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The Importance of Pregorexia Awareness

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ABSTRACT

Objective: Pregorexia is a condition that describes women who reduce energy intake and increase exercise to control weight gain during pregnancy. Although pregorexia is not formally recognized as a medical diagnosis, the term may be considered as an eating disorder during pregnancy, as behaviors such as the fear of weight gain and the worry about body appearance are observed both in pregorexia and in eating disorders. The abnormal weight gain in pregnant women may cause adverse obstetric and fetal outcomes. Eating disorders during pregnancy can cause serious problems for both mother and infant, such as small for gestational age infants, spontaneous abortion, microcephaly, low birth weight babies, maternal hypertension, and anemia. The aim of this review is to increase awareness of eating disorders during pregnancy, particularly pregorexia.

Keywords: Eating disorders, pregnancy, pregorexia, strict diet.

1. INTRODUCTION

A healthy diet is essential at every stage of life and even more important during pregnancy. During this period, the mother's diet should meet both her own and the growing fetus's energy and nutrient requirements. The growth and development of the fetus in the mother's womb are possible through adequate and balanced feeding of mother during her pregnancy. Pregnant women should consume an adequate and balanced diet to meet their own physiological needs (energy, macronutrients, and micronutrients), to keep the balance in the body, to ensure healthy fetal growth and development of the fetus, and to meet the energy and nutrition knowledge required for lactation (1,2).

During pregnancy, the increase in requirement for energy and macronutrients as well as the growing importance of micronutrients distinguishes dietary recommendations from other adults. During pregnancy, it is necessary to consume the food rich in crucial vitamins and minerals (especially iron, folate, calcium) and to supplement vitamin D. Pregnant women should consume green vegetables, citrus fruits, dairy, meat, fish, legumes, oily seeds, grains and fruit-based on individual needs. Weight should be gained according to the age of the pregnancy and maintained a healthy body weight (3).

Premature, low birth weight, dead or mentally and physically retarded babies may be born in case of inadequate and unbalanced nutrition. Also, maternal health may deteriorate (4-6). Eating disorders are one of the factors that cause of mothers' inadequate and unbalanced nutrition.

This review aims to increase awareness about pregorexia, draw attention to the complications of eating disorders in pregnancy, and to provide information about the prevention, treatment of eating disorders in pregnancy.

2. METHODS

General information about pregnancy and eating disorders is explained in the following headings. The following search builders were performed while reviewing information about pregorexia. "Pregorexia (text word) and pregnancy (text word) and eating disorder (text word)" were searched in the PubMed search engine through the advanced search builder. Only one article was found. As the term "allintitle: pregorexia" was searched in the Google Scholar search engine, five articles were found. The articles in which the full-text language is not in English and whose full text is not accessible were excluded. After the articles were eliminated, three articles were left.

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Pregorexia issue is an up-to-date problem, there is not enough research in the literature about this issue. In this context, one of the aims of this review is to provide a resource to the literature and to increase the awareness of healthcare professionals about pregorexia.

2.1. Risks of Eating Disorders in Pregnancy Period

According to the Diagnostic and Statistical Manual of Mental Disorders (DSM-V), eating disorders are persistent disturbances of eating or eating-related behaviors that result in changing consumption or absorption of food and affecting the significant physical health or psychosocial functioning (7). Complications of eating disorders are electrolyte imbalance, gastrointestinal problems, deterioration in cardiac function, abnormalities of bone density, increased risk of mortality (8-10). Types of eating disorders are anorexia nervosa, pica, binge eating disorder, avoidant/restrictive food intake disorder, bulimia nervosa, rumination disorder. Women are known to have a higher prevalence of eating disorders than men (7). Especially during or after pregnancy, women may feel pressure to maintain or lose weight even if they do not have excessive adverse behaviors before (11). The pregnancy period may be a risky process for eating behavior disorders and a turning point for recovery, onset or relapse of eating disorders (12-14). A study showed that body weight dissatisfaction remained unchanged for women with eating disorders during pregnancy; also, body weight dissatisfaction increased for women without eating disorders in pregnancy (15).

Primary risk factors for eating disorders during pregnancy include age <30 years, previously diagnosed eating disorders, a history of sexual abuse, and a significant life stressor or psychological trauma in the past (16).

In pregnancy period – which is a special and complex period of the women's life – metabolic, biological, and physiological changes, as well as social and psychological changes may occur. Pregorexia is one of the pathophysiological issues seen during pregnancy (17).

2.2. Pregorexia

Pregorexia, described in a journal in 2009, is a very new term related to eating disorders in pregnancy (18). This term has been taking place in a few scientific references and there are limited resources (media tools as internet, magazine, news) for the definition of pregorexia. Pregorexia has been considered as an obsession of staying thin by a pregnant woman. Pregorexia term has not been formally recognized in medical diagnosis, and additionally, according to DSM-V criteria pregorexia is not officially classified as an eating disorder (7). This term was used by the media to describe reduced calorie intake and increased exercise to control weight gain in pregnant women (18).

The media is thought to affect pregorexia tendency with pictures of thin pregnant celebrities (11). A large number of women who wish to have low pregnancy weight today are concerned that their weight will continue after pregnancy (19). Some pregnant women may focus on dieting strictly and exercising excessively rather than growing a healthy baby and accepting the change in their body shapes. Besides, pregorexia is described as women who spend hours at the gym and control their diet excessively to keep a svelte figure during pregnancy (20). Signs associated with pregorexia includes a history of eating disorders, talking about the pregnancy as if it were not real, excessively focusing on calorie counts, eating alone, skipping meals, exercising excessively (18,21). There is no internationally accepted definition for pregorexia and its distinctive features have not been identified yet. However, pregorexia is a typical behavior in some pregnant women. It is associated with taking control of the pregnancy period by doing a fairly low energy diet and heavy exercise. Approximately 5% of women experience pregorexia during and after pregnancy (22).

2.3. Effects of Eating Disorders on Pregnancy and Fetal Outcomes

Maternal history of an eating disorder is a risk for both mother and infant (23). When a pregnant woman experiences severe malnutrition especially during the final three months, she faces an increased risk of stillbirth, low birth weight, or infant death (24). Women with a history of eating disorder or current eating disorder have been shown to have a higher risk of obstetric complications, including small for gestational age infants, spontaneous abortion, low birth weight babies, microcephaly (23). A large clinical sample study has found that different maternal eating disorders are associated with increased risk of caesarian sections, small for gestational age, and low Apgar score at 5 minutes (25). Another study showed that the infants of mothers with a history of eating disorders had lower birth weight and lower head circumference as compared with the infants of the control group (26). A follow-up study showed that the risk of low-birth-weight infants was twice high in women with a history of eating disorder compared with women with no eating disorder. The same study reported that in women with an eating disorder, the risk of preterm delivery and small for gestational age was respectively 70% and 80% higher than the rates in women with no disorder (27). In contrast with these, a Swedish national register study did not found a relationship between the history of anorexia and the adverse birth outcomes (28). Although different results can be found, the risks of eating disorders should not be ignored for mother and baby health. Because eating disorders can cause serious complications, as mentioned above.

Besides these, women with a history of eating disorders are at higher risk of negative obstetric outcomes (29). Maternal anorexia nervosa has been related to anemia on the other side; maternal binge eating disorder has been related to maternal hypertension (30). Not only women with a history of eating disorders but also pregnant women with current eating disorders are affected by the risk of obstetric complications. A study reported that women who showed

symptoms of anorexia/bulimia nervosa during pregnancy had a higher frequency of cesarean delivery and postpartum depression than women with no symptoms (31). In a study conducted in Sweden, the risk of hyperemesis of pregnant women with past or current eating disorders was found higher than in the non-eating disorders group (26).

The eating disorders in pregnancy could be associated with health risks in the mother and her child related to sleep quality, maternal nutrition, birth outcomes, and child feeding (32). During the antenatal and postnatal periods, women with both current and past eating disorders could have higher depressive and anxiety symptoms compared with women without a history of eating disorders (33).

Pregorexia could affect pregnancy outcomes like other eating disorders, so healthcare professionals should be aware of this higher risk and pregorexia (21).

2.4. The Prevention and Treatment of Eating Disorders During Pregnancy: Health Professionals' Missions

Clinical potential complaints in pregnant women with eating disorders may be hyperemesis gravidarum after 20 weeks' gestation, absence of weight gain, signs of depression, or dieting (34). It is suggested that women with low body mass index (BMI), fear of weight gain, menstrual disturbances or amenorrhoea, gastrointestinal problems, psychological problems, physical signs of starvation, or repeated vomiting before pregnancy should be screened for risk of eating disorders during pregnancy (35). Precautions should be taken to protect risky women from eating disorders before pregnancy.

Healthcare professionals should be aware of eating disorders in pregnancy for maternal and child health. Screening tools could be used for early diagnosis of eating disorders in pregnancy. SCOFF (Sick, Control, One, Fat, Food) questionnaire, which has been designed specifically for eating disorders, could be used in the screening of eating disorders in pregnancy (36). Pregnant women should be educated about eating disorders and their effects. Pregnant women should be informed about the importance of optimal weight gain management, nutrition, and lifestyle changes during pregnancy.

Management of eating disorders includes nutritional and psychosocial treatments. A treatment team of eating disorders in pregnancy should be comprised of obstetricians, psychiatrists, internal specialists, psychologists, dietitians, nurses, midwives. A specialist team is needed because pregnant patient having an eating disorder care is difficult. In the treatment of eating disorders in pregnancy healthcare professionals should provide education to women. This education should include information about maintaining good mental health and wellbeing, the effects of healthy nutrition and healthy body weight on mothers and infants, the importance of stopping behaviors such as binge eating, vomiting, using laxatives and excessive exercise. Healthcare professionals should monitor and support pregnant women during pregnancy and the postnatal period (36-38). Healthcare professionals could help pregnant women determine an appropriate weight gain during pregnancy, based on her pre-pregnancy weight and BMI. The dietitian should plan the appropriate diet for the pregnancy and follow up on the pregnancy by doing regular interviews about the importance of healthy nutrition. Adequate energy and nutrient intake should be provided for the health of pregnant women and infants.

Health care providers should calculate woman's BMI at the first prenatal visit and advise related to the benefits of optimal weight gain, exercise, nutrition (39). The amount of weight gained during pregnancy can affect the immediate and future health of a woman and her infant. Evidence supported the association between inadequate weight gain and decreased birth weight (40). Weight gain during pregnancy is so important that it helps the health outcomes to be optimal for the woman and her infant. According to the revised gestational weight gain guidelines of the Institute of Medicine (IOM) published in 2009, a woman of normal weight (BMI: 18.5-24.9 kg/m^2) should gain weight in the 11.3-15.9 kg range during her pregnancy (41).

During pregnancy, the mother's diet provides energy and nutrients to both herself and the fetus' growth and for future lactation (42). The maternal diet must provide adequate energy and nutrients to supply the mother's appropriate requirements. Additionally, the needs of the fetus and enable the mother to establish the storage of nutrients required for fetal growth. The energy expenditure of pregnancy was estimated at around 321 megajoules (MJ) (77 000 kilocalories), based on theoretical calculations and data from longitudinal studies. Mothers with a low BMI before and during pregnancy are at arisen risk of having a low birth weight (LBW, birth weight <2.5 kg) infant. Low birth weight is associated with an increased risk of neonatal mortality and morbidity, as well as raised the risk of diseases in later life (43).

Although eating disorders seen before, during, or after pregnancy are rare, it is crucial because they can affect the mother and the baby negatively if not treated. The treatment of eating disorder in pregnancy should include, (35)

– advice general nutritional before pregnancy
– educate about the nutrition and growth of the fetus
– increase awareness of all healthcare personnel about eating disorders related to mother and baby health
– support breastfeeding
– liaise with the health visitor to monitor infant growth and weight gain closely
– watch for postnatal depression and return or worsening of the eating disorder in the postnatal period

3. CONCLUSION

Pregorexia has not yet been formally defined, and its features are not fully determined. Most health providers are unaware of this term. However, it is a fact that some pregnant women suffer from pregorexia. This eating disorder can significantly affect pregnancy outcomes and causes health risks for both mother and infant. For the prevention of these risks, the characteristics of pregorexia should be determined by the health professionals. Differential diagnosis of pregorexia should be made from other eating disorders seen in pregnancy such as bulimia and anorexia nervosa. Future studies should fill the deficiency of literature about pregorexia. Suggestions and guidelines should be enhanced for the treatment of pregorexia as well as other eating disorders in pregnancy.

Although the definition of pregorexia is controversial, screening for eating disorders for pregnant women may be helpful for healthy pregnancy outcomes. The health care team, including a dietitian, should be aware of the eating disorder in pregnancy and especially pregorexia. It is suggested that a specific screening tool should be promoted to identify eating disorders like pregorexia in pregnancy.

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Clinical and Experimental Health Sciences

Investigation of the Relationship Between the Pulp Area and Chronological Age in Patients that Received and Not Received Orthodontic Treatment

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ABSTRACT

Objective: In this study, it was aimed to evaluate the relationship between chronological ages and pulp areas of mandibular canine teeth of patients that received and not received orthodontic treatment.

