Images to Stack). The images were manually drawn to include the area of bone (ROI) to be analyzed (Rectangle Selection). The file containing the areas to be analyzed was converted to black and white image format (Process-Binary-Make Binary). The prepared images were subjected to plugins-bonej-analyse skeleton, anisotrophy, connectivity, fractal dimension, isosurface, structure model index, thickness, volume fraction processes in the ImageJ program. In the evaluation of Connectivity Density parameter in radiological images, in addition to the algorithms applied to other parameters, a purification process has also been conducted as part of the procedure. All procedures were repeated for images of thirty specimens obtained with three different tomography imaging methods.

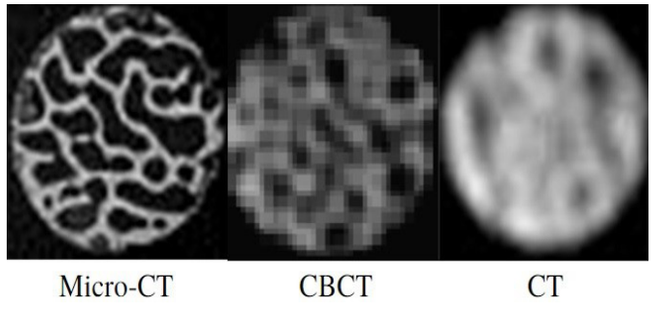

Figure 2. *Images of bone samples taken with three different tomography methods. (Micro-CT: micro computed tomography, CBCT: cone beam computed tomography, CT: computed tomography.)*

2.6. Compression Test Experiment

In order to keep the effect of temperature on the sample's constant, each sample was kept in separate boxes in a – 20 degree freezer. Each sample was placed on the lower jig table of the device with its long axis perpendicular to the ground plane. All compression tests were performed using a universal testing machine (Shimadzu Corporation, Kyoto, Japan). Each sample has been placed on the lower jig table of the device with its long axis perpendicular to the horizontal plane to prevent its movement against the forces and to secure the sample in place, using pink base plate wax of the same thickness as the table at the pre-determined exact center. The device has been calibrated after each setup to ensure it is in the appropriate position for testing. After the device is turned on, a compression test template is created in the software program on the computer by entering the properties of the samples to be tested. In the compression strength test, force is applied in the direction parallel to the long axis of the sample. Before starting the test, the upper jig, which will apply the force, is brought to the allowed shortest distance from the sample without making contact, using the manual movement button. Once the test begins, the applied force has shown a rhythmic and consistent increase. The force increase rapidly decreases and resets to zero after reaching the maximum force at which the samples deform. The compression strength of the sample is recorded, considering the highest force applied by the device before the samples deform.

2.7. Statistical Analysis

The Bland–Altman test was used to evaluate whether the CBCT and CT methods could be alternatives to the µCT method, which is considered the gold standard. Linear regression and multiple stepwise regression analyses were performed to predict compressive strength based on the microstructure parameters. All the analyses were performed using SPSS software (IBM SPSS Statistics 20.0; IBM Co., Armonk, NY, US

3.RESULTS

3.1. Comparison of CBCT and µCT Tecniques

There was a statistically significant moderate agreement between the DA and BV values obtained using the CBCT and µCT methods (ICC = .484 and p < .001; ICC = .537 and p < .05). This parameter was statistically significant between CBCT and µCT but not statistically significant between the CT and µCT values.

There was a statistically significant poor agreement between the BS/TV and BS (Bone Surface) values obtained using the CBCT and µCT methods (ICC = .319 and p < .05; ICC = .294 and p< .05). No statistically significant agreement was observed between the CT and µCT values of this parameter.

There was a statistically significant good agreement between the BV/TV and TV values obtained using the CBCT and µCT methods (ICC = .703 and p < .001; ICC = .673 and p < .001). No statistically significant agreement was observed between the CT and µCT values of this parameter.

3.2. Comparison of CT and µCT Tecniques

There was a statistically significant moderate agreement between the FD values obtained using the CT and µCT methods (ICC = .474 and p < .05). No statistically significant agreement was observed between the CBCT and µCT values of this parameter (Table 1).

Table 1. *Bland and Altman limits of agreement of all assessed parameters.*

	MD	SD	Limits of Aggrement (%95 CI)		ICC (%95 CI)	p
			Lower	Upper		
BS/TV						
CBCT – µCT	0.043	0.042	-0.039	0.124	0.319 (-0.206 – 0.648)	**<.05**
CT – µCT	0.119	0.049	0.022	0.216	0.035 (-0.077 – 0.208)	>.05
BV/TV						
CBCT – µCT	0.027	0.064	-0.098	0.153	0.703 (0.376 – 0.859)	**<.001**
CT – µCT	0.222	0.065	0.094	0.350	0.023 (-0.042 – 0.138)	>0.05
BS						
CBCT – µCT	1965.340	1281.169	-545.752	4476.432	0.294 (-0.208 – 0.645)	**<.05**
CT – µCT	2626.991	1468.520	-251.309	5505.292	-0.047 (-0.208 – 0.186)	>.05
BV						
CBCT – µCT	606.133	3690.823	-6627.880	7840.147	0.537 (0.03 – 0.779)	**<.05**
CT – µCT	778.400	4508.492	-8058.245	9615.045	0.08 (-0.938 – 0.563)	>.05
TV						
CBCT – µCT	-2166.667	5675.618	-13290.879	8957.545	0.673 (0.326 – 0.843)	**<.01**
CT – µCT	-18666.667	9907.619	-38085.600	752.267	0.091 (-0.124 – 0.356)	>.05
FD						
CBCT – µCT	-0.143	0.285	-0.702	0.416	-0.123 (-0.962 – 0.408)	>.05
CT – µCT	0.098	0.199	-0.292	0.488	0.474 (-0.03 – 0.741)	**<.05**
DA						
CBCT – µCT	0.208	0.140	-0.066	0.483	0.484 (-0.236 – 0.798)	**<.001**
CT – µCT	0.180	0.308	-0.424	0.784	-0.386 (-1.398 – 0.27)	>.05

MD: Mean Deviation, SD: Standart Deviation, CI: Confidence Interval, ,BS/TV: Bone Surface/Total Volume, BV/TV: Bone Volume/Total Volume, BS: Bone Surface, BV: Bone Volume, TV :Total Volume, FD: Fractal Dimension, DA: Degree of Anisotrophy. p<.05

The statistically significant parameters are shown in the linear regression model, along with the microstructure parameters obtained using the three tomography methods (Table 2).

Table 2. *Linear regression models showing the relationship between microstructure parameters analyzed by µCT, CBCT, CT and compressive strength.*

	BS/TV	BV/TV	FD	Tb/Th	DA	Con. Dens	SMI
µCT							
R^2	0.279	0.612	0.158	0.389	0.303	NS	NS
F	10.812	44.08	5.262	17.862	12.195	NS	NS
CBCT							
R^2	0.356	0.431	NS	NS	0.302	0.165	NS
F	15.649	21.242	NS	NS	12.123	5.534	NS
CT							
R^2	0.134	NS	NS	NS	NS	NS	0.155
F	4.338	NS	NS	NS	NS	NS	5.133

NS: not significant. BS/TV: Bone Surface/Total Volume, BV/TV: Bone Volume/Total Volume, FD: Fractal Dimension, Tb/Th: Trabecular Thickness, DA: Degree of Anisotrophy, Con. Dens: Connectivity Density, SMI: Structure Model Indeks. µCT: Micro Computed Tomography, CBCT:Cone Beam Computed Tomography, CT: Computed Tomography.

3.3. Multiple Stepwise Regression Analyse of Bone Parameters of µCT Scanning

A multiple stepwise regression model was created to predict the compressive strength of the bone specimens based on the BV/TV and DA values obtained using µCT. The calculated regression model was statistically significant (F (2,25) = 42.255; p < .001), with R^2 = .753. The estimated compressive strength of the bone specimens was equal to – 278.442 + 2825.965 *BV/TV – 49.432*DA. Both the BV/TV and DA parameters, which were analyzed using the µCT method, were statistically significant determinants (Table 3).

BV/TV, DA and Con. Dens values, a multiple stepwise regression model was created to predict the compressive strength of bone specimens. The calculated regression model was statistically significant (F (3, 25) – 16,149, p<.05) and R^2=.619. The estimated compressive strength of the bone specimens is equal to 751.328+750.919*BV/TV-711.786*DA-69490.423*Con. Dens. BV/TV DA and Con. Dens parameters are statistically significant determinants (Table 3).

3.4. Multiple Stepwise Regression Analyse of Bone Parameters of CT Scanning

A multiple stepwise regression model was created to predict the compressive strength of bone specimens based on the SMI value, one of the bone microstructure parameters analyzed by CT. The calculated regression model was statistically significant (F (1, 28) = 5.133, p< .05) and R^2=.125. The estimated compressive strength of the bone specimens is equal to 639,677-132,453*SMI. The SMI parameter, which is one of the bone microstructure parameters analyzed in the CT method, is a statistically significant determinant (Table 3).

Table 3. *Multiple stepwise regression models showing the relationship between microstructure parameters analyzed by µCT, CBCT, CT and compressive strength.*

	β^1 (%95 CI)	SE	β^2	t	p
µCT					
Constant	-278.442 (-536.395-20.489)	125.248		-0.223	.035
BV/TV	2825.965 (2016.424+3635.505)	393.069	0.699	7.189	.000
DA	-349.4321 (-517.603-181.261)	81.655	-0.416	-0.279	.000
CBCT					
Constant	751.328 (174.774+1327.881)	279.943		2.684	.013
BV/TV	750.919 (-26.101+1527.938)	377.279	0.323	1.99	.058
DA	-711.786 (-1162.972-260.599)	219.072	-0.448	-3.249	.003
Con. Dens.	-6940.423 (-126176.934.12803.912)	27523.889	0.379	-2.525	.018
CT					
Constant	63.677 (336.372+942.983)	148.069		4.320	.000
SMI	-132.453 (-252.11-12.696)	58.464	-0.394	-2.266	.031

B^1: Unstandardized beta coefficient, SE: Standard error, β^2: Standardized beta coefficient, CI: Confidence Interval. BV/TV: Bone Volume/Total Volume, FD: Fractal Dimension, Degree of Anisotrophy, Con. Dens: Connectivity Density, SMI: Structure Model Indeks. µCT: Micro Computed Tomography, CBCT:Cone Beam Computed Tomography, CT: Computed Tomography.

4. DISCUSSION

CBCT is commonly utilized to assess bone quality, with a primary emphasis on bone density, as observed in studies such as those conducted by Corpas et al., Ibrahim et al. (3,13). However, to gain a more comprehensive understanding of its impact on the integration of implants with bone tissue, it is imperative to consider bone quality from the microstructural perspective of trabecular bone, as underscored in research by Gonzalez-Garcia et al. (14). Trabecular bone microstructure not only plays a vital role in bone healing and implant stability, as elucidated by Minkin and Marinho, but it also significantly contributes to overall bone strength, as indicated by studies such as Manske et al. (15,16). In this study, we evaluated all the parameters evaluated in the previous studies in three different tomography devices and tried to learn the parameters that best comply with the gold standard and to evaluate the estimation of bone compression strength based on these parameters. We also tried to understand the preferability of the cone beam computed tomography method according to the gold standard.

Limitations of this study can be related with organic components of bone tissue. For example, some authors found an association between bone fracture and bone collagen integrity (17). Optimal ratio of bone organic-inorganic components was reported as a fundamental determinant of bone mechanical quality (18). In some studies, it has

been stated that the ratio of "bound and pore water" is associated with bone fragility (19). Also, deteriorated micro-arrangement between collagen fibrils and apatite crystals was responsible for reduced bone strength (20). As proven by the studies above, organic component is affective on bone strength, However, we did not include a organic bone parameter to this study.

Knowledge about the microstructure and quality of the trabecular bone structure before surgical procedures and in some systemic diseases affecting the bone structure is very important in the success and prognosis of treatment. Radiologic evaluation of bone quality will provide clinicians with very useful information. In recent studies, it is argued that Magnetic Resonance Imaging (MRI) method may be an alternative for trabecular and cortical bone evaluations since it does not contain ionizing radiation (21, 22) and MRI can evaluate organic component and water ratio of bone (19).

With the emergence of new techniques for measuring bone fragility, using not only the mass but also the microstructure of the bone has proven to be very effective in calculating the strength of the bone. Many researchers argue that bone mineral density is still very important in determining the strength of bone. However, many recent studies have shown that microstructure parameters alone, independent of knowledge of mineral density, are sufficient for evaluating bone strength (23).

Goulet et al. used µCT to examine the microstructure of trabecular bone and mechanical tests to evaluate the relationship between the elastic modulus and the total strength of the bone. The researchers concluded that BV, connectivity parameters, and number of trabeculae are highly effective parameters in determining mechanical properties. They stated that increases in the BV parameter are naturally associated with an increase in the density value (24).

In addition, Ding et al. used µCT to investigate microstructural changes in trabecular bone samples taken from patients with early osteoarthritis. They evaluated the relationship of the data they obtained to the strength and failure energy values of the bone. In the regression model they derived, they showed that none of the microstructure parameters could determine the strength and failure energy values of the bone as well as the SMI parameter (25). In the present study, the relationship between the values obtained as a result of µCT evaluation of the microstructure of the bone and the compressive strength of the bone was evaluated, and it was seen that the SMI (R2: 16%) and BS/TV (R^2: 13%) parameters were statistically effective, although the percentage of model identification was low. An increase in the SMI parameter is associated with an increase in the number of rod-shaped trabeculae, whereas an increase in the BS/TV value is associated with an increase in bone mineral density (14,26). In addition, recent studies using the gold standard µCT have shown that some new parameters are statistically more effective in determining mechanical properties than the SMI parameter (27,28).

Maquer et al. proved that the combined evaluation of BV/TV and DA parameters, which are microstructure parameters evaluated by µCT, are the most effective variables in calculating the young's modulus of trabecular bone (27). But in their µCT study on the stiffness and yield strength of trabecular bone, Musy et al. concluded that BV/TV and DA parameters were the best parameters for predicting the mechanical properties of bone, accounting for more than 98% of the regression model (28). Using the histomorphometric method of two-dimensional imaging, Han et al. evaluated the microstructure of samples taken from the knee bones of patients with osteoarthritis (29). They also calculated the Osteoarthritis Research Society International (OARSI) values of these samples. The OARSI classification system is an atlas-based grading system that evaluates osteophyte formation and lateral compartment narrowing (29, 30) concluded that the higher the BV/TV parameter, the higher the OARSI value for bone and cartilage. In the present study, multiple stepwise regression models created with BV/TV and DA parameters obtained by using µCT (the gold standard of ex vivo evaluation) and CBCT imaging (widely used in dentistry applications) were found to be highly effective in determining the compressive strength of trabecular bone. The DA parameter is related to the direction of the stress to which the bone is exposed; it is a numerical indication of the three-dimensional configuration of the bone structure (30). A high DA value means that the trabeculae in the trabecular bone are more frequently aligned in the same direction (31). When trabeculae are aligned in the same direction, the ability to absorb the applied force declines. Thus, there is a negative correlation between the compressive strength of bone and the DA parameter. This alignment also explains why similar microstructure parameters have a similar effect in determining the mechanical properties of bone.

Kang et al. investigated the extent to which implant stability is affected by the microstructure parameters of bones obtained from pigs (26). They used both µCT imaging and CBCT imaging to analyze the microstructure, and they compared their data with the stability according to peak frequency SPF (Implant Stability Criterion) parameter used to determine the primary stability of implants. The BV/TV, BS/TV (BSD), and SMI parameters, from data obtained by µCT imaging, were the variables in the model with the highest descriptiveness. In the present study, among the data obtained by µCT imaging, the BV/TV parameter alone accounts for approximately 58.9% of the compressive strength regression model. Teo et al. reported that a decrease in con. dens. value will not affect the BV and BV/TV values but will lead to a loss of strength in the bone (32). This suggests that the con. dens. parameter should be included in the multiple stepwise regression model between CBCT microstructure parameters and compressive strength. Again, using the CT method to evaluate the microstructure parameters, we observed a negative correlation between compressive strength and the SMI parameter. Kang et al. also found a negative correlation between the SMI value and SPF. This can be interpreted as signifying an increase in the number of rod-shaped trabeculae, which weakens both

the osseointegration between the implant and bone and the compressive strength of the bone to resist forces (26).

Using µCT, Ding et al. analyzed trabecular bone samples obtained from osteoarthritic and osteoporotic human hips and subjected them to compression testing. They concluded that patients with similar BV/TV values did not show similar compressive strength. They argued that the BV/TV parameter is largely effective in determining compressive strength but that factors such as abnormal collagen structure and degree of mineralization may also affect this determination (33). In present study, the parameters affecting the compressive strength were investigated and it was concluded that the parameters such as BV/TV, BS/TV DA Tb/Th and FD evaluated by µCT were statistically effective parameters on compressive strength. In addition, parameters such as BV/TV, BS/TV DA and Con. Dens. In fact, our study supports the study of Ding et al. and clarifies what other parameters are effective on compressive strength.

Müller et al. argued that a resolution thickness higher than 100 micrometers should not be used when analyzing trabecular bone with µCT (34). For this reason, in present study, we performed image acquisition ten times more detailed than the recommended thickness (10 micrometers).

Pauwels et al. investigated how different voxel sizes and kVp values in CBCT imaging have an effect on the microstructural parameters of bones obtained from human mandible. The results showed that the kVp value had no significant effect on most parameters. BV/TV and DA parameters were not affected by voxel size changes. BS/TV, FD and Con. Dens values decreased gradually with increases in voxel size, while Tb/Sp, Tb/Th and SMI values increased (35). In the regression model between bone microstructure parameters and bone compressive strength, BV/TV DA and Con. Dens. parameters were observed. Considering the results of Pauwels et al. study, BV/TV and DA parameters were not affected by changes in voxel size, whereas Con. Dens. parameter changes inversely with voxel size. In the regression model, the Con. Dens parameter and compressive strength in the regression model, it can be interpreted that increases in voxel size will cause an increase in compressive strength.

Diederichs et al. evaluated the microstructure of human calcaneus bone specimens with both MSCT and µCT. They performed MSCT imaging in three different protocols (120 kVp, 200 mAs, 120 kVp, 160 mAs, 80 kVp, 200 mAs). BV/TV and Tb/Th parameter values were statistically significantly correlated with the values obtained with µCT in all three imaging protocols. The Tb/Sp parameter values obtained with only the first imaging protocol (120 kVp, 200 mAs) showed a statistically significant correlation with the values obtained with µCT (36). In present study, only the FD parameters obtained with both methods were found to be statistically significant and moderately concordant (ICC=.474; p< .05). Our imaging protocol was 120 kVp, 69 mAs. As seen in Diederichs et al. study, microstructure parameters are statistically affected by differences between protocols. Therefore, it is thought that the possible difference may be due to changes in mAs level.

Parsa et al. evaluated the microstructure of samples obtained from human cadavers with CBCT, MSCT and µCT. As a result, they showed that there was an excellent correlation between BV value and bone density value evaluated by MSCT and µCT. They also concluded that there is a very strong correlation between the BV/TV parameter, which is considered as the gold standard in bone quality assessments of implant sites, and CBCT gray values (37).

Kulah et al. evaluated cadaveric maxillary bone samples with two CBCT and one µCT devices. As a result of the study; similar to the findings of this study; they showed that the BV/TV and DA parameter values obtained with the CBCT imaging method were compatible with the values obtained with the µCT imaging method (10). In present study, it was observed that the parameters such as BV/TV, BS/TV, BS, BV, TV and DA evaluated with the CBCT imaging method were statistically significantly compatible with the µCT imaging method.

Although µCT imaging method is considered as the gold standard among imaging methods, it has some disadvantages such as not being suitable for clinical use, high radiation content, and not being available in every clinic. At this point, it has been a study that clarifies in which areas the CBCT imaging method, which is now more widely used in our clinics and offers many advantages in terms of radiation amount and procedure time, can be an alternative to the µCT imaging method.

In conclusion, microstructure parameters of CBCT were more compatible with gold standard values comparing with CT parameters. Bone compression strength can be estimated by CBCT and µCT methods in a desired level. BV/TV and DA parameters are significant determinants of bone mechanical property in not only µCT but also CBCT method.

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Peer-review: *Externally peer-reviewed.*
Author Contributions: (Initials only)
Research idea:AK
Design of the study: SK, AK
Acquisition of data for the study: SK, AK
Analysis of data for the study: SK, AK
Interpretation of data for the study: SK, AK
Drafting the manuscript: SK, AK
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Clinical and Experimental Health Sciences

The Relationship Between BMI, KIDMED Score, and Nutritional Habits of Female Adolescents: A Cross Sectional Study

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ABSTRACT

Objective: This study aims to investigate the relationship between the eating habits of adolescent girls and their KIDMED scores and BMIs.

Methods: This cross-sectional and descriptive study was conducted with the participation of 391 female adolescents studying in Istanbul. Descriptive information, the consumption frequency of some foods and dietary habits, and the Mediterranean Diet Quality Index (KIDMED) were asked through a face-to-face questionnaire.

Results: 19.4% of the adolescents were found overweight and obese, whereas 15.1% were underweight. It was determined that the BMIs of adolescents who think that they have an adequate and balanced diet and who consume salad or raw vegetables more than once a day are significantly lower (p<.05). Adolescents' mean KIDMED score was found 4.42±2.44; 35.5% had low and 53.8% had moderate KIDMED scores. It was found that the KIDMED scores of the adolescents who consume fruits and vegetables more frequently, never consume chips, consume products such as ready-made cakes/biscuits/wafers less frequently, drink nine glasses of water a day or more, have more meals, do not skip meals, and have breakfast every day are significantly higher (p<.05). No correlation was found between KIDMED and BMI. It was observed that the diet quality of female adolescents was generally at moderate and low levels, but there were no significant differences in BMI values.

Conclusion: It has been concluded that to improve the diet quality of adolescents, it is necessary to develop nutrition education, knowledge and habits, and to provide opportunities to reach healthy meal options in schools.

Keywords: Adolescent, nutrition, diet quality, Mediterranean diet

1. INTRODUCTION

Adolescence is a transitional period between childhood and adulthood in which physical and mental changes are experienced. The starting time and course of this transition period, which is the second significant growth stage of life after infancy, varies from society to person, and between genders. It is estimated that there are 1.8 billion adolescents in the world, mostly in low – and middle-income countries (1). According to the 2022 data from the Turkish Statistical Institute (TUIK), the adolescent population (10-19 years old) in our country constitutes 7.4% of the total population, and female adolescents constitute 3.6% of the total population (2).

Habits in adolescence affect later periods of life. Considering public health, the evaluation of adolescents as a priority group plays an active role in improving and improving health (3). Understanding the dietary habits of adolescent girls and the factors affecting them is an important public health priority. Adolescence is a crucial transitional period when adult lifestyle habits begin to emerge. Poor dietary habits of adolescents are associated with increased risk for long-term health outcomes in a population with high baseline epigenetic modifications (4).

The high and increasing prevalence of overweight and obesity in adolescents is a major global public health problem. Since the 1980s, the global prevalence of overweight and obesity in children and adolescents has increased by 47%. This trend is observed in both high-income countries and low – and middle-income countries, with very little gender gap. While the rate of increase in overweight and obesity has slowed in high-income countries, the rate of increase is increasing for children and adolescents in low – and middle-income countries (5). Adolescents are vulnerable to several changes in their diet. Studies have found that because adolescents spend more time outside the home, they typically consume more fast foods and sugar-sweetened beverages, and less fruit and vegetables (6). Diet quality and overweight are strongly linked to social inequalities. In Western societies, the rate of overweight and obesity is highest among children and

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adolescents of low socioeconomic status (SES). Therefore, SES is part of the multifactorial obesogenic environment to which children are exposed. In addition, high consumption of energy-providing foods and beverages, long-term exposure to media sources, and parental overweight are relevant risk factors for childhood obesity. These risk factors are linked to the home environment and therefore very difficult to address with preventive interventions, especially in families with low SES (7). Also, during the education period children and adolescents spend most of their waking time at school. Thus, breakfast and lunch behavior is partially dependent on schedule and food availability in the school setting (8,9) In addition, in a study conducted in Sweden reported that an estimated two in three adolescents in the European region and Canada do not consume enough nutrient-rich foods (10). A study conducted in Taiwan also revealed that female adolescents are a unique population and that their nutrition and quality of life are correlated with obesity (11).

Studies on the nutritional habits and knowledge levels of adolescent girls show that the vast majority of them have inadequate and unbalanced nutrition, and further and detailed studies are needed on this subject (12–15). Therefore, this study was conducted to investigate the eating habits of female adolescents living in Istanbul and the relationship between these habits and BMI and KIDMED scores.

2. METHODS

This cross-sectional and descriptive study was conducted in Istanbul, Beykoz in public schools with students from low SES backgrounds between February and March 2020. The sample of the study was calculated as 384 with 95% confidence interval and 5% margin of error. Students who could not be in schools during the study and did not give their consent, those with a chronic or metabolic disease, those on a diet, or those who applied a special nutritional therapy were excluded from the study. A total of 409 female students participated, and 18 of them who answered the questionnaire incompletely were excluded from the research. The study was completed with a total of 391 adolescent girls.

Ethical and scientific approval for this study numbered 10840098-604.01.01-E.66353 and dated 27/12/2019, was obtained from the İstanbul Medipol University Non-Interventional Clinical Research Ethics Committee. In addition, voluntary consent was obtained from the students with the permission of the official institutions to apply the questionnaire to the students for the conduct of the study.

The data of the study were collected through a structured questionnaire. The survey form consists of demographic information and physical activity habits of adolescents, food groups and consumption habits of sugary drinks, number of daily meals and skipping meals, anthropometric measurements, and Mediterranean Diet Quality Index (KIDMED).

The age of the students, their classes, the educational status of the parents, the number of people living in the family and their regular physical activity habits were asked. The type and frequency of physical activity of the students who declared that they did regular physical activity were recorded. Afterwards, the number of main and snack meals, skipping meals and reasons, snack preferences and breakfast habits of the students were recorded. The frequency of consumption of fresh fruit, salad, raw vegetables, cooked vegetables, chips, candy-chocolate, ready-made cake-biscuit-wafer and sugary-carbonated beverages were questioned using the food consumption frequency form.

Before measuring the body weights of the students, ensure that accessories, heavy clothes and shoes were removed and they remained in the lightest clothing, while they were hungry. A scale (Sinbo SBS 4427 Digital Scale) was placed on a horizontal and flat surface, and the measurements were recorded in kg and with an accuracy of 0.1 kg. The height was measured with a non-stretchable measuring tape while standing in the Frankfurt plane. Body mass indexes (BMI) were calculated by dividing the body weight in kilograms by the square of the height in meters and evaluated according to the World Health Organization (WHO) classification (16).

The validity and reliability of the scale developed by Serra-Majem et al. have been demonstrated in Turkish adolescents and its widespread use has been accepted (17,18). The Mediterranean Diet Quality Index (KIDMED) is a diet quality index developed to evaluate the adaptation of children and young people to the traditional Mediterranean diet. It consists of 16 questions. Of the questions included in the KIDMED index, 12 are positive questions and 4 are negative questions, and those who answer yes to positive questions get +1 and those who answer yes to negative questions get – 1 points. Then these scores; are divided into 3 groups as ≥8 points for optimal Mediterranean diet (good), 4-7 points as the need to improve their suitability for the Mediterranean diet (moderate), and ≤3 points as very low nutritional quality (low) (18).

The data of the study were evaluated with SPSS 18.0 (SPSS Inc., Chicago, IL, USA) package program. Descriptive variables are mean (Mean), and standard deviation (SD); categorical variables are given as numbers (n), and percent (%). The Kolmogorov-Smirnov test was performed if the continuous data showed a normal distribution. Non-parametric tests were applied to the data that did not fit the normal distribution. The Mann-Whitney U test was used in two groups, and the Kruskal-Wallis test was used in more than two groups, in the evaluation of the BMI values and KIDMED scores of the adolescents according to their nutritional habits. In the Kruskal-Wallis analysis, the Mann-Whitney U test was used to determine which group had a statistically significant difference. Bonferroni correction was applied in the analysis of multiple comparisons. The correlation analyses of the continuous variables that did not show normal distribution were performed with the Spearman correlation test. The p-value of <.05 was considered significant for statistical differences.

3. RESULTS

Characteristics of adolescents are shown in Table 1. It was determined that the education levels of the mothers and fathers of the adolescents with a mean age of 16.35±1.32 years were primary school at the highest rate with 50.6% and 37.6%, respectively, and the number of people in their families was 4-5 people at the rate of 68.6%. In addition, it was determined that 52.4% of adolescents do not do regular physical activity, and those who do physical activity mostly prefer walking (54.3%). In anthropometric measurements, their BMI was found to be 22.00±3.99 kg/m ^{2}, and 19.4% of them had BMIs above normal.

Table 1. *Descriptive data of adolescents*

Descriptive characteristics		
	Mean ± SD	
Age (years)	16.5±1.32	
Height (cm)	161.73 ± 5.73	
Body weight (kg)	57.37 ± 10.97	
BMI (kg/m^2)	22.00 ± 3.99	
	n	**%**
Mother's educational status		
Primary school	82	50.6
Middle school	52	32.1
High school	26	16.1
University	2	1,2
Father's educational status		
Primary school	61	37.6
Middle school	54	33.3
High school	38	23.5
University	9	5.6
Number of people living in the family		
2-3	15	9.2
4-5	111	68.6
6 and above	36	22.2
Doing regular physical activity		
Yes	186	47.6
No	205	52.4
BMI classification (kg/m^2)		
<18.5	59	15.1
18.5-24.9	256	65.5
25.0-29.9	59	15.1
30 and above	17	4.3
Total	**391**	**100.0**

Adolescents ' consumption frequency of some food groups and the relationship between BMI and KIDMED are shown in Table 2. Accordingly, 47.5% of the adolescents consume fresh fruit, 40% consume salad or raw vegetables, and 24.4% consume cooked vegetables at least once a day. There was no statistically significant difference between the frequency of fruit and cooked vegetables consumption and BMI (p>.05). Adolescents (11%) who consumed these foods more than once a day in salad or raw vegetable consumption were found to have significantly lower BMIs than those who consumed them once a day and less than once a week (p<.0024). 13.9% of adolescents consume chips, 55.2% consume candy or chocolate, 39.8% consume cake/biscuit/wafer, and 30.4% consume sugary-carbonated drinks at least once a day. There was no significant difference between the consumption frequency of these food groups and BMI (p>.05). The KIDMED scores of the adolescents who never consumed chips were significantly higher than the scores of all other consumption frequency groups (except for the occasional/rare consuming group) (p<.0024).

It was observed that adolescents consuming 9 glasses of water or more per day had significantly higher BMI than those consuming 1-2 glasses of water (p<.0083). In addition, it was determined that the BMI of the adolescents in the group who thought that they had an adequate and balanced diet were significantly lower than those who answered sometimes or no (p<.0167). KIDMED averages of those who think that they have adequate and balanced nutrition are statistically significantly higher (p<.0167). It was observed that the mean of KIDMED of those who drank 9 glasses of water or more per day was significantly higher than those who drank 1-2 glasses of water per day (p<.0083).

When the relationship between KIDMED scores and some food groups is examined, it is seen that those who consume more fresh fruit have higher KIDMED scores (p<.001). It was also found that those who consumed salad or raw vegetables more than once a day had a significantly higher KIDMED score. In terms of the frequency of consumption of cooked vegetables/vegetables, it was determined that the KIDMED scores of the groups consuming once a day and 3-6 times a week were significantly higher than those who consumed it occasionally/rarely and those who did not consume it at all.

The relationship between the status of adolescents in each BMI class regarding their adequate and balanced diet and their KIDMED scores is analyzed in Table 3. It was observed that the relationship between thinking about an adequate and balanced diet and KIDMED score was statistically significant in groups with underweight and normal BMI (p<.05). Accordingly, adolescents with lower BMI perceive their diet quality better.

BMI of adolescents and KIDMED scores were analyzed in Table 4 according to meal consumption habits. Accordingly, no significant relationship was found between meal consumption habits and BMIs. (p>.05). When KIDMED scores were examined, it was seen that the KIDMED scores of adolescents who consumed two or three main meals a day were significantly higher than those who consumed one main meal (p<.0083). In addition, the KIDMED scores of those who never had snacks were found to be lower. It was determined that the group that did not skip the main meal had higher KIDMED scores and the group that skipped breakfast had significantly lower KIDMED scores compared to the groups that skipped other main meals (p<.0167). It was determined that the KIDMED scores of the adolescents who had breakfast every day were significantly higher (p<.0167). It was determined that adolescents who had their breakfast at home had statistically higher KIDMED scores compared to those who had it at school (p<.0167).

Table 2. *The relationship of adolescents' consumption frequency of some food groups with BMI and KIDMED*

Variables	n (391)	%	BMI (kg /m^2) Mean±SD	Test result	KIDMED Mean±SD	Test result
Frequency of consumption of fresh fruit						
More than once a day	88	22.3	21.99±3.42	χ 2= 9,096 p =.168	5.18 ± 2.38	χ 2 =34,006 **p <.001**
Once a day	99	25.2	21.61±3.66		4.70 ± 2.36	
3-6 times a week	63	16.2	21.40±4.45		4.85 ± 2.44	
1-2 times a week	70	18.0	21.78±3.86		3.44 ± 2.36	
Less than once a week	5	1.3	21.77±4.69		2.40 ± 2.07	
Occasionally/rarely	61	15.7	23.66±4.79		3.74 ± 2.25	
None	5	1.3	21.50±2.26		3.80 ± 2.48	
Frequency of consumption of salad or raw vegetables	43	11.0	19.96±2.54	χ 2= 13,960 **p =.030**	6.04 ± 2.63	χ 2 =28,110 **p <.001**
More than once a day	114	29.0	22.29±3.53		4.55 ± 2.57	
Once a day	55	14.2	21.93±4.49		4.35 ± 1.90	
3-6 times a week	90	22.9	22.41±4.54		4.10 ± 2.29	
1-2 times a week	8	2.0	23.68±3.05		3.99 ± 2.50	
Less than once a week	61	15.7	22.23±4.09		4.10 ± 2.18	
Occasionally/rarely	20	5.2	21.96±4.34		2.90 ± 2.46	
None						
Frequency of consumption of cooked vegetables/ vegetable meals	34	8.7	21.83±3.70	χ 2= 1.698 p =.945	4.99±2.44	χ 2 =31,034 **p <.001**
More than once a day	61	15.7	21.76±3.86		5.03±2.40	
Once a day	76	19.5	22.02±4.31		4.97±2.16	
3-6 times a week	118	30.0	22.20±4.08		4.28±2.28	
1-2 times a week	13	3.3	21.36±3.52		5.25±2.80	
Less than once a week	59	15.1	21.80±4.04		3.43±2.38	
Occasionally/rarely	30	7.7	22.53±3.79		3.16±2.84	
None						
Chips consumption frequency						
More than once a day	19	4.9	20.88±2.68	χ 2= 6.391 p =.381	3.78 ± 2.07	χ 2 =27,476 **p <.001**
Once a day	35	9.0	22.14±3.79		3.51 ± 2.06	
3-6 times a week	32	8.2	23.58±5.14		3.45 ± 3.09	
1-2 times a week	94	23.9	21.63±3.88		4.29 ± 2.33	
Less than once a week	36	9.3	21.03±3.44		3.99 ± 1.84	
Occasionally/rarely	140	35.7	22.07±3.75		4.78 ± 2.53	
None	35	9.0	22.69±4.93		5.82 ± 2.02	
Frequency of consumption of sugar, chocolate						
More than once a day	110	28.1	21.14 ± 3.39	χ 2= 9,470 p =.149	4.31±2.22	χ 2 =8,981 p =.175
Once a day	106	27.1	22.31 ± 4.17		4.07±2.58	
3-6 times a week	75	19.2	22.10 ± 4.46		4.31±2.53	
1-2 times a week	58	14.8	21.97 ± 3.90		4.91±2.33	
Less than once a week	11th	2.8	23.62 ± 3.69		5.45±2.38	
Occasionally/rarely	28	7.2	23.26 ± 4.16		4.77±2.59	
None	3	0.8	22.83 ± 5.22		6.33±2.51	
Frequency of consumption of ready-made cakes, biscuits, wafers	74	19.0	21.48±3.87	χ 2= 2.727 p =.842	3.87 ± 2.40	χ 2 = 15,540 **p =.016**
More than once a day	82	20.8	22.36±4.06		4.20 ± 2.54	
Once a day	47	12.0	21.57±4.25		4.47 ± 2.50	
3-6 times a week	76	19.5	22.20±3.71		4.70 ± 2.38	
1-2 times a week	27	6.9	21.92±4.27		3.92 ± 2.13	
Less than once a week	71	18.2	22.19±3.69		5.22 ± 2.40	
Occasionally/rarely	14	3.6	22.40±6.06		3.57 ± 2.02	
None						

Frequency of consumption of sugary-carbonated drinks						
More than once a day	51	13.1	21.67 ± 3.42		3.79 ± 2.36	
Once a day	68	17.3	21.77 ± 3.58		4.07 ± 2.43	
3-6 times a week	48	12.4	22.55 ± 4.58		4.58 ± 2.39	
1-2 times a week	68	17.3	22.71 ± 3.74	χ 2= 5.507	4.18 ± 2.34	χ 2 = 11,925
Less than once a week	20	5.1	20.77 ± 3.73	p =.481	3.85 ± 2.60	p =.064
Occasionally/rarely	113	28.9	21.97 ± 4.20		4.86 ± 2.31	
None	23	5.9	21.62 ± 4.97		5.13 ± 3.19	
Thinking that you have an adequate and balanced diet						
Yes	65	16.5	20.66 ±3.49		5.84 ± 2.46	
No	134	34.3	22.89 ±4.34	χ 2= 11,936	3.48 ± 2.27	χ 2 =39,331
Sometimes	192	49.2	21.90 ±3.79	**p =.003**	4.60 ± 2.28	**p<.001**
Daily water consumption						
1-2 cups	83	21.2	21.18±3.30		3.79 ± 2.71	
3-5 cups	125	32.0	21.46±3.60	χ 2= 9,213	4.36 ± 2.39	χ 2 =11.307
6-8 glasses	96	24.6	22.38±4.54	**p =.027**	4.35 ± 2.15	**p=.010**
9 glasses or more	87	22.2	23.10±4.26		5.15 ± 2.46	

** Kruskal Wallis test, p<.05*

***Mann Whitney U test, p<.0024 (for 21 comparisons), p<.0167 (for 3 comparisons), p<.0083 (for 6 comparisons)*

In Table 5, adolescents' average scores of KIDMED, group distributions and the relationship of these distributions with BMI values are included. It was determined that the mean KIDMED score was 4.42±2.44 and only 10.7% of the adolescents had good scores. There was no statistically significant difference in BMI between the KIDMED groups (p>.05).

Table 3. *The relationship between KIDMED scores and the state of adolescents' thinking that they have an adequate and balanced diet according to BMI classes*

BMI (kg/m^2)	Thinking that you have an adequate and balanced diet	n (391)	KIDMED Mean ±SD	Test result
<18.5	Yes	14	5.71 ±2.36	χ 2=12,463
	No	14	2.57±1.74	**p =.002**
	Sometimes	24	4.83±2.54	
18.5-24.9	Yes	41	6.02 ±2.38	χ 2=23,126
	No	69	3.65 ± 2.45	**p <.001**
	Sometimes	113	4.73 ±2.26	
25.0-29.9	Yes	4	4.75 ± 2.98	χ 2=4.957
	No	20	3.00 ±1.94	p =.084
	Sometimes	27	4.29 ±1.81	
30 and above	Yes	one	5.00 ±0.00	χ 2 = 0.237
	No	8	5.25 ±1.38	p =.888
	Sometimes	6	4.83 ±2.04	

** Kruskal Wallis test, p<.05*

***Mann-Whitney U test, p<.0167 (for 3 comparisons)*

Table 4. *The relationship of adolescents' mean KIDMED score and BMIs with KIDMED groups*

Variables	n (391)	%	BMI (kg/m²) Mean±SD	Test result	KIDMED Mean±SD	Test result
Number of main meals per day						
1	56	14.3	21.76±4.19	χ2=2,875 p=.411	3.28 ± 2.53	χ2=16,139 **p=.001**
2	172	44.0	22.17±4.08		4.34 ± 2.21	
3	133	34.0	22.37±4.16		4.96 ± 2.36	
4 and above	30	7.7	21.20±4.02		5.00 ± 2.86	
Number of snacks per day						
0	32	8.3	22.47±4.34	χ2=2,881 p=.410	3.75 ± 1.87	χ2=8,306 **p=.040**
1-2	257	65.6	21.93±4.06		4.24 ± 2.41	
3-4	76	19.4	22.47±4.52		4.91 ± 2.72	
5 and above	26	6.7	20.37±2.44		5.42 ± 2.85	
Main meal skipping status						
Yes	111	28.4	22.30±4.13	χ2=4,129 p=.127	3.81±2.57	χ2=25.386 **p<.001**
No	55	14.1	21.05±3.43		5.92±2.40	
Sometimes	225	57.5	22.09±4.04		4.34±2.24	
Skipped meal (n=336)						
Breakfast	159	47.3	22.16±4.18	χ2=0.735 p=.693	3.57 ± 2.29	χ2=12.982 **p=.002**
Lunch	121	36.0	22.08±3.71		4.49 ± 2.34	
Evening meal	56	16.7	22.77±4.49		4.64 ± 2.41	
Having breakfast						
I have breakfast every day	122	31.1	21.53±3.60	χ2=2.394 p=.302	5.73 ±2.13	χ2=60,779 **p<.001**
Sometimes I have breakfast	220	56.3	22.30±4.17		4.05±2.28	
I never have breakfast	49	12.6	21.78±4.12		2.85±2.38	
Breakfast location						
At home	190	48.5	21.63±3.85	χ2=3,550 p=.169	4.77 ±2.43	χ2=6.312 **p=.043**
At school	163	41.8	22.59±4.34		4.04±2.45	
Out-of-home and school	38	9.7	21.98±3.37		4.27 ±2.29	

**Kruskal Wallis test, p<.05*

***Mann-Whitney U test, p<.0167 (for 3 comparisons), p<.0083 (for 6 comparisons)*

Table 5. *The relationship of adolescents' BMI values and KIDMED scores with their meal consumption habits*

	Mean ± SD (min-max)			
KIDMED score	4.42±2.44 (-4.00-11.00)			
	n (391)	**%**	**BMI (kg/m²) Mean±SD**	**Test result**
KIDMED group				
Low	139	35.5	21.62±3.54	χ2=3,338 p =.188
Moderate	210	53.8	22.43±4.38	
Good	42	10.7	21.16±3.35	

** Kruskal Wallis test, p<.05*

4. DISCUSSION

Adolescence is an age when growth and development accelerate and accordingly, nutritional habits are of great importance (19). In our study, female adolescents with a mean age of 16.5±1.32 years have a height of 161.73±5.73 cm and a BMI of 22.00±3.99 kg/ m². According to the WHO's growth reference values for age, the average height for girls between the ages of 14-19 is 162.7 cm, and the average BMI is 20.9 kg/m². Body weight was not seen as a valid indicator for children and adolescents over the age of ten (20). When compared with the reference values of WHO, it was seen that the adolescents participating in our study had high BMIs and one cm lower than average height. This may be due to genetics and low SES, or it may be because the reference data used in the evaluation are not specific to our population.

In a review study, in 2019, on the dietary habits of adolescents living in North America, Europe and Oceania, fruit and vegetable consumption was examined. Accordingly, the average fruit and vegetable consumption was found to be quite low the recommended daily value of 400 grams or 5 servings in almost all of the populations studied (21). In a study examining the fruit and vegetable consumption frequency of Canadian adolescents participating in the Youth Smoking Survey 2012-2013, it was determined that 38.9% of female adolescents consumed 3-4 servings of fruit and vegetables a day, and 27.7% consumed 1-2 servings of fruit and vegetables a day. These amounts were found

to be considerably lower than the 7 servings of fruit and vegetable consumption per day recommended by Canada's Food Guide for Healthy Eating for girls aged 14-18 years (22, 23). In a study examining fruit and vegetable intake with data obtained from the first follow-up survey of Child and Adolescent Health Research (KiGGS) in Germany; It was determined that 50.7% of 14-17-year-old female adolescents consumed 1-2 servings and 19.9% consumed 3-4 servings of fruit and vegetables per day. It was observed that only 8.1% of 14-17-year-old girls found the recommended amount of 5 servings per day (24). Similar to other studies, it was observed that the adolescents participating in our study were quite low in the daily consumption recommendation of 5 servings of vegetables and fruits. Considering that fruit consumption is not low, it is thought that vegetable consumption is low and the underlying reasons need to be further clarified.

In a study on the water and beverage consumption of children and adolescents in the United Arab Emirates, it was determined that the average daily consumption of plain water in female adolescents was 1002.4 mL. It has been stated that only 31% of female adolescents between the ages of 14-18 consume 1.0-1.15 L/1000 kcal of water, which is the daily recommended water/energy ratio of the European Food Safety Authority (EFSA). The total amount of fluid intake by obese adolescents from foods, plain water and other beverages was statistically higher compared to other BMI classes. However, although there was no statistically significant difference in plain water consumption, it was observed that obese adolescents consumed plain water more than other groups (25). In a review examining the determinants of adolescent water consumption and studies on attempts to increase water consumption, it has been reported that daily water consumption of children and adolescents is below the recommended levels in data obtained from England, Australia, Canada and the USA (26). According to the TNHS 2019 report, when the water consumption of female adolescents in our country is examined, it is seen that the daily average water/mineral water/soda consumption is 1071.1±746.13 mL (27). Similarly, in our study, it was determined that adolescents with high BMI consumed more water. The BMI values of the adolescents who consumed 9 glasses or more of water per day were found to be statistically significantly higher than the adolescents who consumed 1-2 glasses of water. It is thought that this may be because adolescents with high BMI may also feel more thirsty due to their consumption of more food. However, it is seen that the daily average water consumption of the adolescents participating in our study is below the recommended amounts. This may be because adolescents do not know the importance of water consumption and the amount they should consume. Also, another explanation for the differences in the amount of consumed water might be that those who had higher BMI might consume more fast food for instance, including more salt.

In a study, 20.7% of adolescents in the Turkish Republic of Northern Cyprus consume two main meals a day, 79.3% consume three main meals, and 34% of them consume three or more snacks. Also, it was determined that 44% of them skipped the main meal and the ones who skipped the main meal skipped the breakfast meal most frequently at the rate of 71.2%. However, in this study, different from our study, pre-adolescent younger age groups and sex were also included (28). In another study conducted in our country, significant relationships were found between the number of daily main meals and meal skipping status of adolescents and their KIDMED scores. It was observed that the KIDMED scores of both male and female adolescents consuming three meals a day were significantly higher than those consuming two meals (18). In our study, it was determined that adolescents consuming two or more main meals a day had significantly higher KIDMED values than those consuming one main meal. In addition, the KIDMED scores of those who never had snacks were found to be lower. This may be because those who have a higher number of main meals may have more opportunities to diversify their food consumption with different food groups.

It has been known for a long time that having a regular breakfast has important contributions to physical and mental health in adolescence. In a study examining the food consumption habits of adolescents in Brazil, it was determined that 62.2% of female adolescents had breakfast and 54.6% of those who had breakfast had their breakfast at home (29). According to the TNHS 2019 report, the frequency of having breakfast among girls aged 15-18 in our country is 62.3% and 86.4% in women aged 15 and over (27). In a study conducted on adolescents in Malaysia, it was determined that both male and female adolescents who have a regular breakfast every day have slightly lower total cholesterol, LDL cholesterol and BMI levels compared to those who have irregular breakfast habits (30). Arguing that a meta-analysis of 45 observational studies also reported an increased risk of overweight and obesity in those who skip breakfast compared to those who regularly consume breakfast (31). In our study, however, there was no significant difference between the BMI values of adolescents who regularly eat breakfast and those who do not. This may be due to the relationship between meals except for breakfast, overall diet quality and BMI.

In the HELENA study, which was conducted to observe how accurately adolescents could assess their diet quality, a positive correlation was found between the level of perception of diet quality of adolescents and their diet quality index scores. This positive relationship was not valid for obese adolescents but was observed in overweight, normal and malnourished groups. Factors such as gender, pubertal status and parents' education levels were not found to be effective in diet awareness. As a result, it was observed that both male and female European adolescents were able to evaluate their diet quality well, except for those who were obese (32). In our study, it was seen that the relationship between thinking about an adequate and balanced diet and KIDMED score was statistically significant in groups with underweight and normal BMI classes. It was concluded that adolescents with lower BMI values perceived their diet quality better.

One of the limitations of this study is that it was conducted cross-sectionally and only with adolescents studying in public schools. On the other hand, the fact that it was conducted only on female adolescents constitutes a strong aspect in terms of revealing the situation and needs specific to one gender.

5. CONCLUSION

In our study, we found that consuming fresh fruit, salad or raw vegetables and cooked vegetables more frequently, never consuming chips, consuming products such as ready-made cakes/biscuits/wafers less frequently, drinking nine glasses of water a day or more, having a large number of meals, not skipping meals, and having breakfast every day appear to have higher diet quality. BMI values were found low in those who frequently consumed salad or raw vegetables, and higher in those who consumed more water. In addition, those who think that they have an adequate and balanced diet have lower BMI values. On the other hand, no significant relationship was found between the number of meals, having breakfast, KIDMED scores and BMIs. Nutritional habits in adolescence significantly affect both the growth and development of adolescents and their general health in the following years. It has been concluded that the nutrition education of these young people, who will play an important role in raising healthy generations, should improve their knowledge and habits, improve their diet quality, and provide an environment for accessing healthy meal options in schools.

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Clinical and Experimental Health Sciences

Micro-CT Evaluation of the Remaining Endosequence BC Filling Materials and Dentinal Microcracks after Retreatment with D-Race and R-Endo Systems

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ABSTRACT

Objective: To analyze the remaining EndoSequence BC filling materials and microcrack using micro-computed tomography after retreatment with D-Race and R-Endo systems.

Method: The canals of thirty mandibular single-rooted and single-canal premolar teeth were shaped and obturated with EndoSequence BC point and EndoSequence BC sealer. The samples were assigned into two groups and scanned with micro-computed tomography. Then, the canal filling materials were removed with D-Race and R-Endo systems. After all roots were re-scanned, all images were evaluated for the remaining EndoSequence BC filling materials and microcrack. Also, retreatment time was recorded.

Results: The remaining filling material in the R-Endo group was greater than the D-Race group. Both systems caused new cracks formation. However, no significant difference was noticed among them. The D-Race group needed significantly less time in comparison to the R-Endo group.

Conclusion: D-Race system was faster and more efficient compared with R-Endo system. D-Race and R-Endo files caused new cracks on the canal walls.
Keywords: D-Race, Endosequence BC sealer, microcracks, micro-computed tomography, retreatment.

1. INTRODUCTION

When primer root canal treatment fails, non-surgical retreatment is the first alternative to eradicate microorganisms in the endodontic system (1). In the case of retreatment, it is very important to fully remove the root filling materials from the canal walls to perform disinfection, re-shaping, and refilling effectively (2).

Numerous nickel-titanium rotary systems have been manufactured for retreatment procedures, such as D-Race (FKG Dentaire, La Chaux-de-Fonds, Switzerland) and R-Endo (Micro-Mega, Besancon, France) systems. D-Race system is composed of 2 files, DR1 (15mm, 30/.10) and DR2 (25 mm, 25/.04), with alternating cutting edges and triangular cross-section. DR1 file has a cutting tip that enhances penetration of the instrument into the filling material while the tip of DR2 instrument is inactive (3). R-Endo system comprises 4 instruments with inactive tips: Re (15 mm, 25/.12), R1 (15mm, 25/.08), R2 (19mm, 25/.06), and R3 (23mm, 25/.04). These files are characterized by a triangular cross-section and 3 equally spaced cutting edges (4).

Various endodontic applications including root canal instrumentation, root canal obturation, post-placement, and retreatment process can cause microcracks in root dentin (5-7). The retreatment case is a more challenging and more time-consuming procedure in comparison with primer endodontic therapy. Since more dentin tissue is removed from canal walls, more microcracks might occur during this procedure (7,8).

The removal of canal obturation materials and microcracks could be evaluated by radiographic assessment (9,10), sectioning of roots (4,11) and micro-computed tomography (micro-CT) (3,12). However, micro-CT provides more reliable information as it allows three-dimensional imaging (13). There are no available research utilizing micro-CT when comparing the effect of D-Race and R-Endo files on the remaining filling materials and microcrack formation. Therefore, this research aimed to assess the remnant filling material and dentinal cracks on the canal walls after the removal of EndoSequence BC root filling materials (Brasseler,

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Savannah, GA, USA) with D-Race and R-Endo systems using micro-CT. The null hypothesis was that there was no statistically significant difference in remaining filling material and microcrack formation between D-Race and R-Endo.

2. METHODS

G*Power program revealed that at least nine teeth per group were required under the conditions of this study (α=0.05, 1-β=0.8). After ethics committee approval (protocol no: 2019-284), straight, single-rooted human mandibular premolars without previous root fillings, root fractures or immature apices were collected. The periapical radiographs of samples were taken buccolingually and mesiodistally to examine canal configuration. As a result of this evaluation, thirty teeth with a single canal were chosen.

2.1. Root Canal Shaping and Obturation

A single experienced investigator conducted all treatments. Tooth crowns were removed to acquire a uniform sample length. The working length of root canals was adjusted to 0.5 mm shorter from the apex. Each canal was shaped with EndoSequence Xpress files (Brasseler, Savannah, GA, USA) up to a size 35/.04. The instruments were used at 500 rpm/2 Ncm using an endodontic motor (E-connect S Endomotor; Eighteeth, Jiangsu Province, China). The canals were rinsed with 5 mL of 5.25% NaOCl following each file. After the shaping process, final irrigation procedure was accomplished using 5 mL of 17% EDTA and saline. Then, each canal was obturated with EndoSequence BC points (35/.04 and accessory gutta-percha) and EndoSequence BC sealer using the cold lateral compaction method. Following temporary sealing of the canal orifices (Coltosol; Coltene-Whaledent, Langenau, Germany), quality of the canal obturation was affirmed using a periapical radiograph. Each specimen was stored in an incubator at 37°C and 100% humidity for two weeks to complete the setting of EndoSequence BC sealer.

2.2. Micro-CT Scanning

After obturation procedure, each specimen was scanned using micro-CT (SkyScan 1172; Bruker-microCT, Kontich, Belgium) at 100 kV, 100 µA, 13.7 µm isotropic resolution, 180° rotation around the vertical axis, 2.450 ms camera exposure time, frame averaging of 2, with an aluminum and copper filter. The images were reconstructed using NRecon v.1.6.3 (Bruker-microCT) with a beam hardening correction of 65%, a smoothing of 3, and an attenuation coefficient range of 0 to 0.23. The reconstructed images were imported to the CTAn software (v.1.13, Bruker-microCT) for three-dimensional analysis. In each sample, the volume of the filling material (mm^3) was measured for the whole root canal and also for all root canal thirds. Micro-CT evaluation was performed by another experienced and blinded investigator.

After the roots were randomly assigned into 2 groups (n=15), the volume of the root canal filling was statistically analyzed using the Mann-Whitney U test and no significant difference was found between the groups ($p > .05$). This result showed that the specimens were homogeneously distributed.

2.3. Retreatment Procedure

D-Race group: D-Race system includes DR1 (15mm, 30/.10) and DR2 (25 mm, 25/.04) rotary files. DR1 instrument was used at the coronal third of the canals. The canal filling in the remaining part of the canal was removed using DR2 instruments. DR1 and DR2 were operated at 1000 rpm/1.5 Ncm and 600 rpm/1.5 Ncm, respectively.

R-Endo group: R-Endo system includes Re (15 mm, 25/.12), R1 (15mm, 25/.08), R2 (19mm, 25/.06) and R3 (23mm, 25/.04) rotary files. Re rotary files were used in the canal orifice; R1 instruments were used at the coronal third of the root canals, R2 files (19mm, 25/.06) in the middle third and R3 files to the working length. All R-Endo rotary files were operated at 300 rpm/1.2 Ncm.

The new instruments were used for retreatment of each tooth. All retreatment files were used in an up-and-down motion with brushing movements. The canals were rinsed with 2 mL of 5.25% NaOCl following each rotary file.

2.4. Retreatment Time

The time needed to remove the canal filling was noted in minutes using a stopwatch, excluding change of instruments and irrigation time. The timing commenced with the insertion of the initial retreatment instrument into the canal entrance and concluded when the last retreatment file, which reached the working length, was entirely removed from the canal.

2.5. Remaining Root Filling Material

Each sample was re-scanned at the initial micro-CT scanning parameters after the retreatment process. The volume of the remaining filling material was measured and the percentage of the remaining filling material was calculated for the whole root canal and also for all root canal thirds.

2.6. Dentinal Microcrack

The reconstructed images were imported to the DataViewer program (version 1.5.1, Bruker microCT). The transverse cross-sectional images obtained from all scans after obturation and retreatment procedures were examined simultaneously by 2 blinded endodontists with more than 5 years of experience. Any lines extending from the external root surface to dentin or from the root canal lumen to dentin were described as the dentinal microcrack. In case of disagreement, the sections were evaluated until the observers agreed. The microcrack distribution was represented as a percentage.

2.7. Statistical Analysis

After using the Shapiro-Wilk test, the percentage of the remaining filling material in the groups was compared using the Mann-Whitney U and Kruskal-Wallis tests. The Mann-Whitney U test was also utilized to compare the formation of microcrack and retreatment time. The significance level was determined at .05.

Table 1. *Descriptive values of the time (minutes) needed for retreatment procedure with D-Race and R-Endo systems*

Groups	Mean ± SD	Median	Min-Max
D-Race	0.40 ± 0.09	0.37	0.2 – 0.6
R-Endo	1.25 ± 0.14	1.21	1.1 – 1.5
p	***.000***		

SD, standard deviation.