Methods: 102 patients that completed fixed orthodontic treatment and between the ages of 13-24, and 102 age and sex-matched control group subjects were included in the study. A total of 204 dental panoramic radiographs taken with the same procedures and with the same device (Soredex; Cranex Novus, Tuusula, Finland) were evaluated in this study. The pulp areas of the mandibular canine teeth were measured using the Image J software (US National Institutes of Health, Bethesda, MD). Data were analyzed statistically.

Results: In both the orthodontic group (r = -0,511) and the control group (r = -0,592), there was a negative correlation between chronological age and pulp area. There was no significant difference between the groups in terms of the pulp area and gender (p >0.05).

Conclusion: Orthodontic treatment did not result in a significant difference in the correlation between the pulp area and the chronological age.

Keywords: Age estimation, orthodontic treatment, pulp area.

1. INTRODUCTION

Age determination is a frequently applied procedure in forensic and archaeological sciences due to criminal liability, marriage law, illegal migration and mass disasters (1,2). Many tissues are used for age determination (3). Skeletal structures and teeth are the most commonly used tissues for age determination in both dead and living individuals (4,5). The teeth are more preferred biomarkers in age determination than skeletal structures in both dead and living individuals, as teeth are resistant to thermal, chemical and mechanical factors and therefore can protect their existence after death (6,7).

In the literature, it has been reported that parameters of the teeth such as translucency, root resorption, secondary dentin accumulation, dentin rings, cement apposition, retzius lines, and amino acid reamination can be used in age determination (1,2,6,8-10). Using these parameters of dental structures, it has been reported that the age determination is accurately calculated in many methods such as Gustafson's parameters, Johanson's classification, methods based on dental nuclear tests and Demirjian technique (11-13). Secondary dentin accumulation, which is one of these parameters, is a progressive process with age and, is therefore, a dental structure that is frequently used in determining age (8,14). After teeth eruption, the mean increase rates of secondary dentin accumulation around the pulp cavity were reported as 6.5 mm/year for the crown and 10 mm/year for the root. Based on this information, Kvaal et al. (15) revealed a method associated with secondary dentin accumulation and age determination on radiography in 1995. Afterward, Cameriere et al. (10,14) reported a high correlation between the pulp area and the chronological age in determination of age using the pulp area.

Radiographs of dental structures are widely used due to their advantages such as the fact that they do not require an invasive procedure and can be used in both living and dead individuals for age estimation (6). Micro-computed tomography (Micro-CT) allows for the 3-dimensional quantitative analysis of dental structures, but it is an unsuitable method for the prediction of age in living individuals. Cone Beam Computed Tomography (CBCT) is used frequently in age estimation methods and allows the 3D (dimensional) evaluation of dental structures without superposition and distortion (1,5,16-22). However, periapical and panoramic radiography techniques that give a 2D image provide more limited imaging compared

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to CBCT, but it allows the living individuals to be exposed to less ionized radiation (1).

It has been reported that secondary dentin accumulation has increased in patients that received orthodontic treatment (23). Therefore, it can be thought that the correlation between secondary dentine accumulation and chronological age in these treated patients can be affected. In other words, in patients that received orthodontic treatment, age determination based on secondary dentine accumulation may lead to incorrect evaluations.

In our comprehensive literature review, it was found that limited study investigating the relationship between pulp area and chronological age in patients that received orthodontic treatment exists. Therefore, in this study, it was aimed to evaluate the relationship between pulp area and chronological age using panoramic radiographs in patients that received and not received orthodontic treatment.

2. METHODS

A total of 204 panoramic radiographs obtained using the same panoramic X-ray device (Soredex, CranexNovus, Tuusula, Finland, 70kVp, 10 mA, 8-sec exposure parameters) of 102 patients that received fixed orthodontic treatment (mean±standard deviation [SD] age: 17±0.7 years, 45 females, 57 males) and 102 patients that not received orthodontic treatment (mean±SD age: 21±0.3 years, 59 female, 43 male) were included in the study. For the current study, the patients that received orthodontic treatment were treated with only fixed orthodontic brackets and bands and never used any other fixed or removable orthodontic appliance. The mean ±SD duration of treatment for the orthodontic treatment group was 22.5±1.8 months. Radiographs with poor image quality and magnification and distortion in the mandibular canine teeth were excluded from the study. After obtaining the radiographs, the images were recorded in equal dimensions in high-resolution JPEG format (Joint Photographic Experts Group). Subsequently, the images were converted to the image file format (TIFF) and were transferred to Image J version1.3 (National Institutes of Health, Bethesda, MD, USA). The area of the mandibular canine teeth was measured on the radiographs obtained using Image J software (Fig. 1). All measurements were performed by a maxillofacial radiologist with 5 years of experience. To test intra-observational reliability, 40 images were reanalyzed after 10 days the measurements were completed (correlation coefficient= 0.89). Correlation between chronological age and pulp area measurements was analyzed.

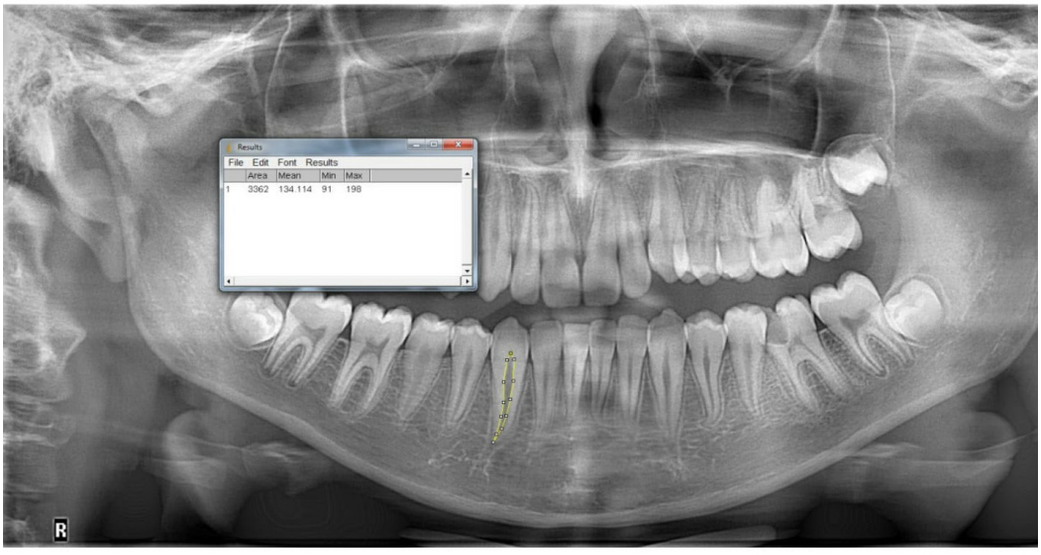

Figure 1. *Measurements of Pulp Area on the Radiograph*

2.1. Statistical Analysis

The independent t-test was used to assess the difference between the pulp area of males and females. The relationship between age and pulp area was analyzed using Pearson's rank correlation test. The coefficient of estimation (R^2) from the analyses was calculated to evaluate the relationship between age and pulp area of mandibular canines. All analyses were performed using SPSS version 21.0 (IBM Statistical Package for the Social Sciences Statistics; New York, ABD). The level of significance was set at $p < 0.05$.

3. RESULTS

There was no significant difference according to gender in the pulp area ($p > 0.05$) (Table 1). In both the orthodontic treatment group ($y=21.28+-2.12E-3*x(R^{2=}$ -0.511, p= 0.000); Fig. 2) and the control group ($y=22.99+-2.51E-3*x(R^2=$ -0.592, p= 0.000); Fig. 3), there was a negative correlation between the pulp area and the chronological age. No significant difference was found in the age estimation with pulp area among the individuals who received and who did not receive orthodontic treatment ($p > 0.05$) (Table 2).

Table 1. *Comparison of the Pulp Area (mm^2) of Male and Female Subjects between Groups (mean ± SD / SE)*

	Male	Female	t value	P value
Orthodontic group	1743.79± 836.12/ 155.26	1754.90± 494.27/ 105.37	-0.05	0.956
Control group	1887.46±597.49/ 163.21	2385.72±638.99/ 184.35	-2.83	0.928

SD: standard deviation; SE: standard error

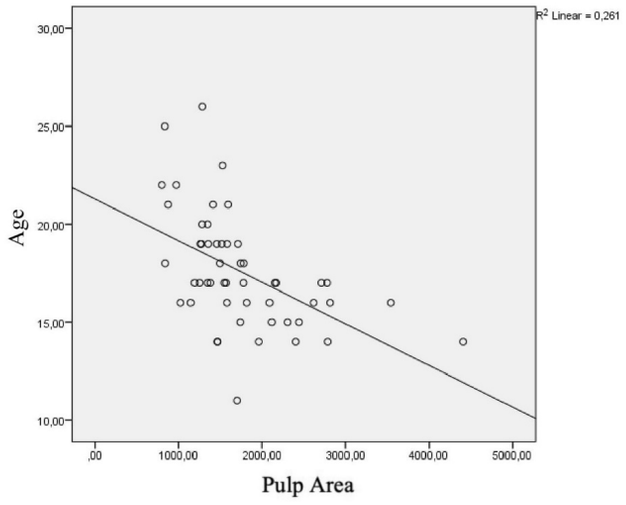

Figure 2. *The Relationship between Chronological Age and Pulp area in the Control Group*

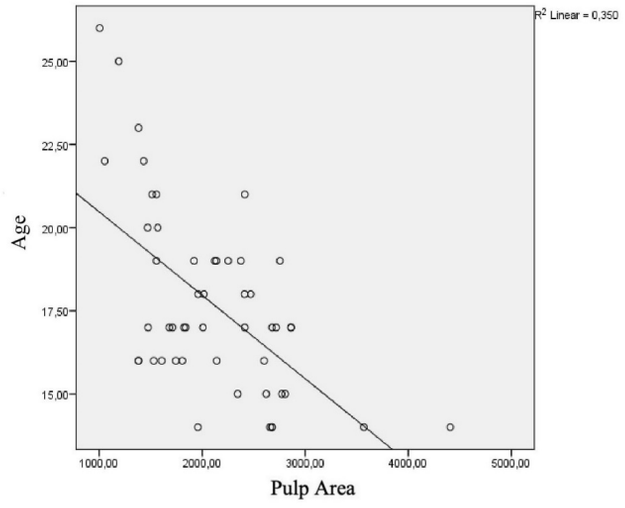

Figure 3. *The Relationship between Chronological Age and Pulp Area in the Orthodontic Group*

Table 2. *Comparison of the Pulp Area (mm2) between Groups (mean ± SD/ SE).*

	Orthodontic group	Control group	P value
Mean ±SD	1748.91± 328.67/ 157.81	2136.602.16/ 171.48	> 0.05

SD: standard deviation; SE: standard error

4. DISCUSSION

Today, age estimation is used in many areas for different reasons. Therefore, there is an increasing interest in the development of age estimation methods which are non-invasive, accurate, cost-effective and simple to implement (24). In the current study, the relationship between the chronological age and the pulp area was investigated based on the accumulation of secondary dentine over time in patients that received and did not receive orthodontic treatment.

It is known that secondary dentin formation is a physiological process that develops with age (15). Therefore, this study was performed on two groups with the same age range to avoid any bias in the study design. Secondary dentin accumulation is an important indicator of age estimation on dental radiography. Many studies on dental radiographs have found a relationship between chronological age and secondary dentin accumulation (15,23,26,27). Orthodontic treatment causes mechanical trauma in the periodontal ligament and induces pulp reactions (28). Histological studies have shown that secondary dentin formation increases after tooth movement (29). Therefore, the size of the pulp chamber decreases due to secondary dentin formation (21). In the light of this information, this study aims to evaluate the effect of orthodontic treatment on the pulp area and age estimation.

In many studies, age estimation was performed on different tooth groups. It has been reported that the complex anatomy of molar teeth may cause difficulties in determining the boundaries of the pulp area and may cause inconsistencies in the measurements (11). Similarly, because of the small pulp volume of the lower incisors and variations in the root canal anatomy, it has been reported that the pulp area can be measured incorrectly using the 2D radiographs (10). For this reason, this study was performed on radiographs of canine teeth which have the largest pulp volume among single-rooted teeth and the longest functional survival rate in the mouth, which is relatively less exposed to occlusal wear and stresses compared to posterior and other anterior teeth (14,25). In this study, the teeth were selected from the left or right side. It has been reported that, there was no significant morphological difference between the permanent teeth on the left or right side of the jaw (15).

In our comprehensive literature review, only one study investigating the effect of orthodontic treatment on age estimation was found. Penaloza et al. (23) showed that using Kvaal method orthodontic treatment did not have a significant effect on age estimation on panoramic radiographs. Similarly, in our study, it was determined that orthodontic treatment did not cause a significant difference in the correlation between the pulp area and the chronological age. The different age estimation methods, the similarity in the results of the study despite the different populations and the different teeth group shows that the secondary dentin accumulation induced after orthodontic treatment does not cause a significant change in the pulp

area. Besides, the results of the study showed that gender had no significant effect on the pulp area of canine teeth. Similarly, Jeevan et al. (30), Singaraju et al. (31) and Dehghani et al. (32) were reported that gender has no significant effect on morphological variables.