Table 2. *Means and standard deviations (± SD) of the percentage (%) values of the remaining filling material after retreatment with D-Race and R-Endo systems in the coronal, middle and apical thirds and total root canal*

	D-Race (n=15)	R-Endo (n=13)	*p*
Coronal (%)	9.67 ± 10.54	15.06 ± 20.30	*1.000*
Middle (%)	3.42 ± 5.79	30.75 ± 33.60	***.043***
Apical (%)	12.64 ±2 7.7	57.31 ± 25.8	***.001***
Total (%)	8.58 ± 17.46	34.38 ± 31.72	***.000***

3. RESULTS

3.1. Retreatment Time

The amount of time (minutes) needed for the retreatment procedure with the D-Race and R-Endo systems is shown in Table 1. The D-Race group needed significantly less time compared to the R-Endo system ($p < .05$).

3.2. Remaining Root Filling Material

The percentage values of the remaining filling material after retreatment procedures with R-Endo and D-Race systems are listed in Table 2. The representative micro-CT images from each group are seen in Figure 1. The D-Race system showed a better performance in removing filling material from the total root canal in comparison to the R-Endo system ($p < .05$). Regarding different root canal thirds, R-Endo group had significantly higher values than the D-Race group in terms of the percentage of the remaining filling material in the middle ($p < .05$) and apical ($p < .05$) third. But there was no significant difference between the groups in the coronal third ($p > .05$).

During retreatment, R3 files were fractured in two teeth and these samples were discarded from R-Endo group. There was no rotary file separation in the D-Race group. Other procedural errors such as ledge or zipping were not observed in either group.

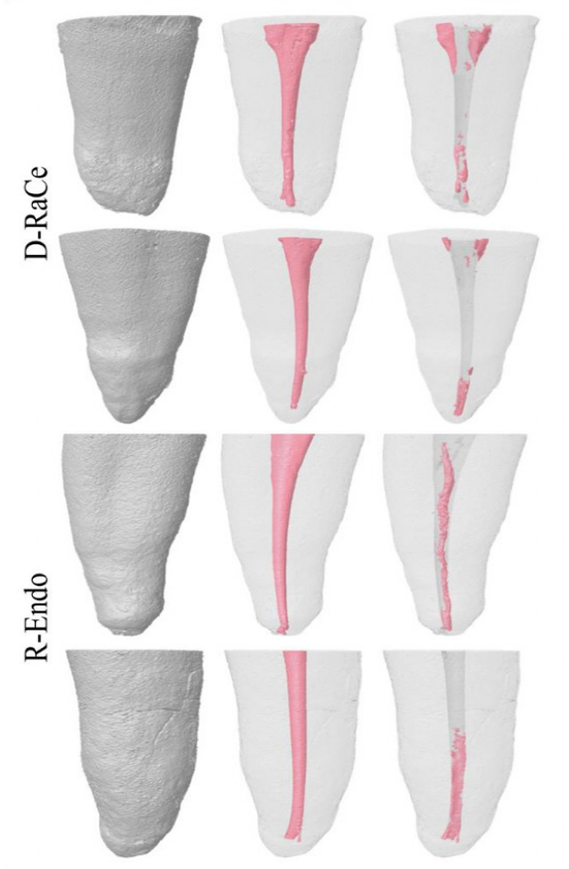

Figure 1. *The filling material in pre – and post-retreatment micro-CT images of representative samples of each group.*

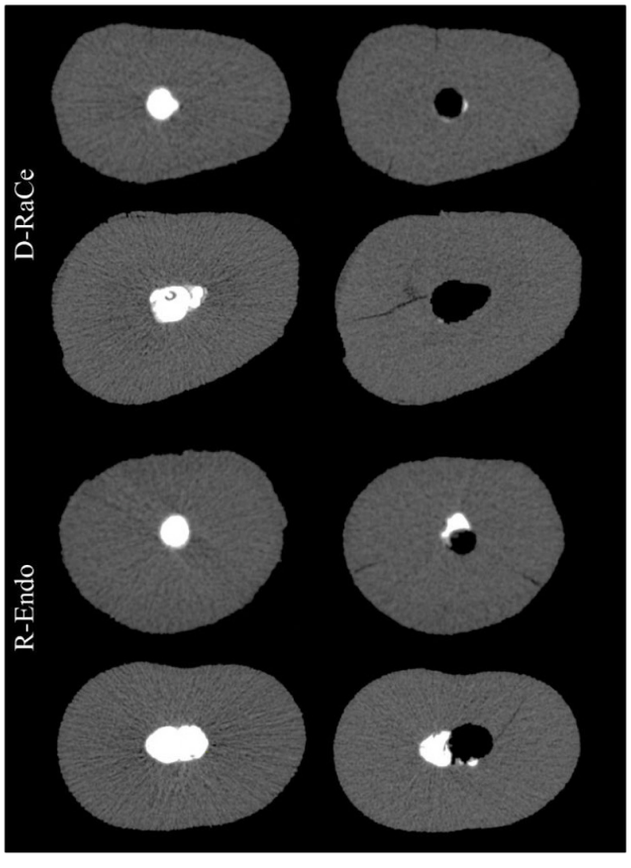

Figure 2. *Microcracks in pre – and post-retreatment cross-sectional micro-CT images of a representative sample of each group.*

3.3. Dentinal Microcrack

The cross-sectional images of all specimens were examined (n = 28.864) to determine the existence of dentin microcracks. Before retreatment procedure, 6.99% (n = 2019) of sections displayed microcracks. Dentin cracks were identified in 3.47% (n = 1002) and 3.52% (n = 1017) of sections in the root filling samples of R-Endo and D-Race groups, respectively. After retreatment procedure, dentinal microcracks were visualized in 26.73% (n = 7717) of sections. These cracks were observed in 14.06% (n = 4059) and 12.67% (n = 3658) of sections in retreatment samples of the R-Endo and D-Race groups, respectively. There were significant differences between the pre-retreatment and post-retreatment images for both systems ($p < .05$) (Figure 2). But no significant difference was noticed in new microcrack formation between the retreatment systems ($p > .05$).

4. DISCUSSION

In this current study, D-Race and R-Endo systems were selected for the retreatment procedure. As far as we are aware, no investigation has been conducted utilizing micro-CT to compare the effectiveness of D-Race and R-Endo systems on the remnant filling material and crack formation after retreatment. According to our results, our null hypothesis was rejected in terms of remaining filling material. D-Race system was more effective in the removal of filling material in comparison to R-Endo system, but both groups showed similar effects on new crack formation during retreatment. In this study, retreatment times of both systems were also measured and D-Race was found to be faster than R-Endo.

Various techniques such as longitudinal splitting of the roots (4,14,15) or radiographic evaluation (9,16) are used to examine the residual filling material after retreatment. But, these techniques have major drawbacks. For example, loss of remnant filling material may occur during the longitudinal splitting of roots and radiographic assessment cannot show all residual filling material and only offers two-dimensional details (17-19). Moreover, micro-CT imaging enables three-dimensional quantitative assessment without damaging dentin and provides high accuracy information (13). In this non-destructive technique, the samples can be evaluated both preoperatively and postoperatively. Since micro-CT was used in the present investigation, the filling material volumes of the groups were statistically compared before retreatment procedure and equal distribution of the samples between the experimental groups, which provided more reliable results, was confirmed.

In the current research, the Endosequence BC filling materials could not be entirely eliminated from the canal walls with the retreatment systems used similar to previous studies (2,3,12). In the literature, only Bedier and Roshdy (15) compared the effects of D-Race and R-Endo systems on the remaining obturation materials after retreatment using the longitudinal splitting method and revealed that D-Race system was more efficient and faster, in compliance with our findings. These results might be due to the alternating cutting edges of D-Race rotary files. Rödig et al (3) indicated that this design may facilitate penetration into the filling material and improve cutting ability. Since both file systems have a triangular cross-section, there may not be any correlation between the cross-section of the files and these findings. Besides, the improved effectiveness of D-Race system might be attributed to the thermoplasticization of the gutta-percha at the higher speed, which makes gutta-percha removal easier. One of the factors that shorten the retreatment time of D-Race system may be active tip of DR1 instrument, unlike R1 file.

Considering root canal thirds, no significant difference was noticed among the retreatment systems used only in the coronal thirds according to our findings. The reason for this may be that the operator can see the obturation materials in the coronal third more clearly in comparison to other thirds. Moreover, in the present study, tooth crowns were removed to acquire a uniform working length similar to most previous studies. Even though decoronation does not represent the clinical practice, it eliminates variables, such as crown anatomy and access cavity. Thus, more accurate data are obtained about the effectiveness of the instruments (20).

The use of mandibular teeth with straight canals in our study resulted in no procedural errors except instrument fractures. Whereas no rotary file separation occurred in D-Race group, two R-Endo files were broken in this study. In contrast, Rödig et al (3) reported the high incidence of rotary file separation in D-Race group, which might be related to their use of teeth with sudden canal curvatures.

In the literature, there is only 1 research comparing the effects of D-Race and R-Endo systems on dentinal microcrack formation. In that study, the number of dentinal defects in the samples before retreatment could not be evaluated because the destructive sectioning technique was used (11). However, extraction and sawing of the samples, as well as initial root canal instrumentation and canal filling procedures, might result in dentin cracks (21,22). Therefore, they chose instrumentation and obturation techniques that minimize these limitations in their study and reported that these two retreatment systems had same efficiencies on dentin defect formation in agreement with our findings (11). This result indicates that the instrument's design and size variations may not be a direct factor for microcrack formation.

In the current study, retreatment procedures with D-Race and R-Endo systems caused new microcrack formations. Previous studies reported that various nickel-titanium retreatment systems trigger new crack formations during retreatment (12,23). In comparison to the initial root canal treatment, more dentin tissue is removed from the root canals and more mechanical manipulation is required during retreatment procedure (7,8,22). Therefore, regardless of the retreatment file systems used, the retreatment procedure may induce the formation of microcracks.

EndoSequence BC Sealer, a premixed bioceramic-based root canal sealer, was used in our study. This highly biocompatible sealer can form a strong chemical bond with dentin hydroxyapatite (24). For this reason, the removal of bioceramic sealers from the canals might be more difficult and may result in more dentin cracks on the canal walls compared to other sealers. However, our results are consistent with similar studies using AH Plus (11,15). Furthermore, previous studies revealed no significant difference between EndoSequence BC Sealer and AH Plus in the remaning filling material and microcrack formation after retreatment (14,25).

5. CONCLUSION

In this study, the Endosequence BC filling materials could not be entirely eliminated from root canals with the retreatment systems used. D-Race system was more effective and quicker at removing of obturation materials than R-Endo system. D-Race and R-Endo files caused new microcrack formations. But both systems showed similar effects on new crack formation during retreatment. Clinicians may prefer the D-Race system to the R-Endo system for a shorter and more successful retreatment.

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Research idea: ŞGZ, DT
Design of the study: ŞGZ, DT
Acquisition of data for the study: ŞGZ, AK
Analysis of data for the study: ŞGZ, SGK, AK
Interpretation of data for the study: ŞGZ, SGK, DT, AK
Drafting the manuscript: SGK, ŞGZ
Revising it critically for important intellectual content: ŞGZ, SGK, DT, AK
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Clinical and Experimental Health Sciences

Respiratory Tract Diseases with Musculoskeletal System Interaction: A Scoping Review

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ABSTRACT

Objective: Respiratory diseases and musculoskeletal disorders are significant causes of morbidity and mortality worldwide, especially among older adults and immunocompromised individuals. Although current guidelines encourage a multidimensional approach to diagnosis and treatment, the interaction between these disease categories has not been adequately investigated. This scoping review aims to provide an overview of current research on diseases related to both respiratory and musculoskeletal disorders and identify gaps for future studies.

Method: Using the PRISMA extension to scope the reviews, we focused on randomized controlled trials and cohort studies published in the last five years. A total of 2003 abstracts were identified in databases such as Scopus, WoS, PubMed, Medline, Cinahl, OpenAIRE and EBSCO. After removing 287 duplicates, 1716 articles were screened based on title and abstract. A total of 470 full-text articles were assessed for eligibility, resulting in 11 articles meeting our inclusion criteria.

Results: The review shows limited but influential studies investigating the intersection between respiratory and musculoskeletal diseases. The findings suggest that musculoskeletal disorders may negatively affect respiratory functions and vice versa. Several studies have demonstrated potential biomarkers, the importance of sleep quality, and associations with multimorbidity.

Conclusion: Although limited to 11 articles, this review highlights the importance of a more detailed understanding of the interactions between the musculoskeletal system and respiratory diseases. This may inform future diagnostic and treatment strategies. However, the limited number of studies in this area indicates that more research is needed, especially which contains interaction mechanism of musculoskeletal and pulmonary pathologies.
Keywords: Musculoskeletal diseases, respiratory tract diseases, respiration disorders

1. INTRODUCTION

Respiratory infections are a major cause of death and illness around the world, and they are especially common and severe in older adults and people with weakened immune systems. Hospitalization ratio also higher in older adults who are affected with different types of respiratory infections (1). Musculoskeletal disorders also a become a worldwide problem in recent years, affects various comorbidities and other diseases such as psychological disorders (2). Its incidence is increasing in completely independent age and occupational groups in many parts of the world (2,3).

In a study which was conducted on 195 countries and areas showed that diseased that leads to or affected by musculoskeletal disorders needs to be diagnosed at early stages. Study also pointed that integrated strategies, prevention strategies and standardized clinical evidence-based treatment when dealing with musculoskeletal disorders (4).

In order to deal with respiratory diseases which has underlying effects of musculoskeletal disorders and comorbidities, clinicians/researchers have to use current guidelines that contains evidence-based and multidisciplinary perspective.

In the musculoskeletal system, controlling the airway opening and airway pressure and performing the coughing maneuver is only possible if the respiratory muscles work smoothly. Pathologies that may impair the function of the respiratory muscles or threaten their neurophysiological integrity may prevent the respiratory system from functioning. These pathologies may cause increased oxygen load, deterioration or change in the structure of the diaphragm, inability to manage the open-air pressure in the lower airways, and oxidative stress (5).

This integrity can lead holistic point of view in many diseases that has a relation pulmonary and musculoskeletal in both ways. Therefore, researchers who prepared this coping review be expectant that this study can enlighten the uncovered areas rapidly, lower the heterogeneity, and lead future studies to focus on this topic productively.

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2. METHODS

2.1. Review Protocol

The PRISMA extension for scoping reviews was applied on this review (6). Main and subheadings were created based on to cover this guideline's flow diagram.

2.2. Identifying the Research Question

Our research question was "What are the current approaches in diseases that related to both pulmonary and musculoskeletal disorders?" In this research, we conducted an analysis to highlight the gaps which are unfamiliar. We aimed to identify gaps in the research on combined musculoskeletal and pulmonary diseases in order to inform future studies on this topic.

2.3. Search Strategy and Selection Criteria

In this scoping review, we searched for only randomized controlled trials or cohort studies which were published in the last five years and conducted with only human participants. Search terms were "respiratory tract diseases" and "musculoskeletal diseases" and they were determined according to MeSH. We have used "and" for search operator. To avoid language barrier, studies written in English language were specified. Articles which were not met these criteria, were excluded.

2.4. Information Sources

Information sources were Scopus, WoS, Pubmed, Medline, Cinahl, OpenAIRE, EBSCO databases accessed via Marmara University VETIS Database Access and Statistics System.

2.5. Study Selection

Two researchers (RUE and TK) conducted a literature search in an electronic database. Researchers (RUE and TK) independently reviewed and screened the abstracts of the articles they found for inclusion.

2.6. Data Charting

A framework for standardized data extraction was developed. Relevant data were extracted independently by researchers (RUE and TK) and including process were applied after the number of the data sources were identified (Figure 1). After identification, extracted data were examined from both authors and articles which were relevant to inclusion criteria for both authors accepted as eligible. Extraction processes were contained reading full text of the article, using inclusion criteria and examining the relevance.

2.7. Data Analysis and Synthesis

Examination processes were contained four parts: 1. General information of studies 2. Study characteristics, 3. Design of the studies 4. Main and secondary findings.

2.8. Consultation Process

Data analyses were applied by researchers and a consultation request was made to a Prof. Tuğba Kuru Çolak, who has an expertise of musculoskeletal physiotherapy and rehabilitation, were examined analysis and synthesis process and assessed for bias, which has been not found.

3. RESULTS

In Figure 1, this study's designing process demonstrated and explained by using PRISMA-ScR. A total of 2003 abstracts identified at the beginning of study selection process. After removal of duplicates, 1246 articles excluded due to several considerations. The number of full-text accessible articles, 470 in number, were examined. 11 articles' data extracted and included into this study.

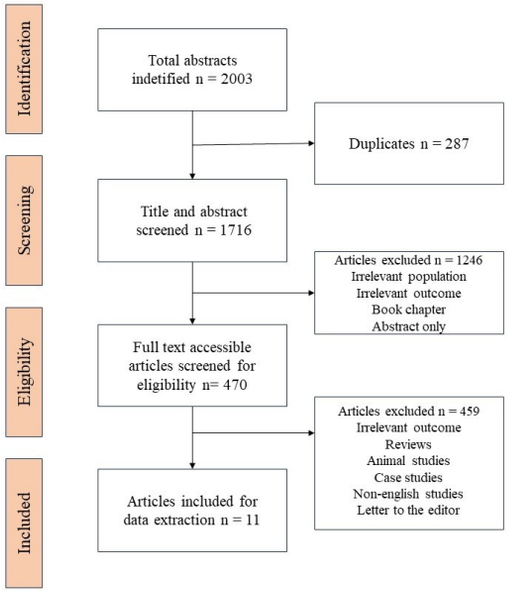

Figure 1. *Selection of Sources (PRISMA flow chart. PRISMA Extension for Scoping Reviews "PRISMA-ScR")*

Table 1 is a compilation of the characteristics and identifiers of the studies. According to Table 1, 8 of 11 studies indexed on Medline. There were 8 articles published in 2021, and there is one article published in each of the years 2018, 2020, and 2022. The studies included in our research consist of 5 different continents. While 5 studies included in our research were conducted in Europe and 3 studies in Asia; there is one study each from Africa, North America, and South America.

Table 2 consists of brief information of selected studies, which are detailed in terms of PRISMA-ScR topics. Our research focused on four different dimensions in each study: study design, outcome data, main results, and other analyses of these studies. Relevance of both respiratory and musculoskeletal disorders' information were highlighted in Table 2 to ensuring integrity of content.

Table 1. *Study Characteristics*

First Author, Ref.	Source of Index	Publisher	Year of Publication	Settings (Country, Continent)
Falksted D et al.[7]	Medline	Int Arch Occup Environ Health	2021	Sweden, Europe
Linden S et al.[8]	Medline	Eur J Cancer Care	2020	Sweden, Europe
Tazzeo C et al.[9]	EBSCO	Age Ageing	2021	Sweden, Europe
Ma KS et al.[10]	OpenAIRE	Eur J Orthod	2022	Taiwan, Asia
Elemary AMM et al.[11]	OpenAIRE	Egypt Rheumatol	2021	Egypt, Africa
Nakamura Y. et al[12]	Medline	Brain & development	2018	Japan, Asia
Katz P. et al.[13]	Medline	ACR Open Rheumatol	2021	USA, North America
Zhu X. et al.[14]	Medline	Front Mol Biosci.	2021	China, Asia
Botman E. et al.[15]	Medline	Bone Rep.	2021	Netherlands, Europe
Senel GB. et al.[16]	Medline	Sleep Breath.	2021	Türkiye, Europe
Paulin F. et al.[17]	Medline	Adv Rheumatol.	2021	Argentina, South America

Table 2. *Design of the Studies*

ID	Study Design	Outcome Data	Main results	Other Analyses
Falksted D et al.[7]	Prospective cohort study. 1,834,555 people between the ages of 44-63 were followed until they reached the age of 55-65. Physical workload, job control and job exposure measures were evaluated.	In participants diagnosed with cardiac and pulmonary disease, a statistically significant relationship was found between physical workload and disability pension status, but this was lower than average.	Swedish workers with higher physical workloads were more likely to receive disability pensions, according to the study	Relationship between disability pension and other status (education years, unemployment history, marital status, and job control) were evaluated in this study.
Linden S et al.[8]	This study examined the prevalence and incidence of comorbidities before non-small-cell lung cancer (NSCLC) diagnosis, as well as the impact of comorbidities on survival and mortality after diagnosis. A population-based cohort of NSCLC patients was identified and followed over time. The prevalence of comorbidities was measured at the time of diagnosis, and the incidence of new comorbidities was measured during follow-up. Median survival and mortality rates were also calculated.	Patients with cancer had more relevant comorbidities and higher incidence rates than the general population. In patients with NSCLC, mortality rates and incidence rates were highly correlated.	The study's findings can help caregivers better prepare to manage cancer patients, who are at increased risk for certain comorbidities and adverse outcomes. In the year leading up to their diagnosis, participants in this cohort were more likely to experience respiratory symptoms, infections, and cardiovascular diseases than the general population.	After diagnosis, patients with NSCLC were more likely to have anemia, central nervous system diseases, and respiratory diseases than comparators. Brain metastases and respiratory diseases were most likely due to the NSCLC itself.
Tazzeo C et al.[9]	Cross sectional and longitudinal cohort study. 2534 individuals were attended and 6-year (n= 2122) and 12-year (n= 2140) longitudinal assessment were applied. Relationship between multimorbidity patterns and frailty in elderly adults were investigated.	Statistically significance were found between physical frailty and mental, cardiovascular, metabolic and sleep disorders after 6 year.	Older adults with a combination of cardiovascular and neuropsychiatric diseases are most likely to become physically frail.	Multimorbidity in older adults is associated with lower quality of life, reduced functional independence, and increased mortality.
Ma KS et al.[10]	Population based 14-year cohort study. 2791 patients diagnosed with juvenile idiopathic arthritis were investigated in terms of obstructive sleep apnea risk. Control group consisted of 11164 matched participants without juvenile idiopathic arthritis.	95 participants diagnosed with obstructive sleep apnea (OSA) and ratio of diagnosed patients were more on juvenile idiopathic arthritis (JIA) group, statistically significance was found. OSA risk was increased after 5 year of JIA diagnosis.	This study found that patients with JIA have a higher risk of OSA and a higher prevalence of OSA. Sleep quality was low in JIA patients, and sleep dysfunction symptoms were common.	This study also explored possible mechanisms causing the increased risk of OSA in JIA patients and evaluated the effects of OSA on treatment strategies and clinical management in JIA patients.

Elemary AMM et al.[11]	Retrospective study based on 100 rheumatoid arthritis patients with pulmonary disorders. It was aimed that to determine the relation between rheumatoid arthritis, and pulmonary involvement type and its frequency.	High resolution computed tomography showed that 68% of patients had abnormalities, and the ground glass were most common (52.9%).	Chest tomography with high resolution was found to be a more sensitive diagnostic tool for pulmonary abnormalities than spirometry in patients with rheumatoid arthritis. Passive smoking, chronic cough, a high Larsen score, and high levels of anti-cyclic citrullinated peptide antibodies were all significant predictors of lung involvement as detected by high-resolution computed tomography.	A significant association was found between pulmonary function tests and high-resolution chest tomography findings. Additionally, age was found to be significantly associated with high-resolution chest tomography-diagnosed pulmonary abnormalities.
Nakamura Y. et al[12]	This study retrospectively evaluated six young adults with Becker muscular dystrophy (BMD) to assess sleep hypoventilation. The goal was to develop predictive estimates of non-invasive ventilation (NIV) initiation in BMD patients by monitoring nocturnal forced vital capacity (FVC), forced expiratory volume in one second (FEV1%), peak expiratory flow (PEF%), peak cough flow (PCF), and average PCO2. Additionally, the researchers investigated the significance of sleep hypercapnia.	Patients with BMD often experience isolated sleep hypercapnia in the early stages, despite preserved waking lung function. However, the significance of respiratory failure in BMD and the ideal timing of NIV warrant further investigation.	Five patients with BMD, three of whom were mobile, experienced elevated PCO2 levels above 45 mmHg during sleep. NIV was initiated in four patients. While one BMD patient with an exon 3-7 deletion had gradual declines in FVC% and PEF, these functions remained stable in other BMD patients. Sleep hypercapnia, which is unexpected based on routine pulmonary function tests, has been observed in patients with BMD.	The average carbon dioxide levels (PCO2) of BMD patients during sleep were lower than the levels measured with the CO2 (end-tidal carbon dioxide, EtCO2) monitor from the esophagus. However, this was consistent with the observation of sleep hypercapnia at younger than expected ages reported in previous studies. Five patients in this study experienced peak PCO2 levels of 52-54 mmHg overnight. Other analyses, such as the causes of sleep hypoventilation, treatment options, and the impact of this condition on the prognosis of muscular dystrophy, are also included in the article.
Katz P. et al.[13]	This article was conducted to investigate the prevalence of asthma and COPD (Chronic Obstructive Pulmonary Disease) in systemic lupus erythematosus (SLE) patients and their effects on the patients' health-related quality of life. Two different patient groups (FORWARD "n=2804" and LOS "n=881") were included in the study.	The findings of this study showed that the prevalence of asthma and COPD is higher in SLE patients than in the general population. In addition, the presence of these diseases has been found to adversely affect patients' physical functioning, fatigue, perceived cognitive function, and pain. These findings suggest that SLE patients should be screened regularly for asthma and COPD and ensure they receive adequate treatment for these conditions.	In the initial observations, 19.8% of the FORWARD group (FORWARD participants were selected primarily from rheumatologists' patients and diagnosed with SLE by a rheumatologist) reported asthma and 8.3% COPD. The LOS (Lupus Outcome Study was a longitudinal observation cohort in which participants completed annual structured telephone interviews. All SLE diagnoses were physician-confirmed), whereas 36% of participants reported either asthma or COPD.	In the FORWARD group, the mean age was 50.5 (±14.1) years, the white non – Hispanic rate was 87.2%, the mean duration of SLE was 15.8 (±12.3) years, the low education rate was 6.3%, and the obesity rate was 36.1%. In the LOS group, the mean age was 46.7 (±12.7), the rate of being white was 68.5%, the mean duration of SLE was 12.6% (±8.5) years, the rate of low education was 19.6%, and the obesity rate was 25.7%.
Zhu X. et al.[14]	This article compares the salivary gland gene expression profiles of patients with (n=36) and without (n=128) primary Sjögren's syndrome (pSS) with interstitial lung disease (ILD).	The article examines the relationship between interstitial lung disease and CXCR2 levels in patients with primary Sjögren's syndrome and the potential of CXCR2 in predicting disease activity. The findings of the article suggest that CXCR2 may be a potential biomarker for monitoring and evaluating the course of the disease in ILD-pSS patients.	As a result of analyzes performed on 36 ILD-pSS patients and 128 non-ILD-pSS patients in total, some genes expressed at high levels in ILD-pSS were identified. The researchers noted that among these genes, CXCR2 in particular could be a potential biomarker to assess the severity of the disease. CXCR2 levels were found to be abnormally increased in ILD-pSS patients and these elevated levels were associated with clinical features.	Other analyzes such as the role of CXCR2, its relationship with other inflammatory markers, its prognostic value and its effects on response to treatment are also included in the article. It was determined that CXCR2 was highly correlated with Erythrocyte Sedimentation Rate (ESR) and ESSDAI scores, which are other parameters that play a role in assessing the course of the disease, and inversely correlated with DLCO.

Botman E. et al.[15]	This study was conducted to evaluate how lung function changes over time in patients with Fibrodysplasia Ossificans Progressiva (FOP) (n=7) and its relationship with the volume and progression of heterotopic ossification (HO) in the lung.	The research shows that respiratory function is impaired at an early age in FOP patients and this deterioration stabilizes over time, and this deterioration is mostly due to small lung volumes and jaw ankylosis.	A decrease in FVC was observed in three patients. The decline was seen during childhood and early adolescence. TLC decreased in 2 patients. In one patient, the discrepancy between FVC and TLC was caused by an increase in the RV/TLC ratio. This rate was higher than expected in all patients. Additionally, FEV1 was observed to decrease over time in 3 patients. In one patient, mouth opening increased to a few millimeters after surgery, resulting in an increase in FEV1. TLC, FVC and RV/TLC ratio did not change due to increased mouth opening.	The patients' FEV1 values were decreased, but the Tiffeneau index was within normal limits. The decreased FEV1 value may be due to decreased lung volumes in FOP patients. Also, jaw ankyloses can affect FEV1 values. FVC and VC values decreased, indicating restrictive respiratory function. TLC values were found to be normal in only one patient. Increased residual volume (RV) was observed in most patients, representing the difference between TLC and VC. In FOP patients with complete ankylosis of the thorax, RV increased, and TLC decreased. In addition, mild abnormalities were seen in the pulmonary parenchyma. In addition, the vertebral integrity of the patients was evaluated, and their kyphosis and scoliosis status were examined.
Senel GB. et al.[16]	The aim of this study is to investigate the effect of obstructive sleep apnea syndrome (OSAS) on cardiac autonomic dysfunction in Duchenne muscular dystrophy (DMD) patients (n=12) (Total N=20).	The study showed that 42% of DMD patients had OSAS and cardiac autonomic dysfunction was more pronounced in these patients. It was also found that heart rate variability (HRV) parameters are abnormal in DMD patients and OSAS plays a role in cardiac autonomic dysfunction as demonstrated through HRV parameters.	Twelve male DMD patients (mean age 9.0 ± 3.1 years, mean BMI 20.6 ± 4.8 kg/m2) and eight healthy men of the same age were included in the study. At clinical evaluation, 58% of patients with DMD had at least one OSAS-related symptom (snoring, observed apnea, or restless sleep). None of the individuals in the control group had any OSAS-related complaints. According to PSG, OSAS was detected in 42% of patients with DMD, while OSAS was not detected in any individual in the control group (p=.004). The mean R-R duration and the mean of consecutive R-R intervals longer than 50% ms were significantly lower in DMD patients than in the control group (p<.006). In DMD patients with OSAS, the LF/HF (low/high frequency) ratio during NREM sleep was significantly higher than in the control group (p=0.005). Higher apnea-hypopnea index and lower oxygen saturation were significantly correlated with higher LF power and LF/HF ratio (p<.001).	In the article, total sleep time, sleep latency, REM sleep latency, sleep efficiency, peak PaCO2 values, periodic leg movements index values, N1, N2, N3 and REM sleep times were determined as additional parameters evaluated.
Paulin F. et al.[17]	This multicenter cross-sectional study investigated the prevalence of pulmonary involvement in patients with early rheumatoid arthritis (RA) without pre-existing lung disease. In the study, 83 early RA patients were included, and lung involvement was evaluated with high-resolution computed tomography (HRCT).	The findings showed that patients with early RA have a high rate of lung involvement affecting the airway and that all types of lung involvement are associated with abnormalities in physical examination findings and functional tests.	83 patients (83% female) were included in the study. The median time elapsed after the diagnosis of RA was 3 (1-6 months). HRCT revealed airway involvement in 57 patients (72%) and interstitial abnormalities in 6 patients (7.5%). The most common change in lung function tests was decreased DLCO (14%). Patients with at least one abnormality on physical examination were found to be associated with lung involvement on HRCT. In addition, FVC% and DLCO% values were significantly lower and RV/TLC values were higher in patients with lung involvement. It was found that any variable associated with joint involvement was not associated with abnormalities in HRCT.	In addition to the parameters evaluated in the study, patients' comorbidities, dyspnea duration, physical abnormality assessments, articular and extra-articular parameters, inflammatory parameters and the presence of anti-Ro antibodies were investigated. The presence of anti-Ro antibody has been associated with interstitial abnormalities in HRCT.

4. DISCUSSION

The interaction between musculoskeletal problems and respiratory tract diseases has been addressed in a limited number of studies. However, examining this interaction is especially important because of its potential effects on an individual's quality of life and overall health. The subject of this research was determined to examine this interaction.

Falksted et al. (7) showed that high physical workload among middle-aged and older workers increases the likelihood of receiving a disability pension. This finding indirectly reveals the potential impact of musculoskeletal problems on the respiratory system. It is conceivable that disability-related retirement may be an indicator of a significant decline in a person's functionality and general health.

At the same time, a study conducted by Linden et al. (8) showed that cancer patients have higher rates of comorbidity than the general population. These comorbidities include respiratory symptoms, infectious diseases, and cardiovascular diseases. This may be further evidence of the impact of musculoskeletal problems on the respiratory system.

Tazzeo et al. (9) revealed that the burden of multimorbidity in older adults has negative effects on quality of life, functional independence and mortality. This study shows that musculoskeletal problems, and especially cardiovascular and neuropsychiatric diseases related to these problems, can have a significant impact on the general health status of individuals.

Studies conducted by Ma et al. (10) and Elemary et al. (11) have shown that certain musculoskeletal diseases (juvenile idiopathic arthritis and rheumatoid arthritis, respectively) can lead to respiratory disorders. These studies show that musculoskeletal diseases can have a direct impact on the occurrence and development of respiratory system diseases.

Katz et al. (13) showed that the prevalence of asthma and COPD was higher in Systemic Lupus Erythematosus (SLE) patients than in the general population. This suggests that SLE, an autoimmune musculoskeletal disease, can have a potentially serious impact on the respiratory system.

The study by Nakamura et al. (12) examined sleep hypoventilation in patients with Becker muscular dystrophy (BMD) and its effects on respiratory functions. The results of the study showed that lung functions were preserved during waking hours, but hypercapnia developed during sleep in BMD patients. It has been mentioned that isolated sleep hypercapnia may develop especially in the early stages of BMD patients. However, it was stated that the optimal timing of respiratory failure and non-invasive ventilation (NIV) should be investigated further.

In another study by Zhu et al., (14) they compared the gene expression profiles of patients with primary Sjögren's syndrome (pSS) and patients with pSS (ILD-pSS) diagnosed with interstitial lung disease (ILD). The findings of this study suggest that CXCR2 may be a potential biomarker to monitor and evaluate the course of the disease in ILD-pSS patients.

The study conducted by Botman et al. (15) focused on how lung function changes in patients with Fibrodysplasia Ossificans Progressiva (FOP) and how this change relates to heterotopic ossification (HO). The findings showed that respiratory functions were impaired at an early age in FOP patients, and this deterioration was mostly due to low lung volumes and jaw ankylosis.

Research conducted by Senel et al. (16) examined the effects of obstructive sleep apnea syndrome (OSAS) on cardiac autonomic dysfunction in patients with Duchenne muscular dystrophy (DMD). The study showed that 42% of DMD patients had OSAS and cardiac autonomic dysfunction was more pronounced in these patients.

The study conducted by Paulin et al. (17) examined the prevalence of pulmonary involvement in patients with early rheumatoid arthritis (RA) without pre-existing pulmonary disease. The findings showed that patients with early RA have a high rate of lung involvement affecting the airway and that all types of lung involvement are associated with abnormalities in physical examination findings and functional tests.

5. CONCLUSIONS

These studies help us better understand the effects of various diseases on the respiratory system and general body functions. The findings show us that the impact of musculoskeletal problems on the respiratory system is important and requires further investigation of this interaction. Such research can provide a more effective approach to respiratory tract diseases of individuals with musculoskeletal problems and help develop strategies to manage these conditions. In addition, research in the literature review contributes to the understanding of various biomarkers and markers that are important in the diagnosis and management of diseases. Especially respiratory functions, sleep quality and gene expression profiles seem to play an important role in the course and treatment of musculoskeletal diseases. However, an important conclusion identified in the studies is that the nature and mechanism of the interaction of musculoskeletal diseases with respiratory tract diseases is still not fully understood and more research is needed on this subject.

Our research covers the studies conducted in the last five years. For this reason, there was a limitation of the studies related to the aim of the research since the previous studies were not looked at.

In the screening conducted for the purpose of our research, it was determined that there were no studies that directly evaluated the "effects of musculoskeletal problems on the respiratory tract and respiratory system". This situation emerged as one of the limitations of the research.

The lack of randomized controlled studies and their inability to use them in this study, due to the limited resources obtained as a result of the search of databases, is another limitation.

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Clinical and Experimental Health Sciences

The Effect Of L-Carnitine And Alpha Lipoic Acid Administration With Exercise In Old Rats On Energy Metabolism Related To Oxidative Stress Parameters

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ABSTRACT

Objective: This study aims to contribute novel insights by investigating the potential positive effects of a combined dietary supplement and exercise program on mitochondrial oxidative stress and energy metabolism in aging. Focusing on the protective impact of Alpha Lipoic Acid (ALA), a potent antioxidant, against exercise-induced mitochondrial oxidative stress in rats, we also assess how L-Carnitine administration affects exercise ability by analyzing resistin and HbA1c levels, indicators linked to insulin resistance and cellular sensitivity.

Method: In this 10-day study, 42 old male Sprague Dawley rats (weighing 400±10 g, aged 15–17 weeks) were divided into six groups (n=7): Control, Exercise, L-Carnitine, Alpha Lipoic Acid (ALA), L-Carnitine+Exercise, ALA+Exercise. Relevant groups received daily oral gavage doses of L – Carnitine (50 mg/ml) and ALA (18 mg/ml). Exercise groups underwent treadmill sessions. On day 10, blood samples were quantitatively analyzed for HbA1c and Resistin levels using a Cusabio ELISA assay kit (China).

Results: ALA supplementation synergistically reduced resistin and HbA1c levels, individually and combined with exercise. Conversely, L-Carnitine supplement, alone or with exercise, increased resistin levels but it caused a decrease in HbA1c levels.

Conclusion: The data indicated a minor, insignificant decrease in resistin levels for the exercise and ALA groups, with a statistically significant difference in HbA1c levels among all groups. Exercise alone positively impacted both HbA1c and resistin levels, suggesting a potential counteraction of age-related oxidative stress and a positive influence on energy metabolism through an appropriate diet and exercise program. Further studies are required to explore specific metabolic pathways and relationships identified in our findings.
Keywords: Aging, exercise, resistin, HbA1c, oxidative stress

1. INTRODUCTION

Aging, a universal stage in living organisms, involves irreversible structural and functional changes at various levels, contributing to degenerative disorders like cancer, obesity, type 2 diabetes, and cardiovascular diseases (1,2). The prevailing oxidative stress hypothesis attributes aging to an imbalance favoring oxidant systems, leading to lipid peroxidation and reactive oxygen species (ROS) release (2). This cellular damage significantly impacts disease pathogenesis. Research on aging primarily concentrates on ROS compounds, emphasizing their crucial role in the aging process (2-4).

Insulin, a crucial hormone in energy metabolism control, undergoes changes in sensitivity and vasodilation in the renal artery during aging, influencing age-related cardiovascular diseases (5,6). Oxidative stress, implicated in damaging lipids, proteins, and DNA, is a key risk factor for age-related cardiovascular and metabolic disorders. Consequently, a positive relationship is suggested between oxidative stress parameters and insulin resistance in old age (6). Mitochondria play a key role in aging, influencing bioenergetics, oxidative stress, cell death regulation, and insulin secretion (4). Research on healthy aging explores therapeutic methods to sustain efficient mitochondrial energy metabolism and reduce oxidative stress. Mitochondrial bioenergetic decline is identified as a crucial factor in age-related diseases, leading to ROS production, mitochondrial DNA damage, pancreatic β-cell dysfunction (7), and diabetes (8) in the physiological aging process.

Exercise involves regular and repetitive physical activity aimed at enhancing the harmony between respiratory and circulatory systems, improving oxygen distribution, metabolic processes, and increasing joint flexibility, muscle strength,

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and endurance (9). Growing evidence from epidemiological and experimental studies suggests that physical activity and exercise can counteract the consequences of aging. Diet and physical factors significantly influence oxidative stress and endothelial function (9-11). Exercise offers various health benefits, especially for the cardiovascular system and muscles, while diet serves as a crucial source of antioxidants. The careful selection of both diet and physical activities plays a key role in preventing cardiovascular and metabolic disorders (10,11).

L-Carnitine is a natural ammonium molecule that can be generated endogenously in all mammalian species in the liver, kidney, and brain from the necessary amino acids lysine and methionine and it is an essential cofactor in the oxidation of fatty acids in the mitochondria. However, since its biosynthesis meets only 25% of the daily requirement, it must be taken into the body through diet or supplementation (12). L-Carnitine, known for enhancing fat burning, improving muscle strength, and endurance, plays a crucial role in treating conditions like diabetes, heart disease, high blood pressure, and a weakened immune system (12,13). Additionally, L-Carnitine functions as an antioxidant, protecting enzymes in the body's antioxidant defense system, such as glutathione peroxidase, superoxide dismutase, and catalase, against advanced peroxidative degradation and age-related diseases (13,14). Numerous studies explore L-Carnitine's impact on exercise capacity and energy balance, enhancing overall physical performance (15). In animal studies, L-Carnitine is suggested to prevent age-related muscle protein breakdown, regulate mitochondrial energy homeostasis, and reduce cellular damage and free radicals (15,16). However, muscle L-Carnitine content declines with age in healthy individuals, hindering its distribution and homeostasis (17,18).

Alpha-lipoic acid (ALA) is a mitochondrial coenzyme, vital for pyruvate dehydrogenase and alpha – ketoglutarate dehydrogenase function (19). As a key cofactor for energy production, ALA is considered a valuable dietary supplement to enhance overall mitochondrial metabolism (19,20). It has demonstrated efficacy in treating disorders related to impaired energy use, including type II diabetes (21), diabetic polyneuropathies, and cardiac ischemia (22) reperfusion injury. The antioxidant properties (23) of (R)-α – lipoic acid contribute to combating increased oxidative stress. Studies also explore the impact of ALA on glucose transport, influencing insulin signaling pathways and blood glucose regulation (24). Given these findings, lipoic acid supplementation is anticipated to offer beneficial effects on energy metabolism, potentially mitigating age-related decline in cardiac metabolism and bolstering antioxidant defense against increased mitochondrial oxidant production with age (25).

The objective of this study was to investigate the protective impact of Alpha-Lipoic Acid, a potent antioxidant, on mitochondrial oxidative stress parameters induced by free radicals resulting from exercise in blood samples obtained from rats under anesthesia. Additionally, we aimed to assess the biochemical enhancement in exercise capacity in animals through the administration of L-Carnitine for energy metabolism. This evaluation involved analyzing resistin and blood glycosylated hemoglobin (HbA1c) levels, known to contribute to insulin resistance by diminishing cellular sensitivity to insulin and impairing glucose uptake. Consequently, the study aimed to explore the interrelation between these variables.

2. METHODS

2.1. Animals and Diet

The number of animals and sample size we used in the study were determined by using similar literature studies based on ethical principles and using the G power analysis method. We obtained 42 male Sprague Dawley rats, aged 15–17 weeks, with a weight of 400 ± 10 g each, from the Düzce University Laboratory Animal Breeding and Experimental Research Center for our research. The rats were housed under a 12-hour light/12-hour dark cycle at a temperature of 22 ± 2°C.

The experimental animals were randomly allocated to cages, with seven animals in each cage. All experimental animals for ten days were fed with standard rat food containing; 88% dry matter, 7% cellulose, 23% protein, 8% raw ash, 2% HCl insoluble ash, 0.9% phosphorus, 1.5% calcium, ,0.7% sodium, 1% salt, 1% lysine, 0.3% methionine. All groups were provided with tap water for drinking, and there were no limitations imposed on the animals' intake of water and feed.

2.2. Ethical Consideration

Our study was approved by Düzce University Animal Experiments Local Ethics Committee (DÜ.ET-2022-02-01).

Table 1. *Content of the Groups and Applications*

Group No	Group Name	Chemicals	Amount	The Delivery Method	Number of Animals
1	Control (K)	-	-	-	7
2	Exercise (E)	-	-	-	7
3	L-Carnitine (L)	L-Carnitine	50 mg/ml	Oral Gavage	7
4	Alpha Lipoic Acid (ALA)	Alpha Lipoic Acid	18 mg/ml	Oral Gavage	7
5	L-Carnitine+Exercise (L+E)	L-Carnitine	50 mg/kg	Oral Gavage	7
6	Alpha Lipoic Acid + Exercise (ALA+E)	Alpha Lipoic Acid	18 mg/ml	Oral Gavage	7

K: Control, E: Exercise, L: L-Carnitine, ALA: Alpha Lipoic Acid

Table 2. *Application Procedure*

Group No	Group Name	Application Procedure
1	Control (K)	• Animals will not be exercised or given any substance. • It will be kept under normal feed-water routine and standard care conditions. • Weight measurement will be made at the beginning and at the end of the experiment.
2	Exercise (E)	• No items will be given. • Weight will be measured at the beginning and at the end. • The following exercise protocol will be applied to the animals on the treadmill without inclination. • Active study (excluding the preparatory period) will last 10 days. • It will be kept under normal feed-water routine and standard care conditions.
3	L-Carnitine (L)	• Animals will not be exercised. • Weight will be measured at the beginning and at the end. • For 10 days, which is the working period, L-Carnitine will be administered at a daily dose of 50 mg/ml in addition to being kept under normal feed-water routine and standard care conditions. • Animals in this group will not be exercised on the treadmill, but the animals will be kept on the treadmill during the exercise to eliminate factors that may occur due to environmental differences.Active study (excluding the preparatory period) will last 10 days.
4	Alpha Lipoic Acid (ALA)	• Animals will not be exercised. • Weight will be measured at the beginning and at the end. • For 10 days, which is the working period, it will be kept under normal feed-water routine and standard care conditions, and in addition to feeding, Alpha Lipoic Acid will be administered at a daily dose of 18 mg/ml. • Animals in this group will not be exercised on the treadmill, but the animals will be kept on the treadmill during the exercise to eliminate factors that may occur due to environmental differences.Active study (excluding the preparatory period) willlast 10 days.
5	L-Carnitine + Exercise (L+E)	• During the study period of 10 days, animals will be given 50 mg/ml L-Carnitine per day by oral gavage 30 minutes before exercise. • The animals will be given the exercise specified on the treadmill, which has no inclination. • Weight will be measured at the beginning and at the end. • Active study (excluding the preparatory period) will last 10 days.
6	Alpha Lipoic Acid + Exercise (ALA+E)	• During the study period of 10 days, animals will be given 18 mg/ml of Alpha Lipoic Acid per day by oral gavage 30 minutes prior to exercise. • The animals will be given the exercise specified on the treadmill, which has no inclination. • Weight will be measured at the beginning and at the end. • Active study (excluding the preparatory period) will last 10 days.

K: Control, E: Exercise, L: L-Carnitine, ALA: Alpha Lipoic Acid

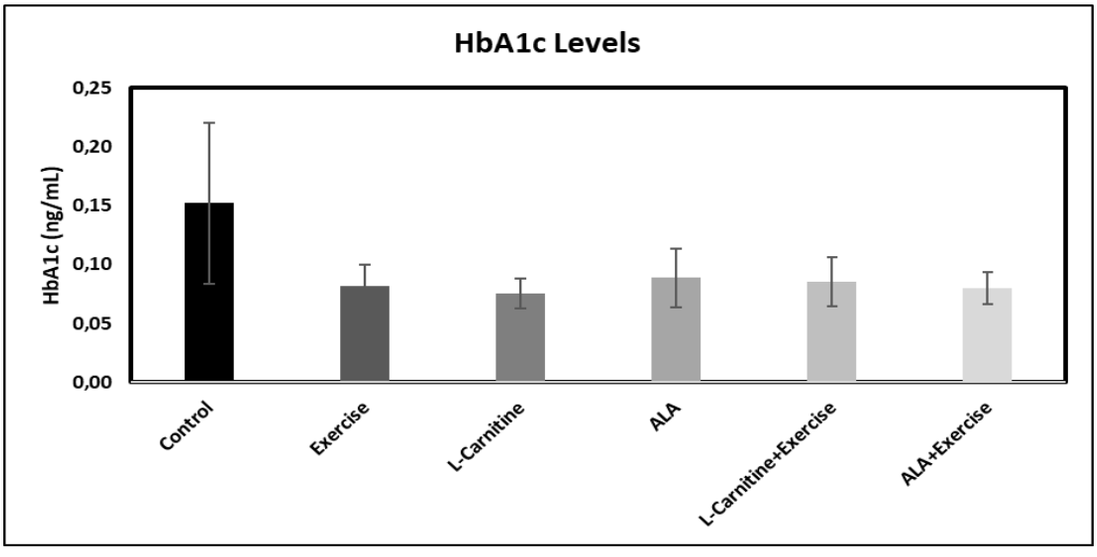

Figure 1. *HbA1c Levels (ng/mL)(ALA: Alpha Lipoic Acid) in groups. Statistical comparisons were run using one-way ANOVA followed by Dunnett's t-test. Statistically significant differences were detected in each group when compared with the control group (p=.001,* p <.05*). The values are represented as mean ± SD.*

Table 3. *Comparison of HbA1c and Resistin levels between groups*

	Control	Exercise	L-Carnitine	ALA	L-Carnitine + Exercise	ALA + Exercise	p*
Resistin	1.1930	1.0529	1.2269	1.1824	1.2490	1.1969	.267
(ng/mL)	±0.2472	±0.1216	±0.0999	±0.1122	±0.1478	±0.1622	
HbA1c	0.1521	0.0819	0.0754	0.0889	0.0856	0.0799	**.001**
(ng/mL)	±0.0686	±0.0177	±0.0124	±0.0250	±0.0207	±0.0135	
p# vs Control		.001	<.001	.004	.002	.001	

*:*One-Way ANOVA, #: Dunnett t test. ALA: Alpha Lipoic Acid*

2.3. Formation of Experimental Groups

In this investigation, six experimental groups (Control, Exercise, L-Carnitine, Alpha Lipoic Acid, L – Carnitine+Exercise, Alpha Lipoic Acid+Exercise) were established, each comprising seven animals. The protocols outlined in Tables 1 and 2 were adhered to for a duration of ten days: At the end of the study, the experimental animals were euthanized under intraperitoneal anesthesia, employing a combination of ketamine (90 mg/kg) and xylazine (10 mg/kg). Cardiac puncture was performed to collect blood samples. Afterwards, the obtained sera were stored at – 80°C.

2.4. Practice of Running Exercise

Features of the Treadmill

All exercise programs applied during our study were carried out using a four-lane experimental animal treadmill branded May TIME 0804 Treadmill Exercise, specially designed for compulsory exercises, fatigue and doping tests.

Preparing Rats for Exercise

All animals were given a trial exercise on the treadmill before the start of the study. Rats that could not run were excluded from the experiment, and the experiment was continued with rats that could run. A 5-day (Monday-Friday) acclimation period was applied for the animals to adapt to the treadmill. Rats were prepared for exercise by running at the lowest speed of the treadmill (2m/min) for 10 minutes.

Exercise Application to Rats

Animals underwent a 10-minute running exercise protocol on a treadmill at a speed of 5 m/min. The exercise protocol was applied for a total of 2 weeks over a 10-day active application period, including the process of acclimating the animals to the treadmill.

Weighing Animals

Weight measurement was made using precision scales at the beginning and end of the experiment. Weighings were made under standard care conditions with normal feeding and water routine.

2.5. Termination of Study

Animals in the groups were sacrificed by taking blood from their hearts by cardiac puncture method under ketamine/xylazine anesthesia after the last administration. After centrifuging the blood samples (15 minutes, 4000 rpm), the serums were extracted and kept at – 80 °C until analysis.

2.6. Measurement of HbA1c and Resistin Levels

After the study, blood samples were utilized to measure HbA1c and Resistin parameters through a quantitative ELISA test kit (Cusabio, China). The spectrophotometric analysis of serum samples was conducted following the protocol provided with a commercially purchased kit, focusing on the specific parameter under investigation. The procedures were carried out in Research Laboratories of Medical Biochemistry Department in Duzce University.

2.7. Statistical Analysis

We conducted statistical analyses using IBM SPSS v.22 software (IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.). The normality assumption for continuous variables was assessed using the Shapiro-Wilk test, and the homogeneity of variances was examined with the Levene test. The comparison among groups was conducted using the One-Way ANOVA test. Dunnett's t-test was applied to identify significant differences between the experimental groups and the control group, while Fisher's LSD test was employed as a post hoc analysis to assess significant differences among the experimental groups. Mean±standard deviation was used to represent continuous variables. Statistical significance was considered at a level of $p < .05$.

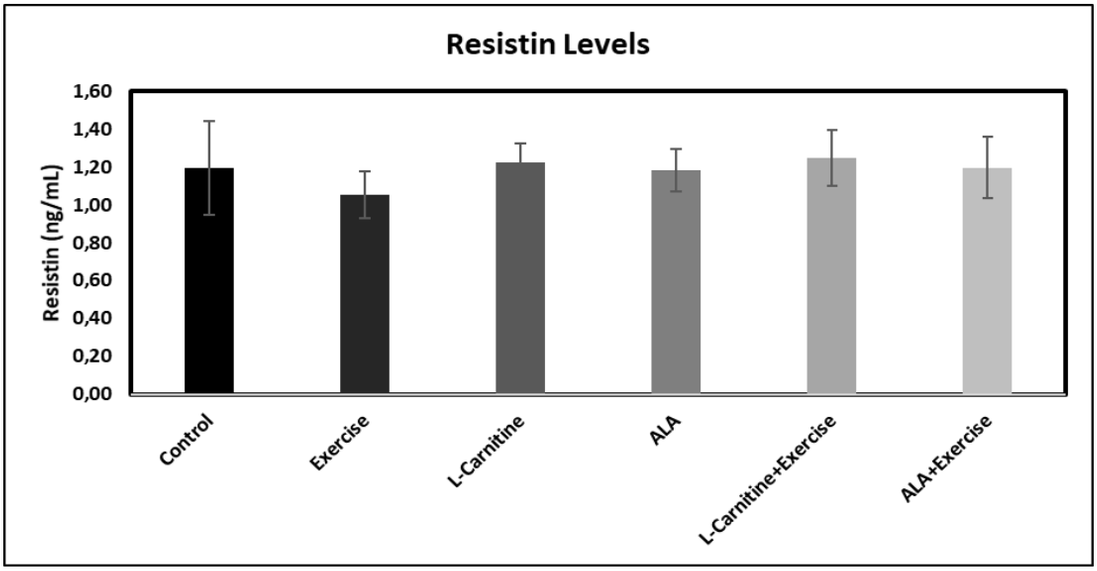

Figure 2. *Resistin Levels (ng/mL)(ALA: Alpha Lipoic Acid) in groups. Statistical comparisons were run using one-way ANOVA followed by Dunnett's t-test. Statistically, no significant difference was detected in any of the groups when compared with the control group (p=.267). The values are represented as mean ± SD.*

3. RESULTS

In all tables and figures, the obtained results are expressed as mean ± standard error.

When the groups' initial and final weights were compared, no significant difference was found. This shows that the 10-day study period is short for us to see the effects of exercise, L-Carnitine and Alpha Lipoic Acid administration on weight changes.

The mean Resistin and HbA1c levels of the experimental groups are presented in Table 3. Analysis revealed that there was no statistically significant difference in resistin levels observed between the groups (p=.267). However, the HbA1c values exhibited significant variability across the groups (p=.001). Post hoc analysis using Dunnett's t-test indicated that the HbA1c level was notably lower in all experimental groups when compared to the control group. Conversely, when the experimental groups were subjected to comparison using Fisher's LSD test, no significant difference was detected among them. Detailed visual representation of the mean HbA1c and resistin levels across the groups can be observed in Figures 1 and 2, respectively.

4. DISCUSSION

In reviewing the existing literature, it is evident that there is a limited number of studies showcasing the potential synergistic effects of applying L-Carnitine and ALA – both known for their individual positive impacts – when combined with exercise in the diet. Our study, designed using an elderly animal model, is anticipated to significantly contribute to the current literature on this subject. We aimed to biochemically evaluate how L-Carnitine administration improves animals' exercise ability by analyzing resistin and glycosylated hemoglobin (HbA1c) levels. The primary objective was to introduce a novel treatment approach, utilizing dietary supplements alongside exercise support, aiming to address the role of mitochondrial oxidative stress and energy metabolism in the aging process. This research endeavors to add valuable insights to the existing body of knowledge in the field.

Resistin is one of the hormones specific to adipose tissue and is a molecule in polypeptide structure. The increase in adipose tissue causes an increase in the level of resistin in parallel. In addition, there is an increase in plasma resistin levels in cases of insulin resistance (26,27). With the advancing age, the body's fat tissue is growing while physical activity is decreasing. In this case, it can be said that the resistin level will result in a high level (28). If there is no change in the fat ratio in the body during exercise activities, it will not be possible for the resistin level to go out of the ordinary (28). As a matter of fact, when the levels of resistin in our study between the groups are examined, it is seen that there is no statistically significant increase or decrease in both the experimental groups and their comparison with the control (Table 3, Figure 2). According to this, it can be concluded that exercise and supplemental alphalipoic acid/carnitine consumption in elderly animals do not cause any negative changes in the fat ratio, and that oxidative stress symptoms do not occur after fat metabolism. The fact that the mentioned supplements do not have negative effects during the application period suggests that the use of the mentioned food supplements in old age will be safe.

L-Carnitine enables cells to break down fat and get energy from stored fat reserves by moving fatty acid chains to the mitochondrial matrix. However, L-Carnitine and its esters help reduce oxidative stress (29). When we interpret it with reference to the fact that resistin is a marker in fat metabolism, the insignificance of the resistin data in our study leads to the conclusion that there is no significant

effect on fat metabolism by administering L-Carnitine to aged rats at the determined dose, duration and exercise application (30). The specific study (30) reported an initial increase in lipid peroxidation and a decrease in antioxidant levels in elderly animals given L-Carnitine supplementation, with subsequent normalization observed after 21 days of administration, attributed to an increase in carnitine status in the body. We referenced this study to highlight existing literature that explores the effects of L-Carnitine on oxidative stress and antioxidant levels in aging animals. The mention of this older study in our publication is intended to contrast with our current research findings, emphasizing potential differences in methodology and study design. This approach aims to contribute to the ongoing discourse in the scientific community and underscore the need for varied research approaches to comprehensively understand the effects of L-Carnitine supplementation in different contexts. Another publication reports that the elderly with reduced muscle carnitine content, decreased lean muscle mass/function, and supplementing with carnitine may have a beneficial effect on mitochondrial dysfunction as well as the benefits of L-Carnitine, especially for the young active population engaged in high-intensity exercise (15). To enhance bioavailability, L-Carnitine supplementation from foods and supplements, supported by exercise, is proposed. Further studies are needed to investigate the current bioavailability of L-Carnitine supplementation.