5. CONCLUSION

The results of the present study showed that orthodontic treatment did not affect the relationship between chronological age and pulp area. To obtain more detailed data on the effect of orthodontic treatment on age estimation, we think new studies with larger sample sizes on different populations and comparison with other age determination methods are necessary.

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Clinical and Experimental Health Sciences

The Prenatal Rating of Efficacy in Preparation to Breastfeed Scale (PREP to BF): A Turkish Validity and Reliability Study

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ABSTRACT

Objective: Breastfeeding self-efficacy is an easy-to-evaluate and easy-to-change variable. The prenatal period is an ideal period of time for women to gain breastfeeding self-efficacy. Valid and reliable measurement tools are needed to assess breastfeeding self-efficacy. The purpose of this study is to examine psychometric analysis of the Turkish form of the Prenatal Rating of Efficacy in Preparation to Breastfeed Scale.

Methods: This is a methodological study. The study was conducted at the obstetrics outpatient clinic of a state hospital in Turkey. The study was administered to 407 pregnant women. The relevant data were collected through the use of a Personal Information Form, The Prenatal Rating of Efficacy in Preparation to Breastfeed Scale. Internal consistency coefficient, split-half reliability and item analysis to assess the reliability of the scale, factor analysis was performed to evaluate the validity of the scale.

Results: Cronbach Alpha coefficient of the scale was 0.96. A 5-factor structure with eigenvalues above 1 that explained 71.267% of the variance emerged. The model fitted the observed data in terms of these fit index values, and that the Turkish version of the scale demonstrated an acceptable level of fit.

Conclusion: The scale can be employed by healthcare professionals to assess prenatal breastfeeding self-efficacy. It can contribute data for the structuring of the content of training and consultancy programs intending to improve breastfeeding self-efficacy.

Keywords: breastfeeding, efficacy, prenatal, reliability, scale, validity.

1. INTRODUCTION

Breastfeeding is an important public health topic, as well as being a lifestyle choice (1,2). World Health Organization (WHO) recommends babies to be fed only with breast milk for the first 6 months from birth. It also recommends starting additional food items from the 7th month on and continuing to breastfeed until the age of two (3,4). Breastfeeding provides optimal health for infants in the first 6 months of life and provides valuable health benefits for the mother (4). The risk of mortality and morbidity is decreased in infants who are breastfed and in women who breastfeed. With the help of breastfeeding, the cost of healthcare is reduced, and damages given to nature due to the production of infant formulas are minimized (5). Although the benefits of breastfeeding are known and many initiatives have been implemented to encourage breastfeeding, breastfeeding rates vary throughout the world. Exclusive breastfeeding rates at 6 months remain low (37% globally) and global suboptimal breastfeeding practices contribute to 11.6% of mortality for children younger than 5 years (6). Although breastfeeding rates in Turkey have shown significant improvement in recent years, it is still not at the desired level. According to Turkey Demographic and Health Survey (2008) data in Turkey, the rate of feeding only breast milk for the first 6 months is 41.6% (7). In Turkey Demographic and Health Survey (2013), the median duration of breastfeeding was 16.7 months, the rate of feeding only breast milk was 30%, and the rate of breastfeeding within the first hour after birth was 50% (8). However, the prevalence of breastfeeding is below the WHO's recommendations.

A better understanding of breastfeeding determinants and barriers to its practice is needed to improve global breastfeeding levels (9). The prevalence of breastfeeding is influenced by individual, social, political, religious and cultural factors (2). Among these factors, the perception of breastfeeding self-efficacy takes an important place. Breastfeeding self-efficacy is defined as the confidence of women in breastfeeding. The concept of breastfeeding self-efficacy is explained by the self-efficacy construct integrating the Breastfeeding Self-Efficacy Theory developed by Dennis with Bandura's Social Cognitive Theory (10,11). According to the self-efficacy construct, people need to believe that they can successfully fulfill a specific task or behavior. People must believe that they are capable of accomplishing a task or behavior (11). Breastfeeding self-efficacy is closely related to a woman's perception of her ability to breastfeed her baby

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and her belief that she has sufficient knowledge/skills to successfully breastfeed her baby (10).

Breastfeeding self-efficacy is an easy-to-evaluate and easy-to-change variable. Analyzing this variable contributes to diagnosing breastfeeding problems early, identifying women at risk, and accomplishing personalized care initiatives. The prenatal period is an ideal time frame for the determination of risk factors that will cause women to experience the perception of inability associated with breastfeeding, the elimination of these factors, and the strengthening of the perception of self-efficacy. In the literature, there is a limited number of measurement instruments to assess pregnant women's self-efficacy and/or attitudes associated with breastfeeding in the prenatal period in Turkey (12-14). In the international literature, however, different measurement instruments assessing breastfeeding with its various dimensions could be found. Some of these measurement tools include: Australian Breastfeeding Knowledge and Attitude Questionnaire Overview (15), Iowa Infant Feeding Attitude Scale Breastfeeding Knowledge (16), Attitude, and Confidence Scale (17), Breast-Feeding Attitude Scale (18), Supportive Needs of Adolescents Breastfeeding Scale (19), The Breastfeeding Self-Efficacy Scale (20), Perceived Breastfeeding Support Assessment Tool (21). The Prenatal Rating of Efficacy in Preparation to Breastfeed Scale (PREP to BF) has been developed by McKinley et al. (22). The scale offers the opportunity to make a detailed assessment of individual, interpersonal, professional and social factors, which have the potential to affect pregnant women's breastfeeding self-efficacy. The scale can be administered in a short time, and it can be interpreted easily, which are some of the most important advantages of it. In this study, it was aimed to determine whether the scale was a valid and reliable measurement instrument for Turkish women as well. The study will contribute to increasing the diversity of measurement instruments that allow to assess Turkish pregnant women's breastfeeding self-efficacy by using objective methods. Moreover, the scale will be available for use as a data collection instrument to determine the scope of training and consultancy services given to pregnant women by healthcare professionals, to structure educational content and to assess the effectiveness of services.

2. METHODS

2.1 Research Type

The purpose of this study is to examine the reliability and validity of Turkish form of the PREP to BF. This study is a methodological research. In the scope of the study, the language and content validity of the scale was assessed first, followed by its psychometric characteristics.

2.2 Data Collection Tools

The relevant data were collected through the use of a Personal Information Form and the PREP to BF.

Personal Information Form: Form was used to identify certain sociodemographic, obstetric and breastfeeding-related characteristics of pregnant women.

PREP to BF: The scale was developed by McKinley et al. for the purpose of measuring prenatal breastfeeding self-efficacy during the process of preparation for breastfeeding (22). The original scale had 39 items, and its Cronbach Alpha coefficient was found to be 0.98. Each factor was found to show a significant and high degree of correlation in a test-re-test analysis. The range of the item–total correlations of the original scale is between 0.54 and 0.78. The scale has 4 factors:

Factor 1. Individual Processes: The Cronbach Alpha coefficient of the first factor with 14 items is 0.88. This factor is related to the self-confidence in cognitive processes involving the goal-setting associated with breastfeeding, the mental preparation, the understanding of situations where breastfeeding can be difficult, fear, stress, and anxiety.

Factor 2. Interpersonal Processes: The Cronbach Alpha coefficient of the second factor with 16 items is 0.89. This factor is related to monitoring and modeling of breastfeeding, the comfort of talking about breastfeeding, and being able to seek advice of friends and family about breastfeeding.

Factor 3. Professional Advice: The Cronbach Alpha coefficient of the third factor with 4 items is 0.91. This factor is associated with the self-efficacy in obtaining professional advice from professionals and following their recommendations.

Factor 4. Social Support: The Cronbach Alpha coefficient of the fourth factor with 5 items is 0.88. This factor is related to social support for breastfeeding from friends and family.

Women who were not pregnant, under 18 years of age or having multiple pregnancies (such as twins, triplets, and so forth) were excluded from the study when administering the original scale. It takes approximately fifteen minutes to respond to all items on the scale. It is very easy to interpret the scale. There is no right or wrong answer on the scale. There is a scoring table just below each statement, with a scale of 0–10, where '0 = never can do it' and '10 = can do it with utmost certainty'. The pregnant woman is asked to read each statement on the scale and to mark a point between zero (0) and ten (10), which she thinks is appropriate for her. As the score on the scale increases, prenatal breastfeeding self-efficacy of pregnant women is considered to increase.

2.3 Content Validity of the PREP to BF

When translating a scale during a scale adaptation study, the 'translation into the target language' and 'translation back to the original language' steps follow each other (23). In this study, two linguists translated the scale from English to Turkish. Texts from both linguists were examined and organized by the researchers into a single text. Following that, this text was sent to two different specialists who were proficient in both languages and did not participate in the first translation process. The text was back translated from Turkish to English by each of the experts independently of each other. The translations in Turkish and English were

compared to the original scale and the text of the scale planned to be adapted was finalized.

2.4 Sampling and Participants

There are different opinions in the literature as to determine the size of a sample in scale development, validity and reliability studies. Factor analysis is a technique for determining the size of a sample. It is recommended in many sources in general that the sample size be at least 300 (24, 25). If factor loads are low, it is recommended that the sample size be increased (25, 26). In this study, 300 was accepted as the lower limit of the number of people in the sample, and the scale was administered to 407 pregnant women.

2.5 Data Collection

A pilot study was conducted on a small group of 20 pregnant women to ensure that the scale was appropriately translated into the culture. After it was understood that the scale was understandable, the actual implementation began. The data collection instruments were administered to the pregnant women who presented to a state hospital in the city center between April and May 2019 to undergo routine pregnancy follow-ups without any health problems. The pregnant women were asked to perform an assessment on each of the statements associated with breastfeeding. The pregnant women were told that it was sufficient for them to circle a score on the 0–10 scoring table located just below each statement. For example:

Thinking about your life right now, how well can you:
Overcome any fear you may feel about breastfeeding?

0 1 2 3 4 5 6 (7) 8 9 10

2.6 Psychometric Analysis of the PREP to BF

After the administration of the scale to the participants, the data were transferred to the computer environment via the Lisrel 8.54 and SPSS 22.0 package programs, and 'reliability' and 'validity' analyses were carried out on the scale. When testing the reliability of the scale, the item–total correlation was assessed using item analysis, the Cronbach Alpha reliability was assessed using reliability analysis, and the Spearman-Brown and Guttman Split-Half reliability coefficients were assessed using the split-half method. Exploratory factor analysis (EFA) was carried out to test whether the construct validity of the adapted scale conformed to the original scale. Confirmatory factor analysis (CFA) was carried out to examine the relationship between factors.

2.7 Ethical Approval

Prior to the validity and reliability studies of the scale, Erin McKinley was contacted, permission was received from the institution, and written permission was received to adapt the scale to the Turkish culture. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Before the scale was administered, the volunteer information form was read to the pregnant women who were to fill out the form, their permissions were obtained, and they were told that the data obtained would only be used for scientific purposes and that the participants' names would be kept confidential.

3. RESULTS

The mean age of the pregnant women was 27.27 ± 5.14 (min: 19, max: 45), 68.3% lived in the center of the city, and 63.3% lived with a nuclear family. Of the pregnant women, 15.7% worked in a wage-earning employment, 3.9% defined their economic status as 'bad', and 5.7% had no health coverage.

Table 1. *Sample Characteristics (n=407)*

Characteristic	n (%)
Trimester	
First	100 (24.6)
Second	140 (34.4)
Third	167 (41.0)
Mean ± SD weight before pregnancy (kg)	62.94 ± 9.41
Mean ± SD weight gain during pregnancy (kg)	7.46 ± 4.74
Planned mode of delivery	
Vaginal	369 (90.7)
Caesarian section	38 (9.3)
Parity	
Nulliparous	312 (76.7)
Multiparous	95 (23.3)
Breastfeeding experience	
Yes	309 (75.9)
No	98 (24.1)
Knowledge related to breastfeeding	
Sufficient	358 (88.0)
Unsufficient	49 (12.0)
Information receiving status on breastfeeding	
Yes	328 (80.6)
No	79 (19.4)
Knowledge source related to breastfeeding (n=328)	
Health professionals	312 (76.7)
TV / book / newspaper	10 (2.5)
Mother / relative	3 (0.7)
Friend	3 (0.7)

SD: standard deviation

Considering the gestational period, 41% of the pregnant women were in their third trimester. The mean weight before pregnancy was 62.94 kg ± 9.41 kg, and the mean weight gained during pregnancy was 7.46 kg ± 4.74 kg. The expected delivery method of 90.7% of the pregnant women was normal spontaneous vaginal delivery, 76.7% of them had a nulliparous, and 75.9% of them had a 'breastfeeding' history. Of the pregnant women, 88% found their level of knowledge of breastfeeding sufficient; 80.6% of them stated that they received knowledge about breastfeeding, and 76.7% of those who stated that they had received knowledge indicated that their source of information was 'health professionals' (Table 1).

Reliability Analysis

3.1.1 Item and total correlations

In our study, the item–total correlations of the scale, which consisted of 39 items, were analyzed. As a result of the item analysis, it was found that there were no items with an item–total correlation coefficient (r) smaller than 0.30, and that the item–total correlation coefficients ranged from r = 0.37 to r = 0.77. The findings that were obtained indicated that the items constituting the scale had sufficient power to represent the scale (Table 2).