Hemoglobin is the protein found in red blood cells that carries oxygen. Hemoglobin A1c, on the other hand, is one of the modified forms of hemoglobin formed as a result of the modifications that take place in the posttranslational stages (31). HbA1c, which is formed as a result of glycosylation of blood sugar in plasma by clinging to hemoglobin, is the molecule that plays an active role in the determination of blood sugar (glucose) level (32). When physical activity decreases, HbA1c level increases (33). In this study, a statistically significant reduction in HbA1c levels was observed in both the exercise groups and the supplemented groups (Table 3, Figure 1). These data reveal the conclusion that the blood sugar level in plasma can be reduced with exercise and supplementation in elderly individuals. In addition, based on the decrease in HbA1c level, it is thought that oxidative stress can be reduced to basal levels with supplementary food and exercise, thanks to decreased blood sugar and metabolism. In a meta-analysis of HbA1c levels, data revealed that individuals engaging in both aerobic (18 studies) and heavy exercise (4 studies), as well as a combination of the two (7 studies), experienced a decrease in HbA1c values post-exercise (34). The data we obtained from the running exercise and supplementary applications in old animals are similar to the aforementioned studies.

In addition to being an effective antioxidant, alpha-lipoic acid (ALA) is a natural compound with pro – oxidant properties that occur with excessive consumption (35, 36). The emergence of disruptions in the biological chain with aging results in an increase in oxidative stress (37). There are scientific studies which show that ALA, which is among the antioxidant substances that can be taken into the body, may be effective in reducing this stress. It has been reported that ALA supplementation applied to mice undergoing swimming exercise helps delay oxidative stress resulting from metabolic activity (38).

Kayali et al., when they investigated the effect of ALA applied to aged rats on oxidative stress parameters in post-mitotic brain and muscle tissues, reported that they encountered an increased oxidative stress and that ALA could have a dose-dependent pro-oxidant effect in aged animal tissues (39). The lack of increase in resistin and HbA1c values in our study may indicate that the dose of ALA administered is the dose that will not cause oxidative stress in aged rats.

5. CONCLUSION

In our study investigating the effects of ALA and L-Carnitine supplements on energy metabolism and oxidative stress in elderly rats, we administered these supplements both with and without exercise. Despite not finding significant changes in resistin parameters related to fat energy metabolism, we observed statistical significance in HbA1c data, a critical factor in glucose energy metabolism. The strength of our study lies in its controlled experimental design, utilizing elderly rat models to mimic aspects of aging and incorporating exercise interventions. The inclusion of both supplements and exercise provides a more comprehensive understanding of their potential impacts.

However, it is important to acknowledge the limitations of our study. Firstly, while rat models offer valuable insights, translating these findings directly to humans requires caution. Therefore, future research employing a more detailed experimental design with human subjects is imperative for clinical relevance. Additionally, the 10-day duration of our study may limit the comprehensive assessment of long-term effects. Future investigations with extended durations and more intensive exercise protocols are warranted to validate and elaborate on the observed positive effects.

In conclusion, our results suggest a potential positive impact of ALA and L-Carnitine supplements on glucose energy metabolism in aging. However, further research with human participants and an extended duration is needed for a more robust understanding of the clinical implications and long-term effects of these supplements. Despite these considerations, our study provides valuable insights into the potential benefits of these supplements in mitigating age-related changes in energy metabolism and warrants continued exploration in future research endeavors.

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Acquisition of data for the study:NŞ,AT,AG,TA,KA
Analysis of data for the study:KKK,AT,AG,TA,KA
Interpretation of data for the study:KKK,NŞ,PY,MA
Drafting the manuscript:KKK,NŞ,KA,PY,MA
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Clinical and Experimental Health Sciences

The Investigation of The Metabolic Effect of High Salt or Western Diet During Pregnancy and Lactation on Rat Dams and Postnatal Offspring Rats

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ABSTRACT

Objective: This study investigated the metabolic effect of maternal high-salt and western low-protein diets during pregnancy and lactation periods on rat dams and adult offspring.

Method: Female rat dams were divided into four groups and fed with a 1% high salt diet, a Western low-protein diet (high fat and sugar and low protein), or a 1% high salt/western low-protein combined diet (WS) during pregnancy and lactation. Afterward, 95 female and male offspring were divided into groups and fed with those diets until 18 weeks of age. The mothers' and offspring rats' body weights and chow intake were recorded periodically. At 18 weeks of age, blood samples were collected from the offspring. Their blood lipid profiles, leptin, and insulin levels were analyzed.

Results: Rat dams had similar weight changes during pregnancy and lactation. Rats exposed to the Western low-protein and WS diet during pregnancy, lactation, and/or postweaning had lower body weights than the control group. Male adult offspring from control dams and fed high salt were heavier and had higher LDL cholesterol than controls. However, rats from high salt dams and fed a high salt diet had lower body weights than the control group. Plasma insulin and leptin of male rats were not significantly different. Female offspring fed Western low-protein and WS diet in the fetal period or in early childhood had significantly low insulin. However, female rats exposed to Western low-protein and WS diets during pregnancy, lactation, and postweaning had similar insulin to control rats.

Conclusion: Maintaining the maternal diet after lactation prevents the detrimental effect of a low-protein diet on insulin levels. Anti-obesity mechanism may develop in offspring exposed to a high salt diet during the fetal period against salt intake in later life.
Keywords: Fetal period, fetal programming, high salt diet, insulin, leptin, western diet

1. INTRODUCTION

Fetal life is a critical developmental period characterized by rapid cell division. While the genes significantly affect fetal development, research shows that this development is affected by the environment, especially by how the mother is fed. Changes in maternal nutrition and endocrine status during the fetal period can result in developmental adaptations that permanently change the physiology and metabolism of the offspring. That is, nutrition during the fetal period may be related to metabolic, endocrine, and cardiovascular diseases in adult life (1).

Experimental studies have shown that the Western diet (high fat and high sugar) during pregnancy and lactation may have long-term effects on the offspring, such as high blood cholesterol, low insulin sensitivity, and liver damage (2,3). The lack of protein may exacerbate the detrimental effects of the Western diet (4).

Excessive sodium intake is related to obesity, low insulin sensitivity, cardiovascular diseases and mortality (5,6). Moreover, excessive sodium intake increases inflammation and is characterized by decreased renal function and hypertension (7). In experimental studies, high salt intake affects health negatively and increases body weight, blood insulin and glucose levels namely metabolic syndrome in rats (8). Excessive salt intake during the fetal period may adversely affect the fetus (9). High salt intake during pregnancy and lactation may also exacerbate the detrimental impact of the Western diet on the offspring (2).

According to the fetal programming theory, impaired fetal nutrition causes an adaptation, increasing the chance of survival of the fetus (10). Therefore, low-protein, high-fat, and high-salt diets during fetal and lactation periods may lead to an adaptation. Their detrimental effects may

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exacerbate or diminish with standard diet in adulthood (11, 12). Due to fetal adaptation, it is also unclear whether maintaining that impaired and unhealthy diet after weaning through adulthood is more detrimental or less harmful than the standard diet (13).

Therefore, this study investigated the metabolic effect of maternal high salt and western diet during pregnancy and lactation period on rat dams and adult offspring rats.

2. METHODS

2.1. Experimental Procedure

The experimental procedures were approved by the Ankara University Animal Experimentation Ethics Committee under protocol number 2018-17-114. The experiments were performed in the Animal Experimentation and Research Laboratory of Ankara University in accordance with relevant guidelines and regulations outlined by the ethics committee.

2.2. Animal and Experimental Design

Based on earlier studies, a power analysis was performed to determine the sample size (2). The results showed that a sample of 24 would be large enough to detect significant differences. The sample consisted of 28 female virgin Sprague Dawley rats. They were fed ad-libitum from weaning until week 12 and maintained at 20-21 °C, 50-60% humidity, and a 12 h light: 12 h darkness cycle. All female rats weighed 200-250 grams. Then, they were randomly assigned to four dietary groups and fed experimental diets ad-libitum for one week before pregnancy. Experimental groups were fed either (1) a Control (C, n = 8) standard rat chow, (2) a 1% NaCl rat chow, high salt diet (S, n = 7), (3) a 27% kcal from fat and 35% kcal from sucrose natural diet, Western low-protein diet (W, n =6), and (4) a 27% kcal from fat and 35% kcal from sucrose natural diet, +1% NaCl natural diet, Western low-protein + high Salt (WS, n = 7) ad-libitum throughout pregnancy and lactation. Female rats were mated with male rats. After pregnancy was detected, they were individually housed. Dams were maintained on their diets throughout pregnancy and lactation. Body weights and chows exposed to four experimental diets were weighed and recorded on the same day every week before pregnancy, during pregnancy (three weeks), and during lactation (three weeks). The baby rats were fed only breast milk until weaning. To ensure standardized nutrition until weaning, the offspring of mother rats with 8-13 pups in a litter were included in the study. The pups not included in the study were killed using 6-8% carbon monoxide in a closed container.

At weaning (three weeks of age), male and female offspring of dams fed control diet were assigned to four experimental groups: (1) Control diet (CC, (M:6, F:6)), (2) high salt diet (CS, (M:6, F:6)), (3) Western low-protein diet (CW, (M:6, F:6)), and (4) Western low-protein and high salt diet (CWS, (M:6, F:6)).

Male and female offspring of dams fed 1% NaCl diet (high salt) were assigned to two experimental groups: (1) control diet (SC, (M:4, F:4)) and (2) high Salt diet (SS, (M:4, F:4)). Male and female offspring of dams fed Western low-protein diet were assigned to two experimental groups: (1) control diet (WC, (M:4, F:4)) and (2) Western low-protein diet (WW, (M:4, F:4)). Male and female offspring of dams fed Western low-protein and high salt diet were assigned to two experimental groups: (1) control diet (WSC, (M:4, F:4)) and (2) Western low-protein and high Salt diet (WSWS, (M:4, F:4)). The experimental design was illustrated in Figure 1.

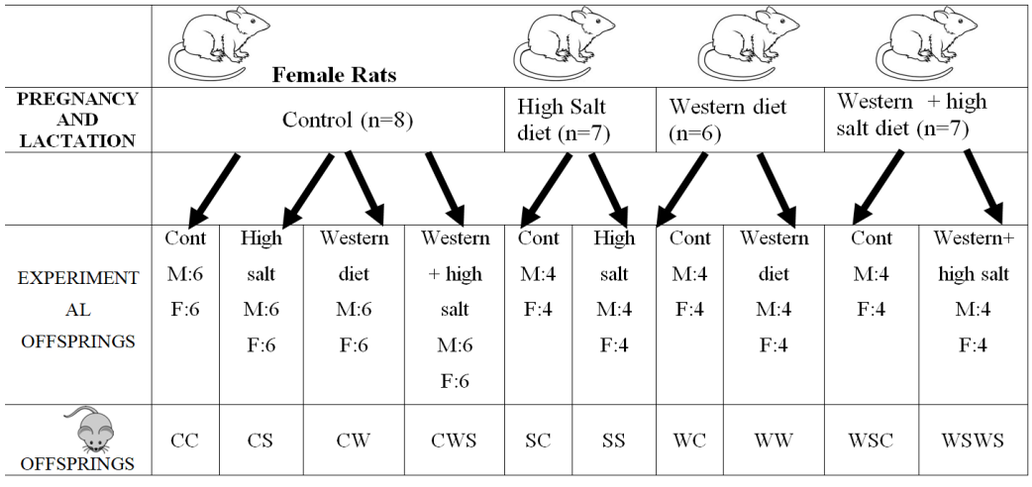

Figure 1. *The experimental design. M, Male; F, Female. CC, rats fed the control diet during pregnancy, lactation and postweaning periods; CS, rats fed the control diet during the pregnancy, lactation periods and high salt diet during postweaning period; CW, rats fed the control diet during the pregnancy, lactation periods and western low-protein diet during postweaning period; CWS, rats fed the control diet during the pregnancy, lactation periods and western low-protein and high salt diet during postweaning period; SS, rats fed the high salt diet during the pregnancy, lactation and postweaning periods; SC, rats fed the high salt diet during the pregnancy, lactation periods and control diet during postweaning period; WW, rats fed the western low-protein diet during the pregnancy, lactation and postweaning periods; WC, rats fed the western low-protein diet during the pregnancy, lactation periods and control diet during postweaning period; WSWS, rats fed the western low-protein and high salt diet during the pregnancy, lactation and postweaning periods; WSC, rats fed the western low-protein and high salt diet during the pregnancy, lactation periods and control diet during postweaning period.*

To assess gender differences, we examined both male and female offspring. After assigning experimental groups to prevent breeding, male and female offspring were housed separately. At 18 weeks of age, blood samples were collected and terminated under anesthesia after overnight fasting. The samples were stored at −20 °C until biochemical analysis.

2.3. Diet and Food Intake

This study used four natural rat chows (MBD animal feed/ Kocaeli). Since comparing the purified Western low-protein diet with the natural standard diet may lead to bias, we also used natural ingredients when preparing the Western low-protein diet. The compositions of chows were prepared based on previous studies (14, 15).

Control diet: Included standard rat chows. It was approx 70% carbohydrate, 18% protein, 12% fat per 100 kcal, 0 mg cholesterol and 0.5 g salt per 100g dry weight. The total energy was 35.5kcal/100g dry chow.

High salt model: Standard rat chow contains 0.2-0.5% NaCl. Therefore, a high salt diet is determined as 1%. It was approx 70% carbohydrate, 18% protein, 12% fat per 100 kcal, 0 mg cholesterol and 1 g salt per 100g dry weight. The total energy was 35.5kcal/100g dry chow.

Western low-protein diet: Consisted of natural ingredients. The composition was formed based on She et al. (14). It was approx 65% carbohydrate, 8% protein, 27% fat, 35% sucrose per 100 kcal, 94 mg cholesterol and 0.5g salt per 100g dry weight. The total energy was 42.3kcal/100g dry chow.

Western low-protein and high-salt diet: Constituted natural ingredients. It was approx 65% carbohydrate, 8% protein, 27% fat, 35% sucrose per 100 kcal, 94 mg cholesterol and 1 g salt per 100g dry weight. The total energy was 42.3kcal/100g dry chow.

2.4. Body Weight

Using a precision scale sensitive to 0.1 g, the rats were weighed every two weeks, from 4 weeks of age until 18 weeks of age. They were weighed in the early evening at the same hour. We did not weigh the rats every week to prevent rats from getting stressed.

2.5. Biochemical Analysis and ELISA Tests

After the experiment, the animals were sedated with Ketamine and Xylazine. Blood samples were collected through an intracardiac puncture after 12 hours of fasting. The sample was centrifuged to obtain the serum and stored at – 20°c. Biochemical variables were determined: fasting glucose, total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), and triglyceride.

Insulin and leptin analyses were performed in duplicate in plasma using the sandwich ELISA principle. The concentrations were determined using rat insulin and leptin ELISA kits (Elabscience, Texas, USA).

2.6. Statistical Analysis

The data were analyzed using the Statistical Package for Social Sciences (SPSS, 21.0) at a significance level of < .05. Normality was tested using the Shapiro-Wilk and Levene tests. In rat dams, for the normally distributed groups, a One-Way Analysis of Variance (ANOVA) test was used. If there was a difference between the groups ($p<.05$), the LSD multiple comparison test was performed for post-hoc analysis. Where the assumption of normal distribution was met, but the assumption of homogeneity of variances was not met, the Welch Test was used. If there was a difference between the groups, the Games Howell Test was used for multiple comparisons. Where the normal distribution assumption was not met, the Kruskal-Wallis Test was performed. The Dunn Test was used for multiple comparisons.

In offspring, a two-way mixed ANOVA test was performed. If there was a difference between the groups, the LSD test was applied. Male and female groups were analyzed separately. We applied a logarithmic transform to normalize offspring energy intake. Since the normal distribution assumption was not met, the Kruskal-Wallis Test was performed to compare offspring blood glucose levels, and then, the Dunn Test was used for multiple comparisons. Mean ($\bar{X}$) and standard deviation (SD) values were given in all tables.

3. RESULTS

Body weight changes during pregnancy and lactation were not significantly different ($p>.05$) (Table 1). Dams fed a Western low-protein diet and a Western low-protein and high salt diet (WS) had significantly higher energy but lower protein intake than dams fed control and high salt diets during pregnancy ($p<.05$). During lactation, the energy intake of dams was not significantly different ($p>.05$) (Figure 2).

Table 1. *Body weight changes of rat dams during pregnancy and lactation and 2nd day weight of offspring (n= 6-8)*

	Control	High Salt Diet	Western low-protein Diet	Western low-protein and high salt diet	p
Weight Changes	**X̄±SD**	**X̄±SD**	**X̄±SD**	**X̄±SD**	
Weight gain during pregnancy (g)	113.75± 34.57	106.57± 21.56	107.0± 26.86	124.57± 32.93	.657+
Weight loss during lactation (g)	-20.875± 7.85	-9.86± 25.35	-11.83± 10.57	-10.29± 29.47	.329*
2nd days weight of offspring (g)	8.81±0.92	7.95±1.52	7.39±1.76	7.52±1.37	.226+

**Welch test; + One-way ANOVA test*

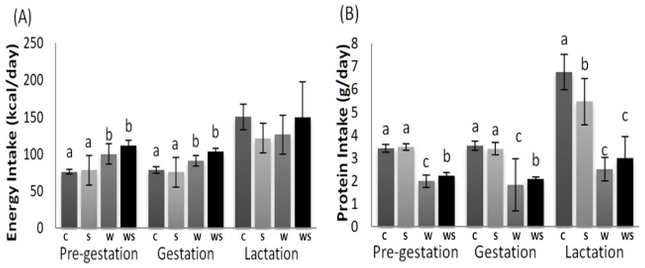

Figure 2. *Energy and Protein Intake of rat dams*
Energy intake of rat dams (n 6-8). (B) Protein intake of rat dams (n 6-8). Values are means, with standard errors represented by vertical bars. a,b,c Mean values with unlike letters were significantly different (p<0.05). C, dams fed the control diet; S, dams fed high salt diet; W, dams fed western low-protein diet, dams fed the western low-protein and high salt diet.

Male offspring groups did not significantly differ in energy intake in the second month. In the second month, female rats exposed to a Western low-protein diet during pregnancy, lactation, and postweaning periods consumed less energy than the CC (control) group (p<.05). In the fourth month, female rats from Western low-protein diet dams and fed a control diet consumed less energy than the control group (p<.05) (Supplementary Table 1). Both male and female adult rats exposed to the WS diet during pregnancy, lactation, and postweaning periods consumed the highest energy in the fourth month (Figure 3).

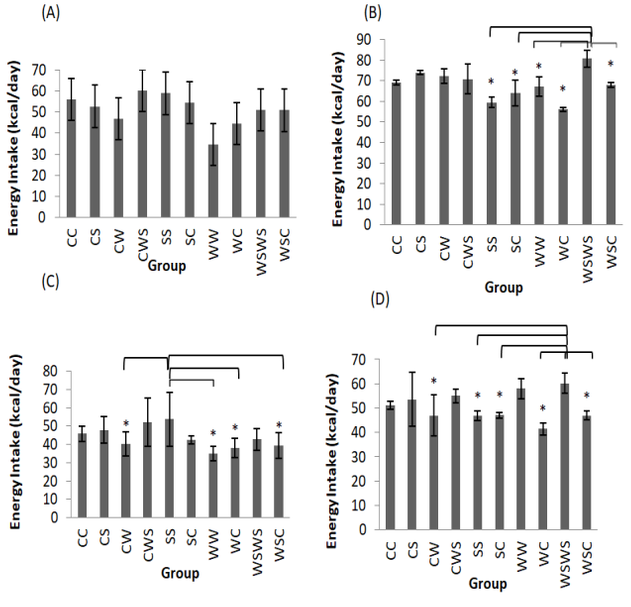

Figure 3. *Energy Intake of offspring*

*(A) Energy intake of males in the second month (n 4–6). (B) Energy intake of females in the second month (n 4–6). (C) Energy intake of males in the fourth month (n 4–6). (D) Energy intake of females in the fourth month (n 4–6). Values are means, with standard errors represented by vertical bars. CC, rats fed the control diet during pregnancy, lactation and postweaning periods; CS, rats fed the control diet during the pregnancy, lactation periods and high salt diet during postweaning period; CW, rats fed the control diet during the pregnancy, lactation periods and western low-protein diet during postweaning period; CWS, rats fed the control diet during the pregnancy, lactation periods and western low-protein and high salt diet during postweaning period; SS, rats fed the high salt diet during the pregnancy, lactation and postweaning periods; SC, rats fed the high salt diet during the pregnancy, lactation periods and control diet during postweaning period; WW, rats fed the western low-protein diet during the pregnancy, lactation and postweaning periods; WC, rats fed the western low-protein diet during the pregnancy, lactation periods and control diet during postweaning period; WSWS, rats fed the western low-protein and high salt diet during the pregnancy, lactation and postweaning periods; WSC, rats fed the western low-protein and high salt diet during the pregnancy, lactation periods and control diet during postweaning period. * displays significant difference*

For all months, offspring fed a Western low-protein diet consumed the least protein regardless of maternal diet. The offspring fed the WS diet come right after those groups in terms of consuming low-protein (Figure 3).

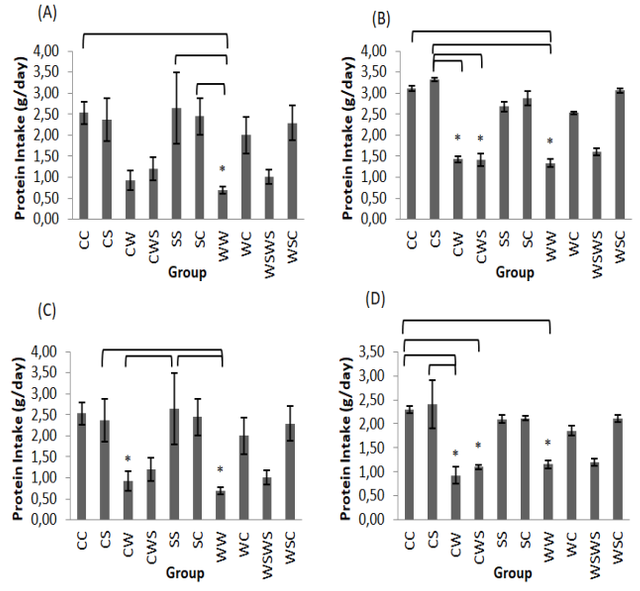

Figure 4. *Protein Intake of Offspring*

*(A) Energy intake of males in the second month (n 4–6). (B) Energy intake of females in the second month (n 4–6). (C) Energy intake of males in the fourth month (n 4–6). (D) Energy intake of females in thefourth month (n 4–6). Values are means, with standard errors represented by vertical bars. CC, rats fed the control diet during pregnancy, lactation and postweaning periods; CS, rats fed the control diet during the pregnancy, lactation periods and high salt diet during postweaning period; CW, rats fed the control diet during the pregnancy, lactation periods and western low-protein diet during postweaning period; CWS, rats fed the control diet during the pregnancy, lactation periods and western low-protein and high salt diet during postweaning period; SS, rats fed the high salt diet during the pregnancy, lactation and postweaning periods; SC, rats fed the high salt diet during the pregnancy, lactation periods and control diet during postweaning period; WW, rats fed the western low-protein diet during the pregnancy, lactation and postweaning periods; WC, rats fed the western low-protein diet during the pregnancy, lactation periods and control diet during postweaning period; WSWS, rats fed the western low-protein and high salt diet during the pregnancy, lactation and postweaning periods; WSC, rats fed the western low-protein and high salt diet during the pregnancy, lactation periods and control diet during postweaning period. * displays significant difference*

Table 2. *Postnatal growth of male and female offspring*

		Body Weight (g)							
		4th week	6th week	8th week	10th week	12th week	14th week	16th week	18th week
SEX	Group	X̄±SD	X̄±SD	X̄±SD	X̄±SD	X̄±SD	X̄±SD	X̄±SD	X̄±SD
MALE	CC	73.3±16.3a	150.2±34.7a	203.8±36.1a	249.8±33.6a	297.8±35.3 b	335.0±36b	372.3±40.3b	386.2±42.9b
	CS	57.7±7.9bc	144.2±12.8ab	221.5±11.7 a	276.0±16.2a	331.7±17.7a	369.3±23.7a	408.0±30.1a	423.0±26.9a
	CW	57.8±4.4b	94.0±10.1 de	133.0±18.9 c	180.4±16.9c	221.8±22.6e	249.4±17.9e	282.0±23.2d	310.6±20.7d
	CWS	54.5±6.5bcd	112.7±17cd	167.8±28.5 b	212.5±29.9b	267.8±26.8cd	294.5±33.7 cd	323.7±33.7c	336.5±32.7cd
	SS	53.5±1.9bcd	114.3±8.3 cd	202.3±12.3 a	252.3±12.7a	284.0±17.1bc	306.0±17.9c	328.5±17.9c	340.8±20.8cd
	SC	55.8±2.2bcd	125.5±5.3bc	208.3±18.2a	260.0±23.7a	303.8±33.9ab	334.8±33.9 ab	347.0±39.4bc	366.3±37.4bc
	WW	29.3±3.7e	52.3±3.2g	87.5±5.9d	116.5±7.0d	163.5±8.4f	198.5±7.7f	193.3±26.2e	239.0±8.8e
	WC	31.3±1.9e	83.5±6.6ef	164.0±13.9b	210.8±13.4bc	245.8±13.4de	273.3±17.6 cde	303.3±15.8cd	320.3±22.9d
	WSWS	47.3±7.4d	94.8±15.9de	167.3±24.1b	207.5±25.8bc	259.0±31.4cd	295.5±36cd	328.5±40.9c	345.3±46.1cd
	WSC	46.3±2.4cd	115.8±8.6cd	205.3±14.5a	255.5±19.7a	310.3±18.8ab	347.8±20.4ab	385.0±22ab	406.8±25.5ab
FEMALE	CC	72.0±13.2a	122.3±16.7ab	167.8±13.3ab	202.8±16a	232.8±16.5a	247.3±17.9a	264.5±18.1a	269.3±15a
	CS	56.7±5.2bc	126.8±8.9a	168.0±16.1ab	201.0±16.8a	219.8±22.5ab	227.2±21.4ab	240.5±25.3ab	248.8±27.3ab
	CW	48.5±3.4de	78.2±6.1ef	106.3±8e	141.8±8.5d	172.3±8.5e	189.8±6.3cd	206.5±8.9cd	217.0±6.5cde
	CWS	47.8±7de	97.3±16.9cd	137.8±16.6cd	169.3±17.1bc	195.3±19.5cd	213.3±23.6bc	222.5±23.8bcd	227.3±22.8bcde
	SS	57.7±4.6b	121.3±4.9ab	175.0±5.2a	199.7±12.3a	215.3±20.6abc	228.3±15ab	245.0±13.5ab	249.7±10ab
	SC	53.0±3.6cd	111.8±6.9bc	159.3±9.2ab	187.3±9.2ab	204.3±8.9bc	213.3±8.2bc	228.0±13.8bc	233.0±12.9bcd
	WW	34.0±8fg	56.3±10.2g	88.0±13.8f	115.3±14.8e	154.5±22.2e	175.0±26.5d	223.0±12.6bcd	201.5±28.9e
	WC	31.8±4.3g	79.8±11.03ef	128.8±17.7d	156.5±23.8cd	172.0±27de	186.8±31.3cd	198.0±32.4d	206.3±31.6de
	WSWS	41.0±4.6efg	86.5±11de	135.8±18.3cd	165.0±22.9bc	198.3±27.7bcd	217.5±29.7b	232.8±26.8bc	238.5±23.9bc
	WSC	42.3±4.8ef	96.0±2.9cd	152.3±4.7bc	183.5±11.3ab	209.0±11.4abc	223.0±13.8ab	235.8±20.9b	242.8±27.8abc

C, control diet; S, high salt diet; W, western low-protein diet; WS, western low-protein and high salt diet; CC, rats fed the control diet during pregnancy, lactation and postweaning periods; CS, rats fed the control diet during the pregnancy, lactation periods and high salt diet during postweaning period; CW, rats fed the control diet during the pregnancy, lactation periods and western low-protein diet during postweaning period; CWS, rats fed the control diet during the pregnancy, lactation periods and western low-protein and high salt diet during postweaning period; SS, rats fed the high salt diet during the pregnancy, lactation and postweaning periods; SC, rats fed the high salt diet during the pregnancy, lactation periods and control diet during postweaning period; WW, rats fed the western low-protein diet during the pregnancy, lactation and postweaning periods; WC, rats fed the western low-protein diet during the pregnancy, lactation periods and control diet during postweaning period; WSWS, rats fed the western low-protein and high salt diet during the pregnancy, lactation and postweaning periods; WSC, rats fed the western low-protein and high salt diet during the pregnancy, lactation periods and control diet during postweaning period.

All male offsprings displayed weight gain over the weeks, all female offsprings displayed weight gain over the weeks except for 18th week ($p<.05$)

^{a,b} Mean values with unlike superscript letters were significantly different($p<.05$).

Table 3. *Blood parameters of adult offspring*

		Leptin (pg/mL)	Insulin (pg/mL)	Glucose (mg/dL)	Total Cholesterol (mg/dL)	LDL Cholesterol (mg/dL)	HDL Cholesterol (mg/dL)	Triglyceride (mg/dL)
SEX	**Group**	**X̄±SD**	**X̄±SD**	**X̄±SD**	**X̄±SD**	**X̄±SD**	**X̄±SD**	**X̄±SD**
MALE	CC	721.49±54.13	2513.33±846.23	193.0±97.74ab	43.83±9.22	12.83±5.67a	58.3±12.43a	60.67±14.56
	CS	629.40±97.38	2941.67±335.58	270.5±122.97ab	54.83±15.11	22.33±7.34bc	66.42±17.7a	89.50±21.27
	CW	734.80±79.06	2400.14±457.76	131.2±16.75ab	47.2±3.83	13.80±1.92ad	68.48±8.83a	87.60±30.23
	CWS	668.20±129.69	2528.69±729.75	108.33±22.3b	55.67±7.71	21.83±4.79bcd	72.33±10.05a	80.0±12.02
	SS	588.04±48.09	2553.57±612.22	353.5 ±52.5a	58.75±10.24	21.50±5.26abcd	72.68±9.92a	88.50±18.16
	SC	626.86±55.12	2372.14±333.40	160.0 ±10.68ab	53.75±5.74	19.25±5.97abcd	72.43±5.87a	77.50±7.51
	WW	654.30±53.35	2139.29±365.57	155.25±19.91ab	53.5±8.96	14.50±7.42acd	87.6±9.96ab	81.50±33.43
	WC	679.72±29.31	3408.93±365.44	126.75±14.17ab	46.25±6.18	14.0±3.37acd	64.85±4.80a	114.25±40.43
	WSWS	617.84±29.62	2578.57±574.75	186.0 ±14.79ab	51.25±6.95	15.5±5.8acd	81.13±6.67b	64.75±10.11
	WSC	678.02±23.12	2620. 36±321.74	157.75±27.1ab	52.75±8.42	26.5±14.15d	63.83±21.29a	75.25±24.85
	p	.72**++**	.122+	**.003**++	.260+	**.035**+	**.038***	.179*
FEMALE	CC	839.08±111.52a	2713.45±597.30a	148.17±36.0abc	54.33±10.13ab	12.83±5.12ab	81.53±10.57a	117.67±43.44ab
	CS	747.94±166.32ab	2501.67±609.42ab	129.5±17.92bc	56.17±9.99ab	12.67±3.08ab	80.08±14.2ab	94.67±22.21ab
	CW	762.43±69.83a	1878.1±621.28cde	106.17±29.38c	44.5±4.28b	7.33±1.86c	83.62±8.44a	53.33±8.52b
	CWS	644.01±113.85ab	1871.55±368.82cde	125.33±27.08bc	55.17±8.52ab	12.0±3.90ab	82.48±8.12a	142.5±29.18a
	SS	690.28±106.83ab	1373.04±158.91e	147.25±24.49abc	59.75±14.5ab	12.75±4.99ab	80.78±5.12ab	159.0±83.32ab
	SC	665.28±48.58ab	2448.21±391.46abc	123.25±13.07bc	51.75±1.26b	9.0±2.16bc	86.63±6.68a	78.25±20.69ab
	WW	775.07±102.3ab	2334.29±518.84abc	113.67±3.06c	48.0±11.14ab	11.0±2.65abc	68.47±11.35b	153.33±58.53ab
	WC	628.71±53.03ab	2088.93±177.83bcd	115.5±5.07c	45.0±9.45ab	8.50±2.38bc	74.23±5.13ab	78.50±34.28ab
	WSWS	798.94±53.7a	2170.0±96.23abc	169.0±2.45a	64.5±0.41a	16.0±0.82a	109.05±1.18c	55.0±0.82b
	WSC	564.19±45.25b	1459.46±472.35de	160.25±8.77ab	51.25±4.99ab	11.75±3.77ab	86.2±1.52a	78.0±28.30ab
	p	**.002***	**.001**+	**<.001***	**<.001***	**.040+**	**<.001+**	**<.001***

**Welch test, +One way ANOVA test; ++ Kruskal-Wallis test; [a,b] Mean values with unlike superscript letters were significantly different(p<.05).*

C, control diet; S, high salt diet; W, western low-protein diet; WS, western low-protein and high salt diet; CC, rats fed the control diet during pregnancy, lactation and postweaning periods; CS, rats fed the control diet during the pregnancy, lactation periods and high salt diet during postweaning period; CW, rats fed the control diet during the pregnancy, lactation periods and western low-protein diet during postweaning period; CWS, rats fed the control diet during the pregnancy, lactation periods and western low-protein and high salt diet during postweaning period; SS, rats fed the high salt diet during the pregnancy, lactation and postweaning periods; SC, rats fed the high salt diet during the pregnancy, lactation periods and control diet during postweaning period; WW, rats fed the western low-protein diet during the pregnancy, lactation and postweaning periods; WC, rats fed the western low-protein diet during the pregnancy, lactation periods and control diet during postweaning period; WSWS, rats fed the western low-protein and high salt diet during the pregnancy, lactation and postweaning periods; WSC, rats fed the western low-protein and high salt diet during the pregnancy, lactation periods and control diet during postweaning perio

As shown in Table 2, among male offspring, the control group had the highest weight in the fourth week. Afterward, male offspring from control dams and fed high salt diet (CS) group gained more weight than the control group and had the highest weight in the twelfth and following weeks. The WSC group also gained more weight than the control offspring for all weeks and had the second highest weight among adult male rats from the 8th week on. The offspring fed the Western low-protein diet had significantly lower weight than the other groups ($p<.05$). Male WC offspring come right after those groups in terms of having low weight.

Among female offspring, the control group had the highest weight for all weeks. Female offspring exposed to the Western low-protein diet during pregnancy, lactation, and postweaning periods had the lowest weight for all weeks, except for one week. The offspring from control dams and fed the Western low-protein diet (CW) had the second lowest weight till the twelfth week. After the twelfth week, the WC group had the second lowest weight. The rats fed WS diet come right after those groups in terms of having low weight. Since the weight gain of the offspring from high salt dams and fed control diet (SC) decreased over the weeks, they were among the low-weight rat groups after the 12th week (Table 2).

Regarding blood parameters, insulin levels of male offspring groups did not vary significantly ($p>.05$). Male rats exposed to the WS diet during pregnancy and lactation but fed the control diet had higher LDL cholesterol levels than the control group ($p<.05$). Triglyceride and total cholesterol levels of male offspring groups did not vary significantly ($p>.05$). The female control group had the highest leptin and insulin levels. The female WSC group had lower leptin and insulin levels than the control group ($p<.05$). The female offspring from control dams and fed Western low-protein and WS diet had lower insulin levels than the control group ($p<.05$). The female offspring exposed to high salt diet during pregnancy, lactation, and postweaning periods had higher triglyceride level than the control group but not significantly ($p>.05$) (Table 3).

4. DISCUSSION

Our results showed that body weight changes in rat dams fed the Western low protein diet and WS diet during pregnancy were not different from the control group (16, 17). In those studies, the daily energy intakes of dams fed the Western diet during pregnancy were not different from the control group. While our rats fed the Western low protein and WS diet during pregnancy consumed more energy than the control group, they gained similar weights. The protein content of our Western low-protein diet was 8.48%. However, the ideal protein intake in rats during growth, pregnancy, and lactation should be 12% of the dry raw material [National Research Council (NRC)] (18). If the protein is around 17-23%, the maximum growth will be supported by 95-100% (19). Therefore, the protein rate in the dry matter of the Western low-protein diet was below the required level. Brito et al. (20) stated that the weight gain of rats fed low-protein (5%) but high-fat and energy diet was lower than control dams. They reported that while energy and fat content were high, fat absorption might be impaired, thus preventing body weight gain. In lactation, similar to Merle et al. (17) and Alexandre-Gouabau et al. (16), weight changes in rats fed Western low-protein and WS diets were not different from control dams. The result may be caused by similar energy intake. The rat dams fed a high salt diet had similar energy intake and body weight change to the control dams (21, 22). While the reason is obscure (23), relatively short exposure time (pregnancy and lactation last six weeks) may have caused this result.

Both male and female offspring exposed to the Western low-protein diet during pregnancy and lactation had less weight than the control groups. A meta-analysis concluded that a maternal diet with a high protein/non-protein ratio leads to higher offspring weight. Postweaning diet had no effect on adolescent and adult weights (24). However, this result was inconsistent with our findings. The body weight of the offspring fed the control diet and having dams on the WS diet were similar to the control group. The offspring from dams on a Western low-protein diet and fed a control diet were lighter than the control group. Maternal high salt intake and a Western low-protein diet may have caused a change in appetite mechanisms. Coupe et al. (25) reported that orexigenic peptide mRNA expressions (NPY and AgRP) in the hypothalamus of offspring of dams fed a low-protein diet were higher than those from control dams. Increased orexigenic peptides cause hyperphagia and high energy intake, closing the weight gap. Our female offspring from dams on the WS diet and fed the control diet had lower leptin and insulin levels than the control group. Leptin reduces food intake and increases energy expenditure (26) Therefore, low leptin levels may be related to higher weight gain. Low insulin in the blood also leads to weight gain by increasing food intake and decreasing energy expenditure by stimulating orexigenic peptides, particularly NPY (27).

Adult male rats from control dams fed a high salt diet had higher weights than the control group. This result was consistent with human studies (28, 29). Salt improves food taste, thus increasing food consumption (29). However, most animal studies reported conflicting results (2, 30, 31). High salt diets in rat models are ≥4%, which is too much to reflect excessive salt consumption in humans (2, 32). The World Health Organization (WHO) and the American Dietary Guide recommend salt consumption to be less than 5 g/day (33). Salt consumption is 10-15 g/day in today's Western societies (34). An adult consumes an average of 1200 g of food daily (35). Salt intake constitutes 0.8%-1.2% of the total food consumption. One g salt in 100 g chow/diet better reflects the salt content in high salt diets. Our male rats fed the high salt diet gained weight regardless of energy and protein intake, as Ma et al. (36) found in humans. Excessive salt consumption, regardless of energy intake, causes more fat storage in the body (37). Chronic high salt intake leads to hyperinsulinemia, excessive secretion of insulin, an anabolic hormone, accelerates the conversion of glucose into lipids. Increased lipogenic activity causes adipocyte hypertrophy and increased body fat (37). Lanaspa et al. found hyperleptinemia

and leptin resistance in adolescent male rats fed with high salt water (1%) (8). Rats gained more weight than the control group by increasing food consumption and decreasing energy expenditure. Male rats from control dams and fed a high salt diet had neither higher leptin nor insulin levels than the control group. Decreased energy consumption or increased testosterone may be the key factor. Like Lanaspa et al. (8), we found higher LDL cholesterol and triglyceride levels than the control group. High body weight is associated with higher LDL cholesterol and triglyceride levels (38). However, we found that female rats from control dams and fed a high salt diet had similar weight, energy and protein intake, and blood parameters to the control group. Female rats handled a high salt diet better than males (39). Therefore, metabolic response to high salt diets may be gender specific.

We found rats from control dams fed a Western low protein diet and WS diet had lower body weights than the control group regardless of energy intake. Low protein intake during the developmental period leads to delayed growth. Postweaning low protein intake is associated with lifelong low offspring weights (40). Despite the low weight, we observed high LDL cholesterol levels in male rats fed Western low-protein and high-salt diets but not Western low-protein diets alone. A human study reported that the LDL/HDL ratio increases up to 12 g/day of salt consumption, reflecting our study's exact high salt model (41). While some researchers indicate that the relationship between salt intake and blood lipids is through hyperinsulinemia and insulin resistance, we found no difference in insulin levels (42). Impaired gut microbiota by a high salt diet may lead to high blood cholesterol. A high-salt diet affects gut health by altering gut microbiota composition. This alteration is characterized by a lack of diversity and increasing *Lachnospiraceae* and *Ruminococcus* but decreasing *Lactobacillus* group (43). *Lactobacillus* species, such as *L. acidophilus* ATCC 314, *L. bulgaricus* FTCC 0411, and L. *casei* MB3, are cholesterol-reducing bacteria, have rate-limiting 3-hydroxy-3-methylglutaryl coenzyme A reductase activity and regulate bile salt hydrolase activity. Deficiency in that species may have caused high blood cholesterol levels (44).

Our female rats from control dams fed the Western low-protein diet (CW) and WS diet (CWS) had lower insulin levels than the control group. Our female rats from dams on the Western low-protein diet, and WS diet and fed the control diet (WC and WSC) had lower insulin levels than the control group. Low protein intake during the fetal period decreases vascularization of the pancreas and reduces the number of islets of Langerhans and β cells. The decrease in β cells causes insufficient insulin secretion (25, 40, 45). In the fetal period and early life/after weaning, pancreas islets, vascularization, and β cells are vulnerable to a low protein diet due to ongoing anatomical and functional growth (40). Rats from dams on high-fat diets have smaller islets size of Langerhans and lower insulin secretion (46). Insulin levels did not differ among male rats. Therefore, we concluded female rats are more vulnerable to low protein intake during critical development periods. We did not observe low insulin levels in female rats exposed to low protein during gestation, lactation, and postweaning periods (WW and WSWS), unlike Gosby et al. (45). This result reminds us of the Thrifty Phenotype Hypothesis, proposing a metabolic adaptation more advantageous if undernutrition sustains. If the postnatal environment supplies abundant nutrition, those individuals will be at increased risk of obesity and Type II Diabetes Mellitus (47). Considering the opposite results of Gosby et al. high fat and high sugar intake during all critical developmental periods may have affected fetal programming (45). While it is speculative, high fat and high sugar and low protein intake may have caused epigenetic modifications in female offspring.

Female rats from high salt dams and fed a control diet (SC) had lower body weight despite similar food intake. A study reported that the mice had lower body weight due to high energy expenditure despite similar food intake (48). Increased brown adipose tissue activity may have contributed to increased energy expenditure in offspring of high salt dams (49). Piecha et al. (50) reported that rats from high-salt dams had higher sodium excretion than the control group due to differences in intestinal absorption. Therefore, low nutrient absorption may be another reason for lower weight gain despite higher food intake (2). Other studies reported those results in male rats (49, 50). There should be more studies to investigate gender factors.

Male adult rats from high salt dams and fed high salt diet (SS) had lower energy intake and body weight than the control group in this study, like Piecha et al. (50). Since we did not find any study searching appetite mechanism in those rats and there was no difference in leptin and insulin levels, drawing a conclusion based on appetite could be speculative. The offspring of high salt dams had high sodium excretion due to low intestinal absorption. The high salt consumption after weaning may also have exacerbated sodium excretion and nutrient malabsorption because they produce more feces than other rats on low sodium diet (50).

5. CONCLUSION

Postweaning a high salt diet leads to higher body weight in male rats. However, a maternal high-salt diet prevents that detrimental effect. We also found regardless of energy, fat, and salt intake, low protein intake during critical developmental period (gestation, lactation, and early childhood) causes growth retardation and low adult body weight in rats. Also, female rats fed a low-protein diet in the fetal period or in early childhood were more vulnerable than male rats and had significantly low insulin levels. However female rats exposed to western low-protein and WS diet during pregnancy, lactation and postweaning periods had similar insulin levels to control group. Maintaining the maternal western low-protein diet after lactation prevents detrimental effect of low-protein diet on insulin levels. Exposure to high salt diet in fetal life may diminish obesity effect of high salt. Further research is needed to examine metabolic responses to maternal diet with respect of gender and fully define the mechanisms of the findings.

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Design of the study: MEÖ, NYA
Acquisition of data for the study: MEÖ
Analysis of data for the study: MEÖ
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Clinical and Experimental Health Sciences

Evaluation of Traceability of Dietary Urine Biochemistry Changes with Commercial Urine Strips

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ABSTRACT

Objective: This research aims to (i) examine the effects of nutrition on urine biochemistry and (ii) compare the two different measurement methods (laboratory and commercial strip). This means it is desired to bring a new direction to the literature.

Methods: The study involved 42 women aged 20-30 from Turkey and examined the urinary excretion of calcium, vitamin C (smoker and non-smoker), sodium, and magnesium based on nutritional status. The collected urine samples were applied onto commercial urine strips, and the resulting color changes were recorded by smartphone; at the same time, it was sent to the laboratory for comparative analysis. The obtained data were used in regression and correlation statistical analysis. All statistical analyzes were performed using IBM SPSS 28.0.

Results: While evaluating the regression analysis results in which the excretion due to nutrition was examined, each nutritional level was compared to the restricted intake. ANOVA sig values <.001, t values 1.96 <, in all metabolites (calcium, vitamin C, sodium, magnesium) evaluations. The following rates (R^2 values) were obtained restricted/optimal nutrition in calcium, vitamin C, sodium, and magnesium: .636, .575, .386, and .209 respectively; restricted/high nutrition in calcium, vitamin C (non-smoker), vitamin C (smoker), sodium, magnesium: .442, .308, .482,.413 and .337 respectively; restricted/supplement in calcium, vitamin C, magnesium: .273, .698 and .799. Calcium and magnesium strips correlated strongly with lab results, correlation coefficients are .679 and .59 respectively. Sodium and creatinine strips correlated very strongly with lab results, correlation coefficients are .876 and .884 respectively.

Conclusion: The study revealed that nutrition significantly affected urine excretion levels for calcium, vitamin C, sodium, and magnesium. Additionally, the results showed that urine strips had a correlation with laboratory results indicating their usefulness for pre-diagnosis purposes.

Keywords: Nutrition, urine biochemistry, urinary excretion, commercial urine strip, statistical analyzes.

1. INTRODUCTION

Metabolic phenotypes, including signals from diet and endogenous metabolism, are the product of environmental and genetic interactions. These products can be used to establish meaningful relationships between health outcomes and nutrients. Longitudinal twin studies quantify the heritability of metabolic phenotypes; it also shows that nutrition has various effects on a person's metabolic phenotype (1,2). Metabolomics can be used to identify dietary biomarkers in nutritional research (2). Urine samples contain a higher amount of food-derived metabolites than blood samples (3). Controlled trials on metabolites found in urine show that urine samples can provide an objective measure of an individual's nutrition status (3,4). Studies have shown the relationship between 46 metabolites in urine and nutrition. Some metabolites have been associated with glucose, fructose, citrus, and vitamin C intake, while other metabolites have been associated with alcohol intake, as well as the consumption of meat (3). In addition, some metabolites such as sodium have also been found to be associated with various health conditions like high blood pressure and obesity (3-6).

Urine analysis, commonly referred to as urinalysis, is a diagnostic test that analyzes the urine in order to assess and monitor different aspects of health. This straightforward procedure provides information about the functioning of the urinary system and can aid in diagnosing various medical conditions. Urine analysis is one of the most crucial aspects of the healthcare, being a multipurpose and also informative diagnostic tool (7). There are many advantages to using urine as a diagnostic specimen over other biofluids. Urine sampling is non-invasive, allowing for repeated collection

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in the desired quantity (8). Among the major goals of urine analysis is to diagnose and detect a wide range of medical conditions. Abnormalities in urine composition may indicate various conditions such as kidney disease, diabetes, UTI, and liver disorders (9,10). Also, the measurement of urine composition is important for body's general wellness.

Creatinine in urine was also examined to aid in evaluating the measurement of calcium and magnesium in urine samples. Calcium (Ca) is the fifth most abundant element in the human body and constitutes 2% of an adult's body weight. The body absorbs about 30-40% of the calcium found in the foods consumed through one's diet, leaving the rest to be excreted through urine and feces (11,12). Vitamin C plays a role in immune defense by supporting the cellular functions of the innate and adaptive immune systems (13,14). Vitamin C protects against environmental oxidative stress by keeping the skin's oxidant cleansing activity and epithelial barrier function (15,16). Sodium is vital for human health and the main cation of the extracellular fluid and is found naturally in foods. The required amount of sodium for the body is determined by the kidneys (17). Magnesium (Mg) is the fourth most abundant cation in the body (after Na+, K+, and Ca2+) and the second most abundant cation in the intracellular compartment after K+ (18,19). The body of a healthy adult contains approximately 24 g of magnesium (19,20). About 65% of this magnesium is found in the mineral phase of the bone, 34% in the intracellular space, and 1% in the extracellular space (19).

With the 'new normal' introduced by the pandemic, this research aims to explain new approaches in telemedicine that enable individuals to use test kits from home to control and diagnose their health. This research investigates (i) examine the effects of nutrition on urine biochemistry and (ii) compares the two different measurement methods (laboratory and commercial strip).

2. METHODS

2.1. Source Population

Data from a diet-induced urinary metabolite excretion study conducted between July 2022 and October 2022 were used.

The subjects were 42 individuals who participated in living in Turkey. Inclusion criteria were that participants had to be 20-30 years old, permanent residents of Turkey, not have any health problems, and not doing sports professionally (Table 1). Groups were assigned based on the number of people who voluntarily wanted to participate in the study.

Table 1. *Information of participants*

Group		Calcium	Vitamin C	Sodium	Magnesium
Age (year)	Mean ± SD	24.8 ± 2.18	24.86 ± 2.13	25.07 ± 2.14	25 ± 1.99
	Range	21-30	21-30	21-30	22-30
Height (cm)	Mean ± SD	162.94 ± 6.14	163.07 ± 5.63	163 ± 6.23	162.86 ± 5.94
	Range	152-177	152-174	152-177	152-177
Weight (kg)	Mean ± SD	57.86 ± 8.86	58.38 ± 9.02	58.96 ± 9.56	57.4 ± 9
	Range	41-78	41-78	45-78	41-78
Gender		Woman	Woman	Woman	Woman
Ethnicity		Turkish	Turkish	Turkish	Turkish
Number of Participants		35 (7 in each group)	42 (7 in each group)	28 (7 in each group)	35 (7 in each group)
** Participants do not have any health problems.*					
*** Participants are not professional athletes*					

The study of Evaluation of Traceability of Dietary Urine Biochemistry Changes with Commercial Urine Strips was accepted by Bezmialem University Non-Interventional Ethics Committee with file number 2022/70. Participants were informed and gave written consent.

2.2. Dietary Record

The applied nutrition programs were prepared by a dietitian who is an expert in her field. The nutrient content of local foods was taken from standard nutritional tables, while the content of commercial foods (e.g. pizza and ready-to-eat foods) was derived from labeled ingredients and nutrients.

The main food groups used are 1) cereals and cereal products (breakfast cereals; bread, rice, pasta, and other cereal-based products); 2) meat products (turkey, chicken, fish, red meat); 3) sdairy products (cheeses, milk, and yogurts); 4) vegetable products; 5) soups; 6) fruits; 7) other foods (pulse, bean, canned fruit, and pickle). The contribution of these 7 food groups to calcium, vitamin C, sodium, and magnesium intake was calculated. Diet lists were based on the amounts of metabolites found in these food groups by our co-author Esra Kozan, who is an expert dietitian.

The diet program was applied for one week for each parameter. For one week, the participants applied different nutrition programs for calcium, vitamin C, sodium, and magnesium. Participants were then given a one week break between each diet program. The experiment was made of calcium, vitamin C, sodium, and magnesium, respectively. Five study groups were prepared for calcium, six study groups for vitamin C, four study groups for sodium, and five study groups for magnesium. The daily intake amounts of each study group are different, these amounts are determined according to the standards (21) (Table 2).

Table 2. *Study groups*

Group	Restricted Diet (mg/d)	Optimal Diet (mg/d)	High Diet (mg/d)	Supplement (mg/d)	Control
Calcium	0-30	1000	1500	Solgar Calcium 600 (Oyster Shell) calcium carbonate (600 mg) + Vitamin D3 (75) mg	were not subject to any diet.
Vitamin C	0-10	90	200 (non-smoker) 250 (smoker)*	Solgar Chewable Vitamin C 500- Vitamin C (500 mg) + Acerola fruit extract (10 mg) + Rosehip fruit powder (4 mg)	were not subject to any diet.
Sodium	500-1000	1500	2000	**	were not subject to any diet.
Magnesium	0-30	310	350	Solgar Magnesium Citrate – trimagnesium citrate (200 mg)	were not subject to any diet.
** This group has been added because vitamin C absorption is less in smokers*					
*** No sodium supplement*					

2.3. Data Collection

The data collection started with the calcium study. Participants were asked to comply with the nutrition programs and pay attention to their supplement intake. On the first, third, and seventh days of the study, urine was collected from the participants three times in the morning, noon, and evening. Urine containers were provided to the participants to collect their urine. Collected urine was transferred to urine tubes (10 ml) and sent to an accredited laboratory for analysis. Analysis with commercial urine strips was also performed. The study was continued for 1 week. After the end of the study, a one-week break was taken and the vitamin C study was started. The same procedures were performed and the study was continued similarly for sodium and magnesium respectively.

Calcium and magnesium were analyzed by photometric method, creatinine by enzymatic photometric method, and sodium by ion selective electrode method in Abbott/Architect C8000 device at the laboratory. As there is no laboratory standard for vitamin C in urine, it has been analyzed only with commercial urine strips. In commercial urine strips, for each strip, one drop of urine was dropped on the parameter being studied, and the color change was photographed by smartphone. The results were determined using a reference color chart. The obtained data (Supplementary A Table 1-60) were used in statistical analysis.

2.4. Statistical Analysis

All statistical analyzes were performed using IBM SPSS 28.0. *P* value of <.05 was accepted to indicate statistical significance. Bivariate Correlation analysis was used to compare laboratory and strip results. Pearson's correlation coefficient was used to determine and test the strength of the relationship. Linear regression analysis was used to examine the association between dependent and independent variables.

There was no dependent-independent variable in the correlation, but there was a reciprocal relationship. In regression, there is an 'effect'. The method of analysis provides information on the interactions between the dependent and independent variables. Impact includes prediction and forecasting. There is a cause-effect relationship. While there is one independent dependent variable in simple regression analysis, there is more than one independent variable and one dependent variable in multiple regression analysis. When performing multiple regression analysis, the first ANOVA table is checked. If the model is found to be significant, then an interpretation is made. If sig(2-tailed) <.05, the established model is significant. Secondly, when the 'model summary' table is examined, the (R Square)x100= gives the power of the independent variable to explain the dependent variable. Third, the "coefficient" table is checked. This table shows the importance of the independent variables on the dependent variable. The higher the 't' value, the higher the degree of importance. If 1.96 < t, it is "meaningless".

The correlation analysis method gives the relationship between two metrics. It is not possible to determine causality where there is a relationship. In general, a correlation between .1-.3 is considered "weak," .3-.5 is considered "moderate," .5-.8 is considered "strong," and .8-1 is considered "very strong." The correlation/correlation coefficient takes values between "-1" and "+1". The correlation weakens as it approaches "0"; the negative correlation increases as it approaches "-1"; and the positive correlation increases as it comes to "+1". (– or + has no mathematical meaning, and only indicates the direction.). There is no distinction between dependent and independent variables, as there is a reciprocal relationship. Pearson Correlation examines the relationship between two variables. If sig(2-tailed) <.05, there is a significant relationship.

3. RESULTS

The study involved 42 participants from Turkey and examined the urinary excretion of calcium, vitamin C (non-smoker), vitamin C (smoker), sodium, and magnesium based on nutritional status. This research aims to (i) examine the effects of nutrition on urine biochemistry by regression analysis and (ii) correlation of the results obtained from two different methods by correlation analysis.

3.1. Regression Analysis

According to the data obtained (Supplementary A Table 1-60), the effect of nutrition on excretion was interpreted using regression analysis (Table 3).

***Table 3**. Regression analysis results between nutrition and excretion*

Group	Restricted/Optimal	Restricted/High	Restricted/ Supplement
Calcium	.636	.442	.273
Vitamin C	.575	.308 (non-smoker) .482 (smoker)*	.698
Sodium	.386	.413	**
Magnesium	.209	.337	.799
** This group has been added because vitamin C absorption is less in smokers.*			
*** No sodium supplement*			

While evaluating the regression analysis results in which the excretion due to nutrition was examined, each nutritional level was compared to the restricted intake. ANOVA sig values <.001, t values 1.96 <, in all calcium evaluations (Supplementary B Table 7-9). In this case, the models are meaningful and can be interpreted. The following effect rates were obtained in calcium: restricted/optimal nutrition, 63.6% (Supplementary B Table 7); restricted/high nutrition 44.2% (Supplementary B Table 8); and restricted/supplement 27.3% (Supplementary B Table 9).

ANOVA sig values <.001, t values 1.96 <, in all vitamin C evaluations (Supplementary Table 10,11,12,13). In this case, the models are meaningful and can be interpreted. The following rates were obtained in vitamin C: restricted/optimal nutrition, 57.5% (Supplementary B Table 10); restricted/high (non-smoker) nutrition 30.8% (Supplementary B Table 11); restricted/high (smoker) nutrition 48.2% (Supplementary Table 12) and restricted/supplement 69.8% (Supplementary B Table 13).