Table 2. *Item-Total Point Correlation of PREP to BF*

Item No	Mean	Standart Deviation	Corrected Item-Total Correlation	Cronbach's Alpha if Item Deleted
BS1	6.705	2.247	0.559	0.963
BS2	6.869	2.020	0.666	0.963
BS3	6.611	2.330	0.562	0.963
BS4	7.029	2.221	0.709	0.963
BS5	6.474	2.292	0.685	0.963
BS6	6.476	2.238	0.659	0.963
BS7	6.702	2.559	0.631	0.963
BS8	6.029	2.760	0.376	0.964
BS9	7.287	2.443	0.681	0.963
BS10	7.132	2.521	0.724	0.962
BS11	6.837	2.527	0.693	0.963
BS12	7.066	2.658	0.725	0.962
BS13	6.990	2.416	0,696	0.963
BS14	7.098	2.429	0.726	0.962
KS1	6.783	2.480	0.588	0.963
KS2	6.518	2.639	0.626	0.963
KS3	6.781	2.595	0.707	0.963
KS4	6.945	2.456	0.698	0.963
KS5	6.098	2.990	0.458	0.964
KS6	6.744	2.539	0.654	0.963
KS7	6.353	2.746	0.517	0.964
KS8	6.552	2.512	0.653	0.963
KS9	4.142	3.341	0.505	0.964
KS10	4.066	3.249	0.485	0.964
KS11	6.100	2.731	0.570	0.963
KS12	6.434	2.472	0.750	0.962
KS13	6.611	2.462	0.729	0.962
KS14	6.815	2.825	0.585	0.963
KS15	6.746	2.680	0.547	0.963
KS16	6.697	2.739	0.506	0.964
PT1	7.245	2.306	0.717	0.963
PT2	7.191	2.313	0.706	0.963
PT3	7.287	2.333	0.770	0.962
PT4	7.081	2.521	0.727	0.962
SD1	5.921	3.058	0.398	0.964
SD2	6.638	2.518	0.618	0.963
SD3	7.199	2.369	0.736	0.962
SD4	7.346	2.398	0.755	0.962
SD5	7.292	2.545	0.714	0.962

PREP to PF: The Prenatal Rating of Efficacy in Preparation to Breastfeed Scale, BS: Individual Processes, KS: Interpersonal Processes, PT: Professional Advice, SD: Social Support

3.1.2 Internal consistency

In this study, the Cronbach Alpha coefficient of the scale with 39 items was 0.96, and the internal consistency of the scale showed it was highly reliable.

3.1.3 Split-half reliability

In this study, the Spearman-Brown and Guttman Split-Half reliability coefficients, which were obtained by using the split-half method, were checked. The Spearman-Brown and Guttman Split-Half internal consistency coefficients of the scale were both 0.88.

Validity Analysis

3.2.1 Construct validity

The Kaiser Meyer Olkin (KMO) value of the scale was above 0.60. The Bartlett test was found to be significant. It was found that the data were suitable for factor analysis (Table 3).

Table 3. *Factor Model of the Prenatal Rating of Efficacy in Preparation to Breastfeed Scale*

Kaiser Meyer Olkin of sampling adequacy		0.910
Bartlett's test of sphericity	χ2	146.14
	SD	741
	p	0.000

3.2.2 Explanatory factor analysis (EFA)

In this study, the adapted scale was determined to have a different factor structure and item distribution than the original scale. While determining the items of the scale through EFA, attention was paid to ensure that eigenvalues were 1, values of item factor loads were at least 0.30, each item loaded only a single factor, and there was at least 0.10 points of difference between the factor loads of the items loading two factors (27,28). A 5-factor structure with eigenvalues above 1 that explained 71.267% of the variance emerged, when 11 items, which had less than 0.10 points difference between the factors, were excluded **(**Factor 1 = 46.445%, Factor 2 = 9.282%, Factor 3 = 5.996%, Factor 4 = 5.063%, Factor 5 = 4.481%). The eigenvalues of the factors were Factor 1 = 13.005, Factor 2 = 2.599, Factor 3 = 1.679, Factor 4 = 1.418, Factor 5 = 1.255. The factor loads of the items varied between 0.46 and 0.82 (Table 4). Following a factor rotation, seven items gathered under the first factor (BS3, BS7, BS8, BS9, BS11, BS12, BS14), nine items under the second factor (KS1, KS2, KS3, KS4, KS6, KS7, KS11, KS12, KS14), five items under the third factor (BS1, BS2, BS4, BS5, BS6), four items under the fourth factor (PT1, PT2, PT3, PT4), and three items gathered under the fifth factor (SD2, SD3, SD4). The items gathering under the 'Individual Processes' factor on the original scale were found to be divided into two factors in the present study. Because the other items were distributed as they were on the original scale, the names of the factors were kept as in the original scale. In this context,

the first factor was named 'Individual Processes' the second factor 'Interpersonal Processes' the third factor 'Mental Individual Processes' the fourth factor 'Professional Advice' and the fifth factor was named 'Social Support'.

Table 4. *The Results of Explanatory Factor Analysis of PREP to BF*

Factors	Item No	Items*	Factor Load	Variance	Eigenvalues
Factor 1 Individual Processes	**BS3**	Set goals for yourself to be successful at breastfeeding your baby?	0.713		
	BS7	Accept that breastfeeding takes time?	0.792		
	BS8	Accept others opinions (positive or negative) about breastfeeding?	0.828	46.445%	13.005
	BS9	Visualize yourself being successful at breastfeeding?	0.742		
	BS11	Accept that breastfeeding will NOT always be easy?	0.799		
	BS12	See yourself as a breastfeeding mother?	0.788		
	BS14	Solve problems that may keep you from breastfeeding your baby?	0.631		
Factor 2 Interpersonal Processes	**KS1**	Ask another breastfeeding mother questions about breastfeeding?	0.702		
	KS2	Obtain opportunities to watch other women breastfeed?	0.763		
	KS3	Talk about breastfeeding with your close friends?	0.689		
	KS4	Talk about breastfeeding with family members?	0.763		
	KS6	Accept advice about breastfeeding from family members?	0.531	9.282%	2.599
	KS7	Locate breastfeeding support in your community?	0.768		
	KS11	Explain the benefits of breastfeeding to another person?	0.672		
	KS12	Discuss breastfeeding with other mothers or pregnant women?	0.641		
	KS14	Talk about breastfeeding with your partner?	0.674		
Factor 3 Mental Individual Processes	**MBS1**	Overcome any fear you may feel about breastfeeding?	0.742		
	MBS2	Overcome any anxiety you may feel about breastfeeding?	0.657		
	MBS4	Mentally prepare yourself to breastfeed your baby?	0.658	5.996%	1.679
	MBS5	Manage the possible challenges that may come with breastfeeding?	0.628		
	MBS6	Overcome any stress you may feel about breastfeeding?	0.620		
Factor 4 Profes. Advice	**PT1**	Gather information to help you make a decision about breastfeeding?	0.685		
	PT2	Find the answers to your questions about breastfeeding?	0.746	5.063%	1.418
	PT3	Accept advice from your health care provider about breastfeeding?	0.797		
	PT4	Talk about breastfeeding with your health care provider?	0.585		
Factor 5 Social Support	**SD2**	Depend on your friends to support the decisions you make about your baby?	0.892		
	SD3	Count on your family to support the decisions you make about your baby?	0.929	4.481%	1.255
	SD4	Count on your family to support the decisions you make about infant feeding?	0.491		

PREP to PF, The Prenatal Rating of Efficacy in Preparation to Breastfeed Scale; BS, Individual Processes; KS, Interpersonal Processes; MBS, Mental Individual Processes; PT, Professional Advice; SD, Social Support; ***All questions began with the root: "Thinking about your life right now, how well can you …"***

3.2.3 Confirmatory factor analysis (CFA)

In this study, it was understood that the fit index values of RMSEA (0.058), CFI (0.86), AGFI (0.89), and NFI (0.94) indicated an 'acceptable fit', and SRMR (0.089) indicated a 'good fit'. It was understood that the model fitted the observed data in terms of these fit index values, and that the Turkish version of the scale demonstrated an acceptable level of fit (Table 5; Path Diagram).

Table 5. *Fit Index Values of Confirmatory Factor Analysis*

Compliance Measures	Acceptable Compliance	Good Fit Measurement	Scale Value
RMSEA	.05 < RMSEA ≤ .08	0 ≤ RMSEA ≤ .05	0.058
SRMR	.05 < SRMR ≤ .10	0 ≤ SRMR ≤ .05	0.089
NFI	.90 ≤ NFI < .95	.95 ≤ NFI ≤ 1.00	0.94
CFI	.95 ≤ CFI < .97	.97 ≤ CFI ≤ 1.00	0.95
AGFI	85 ≤ AGFI < .90	90 ≤ AGFI ≤ 1.00	0.89
x2/df	2 < x2/df ≤ 5	0 ≤ x2/df ≤2	2197.62/680 = 3.23

RMSEA: Root Mean Square Error of Approximation, S-RMR: Standardized Root Mean Square Residual, NFI: Normed Fit Index, CFI: Comperative Fit Index, AGFI: Adjusted Goodness-of-fit Index, χ2: chi-square

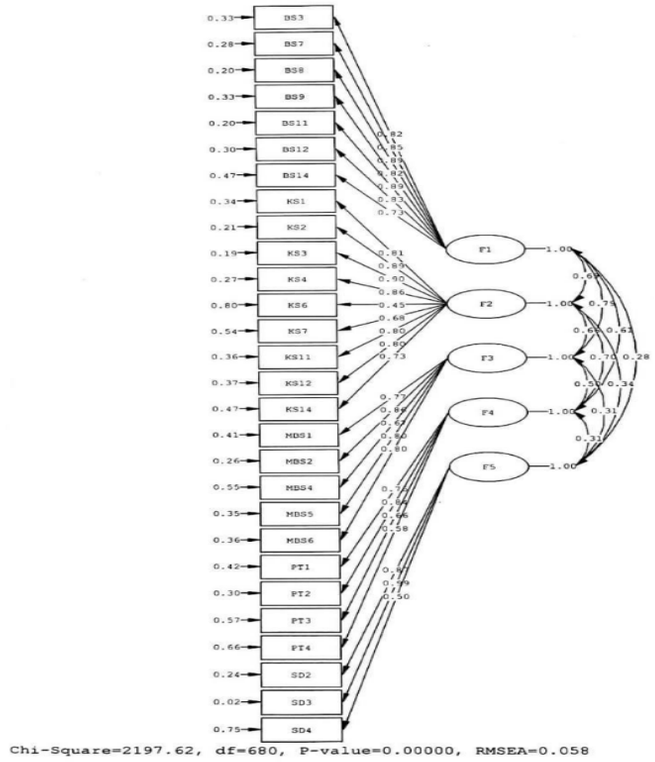

Figure 1. Path *Diagram: Cnfirmatory factor analysis of PREP to BF PREP to PF: The Prenatal Rating Of Efficacy in Preparation To Breastfeed Scale BS: ındividual processes, KS: Interpersonel Processes, MBS: Mental Individual Processes, PT: Professionel Advice, SD: Social Support*

4. DISCUSSION

Item analysis is carried out to identify how powerful each item on a scale is to measure what is desired to be measured with the scale. In the literature, the item–total correlation coefficient is recommended not to be negative and recommended to be greater than 0.30. Additionally, if item–total correlation coefficients are greater than 0.40, it is assumed that the discriminatory properties of the items are good (28,29). In the present study, the item–total correlation coefficients of all items were found to be greater than 0.30. It was found that there were no items with a coefficient of less than 0.30. The item–total correlation coefficients ranged from 0.37 to 0.77. The data showed that the discriminatory properties of all the items that made up the scale were good (Table 2).

The Cronbach Alpha coefficient provides information about the extent to which items constituting a scale are consistent with each other and to what extent they represent the variable (30). There are certain criteria for evaluating the Cronbach Alpha coefficient. Accordingly, the alpha value indicates how reliable a scale is as follows: 0.00–0.40 = not reliable, 0.40–0.60 = poorly reliable, 0.60–0.80 = very reliable, and 0.80–1.00 = highly reliable (27,29,31,32). In this study, the Cronbach alpha coefficient of the adapted scale was found to be 0.96, and the scale was determined to be highly reliable.

Another method that is used to test the reliability of a scale is the split-half method. The split-half method is the most commonly used technique for estimating test reliability. A high reliability coefficient obtained through this method indicates that both forms are reliable, and a coefficient that is not high enough indicates that the reliability of both forms is low (33). In this study, the Spearman-Brown and Guttman Split-Half coefficients were both found to be 0.88 by using the split-half method. In order to decide whether the sample size is adequate in scale studies and whether the scale is suitable for factor analysis, the KMO value should be greater than 0.60, and the result of Bartlett test should be significant (28,31,32). The KMO value of the scale in this study was greater than 0.60, and the Bartlett test was found to be significant. Therefore, the sample was considered to be suitable for factor analysis (Table 3).

When developing a scale, EFA is recommended; however, when adapting a scale from a different culture, only CFA is recommended as the factor structure of the scale is known from the beginning (34). In this study, the adapted scale was determined to have a different factor structure and item distribution than the original scale (Table 4). CFA is used to determine the validity of a predetermined structure (31,32,35). The compatibility of the models established with CFA to the data is evaluated by looking at some fit indexes: χ2 (chi-square) and their concordance index: Root-Mean-Square Error of Approximation (RMSEA), Standardized Root Mean Square Residual (S-RMR), Adjusted Goodness of Fit Index (AGFI), Comparative Fit Index (CFI), Normed Fit Index (NFI) (31,32). It was understood after the CFA that the model fitted the data in terms of the fit index values of the adapted scale and that the Turkish version of the scale demonstrated a good fit (Table 5, Fig 1: Path Diagram).

5. CONCLUSION

In our study, the model–data fit of the Turkish form of the scale was found to be adequate. The scale can be employed by healthcare professionals to assess prenatal breastfeeding self-efficacy. It can contribute data for the structuring of the content of training and consultancy programs intending to improve breastfeeding self-efficacy.

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Clinical and Experimental Health Sciences

Barriers to Women's Pap Smear Testing and Related Risk Factors in Turkey

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ABSTRACT

Objective: This study aimed to identify the barriers to women's Pap smear testing and related risk factors.

Methods: In this descriptive and cross-sectional study, 294 women were included by stratified random sampling. The General Information Questionnaire, The Self-Efficacy Scale, The Health Belief Model Scale for Cervical Cancer, and the Pap Smear Test were used.

Results: About half of the women (47.6%) had a Pap smear test in their lifetime. Residing in a village/non-provincial district (OR = 0.412), not having a Pap smear test because family members/friends did not have one (OR = 7.752), having high Barriers subscale scores (OR = 1.053), and having lower self-efficacy levels (OR = 0.951) were found to be risk factors for not having a Pap smear test. In addition, not knowing the symptoms of cervical cancer, believing that the cervical cancer is a non-treatable condition, expecting to experience vaginal infection, not being able to get permission from her husband, and considering the test as embarrassing were found among the barriers ($p < 0.005$).

Conclusion: A well designed health education program focusing on cervical cancer and the benefits of screening would increase awareness, especially among women with a high educational attainment status. Public health nurses and midwives should provide health educations considering the local cultural environment. To improve access to health services, mobile health services need to be developed, and in hospitals, routine Pap smear test health educations should be conducted and guidance should be provided.

Keywords: Cervical cancer, screening, barriers, self-efficacy, health belief model, Pap smear test.

1. INTRODUCTION

Cervical cancer is globally an important cause of morbidity and mortality in women of all ages (1,2). In Turkey, cervical cancer incidence increases at the age of 40 and over (10.5 per 100.000), and its highest incidence rate (15.7 per 100.000) is seen in 65-70 year-old women (3). Per the recommendation of the World Health Organization (WHO), women in Turkey in the age range of 30-65 years have been screened every 5 years since 1992 with the Pap (Papanicolaou) test (3). Despite this effort, the rate of Pap smear test in Turkey is not at a desired level. According to a report by the Turkish Statistical Institute (TSI), only 30% of the women in the age range of 25-65 had a Pap smear test (4). The rate of having a Pap smear test in the previous year in women over the age of 15 years in Turkey was reported to be 10.9% and the rate of those who had never had a Pap smear test before was 69.3% (5). This rate is expected to be much higher considering the facts that, in the Turkish health care system, Pap smear tests are performed at a very low cost in urban and rural areas, and even they are performed free of charge in Cancer Early Diagnosis, Screening and Education Centers. However, in both national health statistics and surveys conducted in different geographical areas of the country, the rate of having a Pap smear test is very low (4,6,7). In Turkey, studies investigating the reasons why women do not participate in cervical cancer screenings and why they do not have a Pap smear test are very limited and it is very important to find out the barriers to their participation in screening tests. In international studies, barriers to women's having a Pap smear test have been cited as lack of knowledge, religious beliefs, embarrassment, difficulty in access, the fear of a positive result, no health insurance, not having time, and the gender of the healthcare provider (8-10). In a study conducted in Texas, USA involving 524 women, the following barriers were cited: cost (61.6%), fear of a positive result (53.1%), worry (38.7%), embarrassment (25.6%), expectation of pain (23.6%), male obstetrician (19.7%), lack of knowledge (18.8%), language (18.3%), other health problems (16.5%), neglecting to make an appointment (14.9%), and not allocating time (13%) (11).

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In another study conducted in Nigeria, the following factors were reported: the gender of the healthcare provider, the fear of a positive result, the fear of catching a disease at the hospital, discomfort during screening, not being aware of the screening programs, not caring much about cervical cancer, and the need for their husband's approval before screening (9). In order to reduce the incidence of cervical cancer, it is important to understand the perceived structural and personal barriers, misconceptions, and socio-cultural differences in the population in having cervical screening tests. The aim of this study was to identify the barriers to women's having a Pap smear test and related risk factors.

The questions addressed in the study were:

1. Do sociodemographic characteristics of women affect the rate of having a Pap smear test?
2. Is there a relationship between women's having a Pap smear test and their Health Belief Model Scale for Cervical Cancer-Barriers subdomain and Pap Smear Test scores?
3. Do the women's self-efficacy levels affect the rate of having a Pap smear test?
4. Do cultural factors affect the rate of having a cervical screening test?

2. METHODS

2.1. Design

The study was designed as descriptive and cross-sectional.

2.2. Study Setting

The research was carried out at Family Health Centers (FHCs) located in Konya, a province in the Central Anatolia Region of Turkey. There are three metropolitan districts of Konya, namely, Karatay, Meram, and Selcuklu. There are 23, 25, and 24 FHSs in these districts, respectively, with a total of 72 FHSs in the province. Since the women living in these districts have sociodemographic and lifestyle differences and to ensure heterogeneity in the study, women from 3 districts were included in the study. One FHS from each district was randomly selected: FHC-15 from Karatay, FHC-5 from Selcuklu, and FHC-25 from Meram.

2.3. Study Population, Sample Size, and Sampling Strategy

The universe of the study was all ≥18 year-old women who were married or living with a partner and presented to the selected FHCs between 1 February and 1 May 2019. Sample size of the study was calculated using an online tool: A-priori Sample Size Calculator for Multiple Regression (12). The sample size was calculated based on 2 main variables and 21 observed variables, with an effect size of 0.10 (medium), statistical power of 0.90, and α probability of error of 0.05. The minimum required sample size was calculated as 294 using the online calculator (13). During the study period, 350 women were invited to participate, but 56 women rejected to take part due to such reasons as infants' crying, breastfeeding, disapproval of the partner/husband, and lack of time. Therefore, the study was completed with 294 women, who agreed to participate in the study and filled out the forms completely. For this descriptive, cross-sectional research, a total of 294 women were included by means of convenience sampling. The inclusion criteria were being women, being at an age of ≥18 years, having an active sexual life, being literate, being able to speak Turkish, and being volunteer. There was no upper age limit in the participation criteria and women who were in premenopausal or menopausal women were included in the study. Exclusion criteria were being virgin, pregnant, being in the postpartum period, having been diagnosed with cervical preinvasive lesion or cervical cancer, having a mental problem, having an inactive sexual life, and not being able to speak Turkish.

2.4. Pilot Study

To pretest the items in the data collection tools, the tools were administered in 5 women from each FHC and the item with which the women had difficulty in understanding was revised. The answers given by these women were not included in statistical analyses.

2.5. Data Collection

We used a well-structured self-administered questionnaire to collect the data. All participants who properly completed the questionnaire were included in the study. The questionnaire had three sections and took about 15 minutes to complete. The data were collected by the researchers by face-to-face interviews. Women who were willing to participate in the study were asked to fill in the questionnaires in a private room in the FHC. The General Information Questionnaire, the Self-Efficacy Scale, the Cervical Cancer and Pap Smear Test Health Belief Model-Barriers Subscale were used for data collection regarding the women's socio-demographic features and the barriers to the women's having a cervical screening test.

2.6. Measures

2.6.1. The general information questionnaire: This questionnaire was developed by the researcher using the literature. It consists of two parts as 'socio-demographic items' and 'items regarding the barriers to the women's having a cervical test'.

Socio-demographic items: This part comprises 13 items about the educational level, marital status, place of residence, employment status, health insurance status, income level, family type, year of marriage, number of children, chronic disease status (hypertension, diabetes mellitus, etc.), family/individual history of cervical cancer, age, and the age of menarche (9-11,14).

Items regarding the barriers to the women's having a cervical test: This section comprises 9 items, namely, "(1) I don't know the symptoms and indications of cervical cancer, (2) I believe that cervical cancer is not a treatable disease, (3) I don't want to have a Pap smear test because of others' negative comments about it (4) I think that I'll get vaginal infection after the Pap smear test, (5) Because my husband/partner would not allow me to have a Pap smear test, (6) I cannot have a Pap smear test because I cannot afford it, (7) I don't have a Pap smear test because my family members/friends do not have it, (8) I think that Pap smear test is embarrassing, and (9) My religion would not allow me to have a Pap smear test" (10,11,14,15).

2.6.2. The self-efficacy scale: This scale is used to assess women's self-efficacy perceptions regarding the early diagnosis of breast and cervical cancer. The scale was developed by Lechner et al. and reliability and validity studies of the Turkish version of the scale were conducted by Beser et al. Cronbach's Alpha coefficient of the scale is 0.90 (16, 17). The scale comprises seven 7-point Likert-type items. The total score of the scale ranges between -21 and +21. The higher score indicates better self-efficacy perception regarding the improvement of a specific behavior. Cronbach's Alpha internal consistency coefficient of the Self-Efficacy Scale in the present study was found as 0.88.

2.6.3. The health belief model scale for cervical cancer and the pap smear test: The scale was first developed by Champion et al. in 2004 to assess breast cancer screening and the adaptation of the scale for cervical cancer screening was carried out by Guvenc et al in 2008 (18). The adapted version of the scale assesses the effects of the women's health beliefs about cervical cancer and Pap smear test on the rate of having a Pap smear test. Cronbach's Alpha internal consistency coefficient of the scale was reported as 0.62-0.86 (18). Cronbach's Alpha internal consistency coefficient for Pap smear barrier perceptions (14 items) was found as 0.82. The scale comprises 35 items (5-point Likert-type) in 5 subscales. Responses receive points between 1 and 5. Each subscale is evaluated internally. The total score of the Barriers subscale ranges between 14 and 70. Higher scores indicate a higher perception of the barriers. Cronbach's Alpha internal consistency coefficient in the present study was found as 0.80.

2.7. Ethical Approval

At the beginning of the research, research ethics committee approval was granted by the Faculty of Health Sciences of Selcuk University (Ethics permit no: 34/2019). Institutional permission was obtained from the Health Directorate of Konya Province (10.12.2018, 94723667-806.01.03). The primary purpose of the research was explained to all participants. All women involved in this study signed an informed consent form. Furthermore, all the information related to the participants was kept strictly confidential.

2.8. Data Analysis

The statistical package program of SPSS 20.0 for Windows (SPSS Inc., Chicago, IL, USA) was used to analyze the data. Because all skewness and Kurtosis values of all scales were found between -1.50 and +1.50, independent samples *t*-tests were carried out (*19*). As descriptive statistical values, numbers, percentages, means, and standard deviations (SD) were used. A Chi-square test was used in the comparison of the rate of having a Pap smear test and their socio-demographic and obstetric characteristics and in comparing some of the factors in barriers to having a Pap smear test.

Educational status, marital status, the place of the longest residence, employment status, health insurance status, perceived income level, family type, year of marriage, the number of children, chronic disease status, family/individual history of cervical cancer, age, Self-Efficacy scores, The Health Belief Model Scale for Cervical Cancer and the Pap Smear Test – Barriers Subscale scores were treated as independent variables in bivariate analyses. As to the independent variables found significant after performing the bivariate analyses, variables, such as women's educational status (1 = junior high school and under and 0 = senior high school and over), the longest place of residence (1 = village/country, and 0 = city), family type (1 = extended family, and 0 = nuclear family), the number of children (1 = 2 and under, and 0 = 3 and over), the chronic disease status (0 = no, and 1 = yes), age, Self-Efficacy Scale Scores, and The Health Belief Model Scale for Cervical Cancer and the Pap Smear Test – Barriers Subscale scores were included in the logistic regression analysis. Logistic regression analysis with the Backward method was conducted to identify the risk factors causing the barriers to having a Pap smear test. Not having a Pap smear test was assumed as the dependent variable. The assessment was based on having a Pap smear test (1=no) and not (0=yes). In the study, two tailed tests were used, and significance was accepted as $p < 0.05$.

3. RESULTS

The mean (SD) age of the women was 39.9 (10.3) years with a mean (SD) marriage year of 18.4 (11.6) years. The mean (SD) age of menarche was found to be 13.4 (1.2) and the mean (SD) number of children they had was 2.4 (1.2). Of the women, 47.6% (n=140) had at least one Pap smear test in their lifetime and 24.8% (n=73) had the test within the previous 12 months.

Those who were graduates of high school and above (p = 0.014), those living in villages/districts (p = 0.001), those living in extended families (p = 0.018), those with a marriage year of 10 years or less (p = 0.000), those with 2 children or less (p = 0.009), those with no chronic disease (p = 0.004), younger women (p = 0.000), those with lower Self-Efficacy Score (p = 0.000), and those with higher Health Belief Model Scale for Cervical Cancer and the Pap Smear Test – Barriers Subscale scores (p = 0.000) tended not to have a Pap smear test with a significant difference (Table 1).