ANOVA sig values <.001, t values 1.96 <, in all sodium evaluations (Supplementary Table 14,15). In this case, the models are meaningful and can be interpreted. The following rates were obtained in sodium: restricted/optimal nutrition 38.6% (Supplementary B Table 14), and restricted/high nutrition 41.3% (Supplementary B Table 15).

ANOVA sig values <.001, t values 1.96 <, in all magnesium evaluations (Supplementary Table 16-18). In this case, the models are meaningful and can be interpreted. The following rates were obtained in magnesium: restricted/optimal nutrition 20.9% (Supplementary B Table 16), restricted/high nutrition 33.7% (Supplementary B Table 17), and restricted/ supplementary 79.9% (Supplementary B Table 18).

3.2. Correlation Analysis

According to the data obtained as a result of the study (Supplementary A Table 1-15; Supplementary A Table 34-60), comparative analyzes of the two measurement methods used were made and the correlation coefficients were calculated (Table 4). As there is no laboratory standard for vitamin C in urine, it has been analyzed only with commercial urine strips. Therefore, it was not included in the correlation analysis.

***Table 4.** Correlation of laboratory and strip results*

Group	Lab-Strip Correlation Coefficient*
Calcium	.679
Calcium/Creatinine	.604
Magnesium	.59
Magnesium/Creatinine	.511
Creatinine	.884
Sodium	.876
** Correlation of laboratory and strip results with each other*	

As a result of the correlation analysis in which the strip and laboratory results were evaluated, the calcium strip was .679 (Supplementary B Table 1), and the calcium/creatinine ratio was .604 (Supplementary B Table 2), the sodium strip was .876 (Supplementary B Table 3), magnesium strip was .59 (Supplementary B Table 4), and the magnesium/creatinine ratio was .511 (Supplementary B Table 5), the creatinine strip was .884 (Supplementary B Table 6).

Valuable results were obtained in this study on calcium, magnesium, sodium and vitamin C, which are necessary for our body. Based on above results, it is possible to confirm that nutrition affects calcium, vitamin C, sodium and ,magnesium excretion in urine.

When smokers and non-smokers were compared during the vitamin C study, it was found that vitamin C excretion was higher among smokers. It was thus observed again that smoking affects vitamin C absorption. In addition, it is also possible to confirm that the use of supplements seriously affects vitamin C excretion in urine.

This is due to decreased absorption with increased intake of vitamin C, making the bioavailability of vitamin C in supplements less than that of food. When daily doses of 30-60 mg of vitamin C is consumed, almost no vitamin C is excreted in the urine in a 24-hour period. In cases where there is moderate vitamin C intake, approximately 70-90% of the amount taken in is absorbed, while in cases where there is vitamin C intake above 1,000 mg, vitamin C absorption falls below 50%, as unmetabolized ascorbic acid is excreted through the urine (21).

When evaluating sodium results, environmental factors, water and salt consumption should be considered that affect sodium excretion rates.

Although spot magnesium ratios are low in restricted diets, magnesium/creatinine ratios are at optimal levels because of low creatinine excretion, which has been associated with high water consumption. Because of this, the effective rates were lower than expected, but it is still possible to say that nutrition affects the excretion of magnesium in urine.

In addition, it is also possible to confirm that the use of supplements seriously affects magnesium excretion in urine.

4. DISCUSSION

The results of this study significantly contribute to understanding the interrelationships between nutrition and the urinary excretion of crucial elements, including calcium, vitamin C, sodium, and magnesium. Our analysis shows that nutritional status affects the excretion of these elements directly and quantitatively. The regression analysis shows that the levels of nutritional intake significantly influence calcium excretion. The finding that limited diets result in increased calcium excretion relative to optimal or high nutrition implies a counterbalancing mechanism in the body to keep calcium homeostasis. This is important in understanding bone health and the prevention of osteoporosis, especially in diverse populations with different eating patterns (22).

Our data suggest that vitamin C excretion is higher in smokers than in non-smokers. This is consistent with the assumption that smoking increases oxidative stress, leading to increased demand for vitamin C in the body (23). The decreased bioavailability of supplemental vitamin C, as observed through increased excretion with higher supplement consumption, supports the notion that vitamin C levels are tightly regulated, mainly by renal excretion (24). This suggests that a balanced diet should be prioritized over supplementation as the primary source of vitamin C intake. Our results on sodium excretion support the role of dietary intake and environmental effects. These findings emphasize the difficulty of understanding sodium regulation in the body and the need to take into account external factors when evaluating sodium excretion. Our findings of elevated magnesium excretion from supplementation and restricted diets are also interesting. This indicates that, just like vitamin C, the body controls magnesium concentration via urinary excretion, which is based on dietary intake.

Another goal of this study was to assess the accuracy and feasibility of commercial urine strips compared to traditional laboratory analysis methods. It is a very crucial comparison, because it directly addresses the possible implementation in the clinical practice where speed and accuracy are key. Commercial urine strips provide a fast, very inexpensive, and also convenient method for initial urine testing (25,26). These strips are very useful in the situations where quick results are needed, such as emergency medicine or for regular screening in outpatient settings. Their ease of use makes them accessible to non-specialists, expanding the scenarios in which they can be employed in a healthcare setting. But although these strips give quick outcomes, their precision and specificity may be much less than those of the laboratory analyses. The main source of this variance is the semi-quantitative nature of the strip readings and also the possibility of user error in the interpretation. Conversely, laboratory analysis which is carried out using 24 hour urine, despite being lengthy and also costly, provides a greater level of precision and sensitivity. The 24-hour urine test is considered the gold standard method for measuring biochemical parameters, but it is time-consuming and difficult to implement (27). Instead, the equivalent is obtained by determining concentrations of creatinine in spot urine and putting them into proportion with the component. Typically, this method estimates the 24-hour excretion rates of protein, potassium, sodium, magnesium, calcium, urea, and uric acid (28). The comprehensive analysis conducted in a stable setting reduces the many chances of wrong results, making it alot more effective in complicated cases or where the accurate quantification of analytes is essentially essential. However, the time and cost of the laboratory analysis are a constraint in the emergencies or resource-poor settings.

When comparing these two methods, it can be seen that each has its own strengths and also weaknesses. The decision between the use of commercial urine strips and the laboratory analysis is dependent upon the setting of the testing. For example, in a primary care environment where fast screening is critical, commercial urine strips can be a very good first test. But for precise diagnostic purposes, especially when more subtle information is needed, the laboratory analysis remains the better option.

Commercial urine strips and laboratory analysis both play their own role in the medical diagnostics. The choice of the method should be based on factors such as precision, available resources, and the urgency of the situation. This research highlights the necessity of a delicate balance, combining the many strengths of each method to achieve the best possible care for the patients.

5. CONCLUSION

This study provides a new perspective on scientific literature by exploring the impact of nutrition on urine biochemistry, a relatively understudied field.We compare two different urine measurement methods – laboratory analysis and commercial strips of urine. This comparison is useful because it assesses the feasibility and trustworthiness of urine strips relative to the conventional laboratory approach. The conclusions drawn from this study are based on solid statistical analyses, including regression and correlation. The statistical techniques are also appropriate and accurate for the nature of collected data. This research provides evidence for the relationship between nutrition and urine excretion of various metabolites, contributing to a better understanding of this field. Showing the association between urine strips and laboratory findings not only validates the use of these strips but also indicates their ability to pre-diagnosis. This might have great practical implications in the clinical settings or for personal health monitoring.

The focus on a specific age group and location in this study may limit the external validity of the results to other populations. However, the sample size we used is sufficient for conducting the study. On the other hand, a larger sample from different country could give more generalizable results.

Finally, our research provides a useful and unique method of evaluating the effects of nutrition on urine biochemistry. However, the results are constrained by the small sample size and regional scope. Further studies could strengthen these results, by including a larger and more representative sample, a larger number of nutritional and lifestyle factors, and exploring the possible applications in different clinical or health settings.

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Clinical and Experimental Health Sciences

Digital Comparison of Occlusal Vertical Contacts Between Direct Composite and Indirect Cad/Cam Restorations: An in vivo quantitative assessment

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ABSTRACT

Objective: Clinical assessment of the static occlusal vertical contacts for direct composite and indirect Cad/Cam restorations, and evaluation of the clinical experience level's effect on contact registration.

Methods: Sixty restorations on maxillary and mandibular molar teeth were investigated. Postgraduate students performed indirect Cad/Cam restorations (Cerasmart, GC Corp., n=20), and both undergraduate and postgraduate students performed direct composite restorations (Ganeial A'chord, GC Corp., n=20 for each). A single supervisor digitally analyzed the occlusal vertical contacts immediately after by using an intraoral scanner (iTero Element-5D, AlignTech) and OrthoCAD software. The tightness of contacts was assessed by counting the pixel numbers in Photoshop CC (Adobe) software. The statistical analyses were performed by Shapiro-Wilk, One-way ANOVA, Tamhane's T2 test, Kruska-Wallis test, and Dunn tests ($p<.05$).

Results: No significant differences were found for the contacts closer than the yellow code (<0.4mm) among the operator/restoration types ($\geq.05$). Whereas, significant differences were found between the undergraduate direct and postgraduate indirect restorations for the yellow contacts and the lighter ones ($p<.05$). Additionally, no significant contact differences were found either between the direct restorations of undergraduate and postgraduate students or between the direct and indirect restorations of postgraduate students ($p\geq.05$ for both). Considering red, orange, and yellow code contact types together, no significant differences were observed among the operator and restoration types ($p=.069$).

Conclusion: The restoration type was not effective in registering the occlusal vertical contacts for clinicians with equal clinical experience levels. Clinical experience was also not effective in occlusal contacts of direct restorations. Whereas, when the advantages of indirect Cad/Cam restorations are combined with the clinical experience, tighter occlusal vertical contacts might be registered. The potential effects of additional parameters such as the restorative material and the dental technician on the occlusal vertical contacts should be investigated.

Keywords: Clinical experience, cad/cam, direct restoration, indirect restoration, occlusion, vertical contacts

1. INTRODUCTION

Dental occlusion is defined as a static relationship between the occlusal/incisal surfaces of the upper and lower teeth (1). Whereas, it is defined as not only static but also dynamic relationship more recently (2). The contact of all the posterior teeth should be set simultaneously and the occlusal loads should be distributed homogeneously to obtain an ideal, tension-free, balanced occlusion (2). Otherwise, inappropriate occlusal forces on the teeth can lead to the traumatic occlusion mainly due to the primary contacts which may occur following restorative, prosthetic, or orthodontic treatments. However, due to the high occlusal variability of the patient, static and dynamic occlusion patterns are not considered constant and reproducible (2). The imbalanced destructive forces may cause damages on pulp and periodontal tissues, masticatory muscles, temporomandibular joint (TMJ), and also dental hard tissues (3). Therefore, an accurate occlusal analysis before, during, and even after the treatment is very important to avoid such damages, clinically.

The occlusal analysis can be affected by several factors including age, gender, craniofacial morphology, periodontal tissue support, TMJ disorders, presence of pain, the operator/device performing the analysis, and the parameters related to the positioning during the analysis procedure (1,4,5). Moreover, the intensity and location of the occlusal contact can be affected by the position of the mandible which can also be affected by the whole body position of the patient (6).

In terms of restorative dentistry, the type of restoration may have an effect on the occlusion discrepancy of a restoration.

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However, there are only a few scientific evidences in literature for the clinical comparison of the occlusal analysis between direct composite and indirect Cad/Cam restorations. Although some previous studies considered no difference between these two types of restorations regarding the occlusal contacts/relations (7-9), especially larger Cad/Cam restorations (including at least one cusp coverage) can be more advantageous compared to the direct composite restorations, due to the ability of the digitally driven contact guidance clinically.

Moreover, the occlusal analysis technique as well as the level of experience of the clinician may also influence the final occlusion of a restoration. The analysis of occlusion can be done by conventional or digital methods. The conventional method is generally performed by an articulating paper or occlusal spray, while the digital method is performed by the T-Scan analysis system (3). In addition to the digital method, the dental Cad/Cam systems can be used for the occlusal analysis. Thereby, it is also possible to digitally evaluate the estimated occlusion at the design stage before the restorative procedure begins (3). However, there is only a limited number of scientific studies in the literature regarding the use of Cad/Cam systems for the occlusal analysis.

The aims of this clinical study were to digitally evaluate the level of static occlusal vertical contacts in direct composite and indirect Cad/Cam restorations, and also to evaluate the effect of the clinical experience on registration of the occlusal contacts. The null (h_0) hypotheses of the study were; [1] the level of occlusal vertical contacts (contacts, close contacts, and intense contacts) are similar for direct composite and indirect Cad/Cam restorations, among clinicians with similar clinical experience, [2] the clinical experience has no effect on registration of the occlusal vertical contacts.

2. METHODS

This clinical study was conducted with the approval of a local ethics committee (Protocol number: 2023/140). Informed consent was obtained from each patient.

Thirty-three patients out of the entire daily routine undergraduate and postgraduate doctor appointments in the university department of restorative dentistry were selected for the evaluations in this clinical study. The gender and age of the patient were not considered a variable in the study therefore the patients were selected randomly but only with no local or systemic disorders or TMJ disorders that could prevent the bite. A total of 60 posterior restorations (molar teeth involving mesial-occlusal or distal-occlusal surfaces) including the occluding tooth in the counter-arch and with at least one cusp coverage were selected. The undergraduate and postgraduate students in a university clinic performed the restorations. The direct composite restorations (n=40) were performed by the undergraduate and the postgraduate students whereas, the indirect Cad/Cam restorations (n=20) were performed by only the postgraduate students at the same clinic. A nanohybrid composite, Gaenial A'chord (GC Corp., Japan) was used for all the direct restorations. Cerasmart hybrid blocks (GC Corp.) were used for the indirect restorations by using the iTero Element 5D intraoral scanner. All the restorative steps and the final restorations are routinely checked by an experienced clinical supervisor for both undergraduate and postgraduate students at the university clinics. A single supervisor checked and approved all the involved restorations in this study before the occlusal analyses. The occlusal vertical contacts of the restorations were digitally analyzed, immediately after the approval by the supervisor.

The digital impression of each restoration and occluding tooth was taken by using an intraoral scanner (iTero Element 5D, Align Technology, USA) for the analyses. The scanner was used in 'restorative mode' with enhanced intra-oral camera resolution for the study. The occlusal surface of the restoration, the occluding tooth, and the inter-occlusal relations were recorded as a .stl file for each restoration. The .stl data were then processed using the OrthoCAD software (Align Technology, USA) to measure the vertical occlusal contacts quantitatively. OrthoCAD software categorizes the occlusal contacts by specific color codes from tight (0.0 mm) to loose (1.2 mm) contacts such as red (0.0 mm), orange (0.0-0.2 mm), yellow (0.2-0.4 mm), green (0.4-0.6 mm), cyan (0.6-0.8 mm), light blue (0.8-1.0 mm), blue (1.0-1.2 mm), respectively, in 0.2 mm increments for each color code (Figure 1) (10). In terms of the color codes in the OrthoCAD software program, the red color code was defined as the contact; orange and yellow codes were defined as close contacts; green and cyan color codes were defined as intense contact; and light blue and blue color codes were defined as light contacts (3).

Through the OrthoCAD software, 7 different screenshots in .jpeg picture format were provided for each restoration at the same plane, corresponding to the specific color codes for the contact types. In this research, each color code corresponds to a specific occlusal contact type, and every lighter contact overlapped the closer contact/contacts (Figures 2 a-g) (11). For instance yellow code includes the occlusal contacts of yellow and red, while the orange code includes the occlusal contacts of orange, yellow, and red. Then the images were loaded into the Adobe Photoshop CC (Adobe, USA) software program to measure the vertical occlusal contact areas, quantitatively. Starting from the picture of the red color code, each contact type was encircled by using the quick selection tool in the software. Then the number of pixels inside the encircled area was measured by using the histogram tool (Figures 3 a, b). The collected pixel numbers were saved for each contact type.

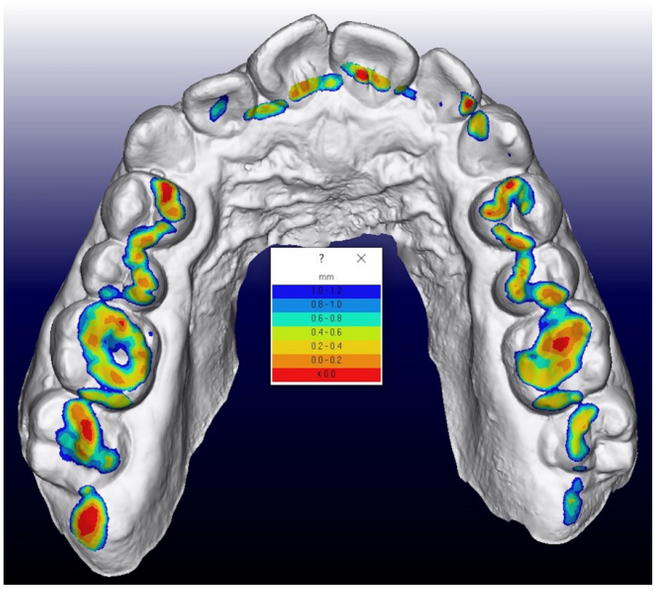
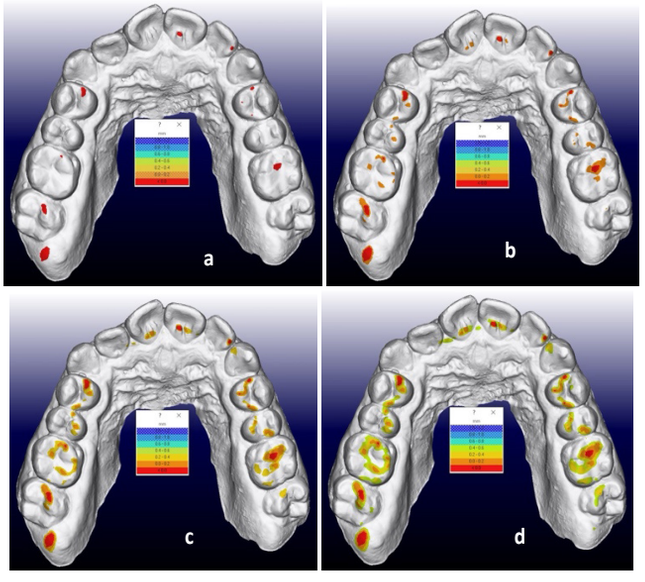
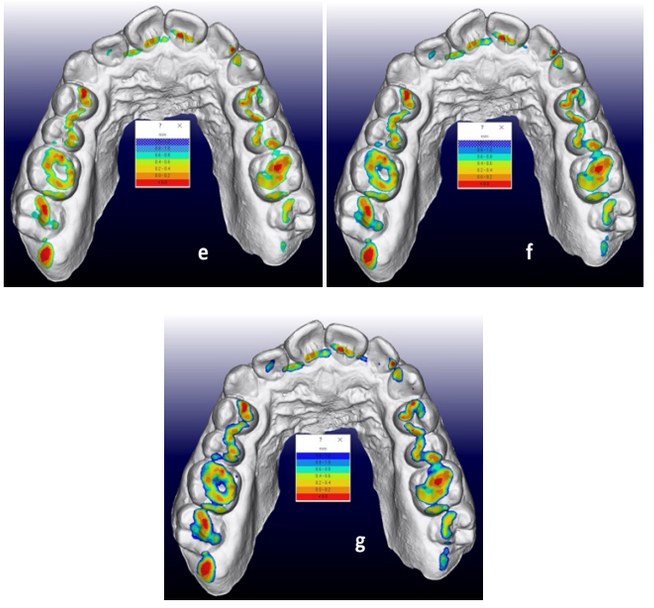


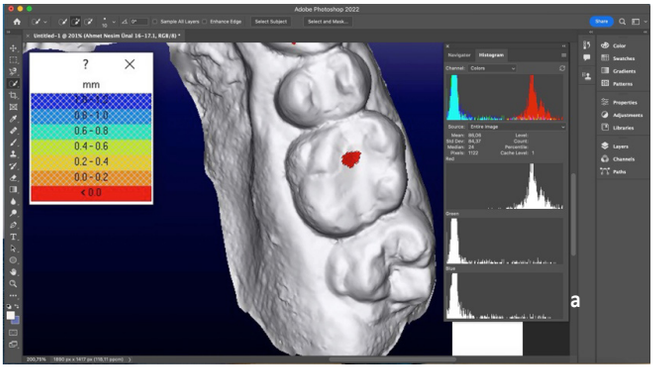
Figure 1. *Occlusal contact color codes in OrthoCAD software program.*

The data were analyzed with the IBM SPSS V23 software program. The distribution of the data was evaluated Shapiro-Wilk test. The normally distributed data were analyzed by One-way ANOVA and Tamhane's T2 test. The rest were analyzed by Kruskal-Wallis test and the Dunn test. The conformity of the contact points and operator groups was analyzed by the conformity analysis. The results were presented as average±standart deviation and median (minimum–maximum). The deemed significance was set at <.05.





Figures 2 *a-g. Collected 7 respective screenshots per restoration, corresponding to each one of the occlusal contact types.*



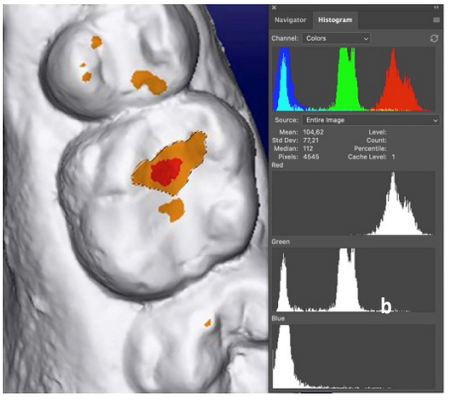


Figure 43a, *b. Measurement of the number of pixels inside the encircled area by using the histogram tool. (a) Measurement of the red code area, (b) Measurement of the orange code area, close-up view.*

3. RESULTS

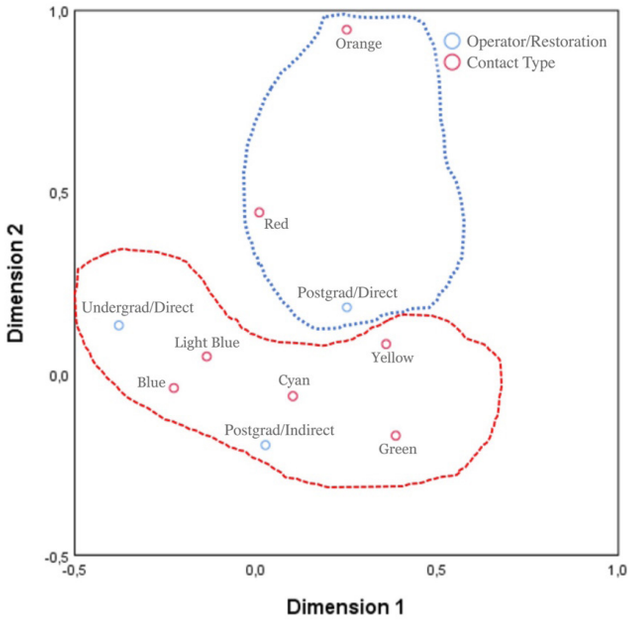


Figure 4. *Map of conformity regarding the distribution of contact types among operator/restoration types.*

According to the map of conformity, red and orange contact types were mostly presented in direct restorations of postgraduate students. Whereas, yellow, green, cyan, light blue, and blue contact types were mostly presented in direct restorations of undergraduate students and indirect restorations of postgraduate students (Figure 4).

The red contact type (0.0 mm) was the lowest number for each operator/restoration type and there was no significant difference among all (p=.401) (Table 1). The yellow contact type (0.0-0.2 mm) was the second lowest number for each operator/restoration type and there was no significant difference among all (p=.438). The orange contact type (0.2-0.4 mm) was the third lowest number for each operator/restoration type. The contact area of direct restorations of undergraduate students was significantly lower than the indirect restorations of postgraduate students (p=.017). However, no significant difference was found among the direct and indirect restorations of postgraduate students (p≥.05). The green contact type (0.4-0.6 mm) was the fourth lowest number for each operator/restoration type. The contact area of direct restorations of undergraduate students was significantly lower than the direct and indirect restorations of postgraduate students (p<.001), of which two were found similar (p≥.05). The cyan contact type (0.6-0.8 mm) was the third highest number for each operator/restoration type. The contact area of direct restorations of undergraduate students was significantly lower than the indirect restorations of postgraduate students (p=.002). However, no significant differences were found among the direct and indirect restorations of postgraduate students (p≥.05). The light blue contact type (0.8-1.0 mm) was the second highest number for each operator/restoration type. The contact area of direct restorations of undergraduate students was significantly lower than the indirect restorations of postgraduate students (p=.003). However, no significant differences were found among the direct and indirect restorations of postgraduate students (p≥.05). The blue contact type (1.0-1.2 mm) was presented as the highest number for each operator/restoration type. The contact area of indirect restorations of

Table 1. *Comparisons between the operator/restoration types and contact types*

Operator/Restoration Type								
Contact Type	**Undergrad/Direct**		**Postgrad/Direct**		**Postgrad/Indirect**		**Test Stat.**	**P**
	Average±SD	Median(Min-Max)	Average±SD	Median(Min-Max)	Average±SD	Median(Min-Max)		
Red	205.5±4028	0.0(0.0-1418.0)	317.8±548.1	0.0(0.0-2097.0)	406.4±1170.0	0.0(0.0-4919.0)	1.826[1]	.401
Orange	1009.9±1119.6	651.0(0.0-3462.0)	1824.0±1734.7	1765.5(0.0-6084.0)	1834.9±2306.5	864.0(0.0-7407.0)	1.649[1]	.438
Yellow	2391.2±2133.5	1879.0(0,0-6839.0)[a]	4549.4±3434.8	4428,(336.0-13397.0)[ab]	6205.1±5309.4	4217.0 (375.0-15574.0)[b]	8.141[1]	**.017**
Green	3942.4±2983.0[a]	3733.0(0.0-11459.0)	7614.7±4881.4[b]	7057.5(1311.0-18531.0)	11316.9±7426.3[b]	11112.0(2381.0-25067.0)	10.615[2]	**<.001**
Cyan	6464.8±4821.5	5897.0(0,0-17201.0)[a]	10364.4±6257.0	8589.0(2886.0-23545.0)[ab]	15735.9±8712.3	17165.0(3711.0-28113.0) [b]	12.445[1]	**.002**
Light Blue	9651.7±6648.3	8274.0 (491.0-23883.0)[a]	13399.6±7679.1	11182.0(3647.0-29027.0)[ab]	20595.7±10795.0	20456.0(4908.0-38252.0) [b]	11.362[1]	**.003**
Blue	11969.2±8141.5	10490,0 (731.0-29662.0)[a]	15622.8±8387.7	14235.0(5027.0-32893.0)[a]	25374.4±12690.3	25840.0(6985.0-51628.0) [b]	13.187[1]	**.001**

[1]Kruskall Wallis H test, [2]One-way ANOVA, a-b: No significant difference between the groups with the same letter.

Table 2. *Comparisons between the operator/restoration types in terms of the tight contacts*

Contact Type	Undergrad/Direct		Postgrad/Direct		Postgrad/Indirect		Test Stat.	P
	Average±SD	Median (Min-Max)	Average±SD	Median (Min-Max)	Average±SD	Median (Min-Max)		
Red+Orange+Yellow	3606.6±3396,6	2848.0 (0.0-11258.0)	6691.3±5414.6	6193.0 (336.0-20371.0)	8446.4±8133.5	4766.0 (484.0-26243.0)	5.353	.069

**Kruskall Wallis H test, a-b: No significant difference between the groups with the same letter.*

postgraduate students was significantly higher than the direct restorations of undergraduate and postgraduate students (p=.001), of which two were considered similar (p≥.05).

No significant differences were observed for the sum of red, orange, and yellow contact types (≤ 0.4 mm) among the operator and the restoration types (p=.069) (Table 2).

4. DISCUSSION

The registration of the occlusion is one of the major challenges during either direct or indirect restoration types. Especially the indirect restorations in which the restoration is cemented in the next appointment may include difficulties in this regard due to the dimensional instability of the impression material, preparation surface changes during the temporary filling, or position changes of the related tooth (7). Even though direct composite restorations have the advantage of a single-visit treatment by preserving the remaining dental tissues more, they may be insufficient to provide proximal and occlusal contacts in cases where tissue loss is excessive (7,12). The proper analysis of the final occlusion should be performed, regardless of the restoration type, to overcome this problem (13). Occlusal analysis can be performed clinically by using conventional and digital methods. The conventional method may only provide the qualitative data that clinically presents the contact point and/or contact area. Although a gold standard has not been defined yet for occlusal analysis, articulating paper is the most common material due to its low cost and ease of use (3,14,15). However, it is not considered a high-sensitivity method because of the physical strength and the thickness of the paper, wet/dry conditions (3,11,16,17).

Digital occlusal analysis methods such as T-Scan and intraoral scanners may provide quantitative and standardized data of the contact point/area and were previously considered relatively more reliable and repeatable methods than the articulating paper (3). T-Scan has widely been used to analyze occlusion in previous studies (11,16-19), but only a few compared it with intraoral scanners regarding accuracy and reliability (3,20,21). The majority of the related studies in the literature were *in vitro* (3). As it was mentioned previously, the thickness of the sensor in T-Scan system might prevent spontaneous closure, and thereby it may misguide the ideal occlusion when used clinically (3). Therefore an intraoral scanner rather than T-Scan was selected for the clinical evaluations. Medina-Sotomayor, P. et al. reported better accuracy for iTero scanner than the Cerec Omnicam in an *in vitro* study even without using iTero with the high-resolution restorative mode (22). In another *in vitro* study Medina-Sotomayor, P. et al. compared the resolution levels for the intraoral scanners. Although they reported the highest resolution for the Cerec Omnicam (79.82 points per mm^2) and the lowest for iTero (34.20 points per mm^2), they used an older version of the iTero scanner in their study (23). Diker and Tak presented no significant difference in trueness between iTero Element 2, Trios 3, Virtuo Vivo, and Prime Scan and in precision among all the scanners used (24). Rene et al. compared intraoral scanners in vitro and presented the order for trueness as 3Shape D800 >iTero >3Shape Trios 3 >Carestream 3500 >Planscan >Cerec Omnicam >Cerec Bluecam and the order for precision as CS3500 >iTero >3Shape D800 >3Shape Trios 3 >Cerec Omnicam >Planscan >Cerec Bluecam (25). However, the exact version iTero scanner they used was not mentioned. With regards to the previous studies, an up-to-date intraoral scanner iTero Element 5D was selected for the present study, for analyzing the occlusal contacts, clinically. The combination of the OrthoCAD (Align Tech, US) and Photoshop CC (Adobe, US) software programs was used to obtain the quantitative contact data. The higher resolution of iTero Element 5D with the restorative mode and direct compatibility with the OrthoCAD software regarding the quantitative calculations of the clinical data were the major reasons for the selection of it in this study.

It was recommended to take the occlusal recordings in the centric relationship when reconstructing a whole new occlusion (26). If the occlusal contact points already exist, the centric relationship may not always occur simultaneously with centric occlusion. Whereas, when the patient bites, the spontaneously provided centric occlusion is a habitual tooth contact and it is the best relationship to record (3). Therefore, in the case of a healthy natural dentition, it is always recommended to consider the centric occlusion contact points as the reference (3,27). Accordingly in the present study, the maximum intercuspation occlusal records were obtained for all the restorations during the centric occlusion with the maximum bite force. It can be argued that the standardization of the bite forces might be controversial among the patients. However, to measure the bite force, sensors should be placed between the upper and lower arches which might prevent the ideal occlusion (28). The

occlusal analysis with the digital systems is based on the principle of opposing jaws in occlusion, therefore, it was not possible to calibrate the bite force clinically in the present study. Moreover, as the inclinations of the patient's head may lead to different occlusal contacts and contact densities (29), the digital recordings were taken from all patients in the same position where the Frankfurt horizontal plane was parallel to the floor, to standardize the head position.

Photoshop software was previously used to measure the area of different fields that were captured by the digital recordings (30,31). Regarding dental research, it was used to measure the number of pixels corresponding to the excessive cement on dental crowns (32,33) and also to perform the occlusal analysis in terms of the pixel count (3). Image J was previously determined as an alternative measurement software program, while Photoshop was considered more convenient and more effective with its various image processing capabilities (30). Accordingly, in the present study, the occlusal contact points/areas were measured by calculating the number of pixels on the restored side of the occlusal plane (mesial-occlusal or distal-occlusal) through the Photoshop CC software. The quick selection tool of the software gives the capability of precisely encircling contact areas when the surface is even not smooth and defining the differences of the colors between the selected pixels by the histogram tool (Figures 3 a, b) (34).

The clinical studies on the use of intraoral scanners for occlusal analysis are limited in the literature. Moreover, the ones which performed it did not analyze the contact point/areas precisely due to the low sensitivity of the analyzing software used or the low number of color codes selected. Abdulateef used Cerec Omnicam *in vitro* to analyze interocclusal recordings and mentioned that it provides sufficient and accurate data (20). Arslan et al. compared Cerec Omnicam data with data from articulating paper through *in vitro* restoration models and presented similar outcomes for the contact points by both methods (21). Abdulateef et al. defined the accuracy of interocclusal recordings of 100 µm on occlusal surfaces as close proximity and of >100 µm as clearance (20). Owens et al. investigated the relationship between the chewing performance and the interocclusal contact areas during maximum intercuspation, and they defined the areas of ≤50 µm as contact areas and 50 – 350 µm as near contact areas (35). In the present study, seven relative color codes differed by 0.2 mm (200 µm) according to the OrthoCAD program presets and formed contact, close contact, intense contact, and light contact groups, which was partially similar to the previous study designs of Owens et al (35). and Bostancıoğlu et al (3). However, the high number of color codes in our study might have provided more sensitive quantitative data for the occlusal contacts. Bostancıoğlu et al. compared the sensitivity of T-Scan and Cerec Omnicam clinically, at the maximum intercuspal position, through the contact tightness with color codes. They considered red as close contact, green as intense contact (green), and blue as light contact, but their color range was not as high as it was in the present study and, therefore can be interpreted as less sensitive (3). They also analyzed the data in Photoshop software quantitatively and observed the blue color code as the most seen contact type which is similar to our results (Table 1). In this study, for both the operator types and the restoration types, the blue contact was the most seen type on the occlusal planes. The recorded areas for the contacts and close contacts were lesser than the intense and light contacts, and they gradually increased consistent with the outcomes of the previous studies (Table 1) (3,20,35).

Although the precision and accuracy of the intraoral scanners and the effect of restorative materials were previously studied, there is a lack of clinically published data on the comparison of the distribution of occlusal contacts among direct and indirect restorations (3,36,37). With regards to the higher accuracy and precision of the intraoral scanners mentioned above, and with the advantages of the digitally driven analysis before the restorative production, the occlusal contact points /areas are thought to be tighter and larger clinically in indirect restorations compared to the direct restorations with articulating paper driven occlusions (3,11). However, in the present study, regarding the similar and well-experienced postgraduate students' direct and indirect posterior restorations including at least one cusp coverage, the contacts (red code / 0.0 mm), close contacts (orange and yellow codes / ≤0.4 mm), and intense contacts (green and cyan codes / ≤0.8 mm) on the occlusal plane were larger in indirect restorations, with no significant difference between the restoration types for all codes (Table 1 and Figure 1). A similar outcome was also observed when the contacts and close contacts gathered as one group like Owens et al. did and named as the near contact areas (Table 2) (35). Accordingly, the first hypothesis of the study was accepted due to the clinically observed similar occlusal vertical contacts between the direct and indirect restorations of the postgraduate students. Our findings were consistent with the previous clinical studies in which indirect and direct restorations were rated with similar success in terms of the clinical evaluation criteria (9,38,39). However, this result is observed for the postgraduate students and it might be interpreted that, the indirect restorative procedures may not have an advantage regarding the vertical occlusion when conducted by the clinicians with similar levels of experience. Whereas, the content and properties of the indirect restorative material might be effective in creating the occlusal relationship and therefore using only the Cerasmart hybrid block can be considered a limitation of this study. The occlusal surfaces of the indirect restorations were also not adjusted by bur after cementation in the present study. Therefore, the result might be due to the higher quality of layering and occlusal adjustment procedures for the direct composite restorations than expected, or even due to the lower quality of the occlusal design for the indirect restorations. It is a well-known fact that many restoration designers (dental technicians or dentists) consciously design the vertical occlusion at light contact levels (light blue or blue) to avoid devastating primary contacts during the restoration, thereby minimizing the possible restoration fractures (40). This is supported by the

result that the larger contact areas in the indirect restorations were significant for the light contacts (blue code / ≤ 1.2 mm) in the present study. However, it was inconsistent with the previous reports of Bostancıoğlu et al., concluding that the intraoral scanners might be sensitive only in the diagnosis of close contacts (3). In conclusion, the technicians' use of low-contact designs to eliminate or reduce the occlusal problems due to the tight occlusal relationship after cementation may affect the results. Whereas, if the occlusal adjustment procedures were performed with an abrasive material after the cementation, closer contacts might be created in the present study.

Regarding the comparisons between the direct posterior restorations by undergraduate and postgraduate students, larger contact areas were registered in the direct restorations of postgraduate students for all the contact types, with no significant difference except the ones for the green code (Table 1). Moreover, when the near contact areas (red, orange, and yellow codes) were evaluated, there was no significant difference between the clinicians at two levels of experience (Table 2). Thus, clinical experience had a minor positive influence on the occlusal registration of posterior direct restorations which was not considered significant. In addition, all the contact areas were significantly larger for the indirect restorations of postgraduate students compared to the direct restorations of undergraduate students, except the full contact areas (red code) and the closest contact areas (orange code) (Table 1). Thus it can be concluded that the higher clinical experience in indirect restorations may lead to significantly better results in occlusal registration. Therefore, the second hypothesis of the study was partially rejected. Clinical experience was not considered effective for the registration of the occlusal contacts in terms of direct restorations, whereas it might be effective when the experience is combined with the advantages of the indirect restoration procedures.

Besides providing useful data regarding posterior occlusal registration, this clinical research may have also some limitations. It might be useful to compare and crosscheck the digital quantitative data with T-Scan data and also with a different intraoral scanner in future studies. Additionally, it might be better to assess the occlusal contacts during the dynamic jaw movements not only vertically, for a more accurate outcome. Also, especially for the indirect restorations, the type of the Cad/Cam restorative material might affect the results, therefore different materials might be investigated in future studies (37). The potential effect of dental technicians can be comparatively investigated.

5. CONCLUSION

Smaller near contact areas (red, orange, and yellow codes) were observed at the occlusal planes of the restorations compared to the lighter contact areas (green, cyan, light blue, and blue codes). The direct and indirect restoration types had no difference in terms of occlusal vertical contacts when performed by clinicians with similar levels of clinical experience. The level of clinical experience was not effective in registering the occlusal contacts of the direct restorations. Whereas, when the advantages of indirect digital Cad/Cam restorations are combined with the clinical experience, tighter occlusal vertical contacts might be provided. Further studies should focus on the potential effects of additional parameters such as the restorative material and the dental technician on the occlusal vertical contacts.

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Clinical and Experimental Health Sciences

The Relationship Between Premenstrual Syndrome and Dietary Habits and Nutrients Intake: Descriptive and Analytical Cross-Sectional Study

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ABSTRACT

Objective: This study aimed to examine the relationship between premenstrual syndrome (PMS) and nutrition in nursing students.

Method: This is a descriptive and analytical cross-sectional study evaluating the relationship between premenstrual syndrome and nutrition in nursing students. In this descriptive and cross-sectional study, relationship between nutrient and PMS was examined by 219 nursing students using personal information form, 24-hour food consumption record and Premenstrual Syndrome Scale (PMSS). The macro and micronutrients consumed by the participants were determined through the analysis of their food consumption records in the Nutrition Information System (BEBIS) program. T test, chi-square test and Pearson correlation analysis test were used in analysis of data.

Results: It was determined that 53.4% of the students had PMS and dietary habits are important in the appearance of PMS symptoms. Breakfast and lunch consumption affected the presence of PMS, but there was no difference between the groups with and without PMS in terms of coffee, salt consumption and skipping meals. The total energy taken daily by the female students with PMS were higher, percentage of energy from protein was lower and difference was statistically significant ($p<.05$). There were significant correlations between PMSS score and daily energy intake, percentage of energy from protein, vitamin E, vitamin B6, magnesium, iron and zinc.

Conclusion: Results of this study was indicated that dietary habits, macro and micronutrient intake are important in increasing premenstrual symptom severity. In female students with PMS, it is recommended to raise awareness about the importance of nutrition in reducing or eliminating symptoms, inform experts about nutrition, and perform further research on this issue.

Keywords: Nursing; nutrition; premenstrual syndrome; university student.

1. INTRODUCTION

Premenstrual Syndrome (PMS) is a situation that causes physical, emotional and behavioral disorders in women. This happens in the late luteal phase of the menstrual cycle (1). Psychological, behavioral and physical symptoms of PMS have been reported (2). Physical symptoms include pain, swelling, breast tenderness, appetite change, nausea-vomiting, fatigue, and insomnia; behavioral symptoms include dizziness, fatigue, decrease in daily activities and reluctance; while psychological symptoms can be listed as emotional fluctuations, crying crises, anxiety and irritability (3). These symptoms negatively affect social life and reduce quality of life (4). It is also reported that nurses are the most affected by PMS among different occupational groups (5). In studies conducted with university students, the prevalence of PMS was between 35.3% and 58.1% (4, 6, 7).

Although the etiology of premenstrual syndrome is not known exactly, it has been stated in many studies that changes in estrogen and progesterone levels trigger these symptoms (8). In addition to hormones, neural, genetic, psychosocial factors and nutritional habits are thought to be effective in the formation of PMS (9). Studies examining the relationship between PMS and nutritional status show that high energy and simple carbohydrate consumption are positively associated with PMS (5). Protein intake was not found to be associated with PMS (10). It was reported that the duration of symptoms is shortened in individuals with PMS fed a low-fat diet (11). Caffeine and alcohol consumption were also reported to be associated with PMS, and a decrease in PMS severity was observed by reducing the consumption of these foods (12). Studies show that there are positive correlation between iron, zinc, calcium, magnesium, potassium, vitamin A, thiamine (B_1), riboflavin (B_2), pyridoxine (B_6), vitamin D deficiencies and PMS (13,14). In a study by Rad et al., it was a significant relationship reported between body mass index (BMI) and PMS, with BMI having a direct or indirect effect on hormone balance and therefore a significant relationship

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with PMS (14). In other studies, it was stated that PMS is more common in obese women than in non-obese women (6, 14, 15).

Although the relation between PMS and different dietary components has been studied, the number of studies examining the role of nutrition as a whole in PMS among university students is limited. In addition, it is reported that nurses in different occupational groups are more affected by PMS (5). For this reason, this study was planned to determine the relationship between nutrition and premenstrual syndrome in nursing students.

2. METHODS

Institutional permit was obtained to conduct the research at the Ordu University where the research was conducted (March 7, 2019.477.38962-663.08/339048). All procedures involving research study participants were approved by the Ordu University Clinical Research Ethics Committee (April 25, 2019-2019/64). Written informed consent was obtained from participants. In this study, the principles of the Declaration of Helsinki were followed.

The population of this descriptive and analytical study consists of female students studying in the nursing department of a university. At the time of the research, there were 261 female students in the nursing department. It was planned to reach the entire universe without selecting a sample. However, 16 students did not meet the inclusion criteria and 26 students refused to participate in the study. The sample of the study consisted of 219 female students who agreed to participate in the research and met the inclusion criteria. The rate of participation was 84%. The criteria for inclusion in the study were volunteering to participate in the study, having a regular menstrual cycle, not using oral contraceptives, and being 18 years or older. Exclusion criteria were dieting in the last 3 months, having a chronic illness, using oral contraceptives, being pregnant or lactating, and consuming any medication or nutritional supplement. Those who scored 111 and above on the PMSS were taken to the pms (+) group, and those who scored 110 and below were taken to the pms (-) group.

The data of the study were collected between June 3 and June 10, 2019. A questionnaire form prepared by the researcher and premenstrual syndrome scale were used while collecting the research data (16). The questionnaire and scale were filled in by the participants themselves, outside of the class hours, under the supervision of the researchers.

Demographic information, physical activity level, smoking status and nutritional habits were questioned in the questionnaire form applied to the participants. Later, the PMS scale was applied. Those who smoked at least 1 cigarette were defined as “smokers”. Those who exercised for 30 minutes at least 3 times a week were defined as “exercising regularly.”

Body weight, height, waist and hip circumference measurements were taken. The weight (kg) was measured with a portable digital scale without shoes and with light clothing, and height was measured (cm) with the help of a wall-hung tape measure. The feet were side by side and the head was in the frankfort plane while measuring the height. While measuring waist circumference (cm), the midpoint between the lowest rib and the crista iliaca was determined; this circumference was measured with an inelastic tape measure. In the measurement of hip circumference (cm), the participant was asked to stand sideways and the measurement was taken from the point where the hip circumference is highest with the help of an inelastic tape measure (17). BMI was calculated by dividing body weight (kg) by the square of height (m^2). BMI classification: <18.5 kg/m^2 underweight, 18.5–24.9 kg/m^2 normal, 25.0–29.9 kg/m^2 overweight, and >30.0 kg/m^2 obesity (18).

The Premenstrual Syndrome Scale (PMSS) was developed by Gençdoğan. DSM-III and DSM-IVR criteria were used in the development of the scale. This scale consists of 44 items (16). PMSS is a Likert type scale and the items of the scale are scored between 1 and 5. The lowest 44, the highest 220 points can be obtained from PMSS with 9 subscales. The subscales of PMSS are depressive feelings, anxiety, fatigue, irritability, depressive thoughts, pain, changes in appetite, changes in sleeping habits and swelling. The higher the score obtained from the scale indicates that the severity of the premenstrual syndrome is intense. If the total score from the PMS scale is 111 and above, it is considered as PMS. In addition, if the subscale scores are more than half of the maximum score that can be obtained from that subscale, the existence of that subscale is accepted (16). Gençdoğan found Cronbach alpha reliability coefficient was between 0.75 for total PMSS and 0.75-0.91 for subscales (16), in this study was found between 0.96 for PMSS and 0.61-0.91 for its subscales. The PMSS asks for last month.

The records obtained from the food consumed in the last 24 hours of the participants through the questionnaire were evaluated with the Nutrition Information System 8.1 (BEBIS 8.1) program, and the amount of macro and micronutrients consumed was determined. The analysis of the data was done on the computer and using a statistical package program (statistical package program for social sciences-SPSS 20.0). In the analysis, descriptive statistical methods, t test, chi-square test and Pearson correlation analysis test were used. Significance level was accepted as $p < .05$.

Normality test was used to determine the normality distribution of the data. The mean ± standard deviation was used for normally distributed data, and median (min-max) values were used for non-normally distributed data. Student t test was used for normally distributed quantitative data in comparison of two independent groups. The Mann Whitney U test was used to compare two independent groups that were not normally distributed. Pearson chi-square test was used to compare qualitative data. Pearson correlation analysis was used to determine the relationship between normally distributed quantitative data. Spearman correlation analysis was used to evaluate the relationship between non-normally distributed quantitative data. The level of significance was accepted as $p<.05$ in all statistical analyses.

3. RESULTS

The data on the general characteristics of the participants are shown in Table 1. Age, anthropometric measurements, smoking status, exercise status, place of residence, daily coffee consumption, salt consumption, meal consumption, and meal skipping status of the groups were compared. It was found that breakfast consumption was higher in the group with PMS. In addition, it was determined that the reason for skipping meals in students with PMS was mostly the absence of ready meals (Table 1).

Table 1. *Distribution of individuals according to their general characteristics*

FEATURES	PMS (+) (n=117)		PMS (-) (n=102)		Total (n=219)		
	Mean ± SD		Mean ± SD		Mean ± SD		p[a]
Age (years)	20.26 ± 1.41		20.42 ± 1.56		20.33 ± 1.48		.412[a]
Body weight (kg)	58.4 ± 10.03		56.96 ± 9.18		57.73 ± 9.65		.271[a]
Height (m)	1.63 ± 0.06		1.62 ± 0.05		1.63 ± 0.06		.092[a]
BMI (kg/m^2)	21.89 ± 3.63		21.68 ± 3.36		21.79 ± 3.5		.654[a]
Waist circumference (cm)	73.01 ± 7.9		72.99 ± 8.47		73.0 ± 8.15		.987[a]
Hip circumference (cm)	98.3 ± 7.56		97.16 ± 6.93		97.77 ± 7.28		.248[a]
Waist:hip ratio	0.73 ± 0.43		0.75 ± 0.49		0.74 ± 0.47		.055[a]
	n	%	n	%	n	%	p[b]
Smokers	11	9.4	6	5.9	17	7,8	.332[b]
Exercising regularly	50	42.7	46	45.1	96	43,8	.725[b]
Place of residence							
Dormitory	91	77.8	73	71.6	164	74,9	
Family home	15	12.8	17	16.7	32	14,6	.656[b]
Home alone	2	1.7	1	0.9	3	1,4	
With friends at home	9	7.7	11	10.8	20	9,1	
Daily coffee consumption more than 1 cup	22	18.8	15	14.7	37	16.9	.421[b]
Used salt without tasting the food	25	21.4	16	15.7	41	18.7	.283[b]
Meal consumption status							
Breakfast	111	94.9	87	85.3	198	90.4	**.017**[b*]
Lunch	79	67.5	82	80.4	161	73.5	.922[b]
Dinner	117	100	102	100	219	100	.922[b]
Snack	77	65.8	67	65.7	144	65.7	.984[b]
Skipped meal status							
Always	56	47.9	41	40.1	97	44.3	.456[b]
Sometimes	58	49.6	53	51.9	111	50.7	
Reasons for skiped meal*							
Lack of time	44	37.6	47	46.1	91	41.6	.204[b]
Lack of appetite	45	38.5	39	38.2	84	38.4	.973[b]
No ready meals	32	27.4	21	20.5	53	24.2	**.009**[b*]
Not a habit	24	20.5	17	16.6	41	18.7	.312[b]
To lose weight	16	13.7	9	8.8	25	11.4	.361[b]

PMS, premenstrual syndrome; BMI, Body Mass Index; cm, centimeter; SD, standard deviation. a Student t-test, b Chisquare test

The average scores obtained from PMSS and its subscales, the ratio and number of female students who scored above 50% according to the highest possible score are given in Table 2. The PMSS total score averages for the university students were 117.18±33.05. PMSS subscale mean scores of the participants were; 19.8±6.54 for depressive feelings, 18.09±5.62 for fatigue, 16.34±6.84 for depressive thoughts, 14.85±5.17 for irritability, 14.42±6.08 for anxiety, 9.24±3.63 for swelling, 8.45±3.23 for pain, 8.37±2.93 for changes in appetite and 7.61±3.17 for changes in sleeping habits (Table 2). The rate of PMS among female students was found to be 53.4%; according to PMSS subscales, 67.6% of the students had swelling, 66.7% had fatigue, 64.8% had depressive feelings, 63.0% had irritability, 61.2% had pain, 59.8% had changes in appetite, 47.5% had changes in sleeping habits, 40.2% had depressive thoughts and 28.8% had anxiety (Table 2).

Table 2. *PMSS subscales scores and total scores of all students (n=219)*

PMSS subscales	Cronbach Alpha	PMSS Min-Max Values	Marked Min-Max Values	Mean	SD	Received a score of over 50% from subscales and total PMSS n (%)
Depressive feelings	0.91	7-35	7 – 35	19.8	6.54	142 (64.8)
Anxiety	0.86	7-35	7 – 35	14.42	6.08	63 (28.8)
Fatigue	0.89	6-30	6 – 30	18.09	5.62	146 (66.7)
Irritability	0.91	5-25	5 – 25	14.85	5.17	138 (63.0)
Depressive thoughts	0.91	7-35	7 – 35	16.34	6.84	88 (40.2)
Pain	0.81	3-15	3 – 15	8.45	3.23	134 (61.2)
Changes in appetite	0.61	3-15	3 – 15	8.37	2.93	131 (59.8)
Changes in sleeping habits	0.81	3-15	3 – 15	7.61	3.17	104 (47.5)
Swelling	0.86	3-15	3 – 15	9.24	3.63	148 (67.6)
PMSS Total	0.96	44-220	51 – 220	117.18	33.05	117 (53.4)

PMSS, premenstraul syndrome scale; SD, standard deviation

The daily intake of energy and nutrients according to PMS groups is presented in Table 3. In the group of female students with PMS, daily total energy intake was higher (p<.001) and the percentage of energy from protein was lower (p<.05). Other nutrient intakes of the groups were similar (p>.05) (Table 3).

The correlations between PMS total score and daily diet energy, macro and micronutrients are shown in Table 4. Accordingly, PMS total score and daily energy intake (r=0.30) had a very weak positive correlation. PMS total score and daily intake of vitamin E (r=0.18), vitamin B_6 (r=0.19), magnesium (r=0.17), iron (r=0.14) and zinc (r=0.14) had a weak positive correlation. There was a very weak negative correlation between the PMS total score and the percentage of energy coming from protein (r=0.15) (Table 4).

Table 3. *Comparison of energy, macro and micronutrients intake according to the presence of PMS among students*

	PMS (+) (n=117)	PMS (-) (n=102)	Total (n=219)	
Energy and Nutrients	**Mean±SD Med (min-max)**	**Mean±SD Med (min-max)**	**Mean±SD Med (min-max)**	**p**
Energy (kcal)	1660.85 ± 1637.52	1446.16 ± 362.72	1560.4 ± 384.86	**< .001**[a*]
Protein (%) [†]	14 (8-24)	15 (8-24)	14 (8-24)	**.012**[b*]
Fat (%) [†]	37.5 (16-63)	38 (11-54)	37.5 (11-63)	.748[b]
Carbohydrate (%) [†]	49 (28-62)	46 (27-67)	48 (27-67)	.185[b]
Fiber (gr)	17.18 (3.23-34.31)	17.26 (6.91-48.8)	17.24 (3.23 – 48.8)	.296[b]
Vitamin E (mg)	11.66 (1.64-36.46)	10.34 (2.05-29.02)	10.94 (1.64 – 36.46)	.296[b]
Vitamin B_1 (mg)	0.77 (0.2-1.43)	0.8 (0.25-2.95)	0.78 (0.2 – 2.95)	.157[b]
Vitamin B_2 (mg)	0.99 (0.26-3.17)	0.98 (0.33-2.24)	0.98 (0.26 – 3.17)	.249[b]
Vitamin B_6 (mg)	1.12 (0.24-2.52)	1.19 (0.34-2.65)	1.15 (0.24 – 2.65)	.252[b]
Calcium (mg)	643.45 ± 245.32	716.81 ± 336.18	677.77 ± 292.99	.071[a]
Magnesium (mg)	242.1 (48.16-410.33)	241.5 (129.4-700.49)	241.5 (48.16 – 700.49)	.498[b]
İron (mg)	9.19 (2.38-19.25)	9.54 (3.21-28.16)	9.35 (2.38 – 28.16)	.547[b]
Zinc (mg)	7.66 (3.13-20.1)	8.64 (3.18-21.49)	8.15 (3.13 – 21.49)	.268[b]

PMS, premenstrual syndrome; SD, Standard deviation

[†] Its contribution to total energy is incicated in %

[a] Independent two-sample t-test, data is normally distributed

[b] Mann Whitney U test, data is not normally distributed

Table 4. *Correlations between PMSS and energy, macro and micronutrient intake scores of students*

Energy and Nutrients	PMSS	
	r	p*
Energy (kcal)	0.30	**<.001***
Water (ml)	0.13	.059
Protein (%)†	-0.15	**.022***
Fat (%)†	-0.11	.116
Carbohydrate (%)†	-0.03	.605
Fiber (gr)	-0.08	.252
Vitamin A (mg)	0.05	.457
Carotene (mg)	-0.02	.762
Vitamin E (mg)	0.18	**.009***
Vitamin B_1 (mg)	-0.09	.165
Vitamin B_2 (mg)	0.11	.114
Vitamin B_6 (mg)	-0.19	**.004***
Folate (mg)	0.09	.179
Sodium (mg)	-0.07	.309
Calsium (mg)	-0.06	.355
Magnesium (mg)	0.17	**.014***
Phosphorus (mg)	0.10	.146
Iron (mg)	-0.14	**.042***
Zinc (mg)	-0.14	**.042***

PMSS, premenstrual syndrome scale

[†] Its contribution to total energy is incicated in %

4. DISCUSSION

PMS, which causes increases in health expenditures, deterioration in social relations, decreases in quality of life and loss of labor in women of reproductive age, is an important public health problem (19). Some studies revealed the relationship between PMS and nutrition (20, 21), but there are also studies that could not find a relationship between nutrition and PMS (10). In this study, the relationship between the frequency of PMS, which affects the majority of women, among nursing students with high health awareness and the relationship between PMS and nutrition was investigated.

In a study by Yoshimi et al., it was found that individuals with PMS skip breakfast more than individuals without PMS (22). In this study, it was found that individuals with PMS eat breakfast more often. However, when the food consumption records of these individuals were examined, they had breakfast with packaged foods such as pastry, bagel, biscuits, instant cake and instant fruit juice (data not shown). It is thought that this result was obtained in the group with PMS as the consumption of these foods that are not included in healthy diets increases the risk of PMS. The dinner and snack consumptions of the groups were similar.

Foods high in caffeine are known to increase PMS symptoms (23). In a study examining the relationship of sociodemographic, diet, and lifestyle factors with PMS among undergraduate medical students, it was stated that caffeine consumption was positively associated with moderate and severe PMS (7, 24). There are also studies stating that caffeine intake is not associated with PMS. In the study of Hashim et al., it was found that there was no relationship between PMS symptoms and caffeine consumption (7). In the study of Desrosiers et al. in young adult women, no significant difference was found between the daily caffeine consumption of women with and without PMS (24). In this study, although it was determined in this study that coffee, a beverage with high caffeine content, was consumed more by students with PMS, this difference was not statistically significant. There is no definite information about the

relationship between caffeine and PMS in the literature. In this study, no relationship was found between PMS and caffeine.

It is reported that excessive salty food consumption is a strong risk factor for PMS (7). Consumption of unprocessed and fresh foods is recommended for the prevention and treatment of PMS. It is emphasized that a diet high in refined carbohydrates, fat and salt increases PMS symptoms (25). In this study, the students' habits of adding salt without tasting the food were examined, and and groups were similar. The difference between the groups may be due to the inability to clearly determine the amount of salt consumed per day. Evaluation of participants' daily salt consumption would have been a better measurement method.

In this study, the total energy intake of the groups and the percentage of energy from carbohydrates are low, the percentage of energy from protein is normal, and the percentage of energy from fat is high (26). However, there is only a difference between energy and protein intakes of students with PMS compared to students in the healthy control group. The total energy intake of students with PMS is higher, and the percentage of energy from protein is lower. It is stated that consumption of foods with high energy, carbohydrate and fat content is a strong risk factor for PMS (7). Looking at the studies conducted, there was a positive relationship between high total energy intake and PMS (6, 7, 27). It was reported that food consumption with high sugar content is significantly higher among those with PMS (27). In a study conducted with university students, high total energy intake was reported to be a strong risk factor for PMS (7). Similar to the literature, in this study, the total energy intake of individuals with PMS was found to be significantly higher than those without PMS. In addition, a positive significant relationship was found between the total energy intake with diet and PMSS total score. Protein intake was not associated with PMS (10). The difference observed between the groups for protein intake in this study is thought to be due to the fact that the 24-hour food consumption record shows only one daily food intake.

Although it has been reported that the amount of dietary fiber should be increased to reduce the symptoms of premenstrual syndrome, studies supporting this information are limited. The role of fiber in the treatment of PMS is unclear. In a study examining the relationship between fiber intake and PMS risk, it was revealed that there was no relationship between fiber consumption and PMS (9). A similar study found no association of different types of carbohydrates and total dietary fiber with PMS (28). However, there are also studies showing a significant relationship between fiber consumption and PMS (29, 30). Since only a 24-hour food consumption record was taken in this study, no clear information could be obtained about the fiber intake of the participants, and therefore, a relationship between the amount of dietary fiber and PMS may not have been found. Studies examining fiber consumption in individuals with PMS in more detail may reveal this relationship more clearly.

It is stated that another reason for the emergence of PMS symptoms is vitamin (especially vitamin B1, B2, B6 and E) deficiencies (31). It is estimated that the deficiency or insufficiency of B group vitamins (vitamins B1, B2, B6) required for the synthesis of neurotransmitters, which play an important role in the pathogenesis of premenstrual syndrome, cause more severe PMS symptoms (31). It is reported that the combined use of magnesium and vitamin B6 reduces premenstrual anxiety (32). However, the relationship between PMS and vitamins is not certain. It was determined that the level of vitamin E in the body was associated only with hand / foot swelling, and no relationship was found between other symptoms of PMS (33). However, these studies do not measure dietary vitamin levels. In the studies, either the levels of vitamins in the blood or the use of supplements were evaluated. In this study, the dietary vitamin levels of the groups were found to be similar. However, there is a weak positive relationship between PMS and dietary vitamin E, and a negative relationship between vitamin B6. Since this investigation is one of the rare nutrition studies that examines the relationship between dietary vitamin levels and PMS, it makes a significant contribution to the literature.

It has been reported that the lack of dietary intake of minerals such as calcium, magnesium, iron and zinc causes premenstrual syndrome (34). In a study by Fujiana et al., individuals whose diets were enriched with calcium, magnesium and iron had improved premenstrual symptoms (35). However, there are studies that claim the opposite of this situation (36, 37). In a study comparing trace elements in the serum of individuals with and without premenstrual syndrome, serum calcium, magnesium, iron and zinc levels of the groups were found to be similar (36). Based on the results of a systematic review and meta-analysis, no significant relationship was found between serum magnesium levels and PMS (37). In this study, as with vitamins, The dietary mineral levels of the groups were similar. However, there is a weak negative correlation between PMS and dietary magnesium, iron and zinc. Since this study is one of the rare nutrition investigations that examines the relationship with PMS by determining the dietary mineral levels of individuals, it makes a significant contribution to the literature.

5. CONCLUSION

In coclusion, it was determined that more than half of the university students had PMS. There was no difference in anthropometric measurements between female students with and without PMS. Breakfast consumption affected the presence of PMS, but there was no difference between the groups with and without PMS in terms of coffee, salt consumption and skipping meals. PMS is affected by energy and nutrient intake. In addition, nutrition is an effective factor in the occurrence of PMS. In female students with PMS, it is recommended to raise awareness about the importance of nutrition in reducing or eliminating symptoms, inform

experts about nutrition, and perform further research on this issue.

This study has some limitations. The first of these is that data on both premenstrual symptoms and nutritional status are based on students' self-reports. No examination or laboratory tests were performed to evaluate premenstrual symptoms and nutritional status. Another limitation is that a 24-hour food consumption record is taken from individuals. Additionally, only students from the nursing department of one university were included in the research. As a result, the data in this study can only be generalized to this sample.

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Clinical and Experimental Health Sciences

The Relationship between Menopause Perception, Body Mass Index, and Waist-Hip Ratio with Menopausal Symptoms in Turkish Women

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ABSTRACT

Objective: The study aimed to investigate the relationship between menopause perceptions, feelings felt, body mass index, and waist-hip ratio with menopausal symptoms in Turkish climacteric women.

Methods: This descriptive and correlational study was conducted in a Family Health Center. The research sample consisted of 220 women in the climacteric period. Data were collected with the survey form and The Menopause Rating Scale (MRS). Body Mass Index (BMI) and Waist Hip Ratio (WHR) were measured and calculated by the researchers.

Results: The women who perceive menopause as a "natural, normal process" had lower somatic, psychological, and general menopausal symptoms, and those who defined it as "the end of sexuality" had higher genitourinary symptoms ($p< .01$). Women who were adversely affected or felt negative emotions about menopause reported that they experienced all menopausal symptoms more severely ($p< .001$). Obese women experienced particularly higher levels of somatic and general menopausal symptoms ($p< .05$-$p< .01$), while women with WHR< 0.72 experienced a higher level of genitourinary symptoms ($p< .05$).

Conclusion: The results showed that menopause perception, feelings felt, obesity, and WHR have an impact on menopausal symptoms and levels.

Keywords: Menopause, symptoms, menopause perception, body mass index, waist-hip ratio

1. INTRODUCTION

Each life stage of women brings its own physical, psychological, and hormonal changes. Menopause, which occurs in the climacteric period, includes perimenopausal, menopausal, and postmenopausal periods, is almost a cornerstone due to its impact on women's lives, and it indicates a transition from the reproductive period to the non-reproductive period (1). World Health Organization (WHO) stated that most women experience menopause between the ages of 45 and 55 years as a natural part of biological aging (2). while some authorities reported 51 (3-5). The average age of menopause is reported to be 48 years in Turkey (6). During menopause, various physical and mental changes occur, especially due to estrogen deficiency and many predisposing factors, and could be experienced symptoms related to vasomotor, psychological, somatic, and atrophic changes (8,9).

Changes in estrogen and its level during menopause affect other hormones and metabolism, altered metabolism leads to increased appetite and reduced metabolic rate. In addition, the tendency to be overweight and obese increases in this process due to decreased physical activity, common emotional eating disorders associated with eating habits and psychological discomfort, and the contribution of environmental factors (8,9). A significant increase in fat mass, especially visceral adipose tissue mass, is observed after menopause, and fat accumulation generally increases in the abdomen, and a transition from gynoid obesity to abdominal obesity occurs with android-type weight gain (10). For these reasons, it is important to measure the Body Mass Index (BMI) and the Waist Hip Ratio (WHR) in terms of this type of weight gain during menopause and to provide recommendations if necessary (11,12). In women, WHR values above 0.72 are considered abnormal, and a value of ≥ 0.80 indicates an increased risk (13). There are also many discussions on the relationship between obesity and menopausal symptoms (14). Some studies have reported an

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association between menopause and BMI, and increased BMI is an important factor in hot flushes (8,14,15). However, some studies reported an inverse relationship between BMI and hot flashes, and hot flashes are more common in women who are underweight compared to BMI and who do not exercise (16,17). It is stated that there is a positive relationship between abdominal obesity, that is increased WHR, hot flashes, and urogenital symptoms (14). On the other hand, attitudes, perceptions, and expectations are also part of the psychosocial phenomenon surrounding menopause (18). Perceptions and attitudes towards menopause are influenced by many cultural and social variables, and it is assumed that these conditions may affect the experiences and symptoms of menopause (18,19). It has been reported that women with a negative attitude towards menopause experience more symptoms (19).

It is seen that further and quality studies are needed that can determine the relationship between menopause perception, BMI, and WHR with menopausal symptoms. The current study aimed to investigate the relationship between menopausal perception, feelings felt, BMI, and WHR with menopausal symptoms experienced by climacteric women. It was thought that the results obtained would contribute to the literature, and develop more effective and personalized interventions and healthcare services for women in the climacteric period. This study is a crucial step towards enhancing climacteric women's health and quality of life by creating more comprehensive strategies based on menopause knowledge.

2. METHODS

The study was reported according to the STROBE Checklist. The STROBE Statement checklist is a tool used to report observational studies, including cohort, case-control, and cross-sectional studies. The checklist consists of items that should be included in reports of observational studies. The checklist is not a prescription for designing or conducting observational studies, nor is it an instrument for evaluating the quality of research (20).

2.1. Participants

This study descriptive and correlational research design was carried out in a Family Health Center. The Finite/Known Populations Formula was used to calculate the study sample size. As a result of the analysis made according to the formula, it was determined that at least 213 women should be sample size and this number was increased. The study sample consisted of 220 women in the climacteric period [Perimenopause n=50 (22.7%); postmenopause n=170 (77.3%)]. Random sampling method was used from non-probability sampling methods in the sampling and women who met the sampling criteria and voluntarily agreed to participate in the study were included. The sampling criteria: Women who were registered at the family health center where the research was conducted, aged between 45 and 65, in the peri and postmenopausal period, able to understand the questions, with no mental problems or serious chronic diseases and not using medication (for such diseases), and volunteering to participate for the research. Those who did not meet the listed criteria were excluded from the sample. No participants withdrew from the research.

2.2. Instruments

Data were collected using the participant's characteristics form prepared by the researcher with expert opinion and the Menopause Rating Scale (MRS). *The participant's characteristics form* has two parts. The first part consisted of 12 questions about socio-demographic, obstetric, and menopausal characteristics and menopause perceptions of women (*What does menopause mean to you? What do you say about your menopause perception and feelings?)*. In addition, women were asked to rate their level of being affected by menopausal complaints from 0 to 10 (0: None, 10: Severe). The second part included height, weight, waist, and hip measurements, and BMI and WHR results calculated by the researchers. *Menopause rating scale (MRS):* The scale was first developed by Heinemann et al. in 1992. The scale consists of 11 items that can determine somatic, psychological, and genitourinary symptoms, and also measure women's quality of life. MRS is a 5-point Likert-type scale ranging between 0 (none at all) and 4 (very severe). The highest score that can be obtained from the scale is 44, and higher scores indicate increased symptoms and adversely affected quality of life (21). The Turkish validity of the scale was conducted by Özlem Can Gürkan and Cronbach's alpha was found 0.84 scale total score (22).

2.3. Ethics Committee Approval

Ethical approval for this study was obtained from the Marmara University, Institute of Health Sciences Non-Interventional Clinical Research Ethics Committee (Date: 11.09.2017; Number of approval: 162), and institutional permission was obtained from the Family Health Center (16867222/604.01.01). Written permission was obtained for the use of the Turkish version of the scale. Verbal and written consent was obtained from the volunteer participants.

2.4. Data collection

A face-to-face interview technique was used to apply the forms to the women included in the study, and the data were collected through self-reporting. After completing the forms, height, weight, waist, and hip circumference measurements were performed by the researchers, and were recorded in the second part of the participant's characteristics form.

2.5. Statistical analysis

Statistical Package for the Social Sciences (SPSS) version 22 was used to analyze the data. The study searched to answer these questions: "*What do BMI and WHR values?*" "*Do the*

menopausal symptoms and/or levels vary according to the menopause perception, feelings felt, BMI, and WHR? And "*What is the relation between them?*" Frequency, mean, chi-square, t-test, ANOVA, and Pearson's correlation test were used in the analysis. Statistical significance was set at p< .05.

3. RESULTS

3.1. Demographic results

Of the women, 22.7% (n=50) were perimenopausal and 77.3% (n=170) were postmenopausal. The average age was 52.7±5.5 years (perimenopause 47.2±2.2; postmenopause: 54.4 ± 5.1), 48.2% were primary school graduates, the majority (82.3%) were married, and 64.1% were unemployed. Taking Hormone Replacement Therapy (HRT) rate was 5.4% (n=12).

3.2. Women's Perceptions of menopause

More than half of women (53.2%) reported a perception of menopause as a "*natural, normal process*", while 41.8% described it as "*the loss of femininity traits*". This expression was higher in postmenopausal women (p< .001). The rate of defining menopause as "old age/aging" and "*increase in health problems/diseases*" was higher in perimenopausal women who are in menopause or will be entering into menopause. (*40.9% of women described their feelings mostly as "unhappiness" and "sadness"*). Women's level of being affected by menopausal complaints was 6.28±1.85 according to the scores between 0 and 10, while this level was higher in postmenopausal women (p<.001). The age of onset of menopause symptoms age was 45.84±3.39. The natural menopause rate was 94.7% in those who entered menopause, the average age of the final menstrual period of these women was 45 years, and the mean age of menopause was 46 years (Table 1).

***Table 1**. Menopausal characteristics of women and their perceptions of menopause*

Variables	Perimenopause (n=50) n (%)	Postmenopause (n=170) n (%)	Total (N=220) n (%)	X^2,p
Menopause perceptions[a]				
The natural, normal process	26(52.0)	91(53.5)	117(53.2)	X^2= 0.03 p= .84
The loss of femininity traits	10(20.0)	82(48.2)	92(41.8)	X^2= **12.66** **p= .000**
The end of fertility	10(20.0)	39(22.9)	49(22.3)	X^2= 0.19 p= .66
Old age/aging	19(38.0)	38(22.5)	56(25.5)	X^2= **5.36** **p= .02**
Decrease or end of sexual activity	5(10.0)	20(11.8)	25(11.4)	X^2= 0.11 p= .73
Increase in health problems/diseases	12(24.0)	22(12.9)	34(15.5)	X^2= **3.61** **p= .05**
Feelings felt *(about being in menopause or entering into menopause)*				
Natural feelings, meeting normal	25(50.0)	62(36.5)	87 (39.5)	X^2= 2.95 p= .06
Negative emotions and negatively affected	25 (50.0)	108 (63.5)	133 (60.5)	
Menopausal symptoms	**Mean±SD**	**Mean±SD**	**Mean±SD**	**p**
Age of onset of menopause symptoms	45.20 ±2.39	46.02± 3.61	45.84±3.39	t= 1.51 p= .13
Duration of experiencing menopause symptoms / years	2.22 ± 1.37	6.74± 5.01	2.11±1.23	**t= 6.29** **p= .000**
Affected level by menopause complaints	5.36 ± 2.14	6.55 ± 1.66	6.28±1.85	**t= 4.13** **p= .000**
Characteristics of women entering menopause (n=170)				
Menopause type	**n (%)**			
Natural	161 (94.7)			
Surgical	9 (5.3)			
	Mean±SD	**Median**		
Final menstrual age	45.66 ±3.76	45		
Menopause age	46.67 ±3.73	46		
Menopause time/years	7.78 ± 5.62	6		

Chi-square and t-test were used

While the rate of experiencing positive emotions was higher in women who perceived menopause as a natural process. It has been found that women who have negative interpretations of menopause *(such as loss of femininity traits, end of fertility, aging, and end of sexuality)* are more likely to experience negative emotions during their menopause period (p< .01 – .001).

3.3. Women's BMI and WHR characteristics

According to BMI, 46.0% of women were obese, and this rate was higher in postmenopausal women (52.3%), while in perimenopausal women the overweight rate was high (44.0%). The abnormal WHR (> 0.72) rate was 93.6% and the increased risk (≥ 0.80) rate was also high at 85.9% (Table 2).

3.4. MRS results according to menopausal perception, emotions, BMI and WHR

MRS total score average was 14.54±6.38, especially somatic symptoms and the level of experiencing overall menopausal symptoms was higher in postmenopausal women (p< .01). Genitourinary symptoms were the least reported. The level of experiencing "somatic", "psychological", and overall menopausal symptoms of women who define menopause as a "*natural, normal process*" was lower than others (p< .01-.001). The women who define menopause as a "*decrease or end of sexual activity*" had a higher level of genitourinary symptoms (p< .01) and those who define it as an "*increase in health problems/diseases*" also had a higher level of psychological symptoms (p< .05). Women who were adversely affected or felt negative emotions about menopause reported that they experienced all menopausal symptoms more severely (p<.001). Obese women had a higher level of experiencing especially somatic and overall menopausal symptoms (p<.05 – .001). The level of experiencing genitourinary symptoms was higher in women with a WHR < 0.72 (p< .05) (Table 3).

According to the Pearson's correlation analysis; as BMI increased, the women's level of experiencing somatic, psychological, and general menopausal symptoms, and affected level by menopause complaints also increased, and there was a significant positive correlation between them (p< .05 – .01). There was no significant correlation between WHR and MRS total and sub-groups (p> .05), but as the WHR increased, the affected level by menopause complaints that women reported was also increasing (p< .01) (Table 4).

Table 2. *Women's BMI and WHR characteristics*

Characteristics	N=220			
	Perimenopause	Postmenopause	Total	p
	(n=50)	(n=170)	(n=220)	
	n(%)	n(%)	n(%)	
BMI				
Underweight (<18.5)	2(4.0)	1(0.6)	3(1.4)	
Normal weight (18.5 – 24.9)	14(28.0)	15(8.8)	29(13.2)	x^2= **23.18**
Overweight (25.0 – 29.9)	22(44.0)	65(38.2)	87(39.5)	**P= .000**
Obesity	12(24.0)	89(52.3)	101(46.0)	
Obesity class I (30.0 – 34.9)	*8(16.0)*	*74(43.5)*	*82(37.3)*	
Obesity class II (35.0-39.9)	*4(8.0)*	*12(7.1)*	*16(7.3)*	
Obesity class III (≥40)	*0(0.0)*	*3(1.8)*	*3(1.4)*	
WHR	3 (6.0)	11(6.5)	14(6.4)	x^2= 0.01
≤ 0.72 (Normal)	47 (94.0)	159(93.5)	206(93.6)	p= .60
> 0.72 (Abnormal)				
≥ 0.80 (increased risk)	39(78.0)	150(88.2)	189(85.9)	x^2= 3.34
< 0.80	11(5.0)	20(9.0)	31(14.1)	p= .06
BMI and WHR averages	**Mean±SD**	**Mean±SD**	**Mean±SD**	
BMI	27.37 ± 4.61	30.16 ± 4.04	29.52 ± 4.33	**t= 4.14** **p= .000**
WHR	0.85 ± 0.08	0.86 ± 0.86	0.86 ± 0.07	t= 0.45 p= .65

Chi-square and t-test were used

Table 3. *MRS sub-groups and overall score averages according to menopause perception, feelings, BMI, and WHR*

Variables		N=220			
		MRS sub-groups and total			
		Somatic score	Psychological score	Genitourinary score	Total score
		Mean±SD	Mean±SD	Mean±SD	Mean±SD
Menopause status					
Perimenopause (n=50)		4.56±2.61	5.58±2.96	1.94±2.03	12.08±6.10
Postmenopause (n=170)		6.48±2.69	6.42±2.77	2.35±2.20	15.27±6.30
Total		6.05±2.79	6.23±2.83	2.25±2.17	14.54±6.38
		t=4.47; **p= .000**	t=1.87; p= .06	t=1.18; p= .23	t=3.16; **p= .002**
Menopause perceptions					
The natural, normal process	Yes No	5.60±2.76 6.55±2.74	5.54±2.62 7.01±2.87	2.04±1.85 2.50±2.47	13.19±5.93 16.07±6.55
		t=-2.54; **p= .01**	t=3.97; **p= .000**	t=1.57; p= .11	t=3.42; **p= .001**
The loss of femininity traits	Yes No	6.28±2.65 5.88±2.88	6.42±2.80 6.10±2.85	2.33±2.18 2.20±2.17	15.04±6.48 14.18±6.31
		t=-1.04; p= .29	t=-0.83; p= .40	t=-0.45; p= .65	t=-0.98; p= .32
The end of fertility	Yes No	5.67±2.80 6.15±2.78	6.40±3.14 6.18±2.74	2.51±2.37 2.18±2.11	14.59±6.69 14.53±6.31
		t=1.07; p= .28	t=-0.48; p= .63	t=-0.91; p= .36	t=-0.05; p= .95
Old age/aging	Yes No	5.82±2.62 6.12±2.85	6.25±2.79 6.23±2.85	2.39±2.37 2.21±2.10	14.46±5.97 14.57±6.53
		t=0.70; p= .47	t=-0.04; p= .96	t=-0.53; p= .59	t=0.11; p= .91
Decrease or end of sexual activity	Yes No	6.84±3.83 5.94±2.62	7.00±3.85 6.13±2.67	3.44±3.61 2.10±1.87	17.28±10.25 14.19±5.65
		t=-1.50; p= .13	t=-1.43; p= .15	t=-2.93; **p=.004**	t=-2.29; **p= .02**
Increase in health problems/ diseases	Yes No	6.50±3.09 5.96±2.73	7.08±3.76 6.08±2.60	2.58±3.37 2.19±1.87	16.17±9.00 14.24±5.76
		t=-1.02; p=.30	t=-1.91; **p=.05**	t=-0.96; p=.33	t=-1.62; p=.10
Feelings felt (*about being in menopause or entering into menopause***)**					
●Natural feelings, meeting normal ●Negative emotions and negatively affected		5.33±2.86 6.51±2.64	5.48±2.67 6.72±2.83	1.79±1.79 2.56±2.34	12.60±5.93 15.81±6.37
		t= – 3.14; **p= .002**	t= – 3.26; **p= .001**	t= – 2.60; **p= .01**	t= 3.74; **p= .000**
BMI					
Normal		4.56±2.38	5.56±3.01	1.81±1.94	11.93±5.53
Overweight		5.97±2.61	6.03±2.81	2.54±2.17	14.55±6.22
Obesity		6.58±2.90	6.62±2.75	2.15±2.23	15.36±6.60
		F=6.76**; p= .001**	F=2.09; p= .12	F=1.51;p= .22	F=3.58; **p= .02**
WHR					
≤ 0.72		6.28±3.26	6.35±2.95	3.35±3.15	16.00±7.50
> 0.72		6.03±2.76	6.23±2.83	2.18±2.08	14.44±6.31
≥ 0.80		6.10±2.71	6.22±2.77	2.22±2.05	14.56±6.14
		t= 0.32; p= .74	t=0.16; p= .87	**t=1.96; p= .05**	t= 0.88; p= .38

t-test and ANOVA were used

***Table 4**. Correlation analysis results*

	Variables	1 *r*	2 *r*	3 *r*	4 *r*	5 *r*	6 *r*	7 *r*
1	BMI	1	**.33***	**.23****	**.15***	.03	**.18****	**.21****
2	WHR		1	-.003	.01	-.01	-.002	**.18****
3	Somatic symptoms			1	**.69****	**.38****	**.87****	**.48****
4	Psychological symptoms				1	**.37****	**.87****	**.43****
5	Genitourinary symptoms					1	**.67****	**.17****
6	MRS total						1	**.46****
7	Affected level by menopause complaints							1

**: p< .05; **: p< .01-p< .001, Pearson's correlation analysis was used; r: correlation coefficient*

4. DISCUSSION

The Turkey Demographic and Health Survey data (23) shows that 84.0% of women aged 40-49 are overweight in Turkey. In the study, the average BMI of women was 29.52±4.33, 44.0% of women in the perimenopausal period were overweight and 52.3% of postmenopausal women were obese. In the literature, there are studies indicating the average BMI of postmenopausal women as 28.98±5.71(24) and 29.5±6.0 (25), and the obesity rate of 20.2% and while the rate of being overweight varies at 42% (26), 36.0% (27). One study, reports that WHR also increases especially in the postmenopausal period (14). In the current study, the abnormal WHR (> 0.72) rate was 93.6% and the increased risk (≥ 0.80) rate was also 85.9%. In the literature, there are studies reporting that WHR varies between 0.76 and 0.84 (28) and WHR ≥ 0.85 is 85.3% (29). The current study results and the literature show that in the climacteric period, especially in postmenopausal women, weight, obesity rate and increase in WHR are serious and significant problems, and women in the climacteric period constitute an important risk group (Table 2).

World Health Organization (WHO) declares the menopause age as 52 (2) while some authorities reported 51 (3,4) and a study conducted in China reported 49 (7). In the current study, the menopause age was 46 years (Table 1). The results support that menopause age varies from society to society. In the study, the rate of women describing menopause as a "natural, normal process" adopting a positive perception was 53.2%. About half of the women reported negative definitions related to menopause, such as "*the loss of femininity traits*", "*end of fertility*", "*aging*", "*decrease or end of sexual activity*" and "*increase of diseases*" (Table 1). Our findings show parallelism with the studies carried out (Table 1) (26,27). The rate of being negatively affected by menopause and feeling negative emotions about menopause was higher in women with a negative perception of menopause. The results of the study by Polat and Karasu (2021) also support our data and they stated that women's negative perceptions of menopause affect their emotions negatively (30). According to Vural and Yangın (2016), Turkish and German women think that menopause is an unpleasant experience for a woman. At the same time, women in both cultures perceive the views that "Femininity is lost in menopause" and "Menopause increases family problems" negatively (31). Women with normal BMI had significantly higher attitude scores towards menopause (32). On the other hand, the study results showed that women with a positive menopause perception experienced general menopausal symptoms except for genitourinary symptoms at lower levels than those with a negative perception. In addition, women who were adversely affected or felt negative emotions about menopause reported that they experienced all menopausal symptoms more severely (Table 3). Some studies reported that the perception of menopause can vary across cultures and negative perception affects the level of experiencing symptoms (30,33). Our results support these literature data. Since there are multifactorial effects in perceptions and approaches to menopause, the results showed the necessity and importance of meeting training and counseling needs in a way that would provide positive perceptions, attitudes, and emotions towards menopause by assessing each woman within her own cultural norms and values. In addition, in the study, high levels of genitourinary symptoms and general menopausal symptoms were also considered a remarkable result in women who defined menopause as a "decrease or end of sexual activity" (Table 3). Whether is the perception of this direct effect on the level of experiencing genitourinary symptoms? or does experienced genitourinary complaints cause this perception? It should be noted that this is important for the sexual function of women, regardless of the direction of this relationship, and it should be taken into consideration during the assessment.

In the literature, it is reported that as BMI and WHR increase, women's levels of experiencing menopausal symptoms also increase (34,35). In the study, as BMI increased, the level of experiencing menopausal symptoms also increased, and the level of experiencing general menopausal symptoms, especially somatic symptoms was higher in obese women. Women with a WHR ≤ 0.72 experienced more severe genitourinary complaints (p< .05) (Table 4). According to the study results, there was a positive relationship between experienced menopausal symptoms and level and BMI and WHR (Table 4). The results of the study are important in terms of indicating that the increase in BMI triggers the increase in the level of experiencing general menopausal symptoms and especially the increase in somatic and psychological

symptoms, that a WHR of ≤ 0.72 affects the increase in genitourinary symptoms, and that women with higher BMI and WHR are more affected by the menopausal complaints they experience. Some studies indicate that there is a positive relationship between obesity and menopausal symptoms (36), and obesity causes an increase in psychological, somatic, and vasomotor symptoms (37), which lead to an increase in heat flashes (17) due to increased weight. The current study results are consistent with the literature. Some studies reported that women with high WHR mostly experience more genitourinary symptoms (36,38). However, the current study showed the opposite, which suggests that further studies are needed regarding this issue.

Limitation: The results of the study cannot be generalized as it was conducted in a specific region.

5. CONCLUSION

The study results indicate that experienced menopausal symptoms and levels can differ according to the state of entering menopause, the perception of menopause, the feelings experienced/felt, BMI, and WHR. The results are valuable in terms of revealing the current situation in climacteric women and guiding the care process to be provided to women in this group. In this context, it is important that climacteric women are evaluated holistically and that an individualized treatment/care approach is adopted.

It is critical to adopt an individualized approach to support the health and improve the quality of life of women in the climacteric period. Health professionals should assess women holistically, taking into account menopausal symptoms, emotional states, body composition, and other individual factors. This assessment will form the basis for creating a customized treatment and care plan for women. In addition, awareness-raising campaigns should be organized to increase the level of awareness about menopause in the community and to make this process more understandable. These campaigns should emphasize that menopause is not only a physical but also an emotional and psychological period and provide information for all segments of society to support women. In conclusion, supported by individualized health services and awareness-raising campaigns, climacteric women can have a healthy menopause and improve their quality of life.

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Clinical and Experimental Health Sciences

An Evaluation Of The Relationship Between Falls, Osteoporosis And The Parkinson's Disease Rating Scale In Patients With Parkinson's Disease

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ABSTRACT

Objective: The purpose of this study is to examine conditions such as balance disorder, risk of falling, fear of falling, vitamin D deficiency and osteoporosis in people with Parkinson's Disease (PD), and their association with the Movement Disorders Society Unified Parkinson's Disease Rating Scale (MDS-UPDRS) sub-components, which are used to follow-up these patients.

Method: The study comprised 38 patients who were followed up for idiopathic PD. All the patients' demographic data, falls efficacy scale, number of falls within the last year, history of fractures, Berg Balance Scale, the MDS-UPDRS sub-components, vitamin D levels, and bone mineral densitometry values were recorded.

Results: There was a positive correlation between the MDS-UPDRS Part I and the number of falls and the history of fractures, and a positive correlation with the Hoehn and Yahr scale, and the MDS-UPDRS Part II, III and total and the Berg Balance Scale, the Falls Efficacy Scale, and the number of falls. Our study found that the bone mineral densitometry values for the femoral neck were lower in women than in men, and there was a positive correlation between the bone mineral densitometry values for the femoral neck and the body mass index. A positive correlation was established between levodopa use and the falls efficacy scale.

Conclusion: Falls, imbalance, osteoporosis are life-threatening conditions in patients with PD. This study established that the MDS-UPDRS, used to follow-up patients, was associated with these conditions. It is believed that this assessment method may also give an idea about these conditions in PD patients who are followed up using this scale.
Keywords: Balance, falls, Parkinson's disease, bone mineral density, vitamin D

1. INTRODUCTION

Parkinson's Disease (PD) is a chronic progressive neurodegenerative disease with motor and non-motor symptoms, characterized by the loss of dopamine-producing neurons in the basal ganglia. The prevalence has been reported as high as 15:1,000 in some populations (1). PD is one of the common neurological diseases that cause recurrent falls, and falls pose a significant problem with these patients (2). Studies show that almost 70% of patients fall at least once a year (3). Falls cause patients to develop various conditions such as fear of falling and fractures, as well as extensive costs to the healthcare system due to fall-related diseases (2). The higher incidence of osteoporosis in patients with PD (4) results in a higher rate of fractures due to these patients' falls. Several studies have been conducted on why osteoporosis is common among these patients. Many factors such as inactivity, loss of muscle strength, reduced body mass index, vitamin D deficiency, and nutrition have been reported to be involved in the increased incidence of osteoporosis (4). Osteoporosis and the increased risk of falls due to various reasons reduce the quality of life of patients with PD, as well as isolating patients due to additional complications. Considering these potential adverse effects, balance disorder, risk of falls and osteoporosis has become extremely important in patients with PD.

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Assessing changes in the health status of patients and determining the severity of the disease are vital in the follow-up of diseases. There are several scales used to assess the motor and non-motor symptoms of patients with PD. Among these are the "Parkinson's Disease Questionnaire-39" to assess the patients' quality of life, the "Non-motor Symptoms Scale" to assess non-motor symptoms, and the "Clinical Impression of Severity Index" to assess the quality of life and disability (5). Likewise, the Unified Parkinson's Disease Rating Scale is of great importance in the follow-up of these patients because it is a comprehensive and reference scale. It was revised in 2008 due to its limited assessment of non-motor symptoms (6). Despite the difficulty of use in practice as it is time-consuming, a complete version of the scale has been created that assesses the disease in every aspect, determines the course of the disease, and is more homogeneous and comprehensive.

In this study, our objective was to examine the association between the sub-components of the Movement Disorders Society Unified Parkinson's Disease Rating Scale (MDS-UPDRS), one of the important assessment scales for the disease, and balance, falls, the falls efficacy scale, history of fractures, vitamin D levels and bone mineral densitometry (BMD) values, which to the best of our knowledge has never been investigated.

2. METHODS

Thirty-eight patients who presented to the Physical and Rehabilitation Medicine Outpatient Clinic and were diagnosed with PD according to the United Kingdom Parkinson's Disease Society Brain Bank criteria were included in the study. The study included patients followed up between 2017 and 2019. A retrospective analysis was made of the patients' demographic data, using the hospital data recording system. Fall history and tests were carried out by inviting patients to the hospital. The number of falls within the previous one year and the history of fractures confirmed by the hospital were recorded. The history of fractures reported by the patients were confirmed by examining their medical records. The study protocol was approved by the University's Ethics Committee (No:02/2021-3).

2.1. Inclusion Criteria

The inclusion criteria were established as follows: ≥ 40 years of age, having idiopathic PD, being followed up by the Neurology outpatient clinic for PD for at least one year and having a stable disease, a Mini-Mental test score of >24, and a PD (Hoehn and Yahr) stage of ≤3.

2.2. Exclusion Criteria

Patients with renal and hepatic failure, patients with thyroid or parathyroid dysfunction, patients diagnosed with cancer, patients with cognitive impairment, patients receiving osteoporosis or hormone replacement therapy, and patients who had deep brain stimulation surgery for PD were excluded from the study. Patients followed up for secondary parkinsonism were not included in the study. Patients with a history of spinal degeneration (stenosis, myelopathy) that may affect falling and balance, and additional neurological disease that may affect balance were not included in the study.

The study patients' demographic data, body mass index (BMI), medications, duration of disease, stage of disease (Modified Hoehn & Yahr scale), comorbidities, assistive devices, number of falls within the previous one year, history of fractures, the falls efficacy scale scores, vitamin D levels, and bone densitometry data were recorded.

The Berg Balance Scale (BBS), developed by Berg et al. (7), is an instrument to assess balance and to determine the risk of falls in patients, and the Turkish validity and reliability study of the scale was conducted by Şahin et al. in 2008 (8). In the 14-item Berg Balance test, the ability to perform the activity in each item is rated on a 5-point scale (0–4), with 0 indicating "unable to do the task" and 4 "able to complete the task safely and independently". The test shows motor functions, disease stage, daily living capacity, postural stability and the risk of falling in PD patients, and has a total score ranging from 0 to 56. Higher scores indicate greater balance. A score of 0–20 indicates balance disorder, a score of 21–40 an acceptable balance, and a score of 41–56 a good balance. Our study used the values of the Berg Balance test that was administered to the PD patients by the same physician.

The Falls Efficacy Scale (FES) is a questionnaire developed by Tinetti et al. (9) to assess the fear of falling, and the Turkish validity study was conducted by Ulus et al. (10). The Falls Efficacy Scale asks the person how confident he/she feels when reaching into cabinets, preparing a meal, walking around the house, getting in and out of bed, answering the door or phone, getting in and out of chair, getting dressed or undressed, doing light household chores, and doing simple shopping. The person is asked to rate each item from 1 to 10 (1: very confident, 10: not confident at all), and the per item ratings are added, giving a total score ranging from 10 (low fall-related efficacy) to 100 (high fall-related efficacy). It has been proven that the validity of this scale is sensitive to changes in fear (11). Our study used the questionnaire forms of the Falls Efficacy Scale that were assessed individually for the patients by the same physician.

The Unified Parkinson's Disease Rating Scale (UPDRS) was developed by members of the UPDRS Development Committee in 1987 and was revised in 2008 due to its limitations (6). The Turkish validity and reliability study of the scale was conducted by Akbostancı et al. (12). The scale determines patients' mental state, activities of daily living, motor functions and treatment-related complications in four parts. The first three parts of the scale are scored from 0 (none/normal) to 4 (severe). The first part consists of 4 items assessing mentation, behavior and mood, the second part 13 items assessing activities of daily life, and the third part 14 items assessing motor examination. The fourth part

assesses motor complications. Higher scores indicate poorer condition. Our study used the data records determined by the same physician through examination.

The vitamin D levels of all patients were measured by the Beckman Coulter UniCel DxI 600 (Beckman Coulter, CA, USA) immunoassay analyzer using the kits of the same brand. Bone mineral density of all patients was assessed by dual energy x-ray y absorptiometry (DXA). The BMD measurements for the L1–L4, femoral neck, and total femur and the T-scores (Hologic QDR 4500 W, Hologic Inc., Bedford, Massachusetts, USA) acquired within the last one year were evaluated.

The DXA scan acquisition and analysis were performed according to the ISCD recommendation for whole body analysis (13). The vitamin D and BMD levels obtained using these methods were used.

Table 1. *Demographic characteristics of Parkinson's Disease patients*

	Total N: 30	Female N: 19	Male N: 19
Age (years)*	68 ± 7.7	67.87 ± 6.4	68.63 ± 9
Levodopa (n ,%)	28 (73)	13 (68)	15 (78)
Comorbidity (n, %)	30 (78)	15 (78)	15 (78)
Surgery (n, %)	24 (63)	6 (31)	11 (57)
Assistive device (n) Canes Forearm crutches	 7 3	 3 3	 4
Length of follow-up (months)*	56.63 ± 55	73.68 ± 69.4	39.58 ± 27.8
BMI – kg/m²**	28.73 (19.72–38.27)	30.29 (20.57–38.27)	28.73 (19.72–36.05)
H&Y stage (n) Stage 1 Stage 1.5 Stage 2 Stage 2.5 Stage 3	 1 3 16 16 2	 1 1 8 8 1	 2 8 8 1

*BMI: body mass index, H&Y: Hoehn and Yahr, *: mean ± standard deviation, **: median (min–max)*

2.3. Statistical Analysis

Statistical analysis of the study data was conducted using software PASW Statistics for Windows, Version 18.0. Chicago: SPSS Inc. The normality of the study data was tested using the Shapiro-Wilk test. The continuous variables were expressed as mean ± standard deviation or median (minimum–maximum) and categorical variables were presented as frequency and percentage. The continuous variables were compared using the Independent Samples t-test or Mann-Whitney U test, while categorical variables were compared using Pearson's Chi-square test or Fisher's Exact Chi-Square test. A p value less than.05 was considered statistically significant for all tests. Pearson's or Spearman's correlation coefficients were used to calculate the degree of association between variables. A 'p' value of <.05 was considered statistically significant. The independent samples t test was used to compare the FES between those who used levodopa and those who did not. The linear multiple regression method was used to examine the relationship between the Berg Balance and Fall Efficacy Scale and the MDS-UPDRS sub-components.

Table 2. *Comparison of the characteristics of Parkinson's Disease patients according to gender*

	Total N: 38	Female N: 19	Male N: 19	p
Berg Balance Scale*	37.45 ± 6.7	37.89 ± 5.8	37.68 ± 7.5	.280
The Falls Efficacy Scale*	58.78 ± 16.0	58.47 ± 16	57.05 ± 16.5	.881
Spine BMD T-score**	-1.2 (-3.6–4.5)	-1.5 (-3.6–0.3)	-1.1 (-3.6–4.5)	.348
Vitamin D (ng/ml)*	20.26 ± 7.3	21.49 ± 8	19.81 ± 6.2	.403
MDS-UPDRS 1**	10 (5–30)	11 (5–25)	10 (7–30)	.769
MDS-UPDRS 2*	14.92 ± 5	14.36 ± 3.6	15.47 ± 5.5	.072
MDS-UPDRS 3*	30.60 ± 12.7	30.42 ± 13.3	30.78 ± 12.5	.975
MDS-UPDRS 4**	0.50 (0.00–8)	2 (0–8)	0 (0–7)	.511
Femoral Neck BMD T-score**	-0.7 (-2.8–2.9)	-1.45 (-2.8–2.9)	-0.6 (-2.1–0.5)	**.032 (p⁺)**

p⁺<0.05, p values of other variables are insignificant
*BMD: bone densitometry, MDS-UPDRS: Movement Disorders Society-Unified Parkinson's Disease Rating Scale, *: mean± standard deviation/ Independent Samples t-test, **: median (min–max)/ Mann-Whitney U test*

3. RESULTS

Of 38 study patients with PD, the mean age was 68 ± 7.7 years and the BMI was 28.73 kg/m². The demographic data of the patients are presented in Table 1.

All patients had osteopenia with a lumbar BMD T-score of –1.2, and had vitamin D deficiency with a vitamin D level of 20.26 ng/ml. Five patients had a history of fractures. Of the five patients with a history of fracture, three were female and two were male. Two of the patients had tibial fractures, one had a hip fracture, one had a T12 compression fracture, and one had a phalangeal fracture.

The mean BBS score was 37.45 ± 6.7, suggesting that the patients had acceptable balance. The patients' stages, according to the Modified Hoehn and Yahr Stage (H&Y) that indicate the PD stage and mean MDS-UPDRS scores, are presented in Tables 1 and 2.

The comparison between female and male genders according to the analyzed parameters is shown in Table 2, and there wasn't significant gender difference in the number of falls (p=.379) and the history of fractures (p=.636). The femoral neck BMD value was significantly lower in female patients than in males (p =.032).

When the relationship between disease stages and osteoporosis parameters was examined, a significant positive correlation was found between femoral neck bone densitometry value and BMI, while there was no significant

association with other parameters; duration of disease follow-up, H&Y stage, and MDS-UPDRS scores.

The patients' BBS score was negatively correlated with H&Y stage, and UPDRS Part II, Part III, and total scores. The falls efficacy scale score was positively correlated with H&Y stage, and MDS-UPDRS Part II score, Part III score, and total scores as shown in Table 3.

The number of falls was positively correlated with H&Y stage, and MDS-UPDRS Part I, II, III, and total scores.

When the relationships among the BBS score, the FES score, and the number of falls were examined, a negative correlation was established between the BBS score and the Falls Efficacy Scale score, and a positive correlation between the Falls Efficacy Scale score and the number of falls. The FES score of the patients using levodopa was found to be significantly higher than the patients not using the drug (p=.034).

When analyzed with the linear multiple regression method, it was seen that MDS-UPDRS Part III score, one of the MDS-UPDRS sub-components, had a high effect on the Berg Balance Scale (p=.03), and the MDS-UPDRS Part II score on the FES (p=.04).

Table 3. *Assessment of characteristics of Parkinson's Disease patients and parameters related to osteoporosis and falls*

	Femoral Neck BMD (g/cm²)	Berg Balance Scale	The Falls Efficacy Scale	Number of Falls
BMI (kg/m²)	r:0,365[1] p⁺	Ø[2]	Ø[2]	Ø[1]
H&Y	Ø[1]	r:-0,614[1] p⁺⁺⁺	r:0,573[2] p ⁺⁺⁺	r:0,351[1] p⁺
MDS-UPDRS I	Ø[1]	Ø[2]	Ø[2]	r:0,426[1] p⁺
MDS-UPDRS II	Ø[2]	r:-0,636[2] p⁺⁺⁺	r:0,568[2] p⁺⁺	r:0,499[2] p⁺⁺
MDS-UPDRS III	Ø[2]	r:-0,694[1] p⁺⁺⁺	r:0,492[2] p⁺⁺	r:0,445[1] p⁺⁺
MDS-UPDRS IV	Ø[1]	Ø[2]	Ø[2]	Ø[1]
MDS-UPDRS Total	Ø[1]	r:-0,553[1] p	r:0,361[2] p⁺	r:0,486[1] p⁺⁺

p< .05, p< .01, p< .001

[1]: Pearson's correlation test, [2]: Spearman's correlation testBMD: bone mineral density, BMI: body mass index, H&Y: The Hoehn and Yahr scale, MDS-UPDRS: Movement Disorders Society-Unified Parkinson's Disease Rating Scale, r:rho, Ø:no correlation

4. DISCUSSION

The mean FES score of the study patients was 58.78 ± 16.01. Considering that the cut-off point is 24 for the Turkish population, the patients had a high level of anxiety (10). This finding suggests that patients have to continue their lives with the fear of falling and the associated problems. Several risk factors have been identified for falls in patients with PD. The examination of medications among the risk factors has revealed that the dose of levodopa and the use of benzodiazepam can be risk factors (14,15) study, there was a positive correlation between the use of levodopa and the falls efficacy scale. This finding may serve as an indicator for informing patients and taking necessary precautions regarding conditions with negative impact on life such as falls in PD patients who are on levodopa.

Studies have been conducted to investigate the risk factors for falls in patients with PD. Previous studies that compared patient characteristics between those who fell and who did not fall reported that the duration of the disease, H&Y, MDS-UPDRS Parts II, III and total might be risk factors (15,16). Our study established a positive correlation between the H&Y stage and the BBS, the FES, and the number of falls in patients who were evaluated similarly, despite the different methodology.

The MDS-UPDRS Part I was positively correlated with the number of falls, suggesting that this assessment scale used to follow-up patients means more than following up the patients' disease criteria. The study by the Parkinson Study Group reported that the UPDRS scoring was used in many studies, and the survey conducted with the experts revealed that the first part of this scale was used at a rate of 60% (17). In fact, the use of this scale may create a chance to benefit from the necessary research, precaution and physical therapy methods in order to prevent falls and fractures before the occurrence of a fall and fracture for patients with an increase in this scale during the follow-up. However, considering that this part of the scale assesses symptoms for conditions such as anxiety, depression, and psychosis, it is believed that the treatment of these conditions may prevent negative situations such as falls and fractures.

There was a negative correlation between MDS-UPDRS Parts II, III and total scores, and the BBS, and a positive correlation between the FES and the number of falls. Similar to our study, the study by Tassorelli (18) et al. established a negative correlation between the MDS-UPDRS total and BBS scores, but did not examine the association with the subscales. The authors found vitamin D levels and the Berg Balance Scale scores to be positively correlated, which was not established in our study. Our study, in turn, determined that the increased Parts II, III and total scores of the patients could be considered as a factor warning physicians about conditions such as falling, risk of falls, and balance disorder.

In our study, the correlation between all MDS-UPDRS parts, bone densitometry and vitamin D levels was examined, revealing no correlation between these parameters. Unlike our study, a study conducted in 2020 established a negative correlation between MDS-UPDRS Parts II, III, H&Y staging and vitamin D levels, femoral neck and spine total bone densitometry values (19).

The mean vitamin D level of the study patients with PD was 20.26 ng/ml, indicating that these patients had vitamin D deficiency. In our study group, the femoral neck BMD value was significantly lower in the female patients. These findings

may suggest that patients with PD are at risk for bone mass loss, with a greater risk in women. A meta-analysis that was conducted in 2013 to evaluate the risk for osteoporosis in patients with PD reported that male patients were at greater risk for osteoporosis compared to female patients in the studies reviewed (20) and that, unlike male patients, female patients had lower hip and spine BMD levels than healthy individuals. Our study also found the femoral neck BMD to be lower in female patients. Studies report lower bone mineral density values in patients with PD than age-matched healthy individuals regardless of gender (21); however, our comparison between both genders revealed that female gender might have an increased risk for hip fracture. Similar to our study, a previous study conducted in Turkey reported higher BMD in men compared to women (22). It should not be forgotten that individuals diagnosed with PD based on these results should be evaluated for vitamin D deficiency and osteoporosis as they are at risk for these diseases, besides the follow-up of neurological diseases.

It has been demonstrated that PD patients with a higher disease severity have a lower body mass index (23), while low BMI is known to be a risk factor for osteoporosis and fractures (24). In fact, a previous study established that female gender, low body weight, and low vitamin D were significantly correlated with hip and spine BMD levels in patients with PD, and were associated with bone loss (25). Our study found a positive correlation between BMI and femoral neck BMD, with lower femoral neck BMD in female patients. Weight loss in patients may be a warning sign for following up both PD and osteoporosis, and the severity of both diseases. Following up the patients for weight loss, and muscle mass loss and recommending appropriate exercise programs to the patient may be one of the important tools in preventing the loss of muscle mass.

The present study shows that patients with PD experience fear of falling, balance disorders, and vitamin D deficiency. It was established that the MDS-UPDRS, which has an important place in the follow-up of PD patients, could guide physicians in predicting conditions such as balance disorders, fear of falling and falls, beyond the severity of the disease. However, the lack of a control group is the major limitation of our study.

5. CONCLUSION

Falls, imbalance, and osteoporosis are life-threatening conditions in patients with PD. This study established that the MDS-UPDRS, used to follow-up patients, was associated with these conditions. In order to prevent these feared situations during the follow-up of patients, it is important to follow up with this scale. It is believed that this assessment method may also give an idea about these conditions in PD patients who are followed up using this scale.

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Clinical and Experimental Health Sciences

A New Perspective to Assess Patellar Surface-Trochlear Sulcus Compatibility: Narrowed Patellar Angle is Associated with Greater Trochlear Sulcus Angle

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ABSTRACT

Objective: In this study, the relationship between patella angle – trochlear sulcus angle (TSA) discrepancy was investigated. A specific cutoff value of patella angle (PA), TSA, trochlear groove depth (TGD), and medial trochlear/lateral trochlear length (MT/LT) ratio for effusion, fat-pad edema, chondromalacia, meniscal and ligament tear were investigated. By doing so, to the best of our knowledge, we bridged the gap in literature since these relationships between the above-mentioned measurements have almost never been examined.

Methods: A total of 446 patients were evaluated on magnetic resonance imaging. PA and TSA were calculated with. the highest specificity and sensitivity in predicting effusion, fat-pad edema, and patellar chondromalacia. A specific cutoff value of PA, TSA, TGD and MT/LT for effusion, fat-pad edema, chondromalacia, meniscal tear, and ligament tear were investigated.

Results: A low-level and insignificant correlation was found between PA and TSA in the negative direction. TSA value. with the highest sensitivity and specificity in predicting the presence of effusion, fat-pad, and chondromalacia was found ≤131, ≤129.6, and >125.8, respectively. Tibial tubercle-trochlear groove (TT-TG) distance. measurement was significantly less in the group with Medial Meniscus (MM) rupture. Increased TT-TG distance posed a risk for quadriceps tendinosis 1.127 times and increased medial trochlea length (MT) posed a risk for quadriceps tendinosis 1.167 times.

Conclusion: Certain cutoff values of PA and TSA may predispose risk for meniscal tear, effusion, fat-pad edema, and chondromalacia. A negative correlation was present between the patella angle and TSA.
Keywords: Knee, MRI, meniscus, chondromalacia, fat-pad edema

1. INTRODUCTION

Knee mal-alignment may be associated with the onset and progression of several pathologies such as medial and lateral meniscal (MM and LM) injuries, anterior and posterior cruciate ligament (ACL and PCL) tear, effusion, fat-pad edema, and patellar chondromalacia. The size and shape of the femur, patella and tibia may also contribute to variations in knee biomechanics (1). There are many angles and measurements that concern the knee joint. The patellar tendon length, patellar height, tibial tubercle-trochlear groove (TT-TG) distance, patella angle (PA), trochlear sulcus angle (TSA), trochlear groove depth (TGD), medial trochlea length (MT), lateral trochlea length (LT), medial trochlear/lateral trochlear length (MT/LT) ratio, lateral patellar tilt angle (LPTA), patella-patellar tendon angle (P-PTA), quadriceps patellar tendon angle (QPA), Insall-Salvati index (ISI), medial trochlear inclination (MTI), lateral trochlear inclination (LTI) are measurements that help to better understand anatomical variations (2). Researchers examining the relationship between pathologies and anatomical variations in this field have followed a certain trend and examined similar issues (2–5).

In our study, the relationship between patella angle-TSA discrepancy and a specific cutoff value of patella angle, TSA, TGD, and MT/LT for effusion, fat-pad edema, chondromalacia, meniscal tear, and ligament tear were investigated. By doing so, to the best of our knowledge, we bridged the gap in literature since these relationships between the above-mentioned measurements have almost never been examined.

2. METHODS

Five hundred patients who underwent knee Magnetic Resonance Imaging (MRI) examination were included in the study and analyzed retrospectively between 2021-2022. Fifty-four patients were excluded from the study and a total of 446 patients' knee MRIs were evaluated. We excluded

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patients with history of surgery involving the lower extremity, chemoradiotherapy, history of known malignancy, prosthesis or ligament graft, severe osteoarthritis, space-occupying mass and systemic diseases causing chronic joint dysfunction such as rheumatoid arthritis from our study. Only MRIs performed for acute or chronic mechanical knee pain were included. Ethical approval was obtained from a local (Clinical Researches Ethical committee, Protocol Code:2021-137) committee.

Patients were divided into three sub-groups: normal, degeneration, and tear according to their meniscus. The patients were also further divided into subgroups such as normal, partial tear, total tear, and mucoid degeneration according to their cruciate ligaments. Fat-pad edema was classified as superolateral (SL) Hoffa, non-SL Hoffa, prefemoral, and suprapatellar according to its location. The presence of patellar chondromalacia was evaluated. The effusion was divided into groups as suprapatellar, retropatellar, infrapatellar, and intraarticular, according to its location. The patellar tendon length, patellar height, LPTA, P-PTA, Q-PA, MTI and LTI, PA, TSA, TGD, MT, LT, MT/LT ratio, TT-TG distance, and ISI were measured for each patient.

The relationship between PA and TSA discrepancy was investigated. PA and TSA were calculated with the highest specificity and sensitivity in predicting effusion, fat-pad edema, and patellar chondromalacia. Our analysis reported that the patella angle value is ≤120.9 with the highest sensitivity (48.92%) and specificity (75.86%) in predicting the presence of effusion with the highest and most significant area under the curve. TSA was evaluated between the groups with a patella angle value of ≤120.9 and >120.9, and it was evaluated whether there was a difference between the measurements.

Patella angle values with the highest sensitivity and specificity were calculated to predict the presence of MM injury, LM injury, ACL, and PCL tear. Predictive values of MT/LT ratio for MM and LM tear were calculated. The cutoff ISI value for fat-pad edema was evaluated. Furthermore, we investigated whether there were significant PPTA and QPA values that could predict fat-pad edema. Additionally, the change in frequency of pathology and measurements with increasing TT–TG distance values were examined. Finally, this research looked at how the frequency of pathology and measurements change with increasing MTI and LTI distance. The change of frequency of the pathologies was evaluated as TSA, TGD, MT, and LT increased or decreased.

2.1. Measurements

ISI, P-PTA, QPA, patellar tendon length, and patellar height were measured in the midsagittal plane where the quadriceps tendon, patellar tendon, and upper and lower ends of the patella are best seen (6). Patella height is a measurement from the distal pole to the proximal pole of the patella. ISI is the ratio of the patellar tendon length to the maximum length of the patella (7). PPTA is the angle between the line connecting the upper-lower pole of the patella and the tuberositas tibia. QPA is the angle between the quadriceps tendon and a line from the upper pole of the patella (5).

The posterior condylar line is selected as a reference line. LPTA is the angle between the parallel line to the lateral patellar facet and the lines drawn along the posterior femoral condyles (8). MTI angle is defined as the intersection between the medial trochlear facet and a tangential line drawn through the posterior femoral condyle. LTI angle is the intersection between the lateral trochlear facet and a tangential line drawn through the posterior femoral condyle (9). The angle between the lines which are parallel to the medial and lateral patellar facets is PA. TSA is the intersection of two lines parallel to the medial and lateral trochlear facets. TGD is calculated by subtracting the distance between the deepest point of the TG and the line parallel to the surfaces of the posterior femoral condyles from the mean of the greatest anteroposterior (AP) distance of the medial and lateral femoral condyles (5). TT-TG is measured as a mediolateral distance from the apex of the tibial tuberosity to the base of the trochlear groove (Figure 1).

MM and LM are evaluated on PD images according to abnormal high signal intensity. Meniscal degeneration is accepted as focal or linear areas of hyperintensity, without an extension to the articular surface. Meniscal tear is accepted as abnormal hyperintensity that extends to the superior or inferior articular surface (10). A thickened and irregular but intact ligament is evaluated as mucoid degeneration. A complete fiber discontinuity and partial tear are evaluated as hyperintensity within the ligaments.

2.2. MRI image protocol

MRI was undertaken using a 1.5-T MRI scanner (Magnetom Aera; Siemens Healthcare, Germany) with a limb matrix knee coil (a Tim coil). The knee MRI protocol was the fat-suppressed PD sequence in coronal, axial, and sagittal planes, and the T1-weighted and T2-weighted sequence in the coronal plane. The coronal plane exam is performed parallel to the midline of the femur and tibia. Each scan examined the knee joint from lateral condyle up to mediale condyle. Phase directions in the axial scans were head to feet.

2.3. Statistical analysis

Statistical analysis of the data was made by using IBM Statistical Package of Social Science (IBM SPSS V26) and MedCalc (Version 19.3.1) package programmes at 95% confidence level. The Shapiro-Wilk normality test was executed to analyze the distribution of data, and Levene's test was used to analyze group homogeneity. Continuous variables are analysed as mean, standard deviation and median values, and categorical variables are presented as number and percentage. T-test and Mann-Whitney U test were used to analyse the effects of the following variables age, sex, ISI, MTI, QPA, and other measurement variables. The relationship between chondromalacia, presence of

effusion, and fat-pad edema groups was investigated by chi-square test. The ANOVA test was used to compare QPA and LPTA between the ACL, PCL, MM injury, and LM injury groups. The relationship between ISI and TT-TG distance and other variables was analyzed by Spearman correlation. Patellar angle and TGD with the highest specificity and sensitivity in predicting effusion, fat-pad edema, and chondromalacia were calculated using ROC analysis, and the Youden index. Logistic Regression Analysis was applied with the variables found to be significant. A value of p less than.05 is considered statistically significant.