Table 1. *Women's Sociodemographic and Obstetric Characteristics and the Rate of Having a Pap Smear Test (n=294)*

Characteristics	Rate of having a Pap smear test		t / x^2	*P value*
	Yes	No		
	n (%)	n (%)		
Educational level				
High school and over	96 (53.3)	84 (46.7)	6.077[a]	0.014
Middle school and under	44 (38.6)	70 (61.4)		
Marital status				
Married	135 (48.7)	142 (51.3)	2.398[a]	0.121
Widowed	5 (29.4)	12 (70.6)		
Place of residence				
Village/district	38 (34.5)	72 (65.5)	12.044[a]	0.001
City	102 (55.4)	82 (44.6)		
Employment status				
Employed	35 (50.7)	34 (49.3)	0.349[a]	0.555
Unemployed/housewife	105 (46.7)	120 (53.3)		
Health Insurance				
Yes	133 (48.5)	141 (51.5)	1.370[a]	0.242
No	7 (35)	13 (65)		
Perceived Income Level				
Poor	31 (53.4)	27 (46.6)	0.984[a]	0.321
Moderate/good	109 (46.2)	127 (53.8)		
Family Type				
Nuclear Family	123 (50.8)	119 (49.2)	5.643[a]	0.018
Extended Family	17 (32.7)	35 (67.3)		
Year of Marriage				
10 year and less	18 (20.9)	68 (79.1)	34.713[a]	0.000
11 year and more	122 (58.7)	86 (41.3)		
Number of Children				
2 and under	65 (40.6)	95 (59.4)	6.884[a]	0.009
3 and over	75 (56)	59 (44)		
Chronic Disease Status				
Yes	49 (61.2)	31 (38.8)	8.187[a]	0.004
No	91 (42.5)	123 (57.5)		
Family/individual history of cervical cancer				
Yes	10 (58.8)	7 (41.2)	0.908[a]	0.341
No	130 (46.9)	147 (53.1)		
Age (mean and SD)	42.6 (7.9)	3 7 . 4 (11.5)	4.546[b]	0.000
The Self-Efficacy Scale (mean and SD)	5.7 (9.3)	0.3 (9.2)	4.982[b]	0.000
The Health Belief Model Scale for Cervical Cancer and the Pap Smear Test-Barriers Subscale (mean and SD)	34.7 (9.1)	39.5 (8.7)	-4.529[b]	0.000

[a]*Chi-square test,* [b]*t=Independent sample t-test, SD=standard deviation*

In terms of barriers to having a Pap smear test in relation with the characteristics of the Turkish society, those who do not know the symptoms and indications of cervical cancer (p = 0.028), those who believe that cervical cancer is not a treatable disease (p = 0.014), those who believe that they would experience vaginal infection after the test (p = 0.019), those who cannot get permission from their husbands (p = 0.000), those who do not have the test because their families or friends do not have it (p = 0.001), and those who believe that the test is embarrassing (p = 0.000) were had a tendency to not having the test with a significant difference (Table 2).

Table 2. *Factors Causing the Barriers to Having a Pap Smear Test*

Factors	The rate of having a Pap smear test		x^2	*P-value*
	Yes	No		
	n (%)	*n (%)*		
I don't know the symptoms and indicators of cervical cancer				
Yes	89 (43.4)	116 (56.6)	4.799	0.028
No	51 (57.3)	38 (42.7)		
I believe that cervical cancer cannot be fully treated				
Yes	89 (43)	118 (57)	5.996	0.014
No	51 (58.6)	36 (41.4)		
I don't want to have the test because I heard negative comments about it				
Yes	62 (48.1)	67 (51.9)	0.018	0.893
No	78 (47.3)	87 (52.7)		
I believe that I will have a vaginal infection after the Pap smear test				
Yes	45 (39.1)	70 (60.9)	5.456	0.019
No	95 (53.1)	84 (46.9)		
I cannot get permission from my husband				
Yes	41 (34.7)	77 (65.3)	13.096	0.000
No	99 (56.2)	77 (43.8)		
I cannot have the test because I don't have health insurance				
Yes	5 (27.8)	13 (72.2)	3.026	0.082
No	135 (48.9)	141 (51.1)		
I do not have the test because my family or friends do not have it				
Yes	11 (24.4)	34 (75.6)	11.440	0.001
No	129 (51.8)	120 (77.9)		
I think that Pap smear test is embarrassing				
Yes	12 (24.5)	37 (75.5)	12.611	0.000
No	128 (52.2)	117 (47.8)		
My religious beliefs would not allow me to have a Pap smear test				
Yes	5 (38.5)	8 (61.5)	0.457	0.499
No	135 (48)	146 (52)		

x^2*= Chi-square test.*

Table 3 presents the results of our regression analyses. Risk factors causing a barrier to cervical cancer screening are:

living in a village/non-metropolitan district (Odds Ratio [OR] = 0.412, 95% Confidence Interval [CI] = 0.227–0.747), having family/friends not having the test (Odds Ratio [OR] = 7,752, 95% Confidence Interval [CI] = 0.244–1.135), Barriers Subscale scores (Odds Ratio [OR] = 1.053, 95% Confidence Interval [CI] = 1.015–1.092), and Self-Efficacy scores (Odds Ratio [OR] = 0.951, 95% Confidence Interval [CI] = 0.919–0.004). The regression analysis showed that living in a village/district, having a family/friends not having the test, and Barriers Subscale score, and Self-Efficacy score increased the risk of not having the test 0.412, 7,752, 1.053, and 0.951 times, respectively (Table 3).

Table 3. *Logistic Regression Analysis of the Risk Factors Causing the Barriers to having a Pap Smear Test*

Risk factors causing the barriers to having a Pap smear test	B	SE	Odds Ratio (OR)	%95 CI		*P*
				Low	High	
Place of residence Village/district (Ref) City	-0.887	0.304	0.412	0.227	0.747	0.004
Having a family/ friends not having the test Yes (Ref) No	2.048	0.980	7,752	0.244	1.135	0.037
The Health Belief Model Scale for Cervical Cancer and the Pap Smear Test – Barriers Subscale	0.052	0.019	1.053	1.015	1.092	0.006
The Self-Efficacy Scale	-0.050	0.018	0.951	0.919	0.984	0.004

**Only significant variables as a result of regression analysis are presented in Table 3. Other variables in regression analysis model are presented in the Methods section.*

4. DISCUSSION

The current investigation evaluated barriers in the cervical cancer screening continuum through assessment of self-conceived obstacles for obtaining Pap smear tests. Almost half of the women (47.6%) had a Pap smear test once in their lifetime. In the previous 12 months, almost a quarter of the women (24.8%) had a Pap smear test at least once. In a study conducted in New Jersey and Southeast Pennsylvania, out of 705 women, 62%-73% were found to have had the Pap smear test once in their lifetime (20). In another study, 79% of the women in an age range of 40-64 years were found to have had a Pap smear test one in the previous 3 years (21). In Turkey, in three different studies conducted in different groups from different regions, these ratios were reported to be 13.5%, 25.2%, and 66.1%, respectively (6,7,14). Considering that Cancer Early Diagnosis Screening and Training Centres (KETEMs) provide screening service free of charge, these rates are expected to be substantively higher. However, the low rate of having a Pap smear test found in this study is in parallel with the results of previous national reports (6,14). However, in several previous reports, it has been stated because of having no health insurance or a low socio-economic status, women could not join the screening programs (22) and medical costs have been reported to be barrier to not having the Pap smear test (23). In a meta-analysis study, poverty was reported to be a strong indicator of screening, diagnosis, treatment, and survival rates (24). However, the present results show that poverty is not the only barrier, and the women's educational level, family characteristics, working status, place of residence, number of children, and age are other barriers. Furthermore, the cultural structure of the society in which the women live should be considered as an integral part, and thereby, their awareness should be raised accordingly.

In the present study, 61.4% of the women were at least high school graduates and the rate of having a Pap smear test was substantially lower in this group. However, Watts et al. (25) reported that the women with at least a high school educational status (56%) had a higher rate of having a Pap smear test. Several other investigations have shown that low levels of education are reliable indicators for screening non-adherence (14, 26). It is considered that the women did not allocate time for the Pap smear test because they were young and they had work- or family-related responsibilities. However, public health nurses and midwives have a great responsibility in raising these women's awareness regarding the Pap smear test.

It was also observed that some cultural characteristics constitute barriers to having a Pap smear test. Women in the present study did not have a Pap smear test because they did not know the symptoms and indicators of cervical cancer, believed that cervical cancer cannot be treated, had the fear of having a vaginal infection after the test, did not get permission from their husbands to have the test, because their family/close friends did not have it, and thought that the Pap smear test is embarrassing. In previous studies, cultural and social barriers included embarrassment to tell people about cervical cancer screening, husband and family not allowing screening, and not knowing what other people would think. This is consistent with other findings from previous studies, where women avoided screening due to attitudes of fear, lack of knowledge, inaccessibility of health services, cultural beliefs, and the belief that cervical cancer is an incurable disease (15, 27).

Living in a village/district (Odds Ratio [OR] = 0.412, 95 % Confidence Interval [CI] = 0.227–0.747) was found to be a risk factor for not having a Pap smear test Similarly, those who have better access to a health center or living in city centers were found to have the test more (8,28). Fernandez et al. (29) also reported that limited access to health centers is an important contributing factor in barriers to having a Pap smear test. Because health centers are not within walking distance in countryside, there is limited mass transportation opportunities, and those living in such areas have low income level, family practitioners and nurses need to provide mobile health services for these women to have a Pap smear test.

In the present study, families and friends play an important role in encouraging the women to have the test. The fact that the women did not have a Pap smear test because their family members/friends did not have it (Odds Ratio [OR] = 7.752, 95% Confidence Interval [CI] = 0.244–1.135) was found to be a risk factor. Women receive recommendations and advices from family members and friends and they receive information from the people they trust, which was found to be one of the important facilitators of Pap smear test. Similarly, it was found that women are informed by their friends or they consult their friends for an advice (30.9%), and they are influenced by their family members or friends (%22) (30, 31). This outcome highlights the importance of conventional communication channels or information sources, and also the information provided by family members and friends raises the women's self-efficacy levels.

The Health Belief Model Scale for Cervical Cancer and the Pap Smear Test – Barriers Subscale outcomes were found to be a contributing risk factor in barriers to having a Pap smear test for cervical cancer screening. Regression analysis showed that the total score of the Barriers Subscale total scores affect the women's having a Pap smear test 1.053 times (Odds Ratio [OR] = 1.053, 95% Confidence Interval [CI] = 1.015–1.092). In a previous similar study in which barriers to having a Pap smear test were investigated, the Health Belief Model Scale for Cervical Cancer and the Pap Smear Test – Barriers Subscale scores were found to inhibit the women from having the test 0.22 times (32). Consistent with previous studies, our finding showed that women with higher scores on the perceived barriers for cervical cancer screening were less likely to have a Pap smear test ever than those with lower scores (26,33). Development and application of education programs based on the Health Belief Model may raise the women's consciousness and reduce the barriers perceived, which may result in an increase in the rates of having a Pap smear test.

Another risk factor for having a Pap smear test is the Self-Efficacy Scale. Logistic regression analysis showed that Self-Efficacy Scale total scores influence the rate of having a Pap smear test 0.951 times (Odds Ratio [OR] = 0.951, 95% Confidence Interval [CI] = 0.919-0.984). In a study investigating the Korean women's having a Pap smear test, a positive correlation was found between Self-Efficacy scores and the rate of having the test (34). Moreover, Fang et al. (20) found a positive correlation between Self-Efficacy results and the rate of having a Pap smear screening test, and Hogenmiller et al. (35) reported that self-efficacy was the primary indicator in having a Pap smear test.

4.1. Limitations

The present study has some limitations. The present study involved women from a few family health centers from city centers only. The results cannot be generalized to the general public, and especially those who do not visit these FHCs may have different barriers. Therefore, the results of this study should be interpreted with caution. The analysis was based on cross-sectional data; thus, causal relationships could not be inferred. The perception of barriers is self-reported, and it is difficult to measure the validity of these responses. On the other hand, one of the strengths of the study is that, it presents a comprehensive account of the barriers to having a Pap smear test, information about knowledge resources, and the women's perception.

5. CONCLUSION

The present study attempted to identify the barriers to women's having a Pap smear test and related risk factors. Almost half of the women (47.6%) in the present study had a Pap smear test once in their life time. Living in a village/non-metropolitan district, having family members/friends not having the test, higher Barriers Subscale scores, and lower Self-Efficacy scores were found to be barriers to having a Pap smear test. In addition, not knowing the symptoms of cervical cancer, believing that cervical cancer is not a treatable disease, thinking that having the test would cause a vaginal infection, not being able to get permission from husband, and thinking that the test is embarrassing were also found to be barriers to having the test. Based on the results of the present study, a well-designed health educational program regarding cervical cancer and the benefits of screening would raise awareness especially among the women with higher education in Turkey. To this end, a multimedia approach enriched with audio-visual materials and presented via personalized communication methods may result in positive results. Public health nurses should provide culturally-oriented health education, mobile health services should be offered for better accessibility to health services, routine Pap smear test health educations should be offered in hospitals, and individualized health education and consultancy should be provided.