Table 1. *Number and percentage of pathological changes detected in patients*

	n	%
Medial Meniscus		
Normal	124	27,8
Degeneration	154	34,5
Rupture	168	37,7
Lateral Meniscus		
Normal	356	79,8
Degeneration	53	11,9
Rupture	37	8,3
ACL Degeneration Grade		
Normal	338	75,8
Partial Rupture	64	14,3
Total Rupture	28	6,3
Mucoid Degeneration	16	3,6
PCL Degeneration Grade		
Normal	440	98,7
Partial Rupture	2	0,4
Mucoid Degeneration	4	0,9

MM: Medial meniscus, LM: lateral meniscus, ACL: anterior cruciate ligament, PCL: posterior cruciate ligament. n: number of the patients

3. RESULTS

A total of 446 patients were included in the study, 258 of them were women. The mean age was 43.50 ± 10.21 years. MM tear was detected in 168 (37.7%) patients and LM tear was present in 37 (8%) patients. There were total tears in the ACL in 28 (6.3%) patients (Table 1). Suprapatellar fat-pad edema was observed in 90 (20.2%) of the patients, and patellar chondromalacia was observed in 340 (76.2%) patients. Forty-two patients had quadriceps tendinosis (Figure 2). The mean values of the measurements are shown in table 2.

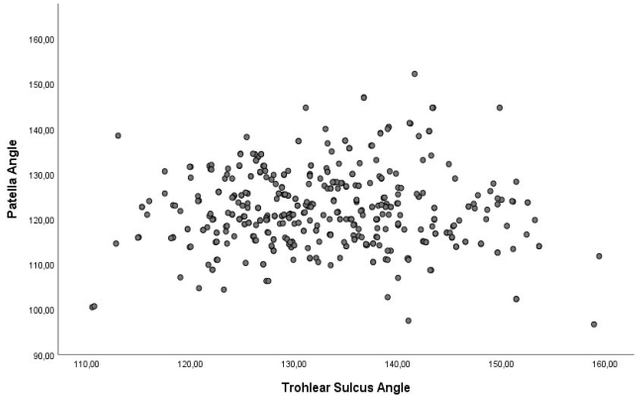
A low-level and insignificant correlation was found between patella angle and TSA in the negative direction (R: – 0.023) (p=.635) (Graphic 1). Patella angle values with the highest sensitivity and specificity in predicting the presence of effusion, fat-pad, and chondromalacia were found to be ≤120.9,>119.8, and <122.9, respectively. The patella angle values ≤117.8 and ≤125.5 show the highest sensitivity and specificity in predicting the presence of MM and LM tears, respectively.

Table 2. *Measurements and mean values of the patients included in the study*

Variables	Mean ± SD	Median
Patellar tendon lenght (mm)	41,72 ± 5,96	41,4
Patellar height (mm)	41,30 ± 4,50	41,0
ISI	1,02 ± 0,17	1,01
TT-TG distance	9,89 ± 4,09	10,3
Patella angle	122,26 ± 8,37	121,3
TSA	132,93 ± 8,77	132,1
TGD	7,06 ± 1,68	7,0
MT	12,92 ± 2,73	13,0
LT	23,80 ± 3,01	23,7
MT/LT	0,55 ± 0,13	0,5
LPTA	12,13 ± 6,00	11,6
PPTA	141,36 ± 6,87	141,8
QPA	132,54 ± 10,66	131,9
MTI	25,90 ± 6,04	26,0
LTI	24,91 ± 4,61	24,7

Mann-Whitney U is used. ISI: Insall-Salvati Index, TT-TG Distance: tibial tubercle-trochlear groove distance, TSA: trochlear sulcus angle, TGD: trochlear groove depth, MT, and LT: medial and lateral trochlea length, MT/LT ratio, LPTA: lateral patellar tilt angle, QPA: quadriceps-patellar tendon angle, MTI and LTI: medial and lateral trochlear inclination. A p value less than.05 is considered as significant.

TSA value with the highest sensitivity and specificity in predicting the presence of effusion, fat-pad, and chondromalacia was found ≤131, ≤129.6, and >125.8, respectively. TSA values>133.6 and>138.9 show the highest sensitivity and specificity in predicting the presence of MM and LM tears, respectively. The patellar angle of ≤125.5 was predisposed risk 2.85 times (CI 1.539-5.277) higher for the presence of meniscal tear (p=.001), and a TSA of >138.9 was pose a significant risk of 2.43 times presence of meniscal tear (CI 1.427-4.012) (p=.001).



Graphic 1. *Relationship between Patella Angle and Trochlear Sulcus Angle*

It was observed that the TT-TG value was significantly lower in the group with MM tear (p=.001). There was no significant difference between TT-TG measurements in groups with ACL and PCL tear (p=.080). The MT/LT values

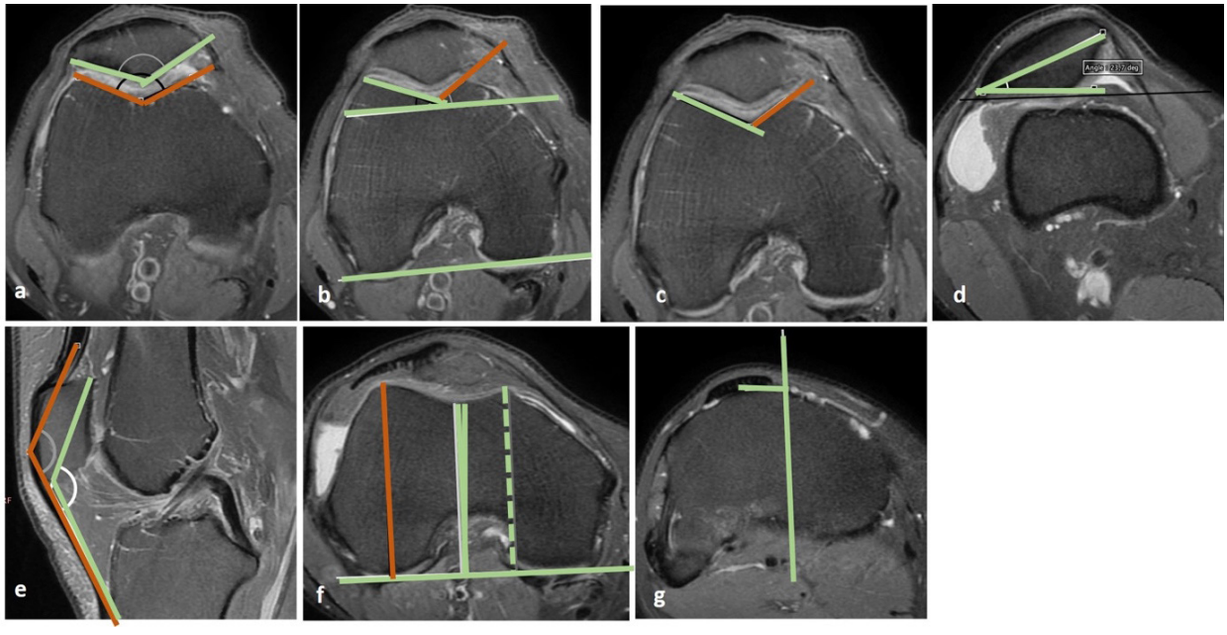


Figure 1. *Measurement methods . On axial proton density images, a.) patella angle is measured as the crossing angle between the lines parallel to the medial and lateral patellar facets (green angle). TSA is measured as the angle of two lines parallel to the medial and lateral trochlear facets (orange angle). b.) MTI is obtained as the angle between the lateral trochlear facet and the transcondylar axis (green angle). LTI is measured as the angle between the lateral trochlear facet and the transcondylar axis (orange angle). c.) medial trochlea length is measured as 26,9 mm (green line) and lateral trochlea length is measured as 15,6 mm (orange line). d.) LPTA is measured as a line parallel to the lateral patellar facet and a line drawn across the posterior femoral condyles (green line). e.) QPA is measured as the angle between the quadriceps tendon and a line drawn from the patella upper pole in the midsagittal plane (green line). PPTA is measured as the angle the between the line connecting the upper-lower pole of the patella and the tuberosities tibia (orange line). f.) TGD is accepted as the width between the most anterior parts of the femoral trochlear facets and the deepest part of the trochlear groove. g.) TT-TG is measured as a distance from the apex of the tibial tuberosity to the base of the trochlear groove.*

with the highest sensitivity and specificity in predicting the presence of MM tear were found to be ≤0.496 and ≤0.656 (p<.001 for both). While MT and LT were significantly higher in the group with fat-pad edema (p=.043, p<.001), no difference was found for TSA and TGD measurements. There was no significant difference in terms of TSA, TGD, MT, and LT measurements in the patellar chondromalacia group compared to the non-patellar chondromalacia group. No significant difference was observed between the groups with and without effusion in terms of TSA, TGD, MT and LT measurements.

In the group with quadriceps tendinosis, TT-TG distance, and MTI were significantly higher (p=.004, p<.001). In the group with quadriceps tendinosis, patellar tendon length, MT and LT were found to be significantly lower (p=.004, p=.012, p=.01, p=.01). No significant change was detected in terms of other measurements. The frequency of fat-pad edema was found to be significantly higher in the group with quadriceps tendinosis (p=.012). There was no difference in the frequency of effusion and patellar chondromalacia in the group with quadriceps tendinosis (p=.287 and p=.276). Increased TT-TG distance has posed a risk for quadriceps tendinosis 1.127 times (CI 1.026-1.237), increased MT has posed a risk for quadriceps tendinosis 1.167 times (CI 1.020-1.335), increased LT has posed a risk for quadriceps tendinosis 1.224 times (CI 1.074-1) (p=.013, p=.025, p=.002).

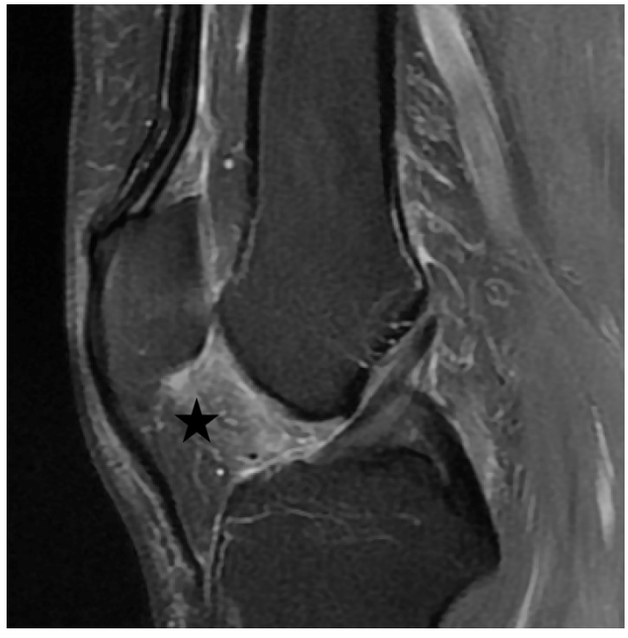


Figure 2. *Hoffa's fat-pad edema. On mid-sagittal proton density images of a 46-year-old male, the high signal in Hoffa's fat-pad (star) and prefemoral fat-pad is seen.*

4. DISCUSSION

This retrospective study examined the relationship between TSA values with the highest sensitivity and specificity in predicting the presence of effusion, fat-pad edema, and chondromalacia, and found that TSA values are associated with knee instability and injury.

Hypoplastic lateral femoral condyles accompanied by a shallow or flattened shaped groove in the trochlea favor trochlear dysplasia (11,12). Yang et al. (13) stated that in the presence of shallow trochlea, patellofemoral joint balance is impaired. A shallow trochlea decreases the stability of the patella from the lateral aspect and causes instability, dislocation, and cartilage loss. Kalichman et al. (14) found that increased sulcus angle creates excessive stress on the medial and lateral condyles. Damgacı et al. (5) suggested that patellar cartilage loss increases in knees with larger TSA and P-PTA and LPTA also decrease in patients with larger TSA. Paiva et al. (15) performed a systematic review through the literature. And they assessed known radiological measurements for trochlear dysplasia. LPTA, TT–TG, and TGD were suggested as useful measurements in evaluating trochlear dysplasia.

Many studies aiming to reveal trochlear dysplasia have determined measurement and angle values. A consensus is still lacking, however, as to which of these measurements is valuable enough to be used in practice. LTI, MTI, TSA and MT\LT ratios are the most commonly used measurements in these studies (15). It has been reported as the most preferred and reliable measurement is LTI. It has been reported that values of 11° and above for LTI can predict trochlear dysplasia with an accuracy of 93% and a specificity of 87% (16). Joseph et al. (17) measured LTI at two different images captured one from the most proximal aspect of trochlear cartilage and the second from the level of posterior condyles. They reported that 2-image LTI has higher reliability compared to single-image LTI and Dejour classification. They also concluded that 2-image LTI or classical LTI measurement demonstrated trochlear dysplasia perfectly with a 91% sensitivity. According to Keser et al. (18) recommended that LTI is above 11 degrees in a sizable proportion of patients with anterior knee pain but for whom no cause could be found, and that it would be beneficial to measure LTI in patients with knee pain of unknown origin. Carrillon et al. (16) suggested that the cutoff value of 11° for LTI was studied in studies that included only a limited number of populations, and therefore should be evaluated in a larger population, especially for patients with patellar instability.

TT-TG distance may be affected by the flexion angle and imaging modality. It has been stated that the intra – and inter-reliability for TT–TG distance is low (15). TT-TG distance greater than 15 mm is defined as pathological in a recent study (19). Imhoff et al. (20) reported that higher grades of trochlear dysplasia demonstrated higher values of TT–TG distance. In the group with quadriceps tendinosis, while TT-TG distance and MTI were significantly higher, the patellar tendon length, MT, and LT measurements were found to be significantly lower in the group with quadriceps tendinosis.

Among the measurements investigating trochlear dysplasia, there are these classically known measurements, and there is no diversity in the publications where different angles are evaluated. Biedert et al. (21) developed a measurement called the patella–trochlear index by dividing the lengths of the patella and trochlea to look at their compatibility, but there are quite a few publications examining the relationship between patella angle and TSA. In our study, the relationship between patella angle-TSA discrepancy was investigated. The compatibility of patella angle and TSA and its change were investigated. In our study, a low level and insignificant correlation were found between patella angle and TSA in the negative direction (R: – 0.023).

The different morphological shapes of the patella will result in the sum of the different kinetics and vectors forces in the patellofemoral joint. Changes in the shape of the patella or trochlear sulcus may restrict patella motion or full extension. The restriction of full extension of the patella may lead to soft tissue being forced to dominate patellofemoral kinematics. On the other hand, increased LTI causes increased patellar flexion and causes an extra load on the posterior part of the patella (22). Jimenez et al. (23) conducted a study investigating whether there is a significant relationship between the shape of the patella and the trochlea. In their study, they found that patellar morphology varied greatly among patients. And they stated that there was a weak correlation between patellar morphology and trochlear dysplasia.

The number of studies investigating how the shape of the patella affects the vectorial forces in the knee joint or the degeneration of ACL tear, PCL tear, MM tear, LM tear, and chondromalacia is extremely few. In addition, there is no study investigating how the importance and normal values of the patella angle change with the shape of the trochlea and how often it is seen with trochlear dysplasia. Damgacı et al. (5) measured normal patella angle values in their patients as 126.5±8.4 and stated that there were significant differences in patella angle in patients with severe chondromalacia. However, they did not examine the associations between patella angle and other variables that suggest trochlear dysplasia. Endo et al. (23) measured patella angle at three distinct levels (the superior, middle, and inferior portions of the patella) known as interfacet angle in a morphometric study. And in this study, no significant relationship was found between patella angle and chondromalacia. In our study, the patella angle value was found to be ≤120.9,>119.8, and <122.9 with the highest sensitivity and specificity in predicting the presence of effusion, fat-pad, and chondromalacia, respectively. The patella angle value with the highest sensitivity and specificity in predicting the presence of MM and LM degeneration-tear was found to be ≤117.8 and ≤125.5, respectively.

We acknowledge several limitations of the current study. First, the retrospective design might have introduced bias to our results. Second, the sample of the study, which included

only patients who underwent knee MRI, is nor representative of the general population. Additionally, the exclusion criteria used may also have limited the sample size and population diversity. Thus, we acknowledge limited genaralizability of our results. Furthermore, the study only examined the association between certain measurements and knee pathologies and did not investigate other factors that may contribute to the development of knee pathologies. We believe that examining other factors that may contribute to the development of knee pathologies in patients with severe gonarthrosis and professional athletes may have beneficial results for knee trauma and rehabilitation and contribute to the literature. Finally, the study did not assess the inter-observer reliability of the measurements taken, which may affect the validity of the results.

5. CONCLUSION

We can conclude that certain cut-off values of patella angle and TSA may pose a risk for meniscal tear, effusion, fat pad oedema and chondromalacia. Although not significant, there was a negative correlation between patella angle and TSA. Patients with narrowed patella angle and shallow trochlear sulcus should be carefully evaluated for meniscal tear. Specific mal-alignancies may be the cause of knee pain and injury.

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Author Contributions:
Research idea: GYO, FZA
Design of the study: GYO, FZA
Acquisition of data for the study: GYO, FZA
Analysis of data for the study: GYO
Interpretation of data for the study: FZA
Drafting the manuscript: FZA
Revising it critically for important intellectual content: GYO, FZA
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Clinical and Experimental Health Sciences

A Preliminary Study on the Effects of 4-Week Training Program with Interactive Floor Support on Plantar Pressure Distribution in Sedentary Individuals

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ABSTRACT

Objective: Our purpose was to investigate on the effects of foot exercises by using interactive floor support as a modality of rehabilitation technology on plantar pressure distribution in sedentary individuals.

Methods: Participants who were aged between 18-35, who agreed to participate voluntarily and had no pathology developed in the lower extremities in the past 6 months were included in this study. In order to determine the plantar pressure distribution change of all participants, the first and last evaluation was measured with Emed® Pedobarography. Balance and proprioception exercises on both lower extremities for 12 sessions (for four weeks, three sessions per week, lasts 15 minutes each) were performed with an interactive floor device.

Results: A total of 15 healthy and sedentary subjects (12 female, 3 male, with a mean of 20,27±0,961 years and 21,31 ± 3,027 kg/m^2 BMI) completed the four-week training protocol. Maximum force of total and hindfoot in both feet significantly decreased after 4-week training ($p<.05$). In addition, changes of total peak pressure and total contact area values after 4-week training program in both feet were not significant ($p>.05$).

Conclusion: Based on our results, the present study revealed that the 4-week training programme with interactive floor support for ankle joint could decrease maximum force of total and hindfoot on the bottom of the dominant and non-dominant side feet in healthy and sedentary individuals. Future investigation should be conducted to clarify the effects of long-term training programs with interactive floor support on plantar pressure distribution in patients with foot deformities.

Keywords: Sedentary lifestyle, technology, exercise, lower extremity

1. INTRODUCTION

Physical inactivity may be characterized by a lifestyle with reduced physical activity, which may lead to a poor posture, posture disorders and decreased energy expenditure. Physical inactivity may also lead to many anatomical and physiological disorders related to impaired health outcomes, such as development of anxiety and depression, obesity, gaining weight, some cardiovascular diseases, and other problems as well (1). In addition of them, because of sedentary lifestyle, many musculoskeletal disorders may occur in all parts of the body. One of the most common musculoskeletal problems is foot posture abnormalities (2).

Feet are the organs that carry body weight and allow walking. One of the main components of the feet is the arch that provides body weight support and plantar pressure distribution (3). Measurement of plantar pressure distribution is clinically useful and important because it can identify anatomical foot deformities, guide the diagnosis and treatment of gait disorders (4). Many researches have investigated that plantar pressure on the bottom of the foot has an important biomechanical meaning (5-8). In addition, plantar pressure distribution can also help physiotherapists interpret the foot deformities and evaluate gait (3).

Research on foot pressure distribution have focused on specific pathologies and deformities (4). In one of the study investigating importance of foot pressure distribution, it has been reported that excessive plantar pressures may be correlated with development of ulcers in neuropathic feet (9). In addition, it has been shown that sedentary lifestyle is correlated with higher plantar pressures under 2–5 toes of the foot (10). It has also been reported that there is a correlation between high plantar pressures and low level of physical activity in a study investigating relationship between plantar pressures, physical activity and sedentariness among preschool children by Mickle et

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al (11). Based on the foregoing assessments, examination of the foot health of sedentary people may be very important (2).

Therapeutic exercises such as foot-muscle training may reduce over pronation and help re-structuring the foot (3). However, there are limited studies investigating the effects of foot-muscle training on plantar pressure distribution, especially in sedentary people. In a study by Kyung Lee, closed kinetic chain exercises significantly changed the contact area and peak contact force of hindfoot in patients with stroke (12). However, it would be better to reveal the effects of foot-muscle training on plantar pressure distribution to understand the underlying mechanism.

In addition to enhancing patient monitoring and adherence, the use of technology by healthcare professionals also help to ease pressure on healthcare services (13). As treatment request is anticipated to extend in future, rehabilitation technology that enables patients to complete training with less therapist involvement will play a crucial role in this (14).

Up to our knowledge, no previous study has investigated on the effects of foot-ankle exercise alone by using rehabilitation technology on plantar pressure distribution in sedentary individuals. Therefore, we investigated whether training programme for ankle joint led to changes in plantar pressure distribution after four-week training program with interactive floor support in sedentary individuals. We hypothesized that foot-ankle exercises using with interactive floor support as rehabilitation technology would change plantar pressure distribution by helping re-shaping the foot after four weeks in sedentary individuals.

2. METHODS

2.1. Ethics

This study was approved by the Non-Interventional Ethics Committee of Marmara University Faculty of Health Sciences (Protocol Number: 52, Date: 28.03.2019). All subjects were informed about the study prior to their participation and written informed consent was obtained from each subject.

2.2. Participants

This study was performed in the Faculty of Health Sciences, Marmara University, İstanbul, Türkiye. Students in the Department of Physiotherapy and Rehabilitation who were aged between 19-23, who were physically sedentary individuals according to their own statements, and who agreed to participate voluntarily were included in this study. Sociodemographic attributes [age, gender, height, body weight and body mass index (BMI)] were collected from participants at the beginning of the study (Table 1).

Participants who were aged between 18-25, who agreed to participate voluntarily and had no pathology developed in the lower extremities in the past 6 months were included in this study. Participants who had a congenital or chronic lower limb pathology and/or participants with acute/chronic problems affecting their visual perception or balance were excluded. The subjects were excluded if they had any orthopaedic problems, such as musculoskeletal pain, limited range of motion, foot or fracture history, any type of injury or surgery in the lower extremity, foot or ankle deformities including hallux valgus, pes planus or cavus, etc. The subjects who participated in sportive and physical activities regularly were also excluded from the study.

2.3. Study Design

In order to determine the plantar pressure distribution change of all participants, the first and last evaluation was measured with Emed® pedobarography device at the beginning and end of the 4-week training program. The balance and proprioception exercises determined by the research team were performed with using an interactive floor device applied as rehabilitation technology.

2.4. Plantar Pressure Assessment

In our study, in order to determine to change of plantar pressure distribution, Novel Emed® pedobarography device was used (Figure 1). This device has been shown to be a reliable and valid method to measure plantar pressure (15). The patients whose plantar pressure were to be assessed walked on the platform where the device was located, and measurements were made five times for each foot. The average of the measurements were determined as the plantar pressure distribution. Most Emed® systems are used in 4 sensor / cm2 high sensor resolution mode and at a frame rate of 50 or 100 Hz (16). With the user-defined narrowed sensor area, this platform area can be scanned at frequencies greater than 800 Hz. Emed® motion analysis provides frame-by-frame input and output signals for digital video and EMG synchronization. All Emed® platforms carry the CE mark for the calibrated Class 1 medical device with a temperature range 10-40°C and a calibrated pressure range from 10 kPa to 1.27 MPa (www.novel.de/products/emed/ Accession Date: 25.08.2021).

Figure 1. *Assessment of plantar pressure distribution using Novel Emed® pedobarography device*

2.5. Training Program

In our study, participants' training program were performed on interactive floor projection device (TTL Technology, Elsa Med. Company, TR). The device which is used in this study contains 3000 Ansilumen LED projection. Its resolution is 1024x768 pixels and has IR/3D sensor system, Windows 7 pro 64 bit OS, Intel I5 Processor, 4 GB DDR3 1333 RAM and 300 Mbps Wireless 802.11b/g/n. The device is placed on the ceiling three meters above the ground. The size of the projection area's is approx. 3 square meters (the length is 2 and the width is 1.5 meters). The participants did balance and proprioception exercises on both dominant and non-dominant lower extremities for 12 sessions (for four weeks, three sessions per week, lasts 15 minutes for each lower extremity) by using games such as Balloon Popping, Ball Collecting, and Shape Crushing on the device (Table 1).

Table 1*. Training program on interactive floor projection device*

Training Game	Training Sessions					
	Week 1					
	First Session		Second Session		Third Session	
	Left Foot	Right Foot	Left Foot	Right Foot	Left Foot	Right Foot
Balloon Popping	3 min.	3 min.	4 min.	4 min.	5 min.	5 min.
Ball Collecting	3 min.	3 min.	4 min.	4 min.	5 min.	5 min.
Shape Crushing	3 min.	3 min.	4 min.	4 min.	5 min.	5 min.
	Week 2, 3 & 4					
	First Session		Second Session		Third Session	
	Left Foot	Right Foot	Left Foot	Right Foot	Left Foot	Right Foot
Balloon Popping	5 min.	5 min.	5 min.	5 min.	5 min.	5 min.
Ball Collecting	5 min.	5 min.	5 min.	5 min.	5 min.	5 min.
Shape Crushing	5 min.	5 min.	5 min.	5 min.	5 min.	5 min.

Balloon Popping

The aim of the balloon popping game is for the participant to explode the virtual balloons which are moving from one way to the opposite by quickly moving both feet in the dorsi and plantar flexion directions. The participant who moves his or her ankle during the popping process also have to move quickly within the interactive floor area, and try to pop as many balloons as possible (Figure 2).

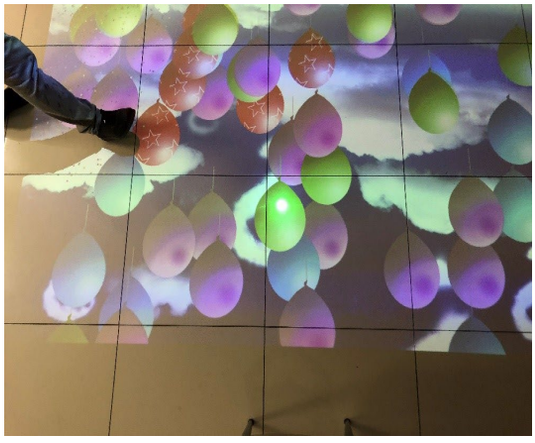

Figure 2. *Training games (balloon popping) on interactive floor projection device*

Ball Collecting

The ball collecting game is played with a maximum of 4 participants. Participants choose a dragon for themselves before the game starts. With the starting signal, they try to collect as many balls as possible by quickly moving their feet in the dorsi and plantar flexion direction. The game ends with a total of 200 balls being shared among the participants (Figure 3).

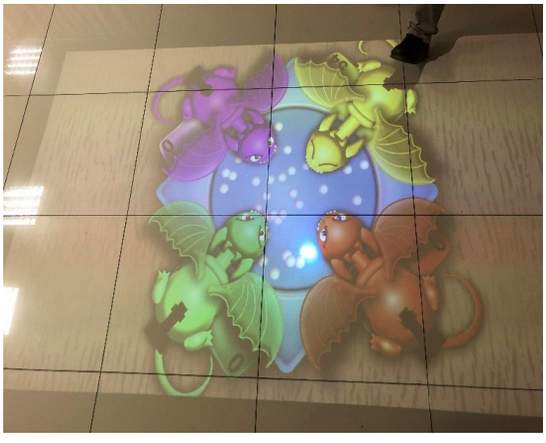

Figure 3. *Training games (ball collecting) on interactive floor projection device*

Shape Crushing

The shape crushing game can be played with a single participant at one time. The participant stands in the

middle of the interactive floor area and tries to crush the virus-shaped objects coming from the environment. The participant, whose one lower extremity is in a stable position, reaches out with the other lower extremity in the direction of the viruses, tries to crush the viruses by moving his foot in the dorsi and plantar flexion direction (Figure 4).

Figure 4. *Training games (shape crushing) on interactive floor projection device*

2.6. Statistical Analysis

In the classification of the data obtained in the study, qualitative and quantitative statistical methods were evaluated with the SPSS 11.5 statistical program in the 95% confidence interval, and the significance were evaluated at the level of p<.05.

Demographic characteristics are presented as frequencies, means and standard deviations (SD). As the distribution of data did not meet the parametric test criteria (data distribution histograms, examining the mean and standard deviation values and Kolmogorov-Smirnov test), the statistical analysis was performed by using non-parametric tests. The comparison of the values of the plantar foot distribution was analysed by using the Wilcoxon Signed Rank Test.

There was no calculated power analysis in the study. The sample size was determined by convenience sampling method in order to availability of participants who intends to participate this study voluntarily (17).

3. RESULTS

3.1. Participants

25 subjects were assessed for suitability and 5 subjects were excluded. Four subjects who were sportive and physically active individuals and one subject stated that he had a fracture in the foot and ankle complex in childhood. The included 20 subjects started for training. During the training period; five subjects were lost to follow-up since they did not join the sessions regularly (Figure 5). A total of 15 healthy and sedentary subjects (12 female, 3 male) completed the four-week training protocol in Marmara University, between October 2019 – January 2020. The dominant extremities of all the subjects in the study were right. The age of participants ranged between 19 and 23 years with a mean of 20,27±0,961 years. Demographic data and general characteristics for the subjects are displayed in Table 2.

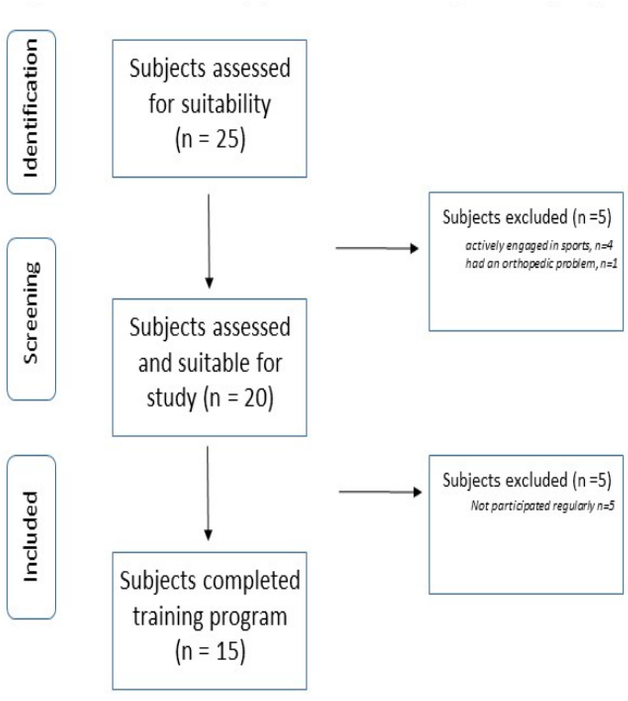

Table 2*. Demographic characteristics of participants*

	n	Mean ± SD	Minimum – Maximum
Gender			
Male	3 (20%)	-	
Female	12 (80%)		
Age (years)	15	20.27 ± 0.961	19 – 23
Height (cm)	15	171.93 ± 9.004	156 – 188
Body Weight (kg)	15	63.27 ± 11.26	44 – 86
BMI (kg/m^2)	15	21.31 ± 3.027	17.26 – 30.11

Values are presented as mean ± SD

n: number, SD: Standard Deviation, BMI: Body Mass Index

3.2. Plantar Pressure Distributions

Table 3, 4 and 5 indicate the pre – and post-training plantar pressure distributions for each foot. The mean values of maximum force for pre – and post-training were shown in Table 3. Maximum force of total and hindfoot in both feet significantly decreased after 4-week training (p<.05). In addition, changes of maximum force of forefoot in the non-dominant leg and changes of maximum force of toes in the dominant leg were significant (p<.05).

***Table 3**. Comparison of maximum force variables at pre – and post-training*

Foot Region			DL		NDL	
Maximum Force	Total	Pre-Training	1570.31±394.86	-%31.7	1612.91±446.56	-%33.1
		Post-Training	1072.57±361.88		1079.43±369.97	
		z	-2.442		-2.101	
		p	**.015***		**.036***	
	Hindfoot	Pre-Training	495.05±108.98	-%8.5	517.66±151.52	-%12.0
		Post-Training	452.75±82.85		455.35±96.56	
		z	-1.988		-2.329	
		p	**.047***		**.020***	
	Midfoot	Pre-Training	202.83±157.16	-%14.9	192.24±120.75	-%10.4
		Post-Training	172.59±71.11		172.19±91.76	
		z	-0.398		-1.250	
		p	0.691		0.211	
	Forefoot	Pre-Training	648.63±148.22	-%4.6	709.66±220.60	-%9.9
		Post-Training	618.90±120.95		639.17±120.33	
		z	-1.306		-1.988	
		p	.191		**.047***	
	Toes	Pre-Training	222.71±100.19	-%19.3	191.37±91.47	-%10,7
		Post-Training	179.68±84.86		170.93±97.59	
		z	-2.442		-1.477	
		p	**.015***		.140	

DL: Dominant Leg, NDL: Non-Dominant Leg

*Wilcoxon-signed rank test, *p<.05*

Values are presented as mean ± SD

The percentage values (%) presents amount of change in the parameters.

***Table 4.** Comparison of peak pressure variables at pre – and post-training*

Foot Region			DL		NDL	
Peak Pressure	Total	Pre-Training	476.33±99.32	+%1.3	508.00±162.33	-%7.3
		Post-Training	482.67±151.80		471.00±96.82	
		z	-0.114		-0.910	
		p	.910		.363	
	Hindfoot	Pre-Training	298.33±82.99	-%7.0	291.33±71.05	+%1.0
		Post-Training	277.33±53.21		294.33±76.45	
		z	-0.228		-0.346	
		p	.819		.729	
	Midfoot	Pre-Training	152.67±123.99	-%12.4	138.00±66.73	+%9.4
		Post-Training	133.67±39.93		151.00±80.36	
		z	-1.323		-1.894	
		p	.186		.058	
	Forefoot	Pre-Training	380.00±122.37	+%5.3	428.33±173.03	-%5.0
		Post-Training	400.00±146.61		407.00±128.22	
		z	-0.767		-0.440	
		p	.443		.660	
	Toes	Pre-Training	376.67±152.14	-%7.3	335.33±177.98	-%13.9
		Post-Training	349.00±183.77		288.67±145.69	
		z	-0.824		-2.345	
		p	.410		**.019***	

DL: Dominant Leg, NDL: Non-Dominant Leg

*Wilcoxon-signed rank test, *p<.05*

Values are presented as mean ± SD

The percentage values (%) presents amount of change in the parameters.

The mean values of peak pressure for pre – and post-training were shown in Table 4. Changes of peak pressure of toes in the non-dominant leg were significant (p<.05).

The mean values of contact area for pre – and post-training were shown in Table 5. Contact area of hindfoot in both feet significantly decreased after 4-week training (p<.05). Changes of contact area of toes in the dominant leg were significant (p<.05).

***Table 5.** Comparison of contact area variables at pre – and post-training*

Foot Region			DL		NDL	
Contact Area	Total	Pre-Training	155.76±37.21	-%5.1	154.79±42.88	-%4.9
		Post-Training	147.73±19.77		147.18±19.25	
		z	-1.477		-0.256	
		p	.140		.798	
	Hindfoot	Pre-Training	38.57±8.71	-%6.5	39.60±14.20	-%10.3
		Post-Training	36.05±5.29		35.52±5.67	
		z	-2.385		-1.988	
		p	**.017***		**.047***	
	Midfoot	Pre-Training	33.16±12.65	-%5.4	32.10±13.60	-%5.6
		Post-Training	31.36±6.14		30.31±6.59	
		z	-0.511		-0.284	
		p	.609		.776	
	Forefoot	Pre-Training	54.84±8.01	+%0.1	55.73±9.75	-%1.7
		Post-Training	54.87±7.37		54.80±7.47	
		z	-0.398		-0.454	
		p	.691		.650	
	Toes	Pre-Training	28.58±11.70	-%12.6	26.57±8.43	-%3.0
		Post-Training	24.97±5.98		25.77±6.02	
		z	-2.385		-0.398	
		p	**.017***		.691	

DL: Dominant Leg, NDL: Non-Dominant Leg

*Wilcoxon-signed rank test, *p<.05*

Values are presented as mean ± SD

The percentage values (%) presents amount of change in the parameters.

4. DISCUSSION

This study conducted to investigate the effects of 4-week training programme for ankle joint via interactive floor projection device applied as rehabilitation technology on plantar pressure distributions in sedentary and healthy individuals. One of the main findings of our study is that maximum force of total and hindfoot in both feet significantly decreased after foot exercises training. According to the results, changes about maximum force between pre and post assessment of dominant leg's toe and non-dominant leg's forefoot area were significant. We think that the 4-week training program for ankle joint decreased the foot pressure distribution by restructuring the foot. In contrast to the present study, Unver et al. reported that maximum plantar force was significantly increased in midfoot after 6-week short foot exercises while maximum force in fore and hindfoot remained similar. We believe that this difference was basically caused because of the subjects who participated in studies. It is known that pes planus leads to higher plantar pressure in medial midfoot (18). In addition, there are some

else significant differences including measuring of plantar pressure region, training protocol, etc between the present study and other one. From this point, we may report from our results that foot exercises via interactive floor projection support in sedentary individuals may help re-shaping the foot and re-distributing the plantar pressure on dominant foot as well as non-dominant foot. Thus, this study setting has shown that the training program which includes balance and proprioception exercises by using technology may lead a better plantar pressure distribution especially on maximum plantar force and even in a population with young and sedentary adults, whose have no deformities on lower limbs.

Another main finding of our study is that changes of total peak pressure and total contact area values after 4-week training program in both feet were not significant. Even more importantly, although maximum plantar force of total in both feet significantly decreased after 4 week, total peak pressure and total contact area were not significantly changed. We could deduce that the 4-week training program by using interactive floor projection support solved the lack of optimal foot pressure distribution which is one of the negative effects caused by physical inactivity by re-shaping the feet. Up to our knowledge, there is no study investigating the effects of interactive floor projection on plantar pressure distribution. Therefore, it is not possible to compare our results with a similar clinical trial. However, there is a study conducted by Braun et al to determine how nursing home residents would respond to the interactive floor projection and it is reported that interactive floor projection may be a promising modality in individuals with nursing home residents to increase their physical activity level (19).

When we examine the general characteristics of the participants in this study, only one participant is obese (Type I) and rest of them are normal or underweight (BMI ≤ 25 kg/m^2). Yıldırım-Şahan et al. reported that increased BMI causes increased plantar pressures (20). Therefore, there was no abnormal score in plantar pressure of participants before the training.

Interactive floor projection is not a brand-new technology but there are very limited studies which ran as a medical treatment plan and based on interactive floor projection device. The study by Takahashi et al. (21) indicates that, this device could use in a unique population such as children with special needs due to its high visual and auditory effects. By using this device, participants may have a higher concentration and cooperation while doing exercises or physical activities that planned to be done. Another study by Hsieh et al demonstrated that interactive floor projection support helped improve the scoring for the scales of behavior and mental health in paediatric patients hospitalized for cancer treatment in Taiwan (22). Consequently, although limited numbers of it, these findings confirm that current technologies such as interactive floor projection support may be beneficial as a therapeutic modality for clinicians. In addition, no side and adverse effects were encountered during the study.

In the present study, there are some limitations which should be addressed. The subjects performed only 4 week – training program. However, it should be 8 or 12 weeks to investigate the effects of exercise program. Another limitations were the lack of follow – up and control group. Moreover, relatively small sample size and gender distribution were the important limitations of this study. We could also add lack of objective measurements for physical activity as a limitation in the study. Therefore, further studies are recommended to investigate the long-term effects of interactive floor projection with a larger sample size and with control group.

5. CONCLUSION

Based on our results, the present study revealed that 4-week foot exercises program with interactive floor projection applied as rehabilitation technology could decrease maximum force of total and hindfoot on the bottom of the dominant and non-dominant side feet in healthy and sedentary individuals. Interactive floor projection support including balance and proprioception games has the potential to provide a better plantar pressure distribution in physically inactive individuals. Future investigation should be conducted to clarify the effects of long-term interactive floor projection training program on plantar pressure distribution in patients with foot deformities such as diabetic foot, ulceration, etc.

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Acquisition of data for the study: OA, RUE, ZS, MGP

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Clinical and Experimental Health Sciences

Bacterial Foodborne Diseases in Sudan: A Review

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ABSTRACT

Objective: To determine the role of the bacteria associated with foodborne diseases in Sudan and to help health policymakers introduce strict intervention measures control.

Methods: The review uses up-to-date data via manual screening of the titles and abstracts of retrieved articles using string foodborne diseases in Sudan and foodborne illnesses as keywords to obtain publications from the electronic databases PubMed and Google Scholar using the publish or perish tool. However, priority has been given to the scientific papers, reports, and literature issued within the past 5 years.

Results: The review reported that many types of research revealed that foodborne infection is a critical, life-threatening health problem in Sudan and that different food pathogens are responsible for people and outbreaks of foodborne illness.

Conclusion: Foodborne diseases are considered one of the main reasons for illness and death, particularly in countries that suffer from poor economic conditions, such as Sudan. The review concluded that the most bacteria that caused foodborne disease in Sudan were *Escherichia coli, Salmonella spp,* and *Staphylococcus aureus*.

Keywords: Foodborne diseases, Sudan, Bacteria, Food safety.

1. INTRODUCTION

Food poisoning results from eating food contaminated with pathogens, toxins, and chemical secretions. Food poisoning occurs when an infection in the digestive system or the presence of neurological symptoms in the injured or in animals that have eaten a meal during the last three days. Undoubtedly, there are harmless and useful bacteria used in making some foods such as yogurt and cheese. There are chemicals among the natural components of food because diseases are transmitted through the food itself, and some of them are accidentally added during the manufacturing processes, either by mistake, neglect, or pollution. Bacteria are responsible for 66% of foodborne disease causes, followed by chemicals (26%), viruses 4%, and parasites 4% (1). Common food-related bacterial pathogens of animal origin are *Staphylococcus aureus*, *Salmonella typhi*, *Campylobacter jejuni*, *Listeria monocytogenes,* and *Escherichia coli*. Since bacteria are among the most important foodborne pathogens because they form spores that make them heat resistant (for example*, Clostridium botulinum*, *C. perfringens*, *Bacillus subtilus*, and *Bacillus cereus* (2). Some types of these bacteria

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can grow under cold conditions or temperatures below 25-45 degrees Celsius (3).

2. BACTERIAL FOODBORNE DISEASES

2.1. Escherichia coli (E. coli)

Escherichia coli (*E. coli*) are Gram-negative bacteria, classified as normal flora of the lower parts of the alimentary tract in animals and humans. However, most of the strains are harmless. However, certain pathogenic *E. coli* strains can infect the gut area and cause severe foodborne diseases. Different types of *E. coli*, such as Shiga toxin-producing *E. coli* (STEC), are responsible for a diarrheal disease that is considered the second cause of death among children worldwide, as well as septicemia, urinary tract infections (UTIs), and neonatal meningitis (4). *E. coli* can affect humans when they consume contaminated foods such as raw meat products, raw milk, and contaminated raw vegetables and sprouts. A pathogenic strain of *E. coli* (*E. coli* 0157:H7) was reported as the causative agent of illness in the two outbreaks in different countries (5). Food handlers and livestock are occasional carriers of pathogenic *E. coli*. However, these carry the facilitation of the introduction of infected serotypes of *E. coli* to the environment through food, crops, and meat contamination (6).

2.1.1. Pathology and Pathogenicity of E. coli

The pathology and pathogenicity of the pliability of *E. coli* have led to the evolution of this bacteria into pathogenic strains that cause illness in humans and animals (7). Nevertheless, *E. coli* is very diverse, as it encompasses both commensal, probiotic (bnign) and pathogenic strains. Various strains pollute water and vEarious kinds of food (8). There are six distinguished varieties of *E.coli* as a source of enteric infection: enteropathogenic *E.coli* (EPEC), enterohaemorrhagic *E. coli* (EHEC), enterotoxigenic *E. coli* (ETEC), enteroaggregative *E. coli* (EAEC), enteroinvasive *E. coli*, (EIEC), and diffusely adherent *E. coli* (DAEC)(9). This grouping is dependent on harmful factors, clinical sickness, phylogenetic profile, and the creation of poisons. Enterohemorrhagic *E. coli* (EHEC), specifically *E. coli* O157: H7, has been identified as one of the microorganisms responsible for foodborne human disease worldwide. This group uses major outbreaks to primarily affect developing countries and is capable of diarrheal sickness in addition to genuine clinical complexities like hemolytic uremic syndrome (HUS) (7). Besides, Shiga poison delivers *E. Coli* (Shiga causes genuine illnesses, which can be perilous).

2.1.2. Escherichia coli as causative foodborne diseases in Sudan

Food poisoning and foodborne disease outbreaks documented in Sudan are very few, although different studies have confirmed bacterial contamination of poultry, fish, camels, and meat in different areas (10).

However, epidemiological studies tracing the level of contamination of products in different areas of Sudan pointed to *E. coli* as quite possibly the most well-known causative specialist of high levels of contamination of some items like meat, milk, and eggs (11),(12). A recent study conducted by Hamid and others in 2020 which reported high contamination of broiler chains in the Khartoum North locality (13). Due to poor hygiene practices, 30% of poultry farms were found positive for *E. coli* contamination. *E. coli* was also isolated from swabs of 30 fresh chicken carcasses in Khartoum State (12).

Analysis of a total of 75 random samples of raw meat collected from different localities in Khartoum, Sudan revealed animal products such as vended red meat as a major source of *E. coli* and other pathogens associated with foodborne illnesses. *E. coli* incidence was 36% and different species were isolated; *E. coli* represented 20%, *E. vulneris* (6.6%), *E. albertii* (5.3%), and *E. fergusonii* (4%) (11). Additionally, pathogenic bacteria were isolated from vending foods in Atbara City (Naher Elneel State), Northern Sudan, and *E.coli* was the most prevalent besides other bacterial strains such as *Staphylococcus aureus* and *Bacillus sp* (14).

2.2. Salmonella spp

Salmonella belongs to the family Enterobacteriaceae, Gram-negative bacteria, oxidase-negative, catalase-positive, non-spore-forming rods, and facultative anaerobes. *Salmonella* are all motile bacteria that live in the environment and cause a variety of diseases in humans and animals. It causes many diseases for humans, such as typhoid and septicemia, and causes local diseases in different types of cells and diseases of the digestive system. Non-typhoid spp. caused about 1027561 and about 387 deaths in the US in 2011. There were 2659 Salmonella serogroups in 2014, with approximately 2639 *Salmonella enterica* and 20 *Salmonella bongori* (15). Salmonella lives in the guts of different animals, and there are several serogroups of Salmonella due to the difference in antigen from one type to another (15). Salmonella was divided into three categories: enteric fever, diarrheal diseases, and invasive non-enteric fever diseases (15) .

Salmonella is an important type of bacteria that may lead to diseases such as typhoid fever in humans and animals. There are about 1.4 million Salmonella cases recorded annually in the United States, and nearly 35,000 cases are isolated by public health laboratories and monitored by the Centers for Disease Control and Prevention (CDC) (16) . The severity of Salmonella sickness differs depending on the type of salmonella serogroup and according to the health status of the infected person. Kids below 5 years of age and the elderly, in addition to people with weak immunity, are more susceptible to contracting the disease compared to healthy people. Most types of Salmonella are considered disease-causing because of their ability to penetrate, live,

and multiply inside human cells, which leads to the disease's becoming a fatal disease (17).

2.2.1. Epidemiology

Salmonella is viewed as perhaps the main source of diarrheal sickness, which prompts central illnesses and lethal infections. In 2010, typhoid fever was recorded as having about 11.9 million cases of illness and 129,000 deaths, while there were about 3.4 million infections and six hundred eighty thousand deaths due to invasive non-typhoidal Salmonella in the same year (18). Enteric salmonellosis is the second bacterial cause of infection related to bacterial foodborne illness. About 95% of Salmonella cases are identified with the utilization of contaminated foods such as milk, poultry, eggs, meat, and seafood. The disease can be cured, but sometimes it leads to serious complications such as bacteremia. Antibiotics are always used to treat diseases (19) .

2.2.2. Resistance of Salmonella to antibiotics

The appearance of resistance in Salmonella to antibiotics is one of the most important health issues all over the world. The first case of Salmonella was recorded as resistant to chloramphenicol in 1960, and then it began to increase the resistance of Salmonella to antibiotics in several states, such as the USA, Saudi Arabia, and the UK. The first conventional treatment for Salmonella was with antibiotics such as chloramphenicol, ampicillin, trimethoprim, and sulfamethoxazole. Then Salmonella began to show resistance to these antibiotics, leading to multi-drug resistance (MDR) (17).

In Sudan, Emad and his colleagues in 2012 conducted studies regarding resistance to salmonella enterica isolated from humans and animals to antibiotics. Experiments were conducted on 119 Salmonella isolated from the faces of humans, camels, poultry, and cattle. The results showed resistance to about 10 types of commonly used antibiotics, such as chloramphenicol, gentamicin, ampicillin, cefalexin, furazolidone, nalidixic acid, ciprofloxacin, colistin, sulfamethoxazole, and tetracycline. 80.67% of Salmonella was resistant to at least one of the antibiotics used and of the 45 isolates of Salmonella, 37.82% had multidrug resistance. In comparison to resistance to antibiotics, the study showed that Salmonella isolated from humans was more resistant to antibiotics than Salmonella isolated from animals. Ciprofloxacin, colistin, gentamicin, and tetracycline were more effective against Salmonella, while the study indicated decreased resistance of salmonella to chloramphenicol, ampicillin, furazolidone, and sulfamethoxazole + trimethoprim (19).

2.2.3. History of Salmonella research in Sudan

The first Salmonella infections in cattle reviewers were reported in Sudan in 1946 by Horgan. Following an outbreak reported by Soliman and Khan in 1959 at Wad Madani City and Dublin, a serovar was isolated from stool samples of two people who became ill after eating a meat meal. The same strain was also isolated from infected calves and some apparently healthy animals. In 1970, Khan conducted a survey in Khartoum city to find out the incidence of Salmonella among the animals, and the result of the survey showed 230 Salmonella isolated from various samples, and 63 serogroups were discovered. Later, 15 serogroups were discovered in Khartoum and Malakal and were added to the Salmonella list in Sudan. In 1971, Sari Eldin recorded the emergence of different strains of Salmonella. Salmonella was also isolated from sheep liver by Salih and Ibrahim. In 1987, Yaqoub and Mohamed isolated 57 strains of Salmonella from chickens slaughtered in Omdurman and North Khartoum. Salmonella Dublin was also isolated from the feces of some animals from Kuku, Omdurman, and Alobeid. In 1999, Al-Tom and others isolated Salmonella enterica from three goats from the city of Omdurman. New strains have also appeared in Khartoum city, which were isolated from 3.4% of stool samples in Khartoum city by Haj Alsafi in 2009 and in 2010 by Elhussain Salmonella enterica can be isolated from both cooked and uncooked foods (20).

There are several other studies that have been done in Sudan regarding Salmonella and salmonellosis that have been observed in animals. In 1992, Mamoun and his colleagues isolated 21 strains of Salmonella from poultry farms from 3 different states in Sudan. *Salmonella enteritidis* was isolated from 1.43% of raw milk samples in 2005 by Yagoub and his colleagues (21) (Mamoun et al., 1992). Also in 2006, Yagoub and others worked on samples of white cheese collected from markets and restaurants in Khartoum and Omdurman. *Salmonella paratyphi* A and *Salmonella paratyphi B* were discovered in 6% of the samples. In 2007, a search was conducted to find out the health and safety of foods sold on the road, and the study was done by taking samples of a Sudanese food sold on the road called Um-Jinger, made from cooked ground pearl millet, sugar, plus yogurt and lemon. Salmonella was isolated in 5% of the samples (22).

Salmonella was isolated from fish by Yagoub in 2009, and the study proved the presence of Salmonella in 6.2% of the samples. There were other studies conducted on food handlers in 2010 by Saeed and Hamed, and it was discovered that 30.1% of food handlers were carriers of Salmonella (20). In 2018, Honua isolated 0.6% of stool samples taken from kids below 5 years of age with acute diarrhea (23) .Also, in 2018, a study was conducted by collecting 30 samples from chicken carcasses, and the project proved the existence of salmonella in 30% of the samples. The study also demonstrated that chicken carcasses are more susceptible to contamination during the manufacturing process than in storage (12) . Mustafa and Hamad in 2016 conducted a study that included factories for the manufacture of poultry and red meat, and the study revealed that processed meat contains different types of bacteria, and the percentage of Salmonella was 10.4% of the total bacteria (24). In 2013, samples were taken from four markets, (Jackson, Mayo, Alfitaihab, and Souk Seta) in Khartoum state. The samples included meat, milk, and

eggs. The study proved the existence of *E. coli* and Salmonella spp. in all samples in different proportions (25).

2.3. Listeria monocytogenes

Listeria species are more widespread in the environment, including *Listeria monocytogenes* that cause Listeriosis, a dangerous disease that is deadly to humans and animals. *Listeria monocytogenes* has been isolated from broilers sold by retailers and served as meals in fast-food restaurants in Khartoum State. The number of samples collected from broiler chickens reached 250 samples. The results indicated that the percentage of Listeria spp. contamination in the total of 250 samples was 95 (38%) (26).

Also, in 2018, a study showed the presence of *Listeria monocytogenes* in 6.7% of samples that were isolated from 30 samples of fresh chicken carcasses compared to 3.6% of frozen chicken carcasses (12). The study done by Mustafa and Hamad included factories for the manufacture of poultry and red meat, and the study revealed that processed meat contains different types of bacteria, and high disease contaminated *Listeria monocytogenes*. 39% of the total bacteria (24).

2.4. Staphylococcus aureus

Bacteria are the most common cause of two-thirds of food-borne disease outbreaks, and the predominant bacteria species that participates in food-borne diseases is *Staphylococcus aureus*, which causes gastro resistance as a result of ingesting contaminated food with enterotoxins produced by staphylococcus (27), (28). *S.aureus* is the most well-known organism that causes staphylococcal food contamination (29). *S.aureus* is typically discovered in the mucous films of individuals and creatures, such as skin and the nose. However, it causes a wide range of illnesses in both humans and animals. It stands out overall due to high mortality related to drug opposition: penicillin (10µg), ampicillin (30 µg) and oxytetracycline (10µg) (30). In Africa. Separates have a high extent of protection from penicillin (31), co-trimoxazole, and antibiotic medication (32).

2.4.1. Etiology

Staphylococcus aureus is a gram-positive, catalase-positive, coagulase-positive, non-motile (26). It can live in a wide range of temperatures from 7–48C, pH (4.2 to 9.3, with an optimum of 7.0 to 7.5). Because of these properties, the bacteria can survive in a wide range of foods, including processed meats, where other organisms cannot (31). Staphylococcal food poisoning is due to the production of enterotoxin by *S.aureus* bacteria. Methods of prevention include safe food handling and processing practices, proper cleaning of equipment, and food should be handled in a way that ensures good hygiene. In a study conducted in Omdurman city and its suburbs, it was found that 30% of people dealing with meat were carriers of pathogenic organisms, mainly *S. aureus* (28). Another study conducted in Khartoum state, the sample size was 400 (milk 100, meat 100, fish 100, and cheese 100). It was found that bacteria were present in 263 of the samples. 137 isolates (34.25%) were *S. aureus*, and the percentage of staph in the different foods is highest in milk, at 63%, and the lowest percentage is present in cheese, at 20% (33). Food handlers who carry Staphylococci contaminate food by direct contact due to a lack of proper hygienic measures (30). In a study conducted in Khartoum state to isolate Staphylococcus aureus from fermented fish (fasekh), 72% of the study sample was contaminated by S. aureus (30). Also, in a study involving 259 people who deal with meat in the Omdurman area, it was found that 71.8% were S. aureus positive (28).

3. FOOD SAFETY AND FOODBORNE DISEASE

Food safety and foodborne diseases are closely connected. Consumption of unhealthy foods causes disease. Cooperation in the food chain between governments and regional border producers, supporters, distributors, and consumers ultimately lead to healthy food supplementation in the future (31). The safety of healthy food supplies has a great economic role related to disease, as well as to economic pressures at the community and international levels. In Africa, mortality has been recorded to be the equivalent of 70% of the outbreak (32). In 2010, the World Health Organization bore the brunt of the burden of foodborne diseases, which resulted in nearly 420–960 million illnesses and 420,000 (95% UI) deaths. The most common foodborne disease is diarrhea, specifically caused by Norovirus and Campylobacter spp. These pathogens were responsible for about 230,000 (95% UI 160,000–320,000) deaths, precisely non-typhoidal Salmonella enterica. The other most important sources of death are foodborne *Salmonella typhi*, *Taenia solium*, *Hepatitis A virus*, and aflatoxin (34).

3.1. The economic impact of foodborne diseases

Foodborne diseases affect people's well-being as well as impose economic impacts (35). It is also considered a major cause of various costs in terms of medical treatment, detection of infestation, recall of food commodities, and loss of public confidence (36).

3.2. Sources of foodborne diseases

In Africa, however, the knowledge regarding clean milk making and the lack of potable water for cleaning purposes were some of the factors that contributed to the poor sanitary standard of milk from farms to collection points in Khartoum State (37). The same finding was noticed among most milk producers unaware of the effect of animal health and environmental circumstances on producing safe milk due to the absence of employee licenses, the absence of technical staff, and the backwardness of milk production and processing systems, besides lack of training (38).