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Clinical and Experimental Health Sciences

Genital Hygiene Behaviors of Women and Their Effect on Vaginal Infections

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ABSTRACT

Objectives: This descriptive study was carried out with the purpose of identifying women's genital hygiene behaviors and the effects of these behaviors on vaginal infections.

Methods: The study included 266 married women, ages 20-49, who consented to participate in the research and were registered at the University Hospital in Trabzon, Turkey. The data were collected with a questionnaire, the Genital Hygiene Behavior Inventory (GHBI), a gynecological examination and the Pap smear test. The results were evaluated by descriptive statistical methods (frequencies, percentages, means, and standard deviation), the Student's t-test, Kruskal Wallis and Mann Whitney U tests.

Results: The mean GHBI score of the women was found to be 77.41±9.05. There were statistically significant differences in genital hygiene behaviors between the women in terms of the women's education, profession, presence or absence of social security, income, husband's work and education, number of pregnancies, total number of children, spontaneous abortion, presence of vaginal discharge and the duration of complaints ($p<0.05$). Of the women, 54.9% reported that they had vaginal discharge; 45.1% had pathological vaginal discharge and odor (34.6%) and burning (35%) complaints. The speculum examination showed that 42.6% had pathological discharge and the incidence of genital infection was 79% in the Pap smear. According to the speculum examination, 71.4% of the women were found to have either pathological or non-pathological discharge.

Conclusions: It was found that the mean GHBI score of the women was slightly above the moderate level and that wrong and inadequate genital hygiene practices increased the women's risk of vaginal infection.

Keywords: Genital hygiene, genital hygiene behavior inventory, genital infection, women

1. INTRODUCTION

The 15-49 years in which women are fertile is a period in which reproductive health problems and issues stand out. One of the most common reasons women in reproductive ages apply to gynecology outpatient clinics is urogenital infection (1). Every year, around one million women in the world suffer urogenital system infections and at least 75% have a history of genital infection (1,2)]. It is reported in regional studies in Turkey that both genital infections (52%-92%) (3-6) and risky hygiene behavior that may cause vaginal infections are common (11-19).

Factors leading to genital infection in women are very diverse. The proximity of the urethra, vagina, and anus to each other is the most important factor creating a predisposition for genital infections. This is accompanied by individual factors that increase the risk of genital infection. These individual factors are a low level of education and socioeconomic status, lack of information about genital hygiene, an excessive number of children, the practice of vaginal douching and the habit of using cloth padding in underwear, IUDs, not washing hands before and after going to the toilet, improper perineal cleaning in the toilet, inappropriate underwear, lack of genital hygiene during menstruation (7-10).

Genital infections do not always threaten women's lives, but their symptoms can lead to serious illnesses (20). These infections may lead to ectopic pregnancy, sepsis, cervical cancer, infertility and congenital infections in the newborn (20, 21). Infections can also cause psychological problems, social isolation, sexual problems, fatigue, and impaired quality of life in women. Time, work and

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economic losses can be experienced depending on the intensity of these factors (22-24). Genital hygiene is the most important method of preventing genital infections and their more serious consequences (25,26). It is known that habits of personal hygiene are important in the control of many infectious diseases. However, research results indicate that women lack knowledge about appropriate genital hygiene practices. It is therefore important to know the risk factors that cause genital infections in women.

The general data on genital hygiene behavior and genital infections in Turkey largely reflect regional characteristics due to the difficulty of conducting studies on the personal matter of genital hygiene in the general population, especially since there are region-specific differences in practices. For this reason, new and current studies need to be carried out to gather information about the relationship between genital hygiene behaviors and genital infection. It was in this context that this study was conducted. The aim was to explore the genital hygiene behaviors of women and the effects of these behaviors on vaginal infections.

2. METHODS

2.1. Type of study

This is a descriptive study.

2.2. Place and time of study

The study was conducted in Trabzon, Turkey, between January 1, 2015, and May 2015.

2.3. Study population and sampling

The study consisted of 10400 women who applied to the gynecology polyclinic of the Farabi Hospital of the Faculty of Medicine for any reason. The sample size was calculated by known population sample selection formula and was set at a total of at least 266 in the 95% confidence interval. The study included women between 20 and 49 years of age who were literate at least, sexually active, non-menopausal, not pregnant or postpartum. Farabi Hospital of the Faculty of Medicine, where the research was conducted, was preferred because of the high potential of patients and the application of the woman from every socio-economic level.

2.4. Data collection instruments

The data of the study were collected through face-to-face interviews. The data were collected via a questionnaire prepared by the researchers and consisting of 32 questions that address socio-demographic and obstetric characteristics, as well as vaginal infection or infection status, and Genital Hygiene Behavior Inventory (GHBI) (14,16). Then, a Pap smear test was taken by a specialist doctor for gynecological examination of the women and the results were recorded by the researcher after 20 days. Following the application of the questionnaire, the women were trained on the issues related to genital hygiene behaviors that women wonder about, they mistreat and lack by the researcher.

2.5. Genital hygiene behavior inventory (GHBI)

The inventory, in the format of a four-level item, was developed by Ege and Eryılmaz to determine the genital behaviors of women aged between 15 and 49 (14). The inventory is single-dimensional and includes 24 positive and 3 negative items. Each item includes the alternative answer “never”, “sometimes”, “frequently”, and “always”. For the items with positive statements, the answer “never” receives “1” point, and the other answers “2”, “3”, and “4” points, respectively. For the items with negative statements, the classification was done in the reverse way. In the inventory, the lowest and highest total points are 27 and 108 points, respectively. The high total points showed good behavior related to genital hygiene. The Cronbach’s alpha value of the inventory was found as 0.86 by Ege and Eryılmaz (14), whereas it was found as 0.87 in this study.

2.6. Data analysis

Statistical analyzes were performed using SPSS 17.0 statistical package program. As well as descriptive statistical methods (frequency, percentage, mean, standard deviation), the Kolmogorov Smirnov test was used to determine if they were appropriate for normal distributions in comparison with the quantitative data, the Student’s t test was used for the data with normal distributions, the Mann Whitney U test and the Kruskal Wals test were used for the data with no normal distribution. The results were evaluated at a 95% confidence interval.

2.7. Ethical considerations

This study was approved by the Ethics Committee (E.3734), and all the procedures were performed under the supervision of the committee and according to the 1964 Helsinki Declaration.

3. RESULTS

The mean GHBI score of women with a discharge and discharge complaints lasting more than 6 months was found to be lower ($p < 0.05$) (Table 1). The mean GHI score of the women was found to be 70.27 ± 10.05 (Table 1).

The mean age of the women was 38.5 ± 7.9 (min: 20, max: 49). 52.6% of the women had primary school and below education level, 70.3% were unemployed, 50.4% had

middle income level, 83.8% had a nuclear family, 92.1% had social security and 63.9% had been married for more than 10 years. It was found that 59.4% of the women had a maximum of 3 pregnancies, 67.3% had 3 or fewer children, 74% did not have a spontaneous abortion, 84.6% did not have self-induced abortion, and 54.9% used a family planning method. 25.9% of those using a family planning method used a traditional withdrawal method. 45.5% and 66.5% of the husbands of the women were secondary/ high school graduates and employed respectively (Table 2).

Table 1. *Comparison of the GHBI scores according to genital complaints characteristics of women*

Genital complaints of women	N	Mean Rank	Median	Test / p
Discharge				
Yes	146	119.83		7119.500**
No	120	144.74		**.009**
The duration of the complaint				
1month and less	63	132.21	69.00	
2-3 months	86	143.42	71.50	7.866*
4-5 months	21	162.71	75.00	**.049**
6 months and over	96	119.07	67.00	
	X	**SD**	**Min-Max**	
Total scores of the GHBI scale	70.27	10.05	46.00-95.00	
** Kruskal Wallis Tests , ** Mann Whitney U Tests*				

It was found that there was a statistically significant difference between GHBI scores and age, education, the occupation of the husband, the education level of the husband, income level, the length of marriage, family type, the number of pregnancies, the number of alive children and spontaneous abortion ($p<0.05$) (Table 2).

The results showed that the GHBI score decreased as the women's age and marriage progressed. As the education level of the women and her husband increased, if the monthly income was perceived as good and if the husbands were employed and if they had nuclear families, GHBI scores increased. There was no statistically significant difference between self-induced abortion, the status of using a family planning method and the type of family planning method GHBI mean scores ($p> 0.05$) (Table 2).

Table 2. *Comparison of GHBI scores of women in terms of socio-demographic and obstetric characteristics*

Characteristic	N(%)	Mean Rank	Median	Test / p
Age				
20-29	51 (19.2)	162.45	77.00	13.096*
30-39	81 (30.5)	140.36	71.00	**.001**
40-49	134 (50.4)	118.33	67.00	
Mean age	38.58±7.9 (min 20, max 49)			
Education				
Primary school and below	140 (52.6)	97.01	65.00	83.433*
Middle-High school	90 (33.8)	156.28	73.50	**.000**
University and over	36 (13.5)	218.47	80.00	
Employment status				
Employed	79 (29.7)	135.44	70.00	7233.500**
Unemployed	187 (70.3)	132.68	73.00	.789
The occupation of the husband				
Unemployed	50 (18.8)	110.07	65.00	17.785*
Employed	177 (66.5)	147.45	72.00	**.000**
Retired	39 (14.7)	100.22	63.00	
Education status of the husband				
Primary school and below	76 (28.6)	73.13	61.50	89.948*
Middle-High school	121 (45.5)	136.87	70.00	**.000**
University and over	69 (25.9)	197.08	79.00	
Income Level				
Satisfactory	65 (24.4)	183.82	77.00	56.616*
Medium	134 (50.4)	132.77	70.00	**.000**
Non-satisfactory	67 (25.2)	83.14	63.00	
Social security				
Yes	245 (92.1)	132.45	69.00	2315.500**
No	21 (7.9)	145.74	74.00	.447
Length of marriage				
1-5	51 (19.2)	150.82	74.00	17.593*
6-10	45 (16.9)	168.04	73.00	**.000**
>10	170 (63.9)	119.16	67.00	
		X	**SD**	
Family type				
Nuclear family	223 (83.8)	72.03	9.61	8.828***
Extended family	43 (16.2)	61.13	6.90	**.000**
Number of pregnancy				
No	28 (10.5)	774.91	74.00	27.330*
1,2 or 3	158 (59.4)	143.78	70.50	**.000**
4 or more	80 (30.1)	98.71	65.00	
Number of alive children				
No	42 (15.8)	163.07	74.00	24.591*
1,2 or 3	179 (67.3)	138.64	60.00	**.000**
4 or more	45 (16.9)	85.46	64.00	
Spontaneous abortion				
Yes	69 (25.9)	108.74	67.00	5088.00**
No	197 (74.1)	142.17	72.00	**.002**
Self-induced abortion				
Yes	41 (15.4)	68.41	10.30	1.286***
No	225 (84.6)	70.60	9.99	.199
Use of family planning				
Yes	146 (54.9)	70.82	10.49	998***
No	120 (45.1)	69.59	9.49	.319
Family Planning method that is used				
Not using	120 (45.2)	128.67	68.50	
Withdrawal method	69 (25.9)	129.49	70.00	5.185*
Condom	13 (4.8)	174.81	79.00	.269
RIA	45 (16.9)	134.08	69.00	
OKS	19 (7.2)	148.95	73.00	
** Kruskal Wallis Tests , ** Mann Whitney U Tests , *** Student's t Tests*				

54.9% of the women were found to have a discharge complaint, 52.6% had pains, 35% had burning sensation, 34.6% had an odor problem, and their complaints lasted for 6 months and over (36.1%). 71.4% of the women had normal vaginal complaints on the speculum examination, but 78.9% of them had vaginal infections in the pap smear test (Table 3).

Table 3. *Distribution of vaginal infections according to microscopic and symptomatic approach diagnosis*

Genital complaints of women	N(%)
Discharge	
Yes	146 (54.9)
No	120 (45.1)
Total	266 (100.0)
Type of discharge	
Clear, odorless*	26 (9.8)
Sour milk-like discharge **	32 (12.0)
Green-yellow frothy discharge **	34 (12.8)
Homogeneous watery, broth-like stream**	29 (9.4)
Purulant discharge/Thin gray/white discharge **	25 (10.9)
Total	146 (54.9)
Other complaints	
Burning sensation	93 (35.0)
Odour	92 (34.6)
Menstrual irregularity	67 (25.2)
Pruritis	55 (20.7)
Bleeding after sexual intercourse	38 (14.3)
Pain	140 (52.6)
The duration of the complaint	
1 month and less	63 (23.7)
2-3 months	86 (32.3)
4-5 months	21 (7.9)
6 months and more	96 (36.1)
Speculum examination	
Normal discharge*	76 (28.6)
White, cheese like discharge**	24 (9.0)
Green-yellow, frothy discharge **	13 (4.9)
Thin gray-white discharge **	27 (10.2)
Purulent discharge **	35 (13.2)
Homogeneous watery, broth-like stream**	14 (5.3)
Touch-cervix bleeds ***	20 (7.5)
Strawberry-like cervix***	2 (0.8)
Erosion/Ulcer***	32 (12.0)
PAP-Smear test results	
Candida**	21 (7.9)
Trichomonas Vaginalis**	2 (0.8)
Bacterial Vaginosis**	3 (1.1)
Mixed Infections**	184 (69.2)
Normal discharge*	56 (21.1)

Non pathologic discharge, **Pathologic discharge, * Pathological image*

4. DISCUSSION

Vaginal infection is an important health problem for women and its prevalence is increasing worldwide. Genital hygiene plays a key role in preventing genital infections (3,13-15,22,27).