Zoonosis such as Malta fever and *Mycobacterium Tuberculosis* are transmitted through unhealthy milk and milk derivatives. These diseases have been recorded in different parts of Sudan (38). A similar study was conducted in the brain area of zoonotic animals to differentiate between vaccinated and infected animals (39).

Brucellosis was also reported among other domestic animals (camel, sheep, and goats) in West Kordofan State (40) . A similar finding revealed the existence of tuberculosis in humans and livestock (41). Furthermore, the presence of brucellosis in milk samples collected from a supermarket in Khartoum State was confirmed (42). Many researchers have provided evidence that *Escherichia coli* is sometimes present as a microorganism in milk for various reasons, including lack of hygiene in handling, storing food besides production, inadequate storage, and post-process contamination (43).

The hypothesis is that human extraintestinal pathogenic *Escherichia coli* (ExPEC) may act as a poultry meat reservoir for humans and is considered the main cause of community and hospital-acquired extraintestinal infections such as urinary tract infections (UTI) (44). Various sorts of food controllers who meet food assume a significant part in the spread and transmission of resistant food-borne sicknesses such as intestinal microbes and parasites, which were discovered to spread more between vendors (41%), followed by restaurant workers' (24.4%), bakers' (24.4%), butchers' (5.1%), milk wholesalers' (2.6%), and organic products/ vegetables dealers' (2.6%) (45)(Ali, 2010). The same findings were seen in 29.4% of people who serve food in Wad Medani eateries who were carriers for intestinal protozoa based on stool tests (23). The study also showed a positive association between personal hygiene, education, and hand pollution among the food handlers (46) .

3.3. Factors contributing to the transmission of Foodborne diseases

Various factors have been evaluated among food handlers, such as food safety knowledge, practices, and educational level among street food vendors in Atbara city. Among the studied groups, 28% were male and 72% were female. 48% of them had a primary school level of education while 42% were illiterate. The most isolated organisms were bacteria from cooked meals, bottled drinks, and fresh juices (14). Other surveyed factors were food hygiene information, disposition, and practices among clinic food controllers in El Managil City. Food laborers express their degree of information, conduct, and practice to be 70.1%, 63.81%, and 74.40%, respectively. The well-being status and the significance of instructional classes added to keeping food from tainting, displaying a low practice rate concerning nail cutting and covering the head during food preparation. A factual examination showed a critical correlation between training level and washing the utensils with sanitizers and wearing gloves while performing work. This examination inferred that food treatment in El Managil hospital expresses a medium knowledge level and an uplifting outlook (47). Food hygiene education is the most effective when messages are directed towards changing behavior, the consequences of which are closely related to foodborne diseases. Factors that control pathogens are personal health, good cooking, avoiding accidental contamination, keeping food at a safe temperature, and separating food from unsafe sources (48).

The application of food hygiene training among food handlers represented a major element in the successful control of foodborne diseases, which could be supported by managers encouraging healthy and safe handling of food during work time (49). Studies have proved that there are several reasons for losing training initiation and implementation of healthy and safe food handling in the catering factory, such as low-paid salaries, staff communication problems, levels of learning, background in quality assurance, large quantities of complex meals prepared, poor access to safe food guidance, facilities, equipment, and shift rotation (50). There is no strict commitment by the food control authorities in the Khartoum State towards food safety issues and there is a lack of awareness and training in employees about meat hygiene and safety that was noticed clearly when we assessed bacterial contamination of sheep carcasses before and after wearing gloves, aprons, masks, and caps. Therefore, bacterial counts from workers' hands after treatment showed a significant reduction compared to control and that the sterilization of knives by warm water (82 °C) decreased the level of viable bacteria. A study conducted on broiler food revealed that contamination of *E. coli* at the slaughterhouse was found concentrated in the defeathering machine chain (13). Moreover, this finding was justified by Mohamed Noor, who attributed this finding to the firm attachment of the bacteria to the poultry skin and rubber fingers (10).

Other studies have shown that weather and temperature play an important role in influencing the outbreak of foodborne diseases (51). However, a similar finding was reported that the rise in temperatures between 25 °C and 37 °C can produce a strong biofilm formation of Vibrio para haemolyticus on shrimp, crab, and stainless steel coupons (52). Moreover, the previous findings show that temperature increases also have a profound effect on bacteria remaining on food, such as *E. coli* O157: H7 and *Salmonella spp*. (53).

4. HAZARD ANALYSIS CRITICAL CONTROL POINT (HCCP) IMPLEMENTATION IN FOOD PROCESSING

The appropriate methods of HCCP when applied during slaughtering operations would reduce the occurrence of these microorganisms. The study reported that Salmonella spp. and Escherichia coli contamination in poultry carcasses at an automatic slaughterhouse in Khartoum State (12), and this would affect the safety and quality of poultry meat. Moreover, the right application of HACCP will greatly reduce the bacterial contamination in poultry plants because it involves constant checking of all stages of the processing (12). It was found that the whole country lacks "in-house" experts and consultation bodies officially registered to

provide the necessary technical support for verification and accreditation of implemented HACCP systems in meat processing operations (12). A study has shown that the implementation of HACCP is hampered by a shortage of money and human resources in small food factories (54). Additional studies supported the same idea that had clearly stated the barriers that obstructed the implementation of HACCP in Sudan due to poor premises construction, poor water supply, indigent farms' hygiene as well as milkers' and the gathering of droppings and animal waste that induced insects to spread (55).

5. DIAGNOSTIC DETECTION METHODS TO ENSURE FOOD SAFETY

Fast and precise detection of foodborne microbes is an urgent necessity for public health monitoring, stopping foodborne infections, and ensuring food safety. The diagnosis of FBD involves an enormous technique that mainly depends on the types of food and samples investigated. However, conventional microbiological methods were used to isolate and identify most pathogenic bacteria. Other studies used enzyme immunosorbent assay (ELISA), along with Nested polymrase chain reaction (nPCR), Rose Bengal test, and single intradermal comparative tuberculin test (SICTT) (45). Parasitic causes of foodborne diseases are detected using direct fecal examination, in which Lugol solution is used to identify undifferentiated protozoan cysts. Concentration techniques such as formaldehyde, floatation, and Biermann's technique help to diagnose larvae of *Strongyloides stercoralis* and hookworm (56). Real-time PCR and reverse transcription-polymerase chain reaction (RT-qPCR) combined with partial sequences were used to investigate viral foodborne diseases such as hepatitis E (57). However, the same technique was used in the detection of rotavirus and adenovirus antigens with an immuno-chromatography test (ICT)(58). Another study has utilized enzyme-linked immunosorbent assay (ELISA) antigens to detect Rotavirus in stool samples, combined with genotyping using nested PCR and sequencing (59).

6. CONCLUSION

Foodborne diseases are among the main causes of disease and death, especially in countries that suffer from poor economic conditions, such as Sudan, where there is a social and economic impact of foodborne diseases, which requires these countries to undertake reforms to reduce them. In Sudan, there is no clear control system, which complicates the problem. Therefore, the health authorities in Sudan must greatly enhance the current system for an effective foodborne disease surveillance system. The review concluded that the most common foodborne pathogenic bacteria in Sudan are *Escherichia coli*, *Salmonella* and *Staphylococcus aureus.*

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Clinical and Experimental Health Sciences

A Review of Sudan's Major Food-Borne Viral and Fungal Diseases

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ABSTRACT

Objective: This review article was conducted to highlight both viral and fungal foodborne situations in Sudan and their health impacts aiming to help health policymakers in introducing strict intervention measures.

Method: The review uses up-to-date data via manual screening of the titles and abstracts of retrieved articles using string foodborne diseases in Sudan and foodborne illnesses as keywords to obtain publications from the electronic databases PubMed, Scopus, and Google Scholar from the year 2000-2020 using the publish or perish tool, the databases were reviewed from January to April 2022.

Results: Foodborne viruses and Mycotoxins produced by certain fungi; are considered among the top priorities and have become of great concern to the food industry over the last few years because their contamination can occur at any point in the nutritional supply chain besides their serious effects on human health both long and short term.

Conclusion: The review on major food borne viral and fungal diseases in Sudan is an important issue to guard against contamination with such micro-organisms and prevent their illnesses.
Keywords: Enteric viruses, Norovirus, Aflatoxins, Mold, Aspergillus flavus.

1. INTRODUCTION

Many serious foodborne outbreaks in the African region result from the consumption of contaminated ready-to-eat foodstuffs informally sold as street foods. Thus, the health aspects and food safety pose a real challenge to the food safety regulators. But because of the shortage of food supplies, people care more about hunger satisfaction than food safety, which negatively affected the global situation. The main predisposing factors for foodborne illness include unhealthy practices, undercooking, and lack of refrigerators, besides environmental causes such as polluted water, unsafe waste disposal, and exposure of food to insects or dust (1). This review article aims to highlight both viral and fungal foodborne situations in Sudan and their health impacts.

1.1. Foodborne viruses in Sudan

Infectious diseases, heavy metals, chemical pollutants, and natural poisons like toxic mushrooms produced foodborne

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illness pandemics. The earliest reported foodborne infection occurred about 323 B.C., when Alexander the Great died of typhoid (2). Foodborne illness was misdiagnosed as poisoning before current microbiology. Before the 1960s, epidemics were frequently poorly recorded and might not have been seen. The 1971 Bon Vivant Outbreak recorded such events. After the Bon Vivant case, many foodborne outbreaks from the 1960s or earlier were not completely written down and may not have been reported until the Jack-in-the-Box Outbreak (3). Foodborne illness outbreaks are most often caused by Norovirus. It was agreed that viral control measures are required all along the food processing chain. However, the effectiveness of such control procedures and how to validate properly their performance still needs to be understood (4). Because viruses do not reproduce in food, their infectivitey depends on virus stability and individual susceptibility. A load of foodborne enteric viral diseases is often difficult to assess since mild illnesses may pass unnoticed, however; such viruses can survive long periods by tolerating fluctuating environmental conditions. And so, testing for water contamination is important to guard against diarrheal disease caused by them. As a result of an enhancement in the sensitivity of detection methods and an increase in reporting, the number of outbreaks has recently increased (4).

1.2. Enteric Viruses

Enteric viruses are microorganisms that spread widely, producing human infections ranging from clinically asymptomatic most often, to gastroenteritis, hepatitis, and even meningitis (5). Such infections are communicated by the faeco-oral route. In the Western world, norovirus (NoV) and hepatitis A virus (HAV) are yet considered at the bleeding edge of human foodborne microbes considering the number of episodes and individuals influenced (6). Other viruses (rotavirus, hepatitis E viruses, and sapovirus) can also represent a risk to mankind (7).

1.3. Foodborne Viral Situation in Sudan

1.3.1. Rotavirus

Worldwide Rotavirus is the main causal agent of severe diarrhea among children under 5 years of age, causing 610,000–870,000 deaths yearly. Class A rotaviruses are the main causative agents of severe diarrhea in children. It is one of the Reoviridae family members that are non-enveloped and has double-stranded RNA. The Rotavirus genus has an 11-segment genome covered (1). In Sudan, viral genotyping was studied based on the viral proteins (VP4 and VP7) by nested PCR and gene sequencing results showed the VP7 predominant G class was G1 (8). Two epidemiological studies conducted on under-5-year-old children infected with rotavirus in Omdurman Pediatric Hospital, Sudan; The first was a setting-based study over one year, which concluded that rotavirus represents a public health problem among Sudanese children younger than five years and that effective control procedures are essential to reduce this problem (9). The other study found that rotavirus prevalence was 25% in children under the age of five, and that rotavirus vaccine, accurate and routine diagnosis of acute child diarrheal infection aids in accurate management, preventing antibiotic use, and reducing the prevalence in vulnerable children (10). Although fecal-oral contact is the known mode for transmission of rotavirus, contaminated hands and surfaces and the respiratory route are also reported (11). Another study targeted the spread of rotavirus and adenovirus, which are responsible for diarrhea among refugees in Khartoum, Sudan. All study population data was collected, and ICT and PCR were used as viral detection procedures. The study results showed that most diarrheal cases under five years of age were caused by rotavirus class A (VP6). It also indicated that most of the patients were using raw water obtained from donkey carts and those who lived in poor environmental conditions with no access to toilets (12). Considering the histological pattern of the upper gastrointestinal tract, rotavirus targeted epithelial cells of the proximal part of the small intestine (enterocytes) where they started to replicate. These cells undergo necrosis and shedding with the loss of their digestive enzymes, leading to primary malabsorption, especially for carbohydrates and proteins. Loss of enterocytes leads to villous atrophy with reactive inflammatory reactions and crypt cell hyperplasia. These changes will increase the severity of diarrhea. Recovery from villi takes 1–10 days. Infected children excrete billions of virus particles with each motion of their stools. These viruses can survive in the environment for several days or weeks (13). The malabsorptive component of diarrhea is due to the accumulation of unabsorbed carbohydrates, water, and other nutrients in the intestinal lumen. Since the revelation of the NSP4 enterotoxin, it has been suggested that there is an extra secretory part in the pathogenesis of diarrhea caused by rotavirus (14). Recovery from the first infectious episode may lower the severity of the following motions but does not induce long-lasting immunity (9). Rotavirus infection has a considerable economic impact on medical or non-medical resources, affecting all healthcare settings and resulting in considerable costs for national healthcare payers, patients' families, and employers (15). Detection methods for such viruses include electron microscopy and nucleic acid detection. Both are highly specific and sensitive. *Rotavirus* viral antigen detection is the most widely accepted diagnostic tool that depends on the revelation of protein antigens on virus molecules in stool samples (16). Molecular-based detection of *Astro-virus* and class A and B Rotavirus was carried out in children less than 5 years old with gastroenteritis in Aljazeera and Khartoum states, Sudan. All samples were tested by RT-PCR amplifications after RNA extraction to detect *Astro-virus*. According to this study, *Astro-viruses* are an important cause of gastroenteritis like rotavirus, and to avoid the spread of nosocomial viral gastroenteritis infections in pediatric units, the detection of rotavirus and *Astro-virus* assays in clinical diagnosis must be carried out (12).

1.3.2. Noroviruses (NOV)

With the universal improvements in the detection methods of viruses in foods, (NoV) is now considered the commonest virus that causes foodborne disease. The role of asymptomatic food handlers who contribute to the outbreak is becoming increasingly apparent, being responsible for up to a quarter of outbreaks (17). Despite their impacts and prevalence, little information about the life cycle and the pathological aspects related to the norovirus-induced disease is available. Intestinal infection is the basic side, but extra intestinal spread and correlating pathologies have also been detected. Preventive and control measures for Norovirus infections are very difficult because of their low infectious dosage, high shedding titer, and environmental stability. The virus can be transmitted by different routes; foodborne and person-to-person are the most significant (18). Hepatitis and Norovirus in Western countries, infections are among the most significant foodborne pathogens in terms of the number of episodes and the impact on individuals., the clinical result of NoV disease is generally gentle. Asymptomatic diseases are far-reaching and may take an interest in the spread of contamination (19). In any case, information is restricted about the rate and epidemiological information of such infections in Sudan. A study was led to examine the sub-atomic based on the study of disease transmission of NoVs in children under 5 years of age suffering from severe gastroenteritis in Al Gezira state, Sudan. It showed that acute gastroenteritis in children under 5 years of age is mainly caused by norovirus. These findings highlighted the importance of introducing routine norovirus laboratory investigation in hospitals admitting children with gastroenteritis (20). Another study was conducted targeting the molecular epidemiology of bacterial and viral causative agents of diarrhea among children in Khartoum State, Sudan. The study investigated the epidemiological and clinical factors of bacterial and viral etiology in children suffering from severe diarrhea in Khartoum State. The collected samples were tested by Multiplex RT-PCR. Results showed that rotavirus was the main causative agent (10.2%) of the isolated enteroviruses, followed by norovirus G2 (4.0%). Outbreak control is dependent on infection control procedures such as hand washing and disinfection, avoiding contact with infectious individuals, and sterilizing the environment (21).

1.3.3. Hepatitis A virus (HAV)

HAV is a small, non-enveloped, SS – RNA virus, it is resistant for acids and thermostable (22). The common route of infection in non-immunized people is the fecal-oral route. The disease is mainly associated with unsafe food or water, poor sanitation, inadequate personal hygiene, and anal-oral sex. HAV can live and persist in the environment, and it resists procedures used routinely in food production for inactivation and/or control of bacteria (23). Hepatitis A viruses multiply in the liver cells disturbing its function. It induces an immune reaction that causes inflammation of the liver. HAV infections aren't very well known in Africa, but the available data shows that the endemic is still high in most African countries, except for some parts of South Africa (18). Rate of HAV in asymptomatic food handlers in Khartoum State was targeted by investigating 70 food handlers using the ELISA method for detection and concluded that the high prevalence rates of HAV antibodies detected in their samples raised the possibility of being a source of HAV infection outbreaks in the community (24). Another study obtained the same conclusion after testing 90 food handlers working in Khartoum cafeterias, serologically using an enzyme-linked immuno-sorbent assay (ELISA) to detect IgG and IgM. The virus doesn't cause only chronic liver disease; but it can cause very bad acute liver symptoms that can lead to death. In the year 2016, WHO estimated that people who died due to hepatitis A globally were 134, representing 5% of the total death rate from hepatitis. A report of a very bad case of the same virus has been made by UN peacekeepers in South Sudan (23). The most widely accepted diagnostic tool for hepatitis A is the discovery of the emergence of antibodies, especially of the IgM type using an enzyme immunoassay. However, the detection of HAV RNA is thought to be a good way to tell if someone has the disease even if they don't have specific antibodies (25).

1.3.4. Hepatitis E virus

Hepatitis E virus is the main causative agent of hepatic toxicity globally (HEV), especially in young people, and it often causes fatal symptoms in pregnant women (10). Due to water pollution, the fecal-oral route is the mode of transmission, but there are also animal sources. The course of the HEV is like other acute hepatitis viruses, and the rate of infection is high in young people. The virus does not have a chronic clinical picture, but asymptomatic infections are common. The death rate among pregnant women reached 25% (26). In the periods between the epidemics of hepatitis E, some studies published individual cases in Africa and some research was conducted on the incidence of the disease in Sudan (in patients from the adjacent states of Kordofan and Darfur, 25% were diagnosed with a mortality rate of 10%, especially among pregnant women). In June 2004, a large epidemic of hepatitis E was reported among the displaced camps in the state of Darfur, western Sudan, and at the borders with Chad, which seem to be an attractive area for researchers. About 5,000 HEV infections were recorded over a 6-month duration. A group of work, sponsored by the non-governmental organization Epicenter in refugee camps in Darfur, Doctors Without Borders, WHO, and the Centers for Disease Control in refugee camps in Chad, conducted a field study of this large epidemic (26). Within the same study population mentioned above, another study was carried out to detect the genetic diversity in the composition of the hepatitis E virus in Darfur, western Sudan, and neighboring Chad. The sequence at the ORF2 site was compared between 23 Sudanese and 5 Chadians who were isolated from the virus, and by presenting the results, it was found that more than 99.7% of the strains belong to Genotype 1 with high similarity. While four Chadian isolates were close to Genotype 2, this sparked widespread debate about the possibility of multiple disease sources (27). A third study was carried out to review

hospital records of hospitalized patients to assess the effects of this virus on pregnant women in Mornay camps, Darfur, Sudan, during the epidemic period. The conclusion showed the drastic effect of this disease on pregnant women, with a particular death rate of 31.1% (26). Another study conducted among the same population aiming to impose the feasibility of comparing the amplification of HEV RNA obtained from dried blood spots or serum to explain the biological characteristics of asymptomatic individuals and patients concluded the first report using RT-nucleic acid amplification of the dry blood spot that was obtained in tropical conditions in the refugee camps at the time of the epidemic spread, and because this mechanism is the easiest for children as it only needs 50 ml of blood and it does not need sedimentation or cooling, it may be the most reliable and accurate way to use it in such research in developing countries (26). The role of water treatment methods was the topic of another study conducted among the same population in Darfur, Sudan. A case and a retrospective cohort study were conducted to detect risk factors for asymptomatic and clinical hepatitis E, respectively. Being a young adult and drinking chlorinated surface water were detected as risk factors for both asymptomatic and clinical HEV infection. Although this was not found to be statistically significant, two donkeys were positive for HEV RNA detected in serum samples, concluding that the current precautions to ensure a safe water supply may not be enough to deactivate HEV and control this epidemic. This study indicates the need to evaluate current water manipulation procedures and to identify other methods adapted to complex emergencies (28). A conclusive study conducted to investigate the serological epidemiology among mothers and neonates in Medani Hospital, Sudan, measured the level of HEV Immunoglobulin G (IgG) antibodies and showed a significant rise of these indicators in pregnant women in central Sudan without considering their age, gestational age, or even the number of their births. These results highlight that optimum preventive procedures should be used against HEV infection (29). The seroprevalence of HEV in South Sudan's homeless was detected in another study. The study indicated that infections with HEV are more prevalent than estimated previously, proposing the likelihood of a higher (yet not detected) burden than even reported (30). In a descriptive and cross-sectional study, using immunological tests (ELISA), the frequency of IgG and IgM antibodies in pregnant females at the Maternity Hospital of Dongola, Northern Sudan was determined in a descriptive and cross-sectional study. The study was conducted in November 2015. The results showed that HEV antibody (IgG) has a low frequency among pregnant women, and they recommended that HEV antibody testing should be performed on pregnant females in Sudan, and that prenatal screening for pregnant women will ensure that doctors can pay more attention to preventing the transmission of HEV in the perinatal period (31). A raised prevalence of HEV in pregnant women in Port Sudan city, Sudan in a cross-sectional study was documented in November and December 2015. It investigated the seroreactivity, and HEV RNA molecular positivity in pregnant women was determined (32). Another study in Port Sudan, Eastern Sudan, reported a Hepatitis E outbreak in the period of November 2010 to March 2011, where maternal mortality was as high as 28.2%. In this study, serological tests were done using ELISA to investigate IgG and IgM Anti-Hepatitis E virus in 39 pregnant women who came to Port Sudan hospital with different signs of viral hepatitis (33) The seroprevalence of hepatitis E in food handlers in Khartoum, Sudan was investigated among females and males who worked in different cafeterias in Khartoum state, using commercial tests with high specificity and sensitivity (ELISA) to detect the virus. The study concluded that HEV was found to be significantly high among food handlers (15.5%) in Khartoum state (34). The symptoms of foodborne illnesses are vomiting, diarrhea, which might lead to dehydration, fever, colic, headache, arthralgia, and myalgia. The final diagnosis can be done by stool culture or by advanced laboratory techniques. Anyhow, empiric treatment must be started before obtaining the laboratory results if a foodborne illness is suspected. Rehydration is a cornerstone if the patient is clinically dehydrated besides antibiotic therapy. Foodborne disease cases should be reported to health agencies (35).

2. CONTROL OPTIONS OF VIRUSES IN FOOD PROCESSING

Control, prevention, and treatment of foodborne illnesses are very costly. Control measures are limited and include that the vomit and/or diarrhea must be cleaned up and disinfected, besides the avoidance of food handling by infected people. The development of diagnostic procedures increases the chances of the identification of enteric viruses, especially in developing countries where the data is still limited (36). Studies on the stability of foodborne viruses are also minimal because most of them don't grow in laboratory cultures. But such studies confirmed that viruses can survive a variety of preservation methods. Intervention procedures that induce the inactivation of microbes are necessary to obtain a 3-log degradation of the level of viruses. Purification of fresh bivalves, on the other hand, is insufficient to prevent viral outbreaks in any case, because the type of food and the virus tested affect the specific food preservation methods (37).

3. FOODBORNE FUNGI IN SUDAN

3.1. Impact of Mycotoxins on Human Health

Mycotoxins are harmful mixtures that a few kinds of mold (fungi) normally produce. Mold, which can excrete mycotoxins, develops in numerous food varieties. It can form either before or after collecting and during storage, on or in the actual food. This typically happens in hot, wet, and damp conditions. Most mycotoxins are synthetically stable and tolerate food preparation (38). In Sudan, the situation of the inability to address contamination with mycotoxins for basic foods makes all measures to reduce the formation of mycotoxins and pollution urgent and necessary for the Sudanese population (39). Mycotoxins of concern in sorghum in sub-Saharan African countries about prevalence, concentration, and

potential exposure to health risks. The results of researchers who conducted a study on these toxins in four countries, including Sudan, indicate an urgent need to study, establish, and implement a coherent pre-and post-harvest management system to ensure safety at all levels in the sorghum production chain in those countries. These countries are encouraged to practice and implement programs to improve the application of good agricultural practices through transportation, storage, and final distribution (38).

3.2. Common Fungi Causing the Foodborne Diseases

Aflatoxins, ochratoxin A, patulin, fumonsens, zyaralenone, nevanol, and deoxy-nephallinol are the most widely recognized mycotoxins that are a source of concern for human and animal wellbeing (40). Mycotoxins show up in the food chain of life because of crop contamination with form both before and after the harvest. Intoxication can happen directly by ingesting contaminated food or indirectly by animals that have been fed with contaminated feed, especially the milk of these animals (41). Mycotoxins are generally found in food varieties, which is why they are of concern. Some foodborne mycotoxins have acute effects and are accompanied by severe clinical symptoms that appear quickly after consuming a contaminated food product, while other mycotoxins are connected to long-term health effects (cancer and immunodeficiency). Ahmed and Al-Bashir (42) examined samples of peanuts and their products that were collected from three Sudanese states: Khartoum in central Sudan, Kordofan in its west, and Gedaref in the east, which represent peanut producing states. The researchers examined the contents of samples of aflatoxin AFB1, AFB2, AFG1 and AFG2 using the method of high-performance liquid chromatography (HPLC) with fluorescence detection. They reported that a sample of peanuts or peanut products was not contaminated with AFG1 or AFG2. The highest numbers of specimens contaminated with mycotoxins are found in 38 analyzed 43 samples of peanut butter from Khartoum state and found 100% contamination.

3.3. Fungi That Producing Mycotoxin

Aspergillus flavus and *Aspergillus parasiticus* are responsible for the production of aflatoxins, the most toxic mycotoxins that grow in soil, deciduous plants, and grains. Crops frequently affected by the multiple Aspergillus species incorporate oilseeds (soybeans, peanuts, sunflower, and cottonseeds), grains (corn, sorghum, wheat, and rice), spices (chili pepper, black pepper, coriander, turmeric, and ginger) and nut fruits (pistachios, almonds, walnuts, and walnuts). M1 aflatoxin can be found in the milk of animals fed with contaminated feed (43).

Kabbashi et al. (2017) (44), collected and analyzed 25 samples of roasted peanuts in Khartoum for their content of aflatoxins. The results of analyzing aflatoxin content showed a high rate of contamination in all samples, and that there were significant differences between the samples collected from Khartoum, Khartoum North, and Omdurman, and that the contamination rate reached 84%, which is a worrying result that shows the risk of consuming roasted beans contaminated with aflatoxin for consumers, especially children. Large doses of aflatoxin can lead to acute toxicity (aflatoxin poisoning) and may be life-threatening, usually by damaging the liver. Aflatoxins have additionally been demonstrated to be genotoxic, implying that they are harmful to DNA and can cause cancer in animal species (45). A few types of Aspergillus and Mycobacterium produce ochratoxin A, which is produced by and is a common mycotoxin that contaminates food. Ochratoxin A is formed during crop storage and is known to cause several toxic effects on animal species. The most sensitive and prominent effect is kidney damage, but the toxin may also affect the development of the fetus and the immune system. On the contrary, while this link has not been proven in humans, the effect on the kidneys was demonstrated in 2016 (46).

Patulin is a fungal toxin produced by a range of molds, notably Aspergillus, Penicillium, and Byssochlamys. Patulin, which is normally found in apples and spoiled apple items, may also be found in natural products, grains, and other rotten food varieties. The primary wellsprings of patulin in the human eating routine are apples and apple syrup, both produced using defiled organic products. In animals, severe manifestations include liver, spleen, and kidney damage, as well as immune system inebriation. With respect to the individual, queasiness, gastrointestinal unsettling influences, and spewing have been accounted for. Patulin is genotoxic, but the potential for causing cancer has not yet been established (47). Fusarium fungi are common in soil and produce several different toxins, including trichococcins such as deoxy-nephallinol, nephallinol, T2 and HT2 toxins, zearalenone, and phaeomonsenate (48). Mold and toxins occur on a range of different cereal crops. Various fusarium toxins are associated with certain types of grains. For example, Zyaralenone is commonly associated with wheat, the T2 and HT2 toxins are associated with oats, and phyomonsenat is associated with corn. Trichococcins can be highly toxic to humans, causing rapid skin or gastric mucosal irritation and leading to diarrhea. Chronic effects reported to occur in animals include suppression of the immune system. At high intake levels, particularly in pigs, zyaralenone has been shown to have effects on hormones and estrogen and may cause sterility at high intake levels. As for the phyomonsens, it is associated with esophageal cancer in humans and liver and kidney toxicity in animals (49). Patulin is genotoxic, but the potential for causing malignant growth has not yet been established (50). Fusarium is a large genus of soil fungi that produces trichococcins such as deoxy-nephallinol, nephallinol, T2 and HT2 poisons, zearalenone, and phaeomonsenate (51). Shape and poisons occur in a variety of grain crops. Different fusarium poisons are related to kinds of grains. For instance, Zyaralenone is ordinarily connected with wheat, the T2 and HT2 poisons are related to oats, and phyomonsenat is related to corn. Trichococcins can be profoundly harmful to people, causing fast skin or gastric mucosal disturbances that lead to the runs. Persistent impacts happen in animals that incorporate concealment of

the immune system. Zyaralenone has been demonstrated to have consequences for chemicals and estrogen and may cause sterility at high admission levels, especially in pigs. The phyomonsens is linked to esophageal cancer in humans and liver and kidney toxicity in animals (49).

3.4. How to Reduce the Health Risks from Mycotoxins

Note that the mold that produces mycotoxins can develop on various crops and food sources and get into food sources deeply, not simply on a superficial level. Normally, mold does not grow on foods that are well dried and preserved, so effective drying of goods and keeping them dry, or storing them appropriately, is an effective measure to control mold growth and the production of mycotoxins (52). Ferreira, et al. (53) reported that the UV-C radiation therapy is efficient in fungal decontamination, photodegradation of mycotoxins, and release of bound phenolics in black and red rice grains after exposure for one hour, without affecting changes in cooking and color qualities.

3.5. Aflatoxins

The effect of heat treatment on the level of aflatoxin Bl on some Sudanese peanut (*Arachies hypogaea* L.) samples and peanut products was investigated. In this study, samples of peanuts from Al-Managel, Sudan (irrigated sector), and Kordofan (rainfed sector) regions were obtained and examined. The fungal count was higher in samples collected from the rainfed sector compared to the irrigated sector. The mean level (ppb) of aflatoxin Bl (AFBI) was 9.33, 4.67, and 3.33 in the Kordfan 1, Kordofan 2, and Al-Managel samples, respectively (Figure 1). However, AFBI was absent in the Gezira samples. Results also showed that roasting lowered the levels of AFBI in most of the samples that were tested. The greatest reduction was achieved when samples were roasted at 175 °C for 20 minutes of Kordofan samples led to a reduction of 19% in the levels of AFBI, while the reduction was 78% by the roasting-blanching method. The traditional confectionery products made from the most contaminated peanut samples displayed low levels of AFBI in Mundaco and Fulia, while Lucom was devoid of any AFBI. This was because the alkaline conditions in the latter were different from those in the first (54).

***Table 1**. Permissible limits for some mycotoxins in food*

Type of mycotoxin	Permissible limit (µg / kg)	Type of crop
Aflatoxin B + G	0-50	Peanuts – corn – other foods
	0-1000	Fodder
Aflatoxin M1	1.0-0.05	Milk
Ochratoxin A	300	Corn – rice – barley – legumes
Zeralone	30 – 1000	All foods
Patulin	20-30	Apple juice

It has been demonstrated that aflatoxin contamination was found to be high in seeds from insect-damaged pods compared to mechanically harvested damaged pods. On the other hand, healthy, intact pods are free from aflatoxin contamination. As regards the contamination of the various peanut products (paste, grey and red roasted nuts), both grey and roasted nuts were highly contaminated when compared to the healthy groundnut seeds (55).

In another study, it was found that chips that were sold in the local supermarkets in Wad-Medani city contained high levels of aflatoxins. These snack foods, which are preferred by children, are more likely to be infected with this type of mycotoxin.

In a study conducted (54), they investigated the levels of aflatoxin contamination with *A. flavus* and the aflatoxin contamination of groundnut samples collected from various locations in Gezira State (Figure 2). Aspergillus flavus development and aflatoxins creation was explored were at their minimum in samples collected from the villages (producing areas) in comparison to elevated levels in samples collected from the supermarkets in Wad Medani city. However, samples collected from local markets in the producing areas were less contaminated. The inhibitory impact of some fundamental oils on Aspergillus flavus development and the creation of aflatoxins was explored (54). The outcomes showed that clove oil was the best inhibitor oil in the diminishing mycelial growth of the organism, concerning both mycelial spiral growth and mycelial dry weight. Also, even though cumin oil was not hindering mycelial development totally, it was second best to clove oil. It gave significantly preferable inhibition over control at all its tried concentrations.

***Table 2.** Aflatoxin contamination of some peanut products (Packed or non-packed) from various Local markets in the Gezira State, Sudan.*

Location	Peanut paste		Roasted Peanut		Fried Peanut	
	Packed	Unpacked	Packed	Unpacked	Packed	Unpacked
Al – Hoosh	6.3	0.1	7.0	0.2	9.2	0.01
Al-Medina Arab	8.0	2.0	9.1	0.5	10.1	0.20
Al_Fao	8.5	3.0	10.2	0.6	9.3	0.33
Soug Merkazi	25.3	5.0	29.5	6.6	25.3	6.1
Malaga	28.5	3.0	29.8	7.0	20.0	8.0

4. CONCLUSION

Foodborne diseases are considered one of the main reasons for illness and death, particularly in countries that suffer from poor economic conditions, as in Sudan. The review reported that many types of research revealed that viral and fungal foodborne infections are critical, life-threatening health problems in Sudan; Hoping that national food safety systems

and protective measures such as awareness and training of employers should be followed and applied in Sudan.

Table 3. *Levels of aflatoxin contamination (ppm) of seeds of certain crops other collected from the two markets of Wad medani, Sudan and stored differently for one month*

Crops	Packed	Unpacked	Packed	Unpacked
Wheat	3.26	0.21	2.91	0.91
Faba bean	5.16	1.16	3.67	1.68
Phaseolus	9.60	3.25	3.67	2.50
Chickpea	6.33	2.33	5.33	2.10
Sorghum	3.50	0.91	1.90	0.80

Source: Abdelrahim, et al., (2012).

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Clinical and Experimental Health Sciences

Comprehensive Review of Nobiletin, a Citrus Flavonoid: Metabolism and Anti-tumor Properties

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ABSTRACT

Nobiletin is a polymethoxylated flavone found in citrus peels. Thanks to its chemical structure and biological activities, nobiletin has been shown to have a positive effect on many diseases. In recent years, there has been a growing interest in research focusing on the impact of nobiletin and its metabolites on different cancer types. Nobiletin exhibits anticancer properties by impeding the proliferation of cancer cells, disrupting the cancer cell cycle, facilitating apoptosis, and regulating signaling pathways implicated in cancer development. In addition, studies have shown that its use with chemotherapeutic agents inhibits multi-drug resistance. This review aims to evaluate the metabolic properties of nobiletin and its possible effects on cancer.

Keywords: Nobiletin, bioavailability, cancer, signaling pathways, antiproliferation

1. INTRODUCTION

Nobiletin, which is also known as 3',4',5,6,7,8-hexamethoxyflavone, is a significant compound belonging to the group of polymethoxylated flavones. It is typically found in the peel of citrus fruits such as Citrus reticulata (tangerine), Citrus depressa, Citrus sinensis (orange), and Citrus limon (lemon). Nobiletin has a molecular weight of 402.399 g and a chemical formula of C21H22O8 (1,2). The concentration of nobiletin within the peel oils of diverse citrus fruits can exhibit variability. For example, nobiletin contains 0.50 g/L in orange, 0.60 g/L in king tangerine, 0.40 g/L in clementine tangerine, and 1.50 g/L in tangerine peel oil. In citrus fruits, nobiletin content is estimated to range from 7 to 173 mg/kg of dry weight, and higher concentrations are found in unripe citrus fruits (3). In addition, the amount of nobiletin in the peels of the same citrus species grown in different geographical regions may vary. Nobiletin was detected at a concentration of 0.43 mg/g in dried orange peels collected from California, whereas orange peels collected from China exhibited higher levels of nobiletin at 7.79 mg/g (4).

Nobiletin possesses a notably lipophilic structure due to the presence of six methoxyl groups located at positions 5, 6, 7, and 8 on the A ring, as well as positions 3’ and 4’ on the B ring in its chemical structure (Figure 1). Many biological effects of nobiletin are associated with its lipophilicity (3). The ability of nobiletin to easily pass through the cell membrane, particularly the blood-brain barrier, and gain access to the central nervous system has been a focal point of investigation to prevent and treat various diseases, including neurodegenerative disorders, cardiovascular conditions, and cancer. These findings suggest that nobiletin holds promise as a potential novel pharmaceutical agent owing to its favorable impact on these medical conditions (1,5,6).

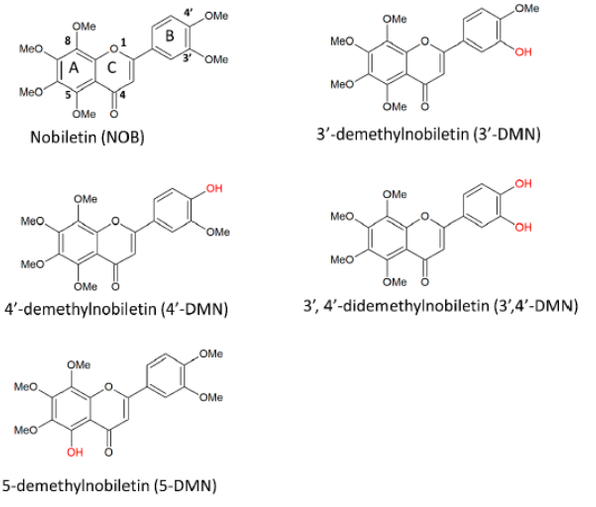

Figure 1. *Chemical structure of nobiletin and its compounds (adapted from source Goh JXH, et al., 2019.)*

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2. NOBILETIN METABOLISM

The bioactivities of a compound vary depending on its structure and metabolism (5). The molecular structure of the compound is one of the important factors affecting its absorption. Due to its lipophilic properties, nobiletin exhibits extensive distribution throughout the body. Nobiletin can be readily detected in organs such as the intestine, liver, stomach, brain, and kidney within four hours after administration, indicating its rapid distribution throughout the body (7). When nobiletin is administered by gastric intubation, it has been found to localize to the mucosa and muscularis of the digestive organs and is completely excreted within 24 hours of administration. The localization of nobiletin is attributed to its high hydrophobicity (6).

Polymethoxy flavones basically have two metabolic pathways: demethylation and conjugation (8). Bioinformatics analysis of nobiletin has unveiled a demethylation pathway leading to the generation of significant metabolites like mono-demethyl nobiletin (DMN). These include various forms such as 3',4',6-, or 7-DMN. Furthermore, di-demethylation processes can convert nobiletin into 3',4'-di-DMN, or 6,7-di-DMN (9). Following the ingestion of nobiletin, several metabolites are generated, with variations depending on the citrus plant source. Three prevalent nobiletin metabolites include 3'-DMN, 4'-DMN, and 3',4'-DMN (10). It has also been suggested that nobiletin may be converted to 5-DMN by gastric acid after oral consumption (7).

Nobiletin metabolites were found to be approximately 20 times more abundant than nobiletin, the main compound in the colonic mucosa. This result shows that nobiletin is immediately metabolized to its metabolites in the body (11). Nobiletin metabolism involves two phases: phase I and phase II metabolism. Cytochrome P450 is involved in phase I demethylation. The conversion of nobiletin to 3'-DMN involves the participation of Cytochrome P450 Family 1 Subfamily A Member 1 (CYP1A1), Cytochrome P450 Family 1 Subfamily A Member 2 (CYP1A2), Cytochrome P450 Family 1 Subfamily B Member 1 (CYP1B1), and Cytochrome P450 Family 3 Subfamily A Member 5 (CYP3A5) enzymes. However, the conversion of 3'-DMN to 3',4'-DMN is primarily catalyzed by CYP1A1 and CYP1A2 enzymes. Phase II metabolism of nobiletin takes place in the small intestine, where it undergoes sulfation and glucuronidation processes (7,10).

3. BIOAVAILABILITY OF NOBILETIN

The bioavailability of therapeutic compounds is a critical factor in their development for disease treatment (3). Comprehending the interactions between the substance and the human body offers opportunities for innovative approaches to addressing the issue, which necessitates the development of new formulations for the effective delivery of nobiletin for chemopreventive purposes (7). When it comes to oral administration, a key factor to take into account is the availability of the active substance. Hence, investigations into nobiletin's pharmacokinetic profile underscore the necessity for gaining a more profound comprehension of the absorption, metabolism, and elimination processes that influence bioavailability. A comprehensive understanding of these mechanisms plays a pivotal role in enhancing the precision of bioactivity prediction (12).

As mentioned in the metabolism section of nobiletin, the most important factor affecting the absorption of a compound is its molecular structure. Proper absorption of the compound is indicated by its solubility and permeability across physiological barriers. The lipophilic property of nobiletin helps it pass through the cell membrane easily. Nobiletin has been shown to have low water solubility (1-5 µg/mL) and minimal oral bioavailability (<1%), resulting in decreased therapeutic and biological activities (13,14).

Despite the proven ability of orally administered nobiletin to cross the blood-brain barrier and exhibit activity in the brain, it's important to note that orally ingested polymethoxyflavones typically exhibit low absorption rates (9). When nobiletin was given orally to rats at 50 mg/kg, the amount of nobiletin in the brain was found to be 3.6 mg/kg (15). In another study conducted with the same dose of nobiletin (>97% purity), it was found that the maximum concentrations in plasma and brain were recorded as 1.78 µg/ml and 4.20 µg/ml, respectively, one hour after administration (16).

Although the water solubility of nobiletin is low, the water solubility of its metabolites was found to be 2-3 times higher than the main compound. This observation may, in part, elucidate the enhanced activity of nobiletin derivatives when compared to the parent compound, as these derivatives possess an increased number of methoxy groups (7). Although the bioavailability of nobiletin is low, it is thought that a large part of its anticancer effect may be mediated by metabolites, so the biological activities of nobiletin metabolites should be further investigated.

As a result of the rapid metabolism and poor bioavailability of nobiletin, new strategies have been the subject of research to increase the bioavailability of nobiletin (10). Many applications such as using ionic liquids, encapsulation strategy, and nanoemulsion methods have been investigated to increase the bioavailability of nobiletin. In a study conducted to increase the bioavailability of nobiletin, it was found that transdermal application by dissolving nobiletin in an ionic liquid containing choline and geranic acid increased its bioavailability 20-fold compared to oral intake (14). As a result of the application of the encapsulation strategy in nobiletin, which is used to change the absorption and solubility of drugs, the release of nobiletin slowed down and the duration of its stay in the stomach and intestine was increased, increasing the absorption (17). Finally, one of the other methods used to increase the bioavailability of nobiletin is the use of nanotechnologies (18,19).

Nobiletin is derived from natural sources and is generally considered safe. Although most phytochemicals are considered nontoxic due to low bioavailability, toxicity

concerns may arise if systemic bioavailability is increased (20). In conclusion, although polymethoxyflavones exhibit promising bioactivities on cancer, research is based on in vitro and animal-based studies; Therefore, it is recommended that health benefits in humans be confirmed based on well-designed clinical studies (12).

4. RELATIONSHIP OF NOBILETIN WITH DISEASES

Nobiletin has demonstrated potential effectiveness in the treatment of certain diseases. The relationship of nobiletin with diseases and metabolic disorders is summarized in Figure 2 (1,5,7,10). Among the neuroprotective effects of nobiletin; are improvement in learning and memory impairment, improvement of ischemia-reperfusion injury, and reduction of dopaminergic neuron production. In the cardiovascular system, nobiletin improves metabolic syndrome, increases locomotor activity, and inhibits platelet aggregation. In addition, nobiletin can reduce insulin resistance, correct lipid metabolic disorders, down-regulate inflammatory and oxidative stress in the digestive system, inhibit osteoclastogenesis in the skeletal system, and reduce bone resorption by maintaining skeletal homeostasis. Among the anticancer properties of nobiletin, anti-angiogenesis, and anti-metastasis activities have been shown (5).

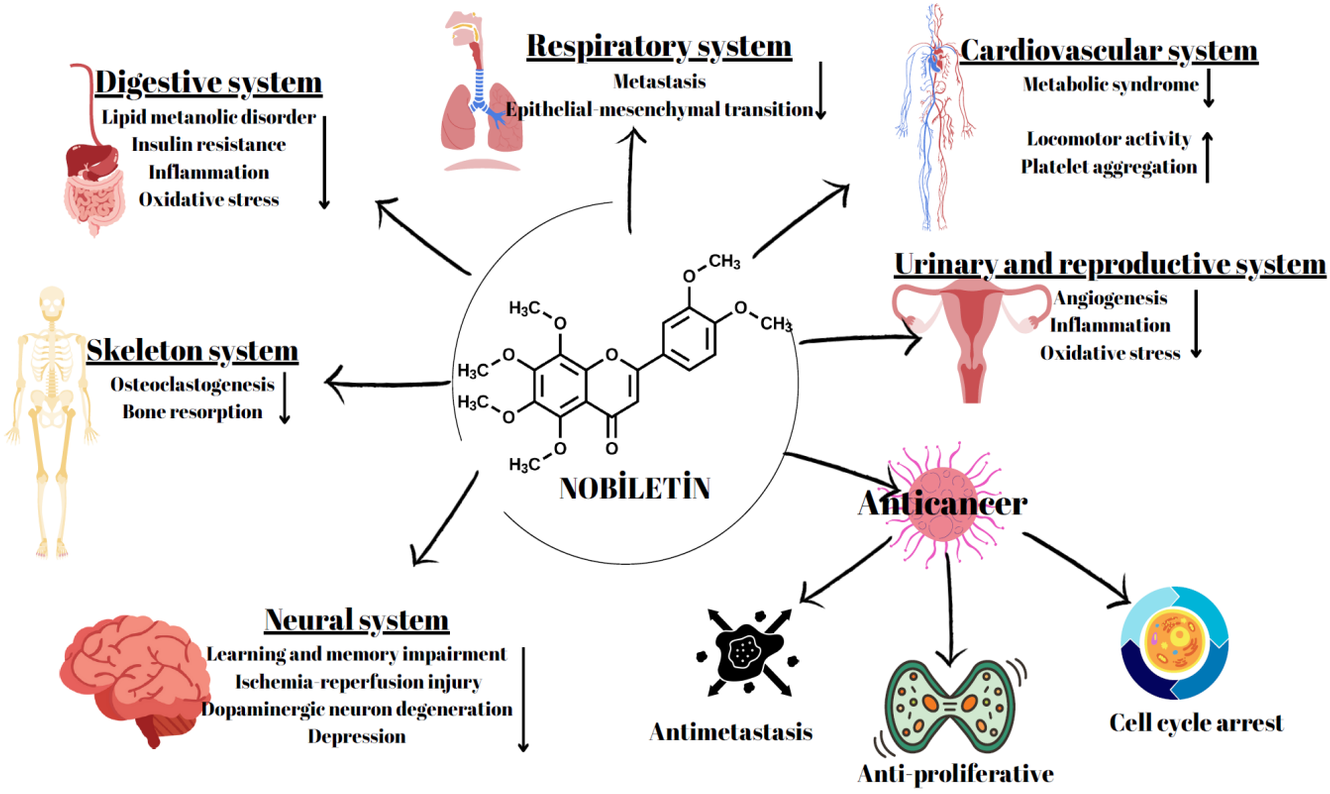

Figure 2. *Association of nobiletin with diseases and metabolic disorders (adapted from Huang H, et al., 2016)*

5. NOBILETIN AND CANCER

Citrus flavonoids have chemopreventive effects on various types of cancer. The anti-proliferative effectiveness of flavonoids depends on various factors, including the configuration and number of hydroxyl groups on the A and B rings of the flavonoid, the presence of methoxy groups, and their inherently low-polarity and planar molecular structure. Citrus flavonoids have attracted significant research attention due to their strong anti-inflammatory and chemopreventive properties, as well as their low toxicity in cellular and animal models (3).

Studies have examined the impact of nobiletin on various cancer types, including breast cancer (21-25), colorectal cancer (26,27), ovarian cancer (28-30), stomach cancer (31-33), lung cancer (34-37), liver cancer (38,39), prostate cancer (40-42), bladder cancer (43), thyroid cancer (44), nasopharyngeal cancer (45,46), kidney cancer (47,48), and bone cancer (49).

Nobiletin demonstrates inherent anti-cancer properties, and its derivatives have emerged as promising chemopreventive agents. Noteworthy derivatives renowned for their anti-cancer activity encompass 3'-DMN, 4'-DMN, 3',4'-DMN, and 5-DMN (7). Colonic metabolites of nobiletin are more potent than nobiletin in inhibiting cancer cell growth, arresting the

cell cycle, inducing apoptosis, and modulating key signaling proteins (11). While nobiletin metabolites, particularly 4'-DMN, and 3',4'-DMN, exhibit more potent anti-cancer and anti-inflammatory properties, it's worth noting that the conversion rate of nobiletin to these metabolites can be subject to variation (11,50).

Nobiletin and its derivatives can affect multiple pathways involved in cancer prognosis. Nobiletin demonstrates significant effects, encompassing cell cycle arrest in cancer cells, suppression of cell proliferation, promotion of apoptosis, inhibition of tumor development, attenuation of inflammatory responses, and limitation of angiogenesis (21,24,26,28,30,33,37,38,43).

5.1. Effects of Nobiletin in Signaling Pathways Playing a Role in Carcinogenesis

Signaling pathways are systems that control apoptosis, cell cycle, and cell growth (51). Alterations in signal transmission disrupt the regulation of cell proliferation and/or survival mechanisms. Consequently, oncogenic signal transduction actively contributes to processes such as tumor development, invasion, and metastasis (52). Signaling pathways altered in cancer affect many important cellular events such as apoptosis/cell cycle, chromatin modification, transcriptional modification, and DNA damage control. Basically, these pathways are; it is effective in processes parallel to the basic features of cancer such as cell proliferation, survival, metabolism, polarity, migration, differentiation, genomic instability, and tumor microenvironment (53).

Studies on nobiletin have shown its anti-carcinogenic effect by acting on different signaling pathways (Table 1), (53, 71). Nobiletin impacts various signaling pathways, including the nuclear factor erythroid 2-related factor 2 (Nrf2), Phosphoinositide-3 kinase (PI3K)/Akt, extracellular signal-regulated kinase (ERK), Myelocytomatosis (Myc), Wnt/β-catenin, transforming growth factor-beta (TGF-β), signal transducer and activator of transcription (STAT), and p53 pathways (10,39,49,54).

Table 1. *Signaling pathways in which nobiletin acts and cellular events in which the signaling pathway is involved*

Signal pathway	Cellular events in which the signaling pathway is involved
Nrf2	Oxidative stress response, cancer chemoresistance
PI3K	Cell proliferation, cell growth, cell survival, cellular metabolic changes, cell migration and polarity
Myc	Cell growth, proliferation, and apoptosis
Wnt	Cell proliferation, tissue homeostasis
ERK (MAP kinase)	Gene expression, cell division, cell viability, apoptosis, metabolism, differentiation, and motility
TGF-β	Cell proliferation and acquisition of stem/progenitor cell phenotype
p53	Cell survival, proliferation, senescence and apoptosis
STAT	Cell survival
JNK	Cell proliferation, apoptosis
Wnt/β-catenin (48)	Cell survival and proliferation, invasion
Src/FAK/STAT3 (24)	Angiogenesis
Cd36/Stat3/Nf-Kb (69)	Angiogenesis, migration, invasion
PARP-2/SIRT1/AMPK (45)	Growth inhibition and apoptosis
PI3K/Akt /mTOR (70)	Cell proliferation, apoptosis, angiogenesis

Akt: Protein kinase B, AMPK: AMP-activated protein kinase, Cd36: Differentiation cluster 36, ERK: Extracellular signal-regulated kinase, FAK: Focal adhesion kinase, JNK: Jun N-terminal kinase, MAP: Mitogen-activated protein, mTOR: Mechanistic target of rapamycin, Myc: Myelocytomatosis, Nf-Kb: Nuclear Factor kappa B, Nrf2: Nuclear factor erythroid 2 associated factor 2, p53: Tumor protein 53, PARP: Poly ADP ribose polymerase, PI3K: Phosphoinositide-3 kinase, SIRT1: Sirtuin 1, STAT: Signal converter and activator of transcription, TGF-β: Transforming Growth Factor Beta

5.2. Anti Proliferative Effect

Uncontrolled and unregulated cell proliferation is one of the most common features of cancer (26). Flavonoids have been reported to possess pharmacological properties that impede tumor progression by inhibiting both tumor cell proliferation and invasion (54).

In studies performed on hepatic, bladder, ovarian, thyroid, gastric, breast, nasopharyngeal, and renal cancer cells, it was found that nobiletin increases inhibition at varying rates depending on dose and time (25,28,31,38,43,44,47). When human ovarian cancer cells A2780 and OVCAR3 were incubated with nobiletin at doses ranging from 0-50 µM for 24 hours, the half-maximum inhibitory concentration (IC_{50}) was 35.31 and 34.85 µM, respectively, and had a strong effect on cell proliferation depending on the dose of nobiletin (30). Based on the results of treatments performed with 100 µl of nobiletin on MDA-MB-468, MCF-7, and SK-BR-3 breast cancer cells for 72 hours, the IC_{50} values were determined as 51.3 µM, 59.8 µM and 86.9 µM, respectively. After 168 hours, these values were determined as 20.3 µM, 39.6 µM, and 59.3 µM, respectively. IC_{50} values decreased as the incubation time with nobiletin increased (21).

Nobiletin exhibited an IC_{50} value of 23.82 ± 5.15 µg/ml in A549 lung cancer cells after 48 hours of exposure, demonstrating a dose-dependent and time-dependent inhibition of cell growth. In contrast, its anti-proliferative effect on the human umbilical vein endothelial cell line (ECV304) was less pronounced, with a 48-hour IC_{50} value of 157.78 ± 95.08 µg/ml (37). These results and other studies show that Nobiletin can show selectivity between cancer cells and healthy cells, and while reducing the viability of cancer cells, it causes less toxicity in healthy cells (37,44,55).

5.3. Effect on Cell Cycle and Apoptosis

Programmed cell death encompasses three distinct types, namely apoptosis, pyroptosis, and autophagy, which are executed by caspases, lysosomal proteases, and endonucleases (56). Apoptosis represents a natural form of cell death, triggered when multicellular organisms respond to internal or external stimuli. In contrast, autophagy represents a cellular process primarily dedicated to the degradation and recycling of intracellular components. Differing in both morphology and mechanisms from other modes of cell demise, pyroptosis is characterized as a pro-inflammatory form of cell death regulated by inflammation and caspase-1 activation (57). Apoptosis is regulated by the balance between antiapoptotic (such as B-cell lymphoma (Bcl)-2, Bcl-XL, Mcl-1) and proapoptotic proteins (such as Bcl-2-like protein 4 (Bax), Bcl2 cell death antagonist (Bad), caspase-3/-9) (47,58). Induction of apoptosis in cancer cells is an important approach to preventing and treating cancer (26). In apoptosis, three key proteins typically play pivotal roles: Bcl-2, Bax, and p53. The balance between Bcl-2 and Bax in the cell dictates the fate of the cell, determining whether it will survive or undergo apoptosis (37). Additionally, the activation of the p38 mitogen-activated protein kinase (MAPK) signal transduction pathway, triggered by the phosphorylation of p53, plays a crucial role in inducing apoptosis in cancer cells (25).

When 20 mg/L nobiletin was administered to liver cancer cells (SMMC-7721) for 48 hours, the cells were found to show signs of apoptosis. The observed morphological changes encompass cell shrinkage, the development of cytoplasmic vacuoles, disruption of the nuclear membrane, pycnosis, anisochromatin, and chromatin margination. Notably, cells treated with 80 mg/L of nobiletin exhibited heightened expression of caspase-3 (38).

Similarly, exposure of human bladder cancer cells to 60 µM of nobiletin led to increased DNA fragmentation, providing evidence of late apoptotic cell death induced by the treatment. This effect was associated with mitochondrial dysfunction, leading to the release of cytochrome C into the cytosol, activation of pro-apoptotic proteins, and suppression of anti-apoptotic proteins. Nobiletin-induced apoptosis was mediated through the modulation of endoplasmic reticulum stress via the PERK/eIF2/ATF4/CHOP pathway and downregulation of the PI3K/AKT/mTOR pathway (43). Furthermore, in SKOV3/TAX cells, nobiletin induced apoptosis by upregulating cleaved Caspase-9/-3 and poly ADP ribose polymerase (PARP). Additionally, it promoted apoptosis by inhibiting autophagic flow. Nobiletin activated the AKT signaling pathway, which played a role in autophagic degradation and apoptotic cell death (28).

Nobiletin also enhances increased reactive oxygen species (ROS) derived from damaged mitochondria, increased caspase 3 activity and PARP cleavage, and/or Beclin-1 triggers apoptosis by releasing mitochondrial cytochrome C into the cytosol to enhance autophagy by activating microtubule-associated protein 1A/1B-light chain 3B-II (LC3-II) and autophagy-related (ATG)5-ATG12 protein expression (30,33,45,56). In addition, nobiletin and its metabolites have effects such as induction of apoptosis and cell cycle arrest through the expression of p21, cyclin-dependent kinase (CDK), cyclin D1, CDK6, CDK4, Bax, and caspase (21,28,30,32,36,38).

Many cytotoxic agents have a cell cycle-arresting effect by acting on the G1, S, or G2/M phase (32). Treatment with 100 µM nobiletin provided inhibition of breast cancer cells through downregulation of ERK1/2, AKT, and the mechanical target of rapamycin (mTOR). Furthermore, there was an increase in the p21 protein in all cell lines, leading to the inhibition of Cyclin-D1, a critical regulator of the G0/G1 cell cycle checkpoint. This suggests that nobiletin arrests the cell cycle at the G0/G1 phase, resulting in reduced Cyclin-D1 levels (21). Furthermore, in the case of SMMC-7721 liver cancer cells, exposure to nobiletin at concentrations of 10, 20, and 40 mg/L for 48 hours led to an accumulation of cells in the G2/M phase. Following treatment with 40 mg/L nobiletin, the percentage of cells entering the G2/M phase increased to 15.13% and apoptosis rates increased to 31.73% (38). In addition, nobiletin induced G0/G1 phase arrest by suppressing growth and proliferation in SKOV3/TAX human ovarian cancer cells; it decreased the G2/M phase with the increase of p53 and p21 (28).

5.4. Effect on Metastasis

Tumor metastasis is a multifaceted process characterized by a sequence of alterations occurring at both intracellular and extracellular levels within cancer cells. As a result of the coordination of several signaling pathways, separation of tumor cells, motility, disruption of the extracellular matrix, invasion, migration, adhesion to endothelial cells, and regrowth at a distant site occur. The process of epithelial-mesenchymal transition is indeed considered a crucial factor in tumor metastasis (39,49). In the course of primary tumorigenesis, genetic and epigenetic modifications within tumor cells, coupled with alterations in the tumor microenvironment, promote the epithelial-mesenchymal transition. This transition enhances the propensity of cells to disseminate from the primary organ site, enter the bloodstream and lymphatic system, invade neighboring tissues, and ultimately establish secondary tumors in distant organs (59).

This can be attributed to the dysregulated control of various pathological processes related to tumor invasiveness and the activation of intracellular signaling molecules like mitogen-activated protein kinases and tyrosine kinases. (54).

Matrix metalloproteinase (MMP)-2 and MMP-9 are proteolytic enzymes responsible for the degradation and modification of the extracellular matrix. They also interact directly with cell surface molecules and facilitate the activation of the epithelial-mesenchymal transition process. Focal adhesion kinase, a non-receptor tyrosine kinase, is predominantly situated at sites of cell-matrix adhesions, where it functions as a central regulator of focal adhesions, thereby influencing cell adhesion and metastasis. Focal adhesion kinase is closely linked to the imbalance of E-cadherin during the epithelial-mesenchymal transition (35,49).

Studies have shown that nobiletin can regulate MMP expression in different cancer cells (35,39,46,48,49,54) (Figure 3).

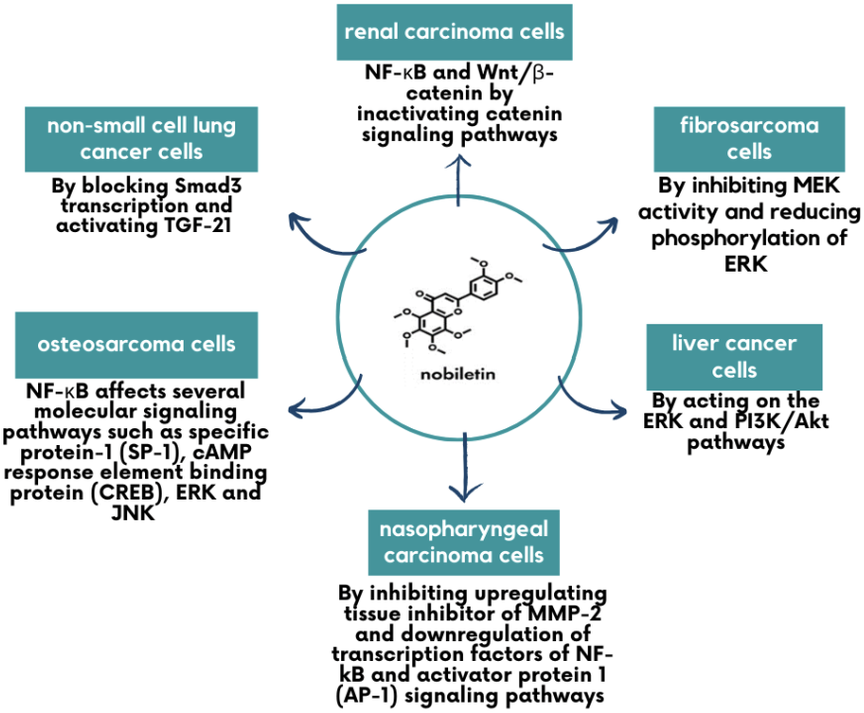

Figure 3. *Effects of nobiletin on MMP expression in different cancer cells. AKT: Protein kinase B, AP: Activator protein, cAMP: Cyclic adenosine monophosphate, CREB: cAMP response element binding protein, ERK: Extracellular regulated kinases, JNK: c-Jun N-terminal kinase, MEK: Mitogen-activated protein/extracellular signal-regulated kinase, MMP: Matrix metalloproteinase, NF-κB: Nuclear Factor kappa B, PI3K: Phosphoinositide-3 kinase, SP-1: Specificity protein 1, TGF: Transforming growth factor*

5.5. Drug Interactions and Use with Chemotherapy Agents

5.5.1. Drug Interactions with Nobiletin

Natural components found in foods can change the pharmacokinetics of drugs by affecting the activities of enzymes (cytochrome P450 (CYP)) that metabolize drugs or transporters (such as P-glycoprotein) that play a role in the absorption, distribution, metabolism, and excretion of drugs (60). Fruit juices are known to cause many food-drug interactions. It is reported that citrus juices, especially grapefruit juice, have more than 85 drug interactions because they inhibit CYP3A4, CYP1A2, and P-glycoprotein. Other juices may interact with medications; however, it is generally emphasized that there is not enough consumption to create interaction (61).

There is no clinical study yet on food-drug interaction for nobiletin. However, an in vitro study conducted to determine the drug interaction of nobiletin, sinensetin, and tangeretin compounds in clementine juice showed a possible inhibitory effect of nobiletin on CYP1A2 and CYP3A4. However, it has been reported that CYP3A4 inhibition is likely to be the result of additive or synergistic effects caused by various compounds. This research for nobiletin is the first report assessing interaction with CYP1A2 (62).

Predicting potential drug interactions caused by fruit juices is difficult due to the unknown number of phytochemicals found in fruit juice, unknown doses, and individual differences among individuals who consume them along with their medications (60). As stated in the literature, although there is evidence that some fruit juices may affect

drug distribution and therefore interact with drugs, there are not enough clinical studies to evaluate their role in drug interactions. Consequently, further research is needed to better understand the mechanisms behind nobiletin drug interactions.

5.5.2. Nobiletin Use with Chemotherapy Agents

New treatment strategies are needed to increase the therapeutic effects and reduce the side effects of drugs used in cancer treatment. For this reason, the potential effects of the combination of chemotherapy drugs and natural compounds have been frequently investigated recently (55,63). With the application of 4'-DMN (36 µM) or atorvastatin (18 µM) separately to HT-29 human colon cancer cells, growth inhibition was observed in these cells at rates of 25.89% and 20.89%, respectively. However, when these compounds were given in combination (7.2 µM atorvastatin with 14.4 µM 4'-DMN), a significant increase in the inhibition rate (53.84%) was found. This combined treatment triggered cellular apoptosis by synergistically elevating the expression levels of p53 and cleaved caspase-3, leading to stronger inhibition of cancer cells (26). Similarly, the combination therapy of paclitaxel and carboplatin with nobiletin demonstrated a synergistic preventive effect against the proliferation of A549 and H460 cell lines. Additionally, it was observed that as the ratios of nobiletin, paclitaxel, and carboplatin increased, the percentage of apoptotic cells decreased (36). In another combination therapy, the co-administration of sorafenib and nobiletin induced heightened apoptotic cell death and cell cycle arrest at the G0/G1 phase in PC-3 prostate cancer cells. This effect was characterized by the upregulation of Bax, Rb1, and CDKN1A (p21) levels compared to the treatment with nobiletin or sorafenib as individual agents (55).

Chemotherapy is often used to treat many types of cancer, but multi-drug resistance (MDR) often causes chemotherapy failure and kills the majority of patients (64). MDR phenotypes, both classical and non-classical, are accountable for the cellular mechanisms that lead to drug resistance. P-glycoprotein (P-gp) functions as an active component of the blood-brain barrier, serving as an ATP-dependent efflux pump. It is encoded by the MDR1 gene and plays a pivotal role in regulating the passage of diverse molecules across the blood-brain barrier (9,10). Overexpression of P-gp is associated with poor prognosis in many types of cancer and is frequently seen in clinically recurrent tumors (65). Increased expression of P-gp enhances the aggressive behavior and progression of cancer cells through its role in promoting the epithelial-mesenchymal transition. Anti-cancer drugs exert their inhibitory effects on the proliferation and survival of MDR cancer cells by targeting and suppressing the activity of P-gp (10). Nobiletin has been shown to work as an inhibitor of MDR-flux proteins by competing with chemotherapy drugs for the same P-gp binding site, thereby enabling in vitro enhancement of the efficacy of cancer chemotherapy (63). In the paclitaxel-resistant ovarian tumor cell line (A2780/T) and its parental line (A2780), nobiletin at a dose of 9 µM reversed the multidrug resistance of ovarian tumor cells of resistant A2780/T and rendered resistant cells 433 times more susceptible compared to A2780 cells (63).

A compound formed by the synthesis of nobiletin, 29d, exhibited 280 times greater water solubility than nobiletin and in a drug-resistant A549/T xenograft model, it has been shown to inhibit tumor growth more effectively than nobiletin and significantly increase the concentration of paclitaxine in tumors when administered together with paclitaxel (15 mg/kg) at a dose of 50 mg/kg (64). It has been shown that 0.5 µM adriamycin used in combination with 50 µM nobiletin in non-small cell lung cancer cells (parental and adriamycin-resistant A549 cells) has a good synergistic effect, and the combined treatment reduces tumor volume by 84%. It has been found that nobiletin suppresses the Akt/GSK3/β-catenin/MYCN signaling pathway and inhibits the expression of MDR1, resulting in increased accumulation of adriamycin. It has also been reported to potentiate apoptosis with increased caspase-3 activation, PARP cleavage, and sub-G1 accumulation compared to treatment with adriamycin alone (34).

6. RECOMMENDED DOSE-TOXICITY STUDIES

Many compounds from natural sources have become promising candidates for the development of a new drug because of their pharmacological benefits (1). Assessments of oral acute and chronic toxicity play a crucial role in evaluating the safety of pharmaceuticals and botanical substances for human consumption. It's worth noting that there is a scarcity of comprehensive toxicological data available regarding polymethoxy flavones (9).

Alterations in body weight are frequently employed as an indicator of potential toxicity. If any toxicity occurs, body weight is expected to be significantly reduced (7). In a research study examining the chemopreventive properties of nobiletin and its metabolites in colon cancer, it was found that after treatment of cells with 40 µM or oral ingestion of 0.05% concentration of nobiletin for 20 weeks, there was no significant change in body weight, liver weight, spleen appearance and behavior in rats and did not cause any adverse side effects (11). According to an in vitro study on prostate cancer cells, no toxic effect of nobiletin was observed in human umbilical vein endothelial cells at a concentration of 80 µM (66). In the study on lung cancer cells, mice were administered 100 mg, 200 mg, or 300 mg of nobiletin per body weight, and no signs of toxicity were observed by monitoring body weight in mice (37). Likewise, to assess the potential toxicity of nobiletin, doses of 200 and 400 mg/kg/day were administered to C57 mice via gastric lavage. The results of the study indicated that there were no substantial reductions in the mice's body weight following the nobiletin treatments. Furthermore, no noteworthy pathological alterations were detected in the heart, liver, kidney, spleen, or intestines of the mice (47).

In a research study, a liquid extract containing a high concentration of polymethoxy flavones obtained from orange peels, which included nobiletin (19.8 mg/g), sinensetin (17.4 mg/g), scutellarein tetramethyl ether (10.8 mg/g), and tangeretin (3.88 mg/g), showed similar IC_{50} values in both normal thyroid cells and anaplastic thyroid cancer cells. This extract demonstrated significant effectiveness in inhibiting the metabolic activity of cancer cells, but it also displayed some level of cytotoxicity towards normal cells (44). This study increases the importance of the application of nobiletin alone to selectively target cancer cells without toxic effects on healthy tissues. Taking into account both a mixture of polymethoxylated flavones containing 32.5% nobiletin and the in vivo mutagenic tests conducted with C. reticulata peel extract containing 50.3 mg/g nobiletin and 18.7 mg/g tangeretin, EFSA (European Food Safety Authority) has expressed the opinion that there are no concerns regarding genotoxicity related to polymethoxylated flavones (67). In a 90-day experimental study involving rats, a test substance characterized by its richness in nobiletin and tangeretin, containing 69.7 mg/g of nobiletin and 29.5 mg/g of tangeretin, was administered at varying doses of 54, 180, or 540 mg/kg body weight (bw) per day. Among male rats receiving the highest dose of 540 mg/kg bw per day, an occurrence of hyaline droplet nephropathy, a condition typically observed in adult male rats, was noted. However, this condition was not considered a relevant endpoint. No other adverse effects were observed throughout the study (68). The EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances Used in Animal Feed) identified NOAEL (No Observed Adverse Effect Level) values of 38 mg/kg bw per day for nobiletin (67). Due to differences between biological systems, certain doses may be beneficial for some people and harmful for others. Therefore, although the effects and mechanisms of nobiletin on various types of cancer have been demonstrated, further research is needed to develop individualized recommendations. In nobiletin studies, factors such as dose-effect relationship, dose interval, and time-effect relationship should be taken into consideration and appropriate models should be used to evaluate possible effects (20).

7. CONCLUSION AND RECOMMENDATIONS

In recent times, there has been a growing interest in utilizing plants and fruits for the treatment of various diseases. This inclination is rooted in the existence of advantageous natural compounds within plants and fruits, along with their metabolites. These compounds, upon absorption into the body, engender health-promoting benefits. The discernment, extraction, and refinement of these natural chemicals hold significant importance, particularly in the context of addressing diseases, notably human malignancies. A multitude of research endeavors have been dedicated to exploring the potential of natural plant-derived compounds in the treatment of cancer.

As nobiletin is derived from citrus peels, it is naturally abundant and demonstrates that its utilization in disease treatment represents a cost-effective approach. Both itself and its metabolites have important anti-cancer properties. It prevents cancer cell growth, proliferation, and metastasis by affecting important signaling pathways. It has been shown that nobiletin targets only cancerous cells and has little effect on healthy cells. However, although there are some suggestions that lower doses may affect cancer, it is necessary to increase the bioavailability of nobiletin. It is thought that it is not only limited to cancer but may have the potential to be a new drug due to its positive effects on other diseases. Despite its natural origin and the prevailing perception of safety, nobiletin's toxicity remains insufficiently investigated across various cell types and tissues. Acute and chronic toxicity studies are needed for oral consumption.

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Clinical and Experimental Health Sciences

Disease Management in Individuals with Phenylketonuria

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ABSTRACT

Phenylketonuria (PKU), one of the most common metabolic diseases, is a recessive, congenital and hereditary disease that occurs with the absence or deficiency of the enzyme called phenylalanine hydroxylase, which converts phenylalanine to tyrosine. Therefore, the aim of this review was to discuss the disease management in individuals with phenylketonuria. As a result of the inability to metabolize phenylalanine amino acid in patients with PKU, the level of phenylalanine increases in the systemic circulation and brain, which may lead to neurocognitive activity and psychosocial dysfunctions and various disorders. If infants with PKU, who are indistinguishable from healthy babies at first birth, are fed like normal babies, phenylalanine accumulates in the body and symptoms occur and gradually worsen. To keep the blood sugar phenylalanine level at the desired range various medical treatment methods (pharmacological treatment and gene therapy), especially nutritional therapy, can be preferred. However, alternative treatment methods should be carried out in combination with diet therapy. Some dietary restrictions are maintained for life, while patients follow a strict diet in dietary protein intake to prevent high plasma phenylalanine levels and neurological damage. In this review, the definition, classification and phenylalanine-restricted diet treatments of phenylketonuria are discussed.

Keywords: Phenylketonuria, phenylalanine restriction, phenylalanine hycroxylase, enzyme deficiency, nutritional therapy

1. INTRODUCTION

Phenylketonuria (PKU) is an autosomal, recessive disorder associated with the deficiency or absence of hepatic phenylalanine hydroxylase (PAH) enzyme, which catalyzes the conversion of phenylalanine (Phe) to tyrosine as a result of a genetic defect in the PAH (1,2). The absence or deficiency of a functional PAH enzyme that catalyzes the hydroxylation of phenylalanine to tyrosine causes increased production of phenylketone bodies and thus increased Phe levels. These untreated Phe accumulations can lead to serious physiological, neurological and mental disabilities (3,4). While intellectual disability, hyperactive behaviors and autistic features can be seen, it can also lead to significant delays in developmental milestones. If treatment is started in the first 4 weeks of life, babies with PKU can be evaluated as normal babies and can lead their lives independently, provided that metabolic controls are made. Diagnosis and modern treatment of PKU were made by three key findings (5):

1. In the 1930s, Asbjorn Folling identified high phenylalanine levels (hyperphenylalaninemia) in the blood as the cause of neuropsychological disorders.
2. Horst Bickel recommended the treatment of a low phenylalanine diet to treat Phenylketonuria in the 1950s.
3. In the 1960s, Robert Guthrie introduced the Guthrie Test, which is suitable for mass screening for hyperphenylalaninemia, which is used in the diagnosis of PKU in many countries around the world today.

In metabolic diseases, metabolites that can cause toxic effects due to the absence or deficiency of enzymes in the synthesis or catabolic pathways of macromolecules such as carbohydrates, proteins and fats accumulate, or the final product cannot be produced (6). PAH is an enzyme produced in the liver that converts phenylalanine to tyrosine and its cofactor is tetrahydrobiopterin (BH4). Phe exists in nature as D and L enantiomers, and especially L-Phe is an essential amino acid for protein synthesis (7). This amino acid, which

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is indispensable for the continuity of protein production and homeostasis for human metabolism, is converted to tyrosine by being metabolized in the liver by PAH when it is not included in protein production. This essential amino acid, which is obtained not only by diet and protease activity, takes part in protein synthesis and is converted to tyrosine, but also is important for the synthesis of substances such as dopamine, norepinephrine and melanin. The main metabolic pathway of Phe refers to its hydroxylation to tyrosine by the enzyme PAH found in the liver and kidney (4,8).

Studies and data presented confuse clinical symptoms in untreated patients with blood Phe levels of 360 – 600 µmol/L, regardless of central nervous system damage (9). In addition to the normal results, low levels of neurocognitive disorders were also detected. Although it does not draw a definite limit, treatment is not recommended in infants with blood Phe concentration of 120 – 360 µmol/L, and it is recommended to be followed for at least the first 2 years of their lives. For this reason, trying to keep the blood phenylalanine level within these ranges is the first goal of all treatment methods. In Table 1, the classification of PKU based on blood Phe levels was demonstrated (10,11).

Table 1. *Blood phenylalanine values and daily tolerance classification for 5-year-old PKU patients (10,11).*

PKU Types	Blood Phenylalanine Levels	Daily Tolerable Phe Values
Classic Degree PKU	1200 µmol/L	20 mg/kg and below
Intermediate PKU	900 µmol/L – 1200 µmol/L	20 – 25 mg/kg
Mild PKU	900 µmol/L	More than 25 – 50 mg/kg
Mild Hyperphenylalaninemia	600 µmol/L	Nutritional therapy may not be needed.

PKU infants, who appear normal at newborns, will only show nonspecific symptoms such as feeding difficulties, hypotonia, and the characteristic musty odor due to phenylacetic acid ingestion if not treated in the first weeks of life (12). The retardation in psychomotor development, which is the most important and constant feature in phenylketonuria, progresses continuously and occurs in the 4th-6th phase of life. It starts to appear after a month. This period of neurocognitive damage, in which seizures can also begin, reaches its peak around the age of 3-4 years. They lag behind their peers in acquiring basic skills such as sitting, walking and speaking. Babies with PKU who are indifferent to their environment may also exhibit hyperkinetic and autistic behaviors (13).

1.1. Aim of the Review

In this review, it was aimed to discuss the disease management in individuals with phenylketonuria including the phenylalanine-restricted diet treatments of phenylketonuria.

2. PKU TREATMENT METHODS

For most individuals who begin treatment shortly after birth, individuals generally fall within the normal range of general cognitive ability (14). Treatment of phenylketonuria should be initiated as soon as possible (15). This shortest possible period is usually the first 2 weeks of life, and during these first 2 weeks blood Phe values should be suitable for initiation of therapy (16). It is aimed to reduce blood Phe levels to the desired range as soon as possible in babies who are diagnosed and treated. In the first place, a Phe-restricted diet is started depending on the blood Phe levels and Phe is removed from the diet until the Phe values fall into the desired range (17). Breastfeeding is usually possible with medical support (18). It is possible to start treatment early with diagnosis and follow-up tests. Communication between families and primary health care services and access to specialist physicians are very important in this process. Although many treatment centers in North America starting treatment at blood Phe levels of 360 µmol/L and above, the accepted opinion in Turkey and most other countries is that infants whose blood Phe levels exceed 600 µmol/L should be start the treatment. In addition, clinical outcomes for untreated infants with blood Phe values in the 360 – 600 µmol/L range are mixed. Clinical evaluations and consultations with families suggest that infants with Phe values >360 µmol/L are at risk from a neuropsychiatric point of view and should begin the treatment. Although no negative threshold is specified for blood phenylalanine levels, it is thought that infants with phenylalanine levels of 120-360 µmol/L do not need treatment but should be monitored for at least the first 2 years of life. At the same time, blood Phe monitoring should be performed annually or biennially in subsequent evaluations so that Phe levels do not shift to higher levels (15). In Table 1, blood Phe concentration control and clinical monitoring frequency in PKU are given.

2.1. Medical Nutrition Therapy

As with all congenital metabolic diseases, medical nutrition therapy has a great role in the management of PKU, and medical nutrition therapy is a long process that must be continued throughout life (19). The basic principle of diet therapy is to restrict and remove foods containing the amino acid phenylalanine, which cannot be converted to tyrosine due to the absence of PAH, and to support the body's need for tyrosine amino acid (20). The main aim of the treatment of PKU, especially nutritional therapy, is to keep blood Phe levels at desired, reliable levels (21). Principles of nutritional therapy have 3 goals to be followed:

1. Natural protein/phenylalanine intake should be strictly monitored and excessive Phe accumulation in the blood and brain should be prevented (22).
2. Instead of natural proteins eliminated from the diet, synthetic proteins, amino acid mix supplements or safe proteins called protein substitutes should be used (23).

3. It is important to ensure the continuity of the normal growth and development process along with nutrition. This is only possible with a balanced distribution of all nutrients and energy in the diet. At this stage, vitamin – mineral supplements that play a role in growth and development are added to protein substitutes or given separately (24).

2.1.1. Foods to Avoid on a Low Phe Diet

In most individuals with phenylketonuria, natural protein intake and phenylalanine are limited to 25% or less of normal to keep blood Phe concentrations within the target Phe ranges of the European PKU Guidelines (25). The amount of Phe allowed in PKU, which is a congenital metabolic disease, is quite low compared to healthy individuals. For this reason, all protein-containing foods are given in a limited way (11). It is necessary to avoid high-protein foods such as those listed below or to limit high-protein foods (1,6):

- animal foods such as meat, chicken, fish
- egg
- cheeses made from animal milk such as cow, goat or sheep
- nuts, seeds, quinoa, wheat, oats, rye, barley
- meat substitutes made from fungal protein
- soy, tempeh, pulses/lentils
- plant algae such as gelatin and spirulina
- sweeteners (aspartame)

2.1.2. Aspartame Content of Food and Beverages

Aspartame is an artificial sweetener with the food additive code E951 and 50% is converted to the free phenylalanine molecule (26). For this reason, it should not be included in low Phe diets. Aspartame is often added to soft drinks, chewing gums, desserts, jellies, and candies. Although it mentions that it contains aspartame on food labels, the amount is often not specified (1).

2.1.3. Foods Containing Phenylalanine

Phenylalanine is an essential amino acid found in natural proteins (14). Different foods contain different amounts of phenylalanine. Meat, chicken, fish, eggs and milk from animal proteins as well as wheat flour and breakfast cereals, which are among the cereal protein sources, 1 g protein amount generally enters our body as 50 mg phenylalanine. Animal and cereal proteins contain about 5% of the amino acid phenylalanine. This situation leads to the conclusion that food protein labeling can be done without knowing the phenylalanine content of foods. In addition to animal and cereal proteins, fruits and vegetables have a variable phenylalanine content of 20–40 mg per 1 g of protein (27). Just like animal and cereal proteins, the phenylalanine content of fruits and vegetables cannot be accurately calculated in this way from a nutrient analysis label on a package/container that declares protein content alone. Looking at the protein content alone, it can be assumed that these foods have a high phenylalanine content. There are vegetables that are exceptionally high in phenylalanine/protein. These are: spinach, peas, seaweed, cabbage and corn (25).

Phe intolerance is the amount of phenylalanine that can be consumed by an individual with PKU while keeping blood Phe values within the target treatment range (14). The American College of Medical Genetics and Genomics guidelines on PKU recommend maintaining blood phenylalanine levels between 120–360 µmol/L in all patients (17). The amount of dietary phenylalanine tolerated, based on the severity of PKU for each individual patient, as well as depending on the dose, compliance, and daily distribution of protein substitutes (20). Medication, Pegvaliase (used for patients ≥18 years of age) and sapropterin are also part of the treatment (17, 28). In addition, conditions such as pregnancy, growth and development during the disease are affected in the catabolic state. Most nutritional therapy patients tolerate less than 500 mg/day Phe. Patients sensitive to sapropterin are expected to at least double their tolerance to phenylalanine or be able to tolerate the safe protein intake defined by the WHO/FAO/UNU (20).

Control of blood Phe value by nutritional therapy requires a low-protein diet that provides limited but necessary Phe intake, together with natural and specially produced low-protein food sources (29). Diets low in protein lead to insufficient intake of not only Phe but also other amino acids. This leads to the conclusion that the diet should be supplemented with protein substitutes (amino acids). Foods made with low Phe are universally available and provide an important dietary source of Phe (30). For this reason, it is very difficult to control blood Phe values without specially produced foods that are poor in Phe amino acids (29).

2.1.4. Intake of Dietary Free Nutrients

Foods that are free in the diet can be defined as foods with a Phe value that is allowable without being measured (30). They are both naturally occurring and have very low Phe values. Although free foods show some variation in different treatment centers in different countries, some free foods that basically all treatment centers agree can be itemized as follows oils, some candies, honey, jam, spices, and apple juice (31).

2.2. Medical Nutrition Therapy of Babies

Infants with blood Phe >360 µmol/L are treated with a diet low in phenylalanine (32). Protein intake is tried to be controlled by giving a small amount of breast milk and a standard infant formula along with infant formula that does not contain Phe (25).

Some studies have shown that acceptable blood Phe values, growth and weight gain can be achieved when a Phe-free infant formula and breastfeeding are combined (33). Breastfeeding is advantageous in many ways compared to standard formula. These advantages are as follows:a)

a) It has a low Phe value of 46 mg in 100 mL and contains long chain polyunsaturated fatty acids.

b) Breastfeeding helps to establish a strong mother-baby bond.

c) Breastfeeding includes the mother in the feeding process and gives her control.

d) Breastfeeding is a process that takes place in line with the baby's demand and reduces the number of bottle feedings.

Breastfeeding of infants with phenylketonuria is known to be relatively beneficial (30). Breast milk is low in Phe and is also a good source of BH4. In the long term, some studies have shown that breastfed babies are healthier than formula fed babies. The recommended and widely used method of breastfeeding is based on the principle of giving Phe-free infant formula before each feed. This situation indirectly reduces the baby's appetite and the desire to suck. Thus, the amount of breast milk taken decreases and Phe intake decreases (1).

2.3. Eating Behavior in Young Children with PKU

Nutritional problems are common in young children with PKU. The problems at the root of these problems are limited variety of food consumption and loss of appetite (20). Other causes of nutritional problems are:

- Energy content of the protein substitutes used: Some of the frequently used protein substitutes help to obtain additional energy from carbohydrates and fat (20). They provide up to 25% of the energy needs of children aged 1-3 years. Therefore, parents have unrealistic expectations about how much food their children should eat and may force their children to eat even though they are not hungry (34).
- Refusal of the nutrients provided by the Phe allowance: Parents may particularly insist on feeding their children all the foods provided by the Phe allowance (20). This leads children to repeated food refusal behavior.
- Preparing special meals for children at mealtimes: Families preparing two kinds of meals at mealtimes or giving meals to children at different times can have adverse effects on the nutrition of children with PKU and cause children to acquire isolated eating behavior (35).
- Difficulty in consuming protein substitutes: After a while, negative attitudes such as retching, vomiting and deliberately spilling products can be seen when consuming protein substitutes in young children (35).
- Food neophobia: It is a common behavior in childhood in general. It is also seen at high rates in children with PKU (36). Children prefer familiar foods than experiencing new ones. This is due to reasons such as not being exposed to various nutrients during the weaning period, and poor associations with protein replacement (35).

3. PKU AND CHANGES IN BODY METABOLISM

Although the pathophysiological mechanisms of the destruction of brain function found in patients with PKU are not yet clearly understood, there is ample evidence of metabolic changes in studies in both patients and animal models (37). Such changes include energy metabolism disorder, oxidative stress, damage to lipid and protein metabolism, and disruption of calcium homeostasis and neurotransmitter synthesis in the brain (20).

3.1. Neurotransmitter Metabolism

Neurochemical and behavioral studies have shown that animals fed diets rich in Phe have reduced brain serotonin levels and impaired certain problem-solving abilities (38). Recently, it has also been noticed that these patients are more susceptible to neurological manifestations caused by cerebral dopamine deficiency, such as Parkinsonism (39). Various studies have also shown that high Phe levels are associated with decreased dopamine, serotonin and norepinephrine levels in human and mouse PKU. The decrease in the levels of these neurotransmitters was thought to be related to the effect of high Phe concentration on amino acids transported through the blood-brain barrier (BBB) or on enzymes involved in neurotransmitter synthesis (40). It is important to emphasize that the large neutral amino acid (LNAA) transporter has a high affinity for Phe, competing with other amino acids to cross the BBB, ultimately reducing the amount of Trp and Tyr available for neurotransmitter synthesis (37).

3.2. Oxidative Stress

Oxidative stress is defined as the imbalance between the production of reactive oxygen/nitrogen molecules and the antioxidant system (41). This imbalance can cause oxidative damage to proteins, lipids or DNA. Including oxidative stress, it has been also associated with the pathophysiology of many neurodegenerative diseases such as Parkinson's and Alzheimer's diseases, epilepsy, and demyelination. In recent years, oxidative damage to macromolecules has been the subject of research in animal models of HPA and biological samples from PKU patients (39). It has been found that high Phe concentrations in patients with PKU are associated with DNA, protein and lipid damage as well as decreased antioxidant defense.

3.3. Protein Metabolism

Blocking and inhibiting the neutral amino acid transporter by Phe negatively affects protein synthesis (37). Because amino

acids, which are essential for the production of neutral amino acids, are not found in sufficient quantities. These patients have low levels of IgG and IgA, and a decrease in the production of antioxidant enzymes was also seen. This was associated with more cellular oxidative stress and therefore less protein production (20).

3.4. Lipid Metabolism

A study of lipid metabolism in PKU demonstrated impaired lipid metabolism in PKU, which has the potential to cooperate with hypomyelination found in patients (42). It showed that phenylketonuria patients have altered serum lipoprotein levels, including low total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), and apolipoprotein AI/A-II levels (39).

3.5. Lipid Metabolism

Brain and energy metabolism changes play an important role in the pathophysiology of many congenital metabolic diseases (39). Related to this condition, impaired energy metabolism has been reported in HPA animal models and patients (42).

3.6. Calcium Metabolism

Calcium balance is very important for brain functions and has been the subject of studies (39). One of the types of metabolism that is impaired with PKU is calcium metabolism. In this context, parathyroid hormone, osteocalcin, and dehydrocholecalciferol, which are responsible for regulating calcium metabolism, were found to be increased, while calcitonin was found to be decreased in serum samples of infants with PKU. Studies have found that Phe can change the intracellular calcium concentration and affect the activity of the calcium ATPase enzyme in neurons (37). For this reason, it has been shown that Phe affects both the intracellular calcium concentration and the activity of the calcium ATPase enzyme in neurons, as the reason for the elevation of dihydroxycholecalciferol, parathormone (PTH) and osteocalcin levels in PKU patients. As a result, it was found that calcitonin hormone decreased in blood concentration controls due to these negative effects. As predicted, an increase at the levels of these hormones causes a decrease in bone density whereas the serum calcium value increases. Therefore, it was concluded that it may be related to neuropathological conditions together with PKU (39).

4. PKU COMORBIDITIES

There are not many studies on PKU comorbidities in PKU patients, for whom diet therapy is considered the gold standard, except for diseases caused by the diet brought on by a restrictive diet (14). Risk factors that may occur due to nutritional deficiencies can be listed as follows (32):

- Brain development
- Mental health disorders
- Diabetes and cardiovascular risks
- Kidney ailments
- Bone health
- Sarcopenia and fragility
- Gastrointestinal problems
- Inflammation and immune system

4.1. Brain Development

Although it is not known exactly how PKU affects brain functions, several different causes are discussed (43). High Phe levels in the blood can have a direct toxic effect on the myelin sheath, which is responsible for increasing the conduction velocity by wrapping the axons in the brain cells. In addition to all these, absence or limited PAH activity may reduce the amount of important neurotransmitter substances such as serotonin and dopamine (44). Because dopamine is synthesized from tyrosine and competes for tyrosine in the body (45). Phe competes with essential amino acids such as tryptophan, which is responsible for the synthesis of the hormone serotonin. Finally, high blood Phe levels are thought to inhibit the activities of tyrosine hydroxylase and tryptophan hydroxylase enzymes that function in the synthesis of dopamine and serotonin, respectively. Individuals with PKU may experience cognitive deficits due to minor neurological disorders even when treatment is initiated (46). These may result from demyelination due to damage to the myelin sheath in the central system and disruption in the synthesis of neurotransmitter substances. These disruptions in neuropathophysiology have been associated with impairments that should be seen in the natural aging process but seen at younger ages.

4.2. Mental Health Disorders

With phenylketonuria newborn screening, babies with PKU can be identified and treatment can be started immediately after birth (47). This treatment is a Phe-restricted diet therapy, which is widely used and has good results. Studies have shown that patients with PKU have lower IQ and lower cognitive functions than healthy individuals, despite early initiation of diet therapy (48). Although the most severe symptoms of the disease are tried to be eliminated by treating with diet, it is seen that individuals with PKU who start Phe-restricted diet therapy in the early period and are treated continuously have psychiatric problems such as attention deficits, behavioral and emotional problems (47). These psychiatric problems are thought to be due to fluctuations in blood Phe levels during the treatment process. High blood Phe levels can lead to both acute and chronic neuropsychiatric problems (49). While impairment in psychiatric, behavioral and neurocognitive functions may be due to the emotional burden of having a chronic disorder, the main consideration is the time, duration and intensity of Phe

exposure. In a meta-analysis study investigating the effect of high blood Phe levels on neuropsychiatric symptoms, it was stated that inattention, hyperactivity, depression, and anxiety symptoms were higher than the general population estimates. In addition to all these symptoms, which may be caused by disorders in neurotransmitter synthesis, discomfort from socializing, low self-perception and decreased positive emotions were included in the studies (48). Table 2 shows that untreated individuals and individuals who started treatment at different times may show similar symptoms of neuropsychological disorders (14).

Table 2. *Psychological disorders in children, adolescents and adults with PKU (14,49).*

Untreated PKU	Children and adolescents who start treatment early	Adults treated early
Psychological symptoms	Attention problems	State of depression
Autistic behaviors	School problems	Social isolation/isolating oneself from society
Hyperactivity	Low achievement motivation	Generalized anxiety
Aggression	Low self-esteem	Lack of social maturity behavior
Anxiety	Decrease in social skills	Decrease in positive emotions
State of depression	Difficulty with autonomy	Low self-esteem
Social relationships impaired by intellectual disability	No data	Lack of autonomy

4.3. Diabetes and Cardiovascular Risks

The use of a Phe-restricted diet for the treatment of phenylketonuria leads patients to prefer foods containing more carbohydrates and less fat (50). It is stated that individuals who comply with the diet are vulnerable to the risk of developing diabetes, cardiovascular diseases and metabolic syndrome (32).

4.4. Kidney Ailments

As with diabetes and cardiovascular diseases, kidney disorders can also be diet-related. Since there is high Phe exposure when trying to take protein naturally, these patients are helped by synthetic amino acids that do not contain Phe (51). Since this situation can bring about high protein intake, kidney function disorders can also be encountered (32).

4.5. Bone Health

High Phe concentration in the blood disrupts the balance between bone formation and resorption (52). Although the relationship between bone mineral density and high Phe levels is not clear, dietary deficiencies and genetic factors are also effective on bone health, as well as restriction of protein intake (14).

4.6. Sarcopenia and Fragility

Sarcopenia is a progressive and common skeletal disorder (50). Although sarcopenia, also called muscle insufficiency, may occur in advanced ages, it can also occur in the early stages. It increases the risk of falls and fractures for human health and may interfere with life activities. Preservation of muscle mass and prevention of loss of strength through adequate dietary protein intake are important for sarcopenia. However, decreased natural protein intake, oxidative stress, and vitamin D deficiency are risk factors for sarcopenia in individuals with PKU (53,54).

4.7. Gastrointestinal Problems

Although there are no studies showing a direct link between gastrointestinal pathology and PKU, it is thought that a Phe-restricted diet may cause some digestive system problems (50). Conditions such as a low-fiber diet, the use of protein substitutes with high osmolality and acidity can cause constipation and indigestion (32).

4.8. Inflammation and the Immune System

Most studies on phenylketonuria and the immune system have reported increased concentrations of pro-inflammatory factors and decreased antibody (IgG and IgA) concentrations in the blood plasma of children and adults (≤ 22 years) with PKU (50,55).

5. MATERNAL PKU

When women with PKU who become pregnant without their blood Phe values are kept under control, there are undesirable risks of this situation when they stop their diet (56). It is known that more than 90% of babies born after conception in this way have mental retardation. Congenital heart diseases and microcephaly can also be seen in these babies, who usually have low birth weight (57). The reason for all these bad situations that may occur is due to the teratogenic effect of the mother's high blood Phe level (56). Maternal PKU risks can be prevented to a large extent if diet is controlled before pregnancy and if control is not left during pregnancy (57). However, the fetal morbidity is associated with HPA in individuals with PKU, blood Phe concentrations should be checked more frequently in mothers with PKU (7). Affected mothers require a pre-pregnancy diet with a Phe value of 100-360 µmol/L. At the same time, it is important to monitor blood Phe values weekly and keep them at reliable levels (56).

6. PKU AND SPORTS

Since there is little information on how a diet low in phenylalanine affects sports performance, the nutrients are essential for general sports nutrition and therefore adapted to PKU as follows (58):

- A diet rich in carbohydrates should be maintained. Carbohydrate-rich foods should be recommended before and after exercise, and low-fat, low-protein foods, such as low-protein pastas, should be preferred.
- Since sports drinks (without aspartame) will contribute to carbohydrate loading, the target carbohydrate intake should be 30–60 g/h for 1–2.5 hours of endurance exercise.
- Great attention should be paid to the hydration status.
- A dose of protein substitute should be taken during the recovery phase immediately after exercise. Approximately 20 g protein equivalent of protein replacement should be taken after exercise.

In addition, there is research suggesting that brief acute exercise does not affect blood Phe concentrations, but the effect of endurance exercise has not been studied (59).

7. WEIGHT MANAGEMENT IN PKU INDIVIDUALS

Being overweight is not uncommon in individuals with PKU. However, trying to lose weight quickly can lead to catabolism and loss of control of blood Phe values. Healthy weight loss behavior can be gained by making the following dietary changes (1):

- Sugary foods should be replaced by foods without sweetener/aspartame.
- Drink plenty of water.
- The carbohydrate/energy content of the protein substitute must be constantly checked. It should be ensured that ready-made medicines available as powders are prepared with only water.
- Special milks with low protein containing ≥ 60 kcal/100 mL should be replaced with plant milks with lower energy.
- Food should be eaten in smaller portions at mealtimes, but still be encouraged to eat 3 meals a day using low-protein pasta, rice and low-protein bread with a lower fat content.
- Candies, sweets, chips, vegetable chips, jams, honey, low protein chocolates or biscuits should be limited.
- A minimum amount of oil (preferably olive oil) or 'light' oil cooking sprays should be preferred while cooking.
- If possible, try to do physical activity such as light-paced walking for 30 to 45 minutes every day.

Table 3 lists foods and beverages that patients with PKU can consume safely and should not consume (14,27).

Table 3. *Foods and beverages that patients with PKU can consume safely and should not consume (14,27).*

Things not to be eaten	Freely consumable foods	Foods that should be consumed in limited amounts	Medical foods
Milk and dairy products (milk, yoghurt, buttermilk, tzatziki, cheese and its varieties, all foods made with them)	Corn starch	Vegetables	Low protein drink
Egg	Plain Turkish delight, plain hard candy	Fruits	Low protein pudding
Meat and meat products (red meat, chicken, fish, turkey, salami, sausage, sausage, pastrami, roasted meat, shellfish, mussels, etc.)	tea, linden, sage	floury foods	Low protein cereal
Internal organs of the animal (brain, liver, kidney, etc.)	Oil	Olives	Low protein pasta
Regular bread (wheat, rye, oat, corn breads)	Tea sugar	Margarine	Low protein bread
Dried nuts (hazelnuts, peanuts, roasted chickpeas, seed varieties, almonds, walnuts)	Apple juice, compote water	Butter	Low protein cookies
Dried legumes (dry beans, chickpeas, lentils, broad beans, soybeans, dried kidney beans)	Soda, cola drinks	Honey, molasses	Special gel for yoghurt making, milk sugar for yoghurt making
Ready-made food (crackers, biscuits, cakes, cookies, pastries and all prohibited foods)	No data	No data	Low phenylalanine cheese
All drinks, gums and foods containing aspartame and phenylalanine.	No data	No data	Low protein semolina

8. CONCLUSION

Some nutritional deficiencies in PKU individuals can cause different comorbidity. Breastfeeding has advantageous in many ways compared to standard formula. On the other hand, the results of the studies show that basic recommendations for a balanced and healthy life are possible for individuals with PKU. The protein substitute and phenylalanine dose to be taken should be divided into certain times of the day and included in the diet. Fruits and vegetables containing 75 mg or less Phe per 100 grams should be preferred. Care should be taken to consume 5 servings in total, with at least 1 serving at

each meal. Foods such as low protein bread and pasta can be preferred in most meals in order to close the calorie deficit, provide satiety and create variety. It should not be neglected to have blood Phe levels checked regularly, at the same times and on an empty stomach.

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Clinical and Experimental Health Sciences

Human Biting Plant Bug *Campyloneura virgula* (Hemiptera: *Miridae*): First Case Report in Türkiye

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ABSTRACT

Campyloneura virgula is a plant bug found in many parts of the world. While information about the human biting of this insect is available in the literature, this is the first case report of such an incident in Türkiye. The patient was a 44-year-old man who was bitten by *Campyloneura virgula* in June of 2021. Clinical signs were recorded, and the bite's effects were reduced within a few days. Possible reasons and outcomes of the incident were discussed.

Keywords: Campyloneura virgula, plant bug, human bite

1. INTRODUCTION

Campyloneura virgula is a predatory insect that feeds on a variety of plants and small insects and is commonly found in Europe, Africa, and Asia. It belongs to the *Miridae* family, and adults can reach a length of 4-5 millimeters. The insect's pale-yellow body has a black head, long antennae with red bands, and bright yellow cuneus tips (1, 2). Adult *C. virgula* have yellow scutellum, pronotums with a red edge stripe, and light green transparent hemelytra, while nymphs have a red stripe along the pronotum edge and are yellow. The rarity of males suggests that the insect is possibly parthenogenetic (3).

Campyloneura virgula is truly omnivorous and can survive by hunting small insects, such as aphids and red mites, as well as plants, making it a destructive pest (2). Adults can be found on and around a variety of deciduous trees from July to October, while the nymphs emerge in May. Mirids which *C. virgula* was classified, are also known as small plant bugs that feed on plants. Although they can bite humans, it is relatively uncommon. The reasons for this behavior are unknown, and the transmission of pathogens from mirids to humans was not confirmed. The possibility of any diseases related to *C. virgula* is also unknown (2, 4, 5). While there have been a few reported cases of mirids biting humans, little information is available about the clinical effects and consequences of *C. virgula* human biting and it is the first case report of human biting *C.virgula* in Türkiye.

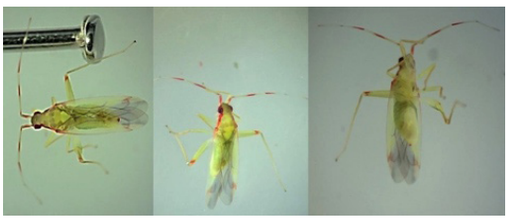

Figure 1. *Stereo-microscopic appearances of Campyloneura virgula*

2. CASE REPORT

We present the first case report of a human bitten by *C. virgula* in Türkiye, confirmed by stereo-microscopic examination (Fig.1). The patient was a 44-year-old man who reported being bitten below the elbow. Since he was a researcher in parasitology field, he had the chance to observe the whole course in detail. He was bitten in Tekirdağ Namık Kemal University Campus in June, humidity and the temperature in the location were 77% and 21.6°C respectively. The initial biting lasted approximately two minutes, followed by repeated biting for about one minute (Fig.2-A). The patient described the bite as very painful, worse than a mosquito bite, and the feeding behavior was similar to that of Cimex spp., occurring in a train-like pattern (Fig. 2 – B).

After the bite, the patient experienced itching, which lasted for about 30 minutes. Local swelling was observed for a day,

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and redness persisted for five days (Fig.2-C). After three days, the redness began to spread beyond the initial bite site, but there were no clinical manifestations such as fever or malaise. No local treatment was administered (Fig.2-D).

It is worth noting that there is no report of *C. virgula* biting humans in Türkiye prior to this incident.

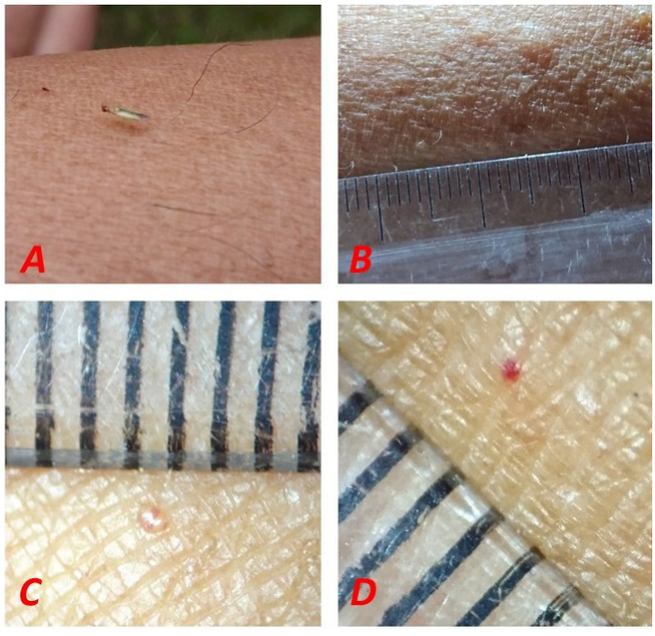

Figure 2. *A-Campyloneura virgula on biting site, B – Train like pattern on biting site, C – Local swelling in biting site, D – Redness in biting site after 5 days*

3. DISCUSSION

Miridae family is known as plant leaf bugs that feed on the many types of trees and plants. Some of the *Miridae* bugs, such as C. virgula, have zoophytophagy behavior in feeding, and they can feed on spider mites, aphids, psocids, and chrysomelid larvae (1, 5). Mirids have active flying behavior and can be seen nearby human populations. Several mirids are known to bite humans and their bites are described as more painful than other bloodsucking arthropods, for instance, mosquitos. However, the reason for the blood-sucking behavior is still unknown (5). Case reports on human biting mirids, namely *Blepharidopterus angulatus* and *Phytocoris buenoi,* and clinical manifestations were reported (6, 7). Most of the human-biting *Miridae* cases were seen in northern parts of America, but cases also have been reported from other regions such as the UK, Japan, South Africa, and Nigeria (5). There is no report about the transmission of pathogens or post-biting systematic allergy or any medication recommendations described specifically. The close proximity of the human residential areas to mirids' natural habitats and their zoophytophagic feeding behavior was suggested as the reason for human cases (2). On the other hand, due to the dominant feeding behavior of *C.virgula*, it is highly unlikely that this organism would be able to act as a vector for pathogenic microorganisms that could be harmful to humans. It is unclear whether these bites can cause any serious health complications or if they are simply an annoyance. Further research is needed to better understand the potential risks associated with mirid bites and to develop effective strategies for preventing them. In the meantime, it is recommended that people avoid handling or coming into close contact with mirids in order to reduce the risk of being bitten.

4. CONCLUSION

To date, no research has been conducted to determine the ability of Mirids to transmit human diseases. However, the identification of *C. virgula* and its documented biting behavior in specific regions raises concerns about the possible public health risks associated with this insect. Further investigation is therefore suggested to evaluate the potential for disease transmission and to develop appropriate measures for mitigating any associated health threats. In the field of agricultural research in Türkiye, various studies have been conducted on the *Miridae* family. However, there is currently no research available on the ecology of *C.virgula* or its host and parasite interaction.

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Clinical and Experimental Health Sciences

Pseudotumor Cerebri with Familial Mediterranean Fever in an Adult Patient

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ABSTRACT

Familial Mediterranean fever is an autosomal recessive disease characterized by recurrent episodes of fever, peritonitis, pleuritis and arthritis. Neurological involvement in FMF is rare but serious. Headache, seizures, demyelinating lesions, stroke, posterior reversible leukoencephalopathy syndrome, aseptic meningitis and cranial neuropathy have been reported in the literature. In this paper, we present a combination of FMF and pseudotumor cerebri in an adult patient.

Keywords: Familial Mediterranean Fever, pseudotumor cerebri, headache

1. INTRODUCTION

Pseudotumor cerebri (PTC) is characterized by the presence of intracranial hypertensive symptoms despite the absence of any detectable mass lesion on radiological imaging. Headache, nausea, and vomiting are frequently encountered as the primary symptoms of the disease. Patients with PTC may also experience diplopia, blurred vision, and pulsatile tinnitus (1). Neurological manifestations commonly observed in PTC include bilateral and symmetric papilledema and unilateral or bilateral partial or complete sixth nerve palsy. Partial or complete loss of vision is frequently observed in the advanced stages of the disease. According to the updated criteria for PTC by Friedman et al., the disease is categorized into two groups: primary (idiopathic) and secondary. The primary form refers to cases where no identifiable underlying cause is found, while the secondary form encompasses cases where PTC is attributed to various causes, ranging from medication-induced factors to cerebral venous sinus thrombosis. Friedman et al. prefer the term “pseudotumor cerebri syndrome” (PTCS) to “benign intracranial hypertension” or “idiopathic intracranial hypertension” (2). Familial Mediterranean Fever (FMF) is an autoinflammatory disease characterized by recurrent fever and polyserositis, including peritonitis, pleuritis, and synovitis. It is inherited in an autosomal recessive manner. Although neurological manifestations in FMF are infrequent, they may manifest as seizures, recurrent episodes of aseptic meningitis, cranial nerve involvement, pseudotumor cerebri (PTC), ischemic stroke, vasculitis, and demyelinating lesions (3,4).

There are only few case reports on the relationship between FMF and PTC in adults (5,6). This paper presented an adult patient with FMF and PTC.

2. CASE PRESENTATION

A 30-year-old male patient presented to our outpatient department with complaints of headaches in both frontal regions and blurred vision that began approximately one month ago. He experienced a dull and intense headache that did not show any improvement with the use of painkillers. He also reported experiencing what is commonly known as pulsatile tinnitus. He has been on colchicine (60 mg/day) as he has had FMF for ten years. During the examination, the patient exhibited hypertension, while the rest of the vital signs were within the normal range. Both his eyes had grade 3 papilledema (Figure 1). His right visual field was narrowed. Visual acuity was bilateral (0.3). No abnormalities were detected during the neurological and physical examination. His brain magnetic resonance investigation and magnetic resonance venography were normal (Figure2). We performed lumbar puncture (LP). The cerebrospinal fluid (CSF) pressure was 270 mm H2O. CSF protein was high (40.7 mg/dl). Otherwise, his CSF biochemistry and cell count were normal. We performed a second LP to drain the CSF. The opening pressure dropped to 190 mm H2O. His complaints of headache and blurred vision showed significant improvement.

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Figure 1. *The papillae borders are obscure in both fundi of the case.*

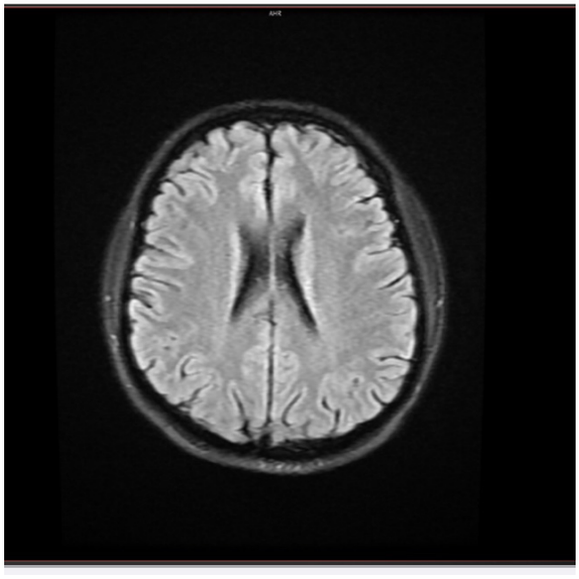

Figure 2. *MRI scan of the case was normal.*

The protein/creatinine ratio in his spot urine was high, with +++ proteinuria (9.74 mg/dl). We conducted a renal ultrasonography. Grade 1 growth was present in his kidneys. Both serum amyloid A (15.4 mg/dl) and protein C (189 mg/dl) were positive. We performed a kidney biopsy. The diagnosis was secondary amyloidosis due to FMF. Hyperlipidemia (total cholesterol: 271 mg/dl, LDL: 190 mg/dl), proteinuria (+++), and hypoalbuminuria (2.4 mg/dl) were present with FMF-related amyloidosis. Due to the presence of nephrotic syndrome, the patient was started on an angiotensin 2-type 1 receptor antagonist. He was also started on 500 mg/day acetazolamide. The treatment was effective. At the second LP, the opening pressure dropped to 190 mm H2O. He experienced a significant improvement in his complaints of headache and blurred vision and remained symptom-free throughout the two-year follow-up period.

3. DISCUSSION

Familial Mediterranean fever is a genetic disorder that is commonly observed among individuals of Armenian, Arab, Jewish, and Turkish descent. Referred to as MEFV, the gene responsible for FMF is located on chromosome 16p13.3. Its product is called pyrin, a protein including 781 amino acids (7,8). Research shows that pyrin plays a vital role in the innate immune response (9,10). Although FMF involves many organs, it rarely affects the central nervous system (3,8,11-13). From a neurological point of view, headache is the most common symptom. The majority of neurological manifestations in FMF can be attributed to the disease itself. However, neurological involvements in FMF can also arise from amyloidosis, which is the most significant and potentially fatal complication of the disorder, or as an adverse effect of treatment (4, 14).A recent review explores the relationship between increased intracranial pressure and nephrotic syndrome (NS). The researchers highlight the notable occurrence of cerebral venous thrombosis in a majority of patients presenting with both conditions. They also report hyperaldosterinism as a possible etiological factor. A nephrotic syndrome due to amyloidosis is known to develop during the course of FMF. However, as mentioned in the review, the available data and evidence are currently insufficient to establish NS as a definitive secondary cause of PTC (15). In the case of our patient, cerebral venous thrombosis was ruled out as a normal MR venography did not reveal any abnormalities. The patient was admitted to our clinic exhibiting classic symptoms and signs of PTC, including headache, blurred vision, pulsatile tinnitus, visual loss, and disc edema. We performed investigations for differential diagnosis but did not identify any pathological conditions that could account for the observed increase in intracranial pressure. He had a CSF pressure of 270 mm H_2O. Based on the presented clinical criteria, we diagnosed him with PTC.

Currently there is no consensus regarding the underlying pathophysiology of PTC associated with FMF. Etiological factors considered in relation to PTC associated with FMF include increased cerebrospinal fluid (CSF) production, venous outflow obstruction, defective CSF resorption, parenchymal edema, and expanded cerebral blood volume. As pointed out in a recent review (15) and observed in our case, it is possible that nephrotic syndrome, which can occur in individuals with FMF, might have contributed to the development of PTC. The classical treatment guidelines for pseudotumor cerebri (PTC) typically involve weight loss strategies, along with medical interventions, such as acetazolamide intake. Additionally, topiramate can be used as monotherapy or as an add-on therapy for managing the condition. Surgery (optic nerve sheath fenestration, venous sinus stenting, ventriculoperitoneal and lumboperitoneal shunt, etc.) is an option for patients unresponsive to medical treatment or with fulminant course.

4. CONCLUSION

PTC is a rare neurological manifestation of FMF. Given the potential for irreversible permanent visual loss and significant disability associated with PTC, early diagnosis and regular multidisciplinary follow-up are of utmost importance. We believe that our case will emphasize the need to consider FMF as a possible underlying condition in patients presenting with PTC, thereby expanding the list of secondary causes associated with PTC.

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