The study showed that incorrect and inadequate genital hygiene behavior increases the risk of vaginal infection. The mean GHBI score of the women in the study was found to be 70.27 ± 10.05. At the same time, a significant relationship was found between genital discharge complaints and GHBI score hygiene behaviors ($p<0.05$). In other studies, inadequate genital hygiene behaviors are mentioned as being among the most important causes of vaginal infection (3-6, 26). For example, the mean score of GHBI was found to be 80.90 ± 10.54 in Orak and Canuygur's study (7), 77.7 ± 12.8 in Ege and Eryılmaz's study (14) and 75.01 ± 11.63 in Çankaya and Ege's study (26). It should be noted that the rates of positive genital hygiene behaviors have been generally found to be low in the studies of genital hygiene behavior conducted in Turkey. It is imperative in terms of protecting against genital infection that genital hygiene practices are implemented correctly.

A significant relationship was found between the basic individual risk factors of age, education levels, the duration of marriage, and the women's GHBI mean score. That is, as women's ages and the duration of their marriages increased, the mean GHBI score decreased. The findings of Polat et al. and Yasmin and Mukherjee confirm this in that the authors showed that the incidence of genital infections increased with age and years of marriage (28,29). In addition, T. vaginalis infections were reported to be more common among women of ages 30–40, the period in which women are sexually active (30). Another study however reported no correlation between the mean GHBI score and women's ages (14). Still another study conducted with 134 pregnant women in Izmir revealed that there was no correlation between the mean GHBI score and women's ages (16). The differences seen here may be attributed to the fact that these studies were conducted in regions of different socio-cultural and economic levels, resulting in an increased mean GHBI score due to the young women's having more access to information.

The mean GHBI score was found to be higher in women who had a good monthly income level. This result suggests that as their average monthly income increases, women enjoy better conditions and the likelihood of accessing services and carrying out appropriate hygienic practices increases. In a study by Dan et al. (31), vaginal infections were seen to be higher in those with poor socioeconomic status, supporting the finding of the present study.

When the mean education level of the women and their GHBI scores were examined, it was seen that while the women with an education level of primary school or less had a lower GHBI score, graduates of high school and university had higher GHBI scores. Yağmur (11), Ege and Eryılmaz (14), Koştu and Beydağ (15) reported that high school graduates had higher GHBI scores, which supports the findings of the present study. This outcome suggests that the higher the education level of a woman, the better knowledge of

genital hygiene she has and the more she is sensitive toward preventing genital infections.

It was seen in the study that the GHBI mean score of the women with more education and whose husbands were employed was higher. Çankaya and Ege's study similarly showed that women whose husbands were primary school graduates and without a permanent job had lower scores than those whose husbands were graduates of high school or more or were civil servants (26). In another study, the mean GHBI score of women with husbands having a higher level of education and who are civil servants is high (14). Having a husband with a permanent job implies having a constant income and health insurance. This has a significant impact on creating a certain level of prosperity, interactive relations in a social environment, relatively better economic conditions, and the development of health consciousness and correct genital hygiene behaviors.

The study showed that the mean GHBI score of the women living in a nuclear family was higher than the women living in an extended family. Similar to our data, Ege and Eryılmaz (14) demonstrated in their study that mean GHBI scores were higher in women living in nuclear families. It can be said that it is important to ensure an individual's need for privacy if adequate hygienic habits are to be maintained.

As the total number of pregnancies, the number of living children, and the number of spontaneous abortions increased in the study, the mean GHBI scores decreased. Supporting our data are the studies by Özkan and Demir (24), who indicated that spontaneous abortion raised the risk of vaginal infection, while studies by Çankaya and Ege, Patel et al. and Prasad et al. (26,32,33) revealed that the number of pregnancies and births had an impact on vaginal infection risk. Prasad et al. (33) too reported an increased risk of vaginal infection in the same context. It can be said then that women's experiences influence their susceptibility and vulnerability towards risk.

The use of an intrauterine device (IUD) has been associated with genital system infections (27,35). Besides studies that have pointed to increased risk of sexually transmitted diseases and even more important, of pelvic inflammatory disease (PID) with the use of IUD's, there are also studies that refute this association (35-38). Our research however did not reveal any significant difference in GHBI scores in terms of any type of birth control method or specifically associated with the use of an IUD.

Researchers have indicated in previous studies that inappropriate perineal hygiene may lead to genital infections (7,9, 12,39). The incidence of genital infection in the women in our study was found to be 79% in the Pap smear test. The study by Hamed found a higher frequency of genital infections among participants who practiced incorrect techniques (27). In another study, the authors reported the frequency of genital infections as 35.1% among participants who practiced correct genital hygiene and 38.1% among those who cleansed the genital area incorrectly (25). Similarly, the incidence of genital infection was determined as 71.1% in a study by Hacıalioğlu et al. (34), and 65.6% in a study by Öner et al. (40).

The literature reveals that the majority of women experience a genital infection at least once in their life (2,17,20,26,30). In this study, the rate of women who stated that they had vaginal discharge was 54.9%. Of these women, 45.1% stated that they had a pathological condition, odor (34.6%) and burning (35%). The speculum examination determined that vaginal discharge was pathological in 42.6% of the women. In the study by Ortaylı et al. (41), the vaginal discharge rate was 60.5% while 49.3% of women had odor. Furthermore, the mean GHBI score was lower among women who currently had discharge that had lasted for more than 6 months. In Çankaya and Ege's (26) study, 54.8% of the women were found to have vaginal discharge with odor and in the women who complained of having malodorous discharge in the last one year, GHBI mean scores were found to be low, which supports our findings. Similar to our results, an increase in vaginal infections and vaginal discharge, malodor, vaginal itching, redness in the genital tract, pain in the lower abdominal region, and burning in the vagina were observed in the studies performed by Kısa and Taşkın's (42), Bezircioğlu and Öniz (43) and Karer et al. (44). In our study, 71.2% of the women were found to have either a pathological or non-pathological discharge. This high rate is significant in terms of women's health and points to the need for more education and awareness programs.

5. CONCLUSION

Early recognition of vaginal infections, initiating appropriate treatment and taking necessary precautions are essential in protecting and improving women's health. In conclusion, it was determined that women's genital hygiene behaviors are inadequate and they lack information on appropriate and inappropriate genital hygiene practices. It is important for the protection and improvement of female reproductive health that women acquire correct genital hygiene habits. Both at the individual level and in collective training, women should be given health education on genital hygiene practices and abnormal genital discharge.

Limitations of the study

The study has some limitations. First, the results cannot be generalized because the sample size is small, the study was conducted at a hospital and answers were self-reportedly given. Therefore, multicentric studies with large sample sizes are required.

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Clinical and Experimental Health Sciences

Investigation of the Knowledge and Behaviors of Pregnant Women on Early Diagnosis Methods of Breast Cancer and Risk Factors

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ABSTRACT

Objective: To investigate the knowledge and behaviors of pregnant women about early diagnosis methods of breast cancer.

Methods: The study has a descriptive, cross-sectional design. Data collection was performed by using a questionnaire created by the researcher in light of the literature. Data were collected in a state hospital between October and December in 2018. The study population included all pregnant women presenting to the study hospital and experiencing their second and third trimesters at the time of data collection. The study sample involved 194 pregnant women accepting to participate in the study.

Results: Age at the first pregnancy was 30 years or older in 4.5% of the women, 13.6% of the women was nulliparous and 8.8% of the women had a familial history of breast cancer. Thirty-two-point five percent of the women heard about breast self-examination and 61.3% of the women performed breast self-examination before their pregnancy. During their pregnancies, 74.7% of the women did not perform breast self-examination and 95.9% of the women did not have clinical breast examinations.

Conclusion: Evaluation of risk factors and performing clinical breast examination and ultrasound in the first prenatal follow-up visit will help to make early diagnosis of breast cancer. Pregnant women should be informed about breast self-examination to raise their awareness about pregnancy-associated breast cancer.

Keywords: Breast cancer, pregnancy, risk factor, early diagnosis

1. INTRODUCTION

Pregnancy-associated breast cancer involves breast cancer emerging in pregnancy, in postpartum one year or at any time during lactation (1,2). Although pregnancy-associated breast cancer is rare, it is the most frequent cancer appearing during pregnancy (3). Duranni et al. reported that it has an incidence of one in 3000 pregnant women and can reach up to 3% (1). American Cancer Society (ACS) categorized risk factors of breast cancer into four: unchangeable risk factors, lifestyle-related risk factors, uncertain risk factors and unproven risk factors. Unchangeable risk factors are the female gender, advanced age, history of breast cancer, breast tissue density, benign breast diseases, early menarche, late menopause and receiving radiation to breasts. Lifestyle-related risk factors are alcohol intake, obesity or being overweight, lack of physical activity, not having children, not breastfeeding, contraceptives, hormone replacement therapy after menopause and breast implants. Uncertain risk factors are diet, vitamin intake, chemicals in the environment, smoking and working at night. Unproven risk factors are antiperspirants, wearing bras and induced abortion (4).

Personal characteristics of women, family history, breast cancer gene mutation and the number of deliveries in addition to age play an important role in development of breast cancer in pregnancy (5,6). At present, women tend to postpone becoming pregnant due to their work life and personal choices. Many women become pregnant when they are in their thirties and forties and this increases the incidence of breast cancer (7). The median age of pregnancy-associated breast cancer has been reported to be 33 years (8). For these reasons, it is predicted that the incidence of breast cancer increases in pregnant women and that pregnancy-associated breast cancer becomes an important problem (7,9).

It can be difficult to diagnose breast cancer in pregnancy. It may be that physiological changes in breast tissue during pregnancy are considered as normal and that women are unable to discriminate abnormal masses from normal tissue (10,11). Diagnosis of breast lumps can be considerably delayed in pregnant women compared to unpregnant women. Pregnant women can consider physiological changes in their breasts as pregnancy-associated benign

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changes (12). An increase in density and firmness of breast tissue is another factor preventing recognition of breast masses (10,13). Pregnancy-associated breast cancer is most frequently diagnosed with palpation of masses. Therefore, it is recommended to perform a clinical breast examination (11,14). It is suggested that this examination should be carried out in the first prenatal visit in the first trimester (2). Although breast self-examination (BSE) does not help to make a differential diagnosis, it causes women to recognize changes earlier and see their doctors (10,11). In summary, so that pregnancy-associated breast cancer can be diagnosed earlier, clinical breast examination (CBE) and ultrasonography should be carried out in the first prenatal visit. In addition, women's awareness should be raised about performing BSE during pregnancy and lactation. These attempts can allow early diagnosis and treatment of pregnancy-associated breast cancer (15). There have not been any studies directed towards examining the knowledge and behavior about early diagnosis methods. Therefore, the aim of this study was to investigate the knowledge and behaviors of pregnant women about early diagnosis methods of breast cancer.

Research Questions

1. What are the risk factors of breast cancer in pregnant women?
2. What is the knowledge and behavior of pregnant women about early detection methods of pregnancy-associated breast cancer?

2. METHODS

2.1. Participants

The study is descriptive and cross-sectional and was conducted in a state hospital in a city located in Central Anatolia Region. Data were collected between October and December in 2018. The study population comprised of the pregnant women presenting to the gynecological and obstetric outpatient clinic of the hospital and experiencing the second and third trimesters at the time of data collection. The study sample involved 194 pregnant women volunteering to participate in the study.

Inclusion criteria were being pregnant, experiencing the second or third trimester and not being diagnosed with breast cancer before (2).

2.2. Data Collection and Instruments

A questionnaire prepared by the researcher in light of the literature was utilized to collect data (4,9,16).

The questionnaire was composed of four questions about socio-demographic features (age, occupation, education and income), 12 questions about risk factors of breast cancer (age at the first pregnancy, working at night, alcohol intake, smoking, age at menarche, breastfeeding, duration of breastfeeding, familial history of breast cancer, having children and benign breast diseases), six questions about information and behavior concerning early diagnosis of breast cancer before pregnancy (four questions about BSE and two questions about ultrasonography and mammography) and seven questions about knowledge and behavior of women regarding early diagnosis methods of breast cancer during pregnancy. After the pregnant women were informed about the study, those accepting to participate in the study were asked the questions in the questionnaire and data were collected by the researchers. It took about ten minutes to fill in the questionnaire.

2.3. Data Analysis

Data were analyzed with Statistical Package Program for Social Sciences 22 by using numbers, percentages, mean, standard deviation (SD) and minimum and maximum values.

2.4. Ethical Considerations

Ethical approval was obtained from the ethical committee of Çankırı Karatekin University (Approval number: 2018/47). Written permission was taken from the administration of the state hospital where the study was carried out. The participants were assured that the obtained data would be used for scientific purposes and published, and their oral consent was obtained in accordance with the Declaration of Helsinki.

3. RESULTS

3.1. Participants' Characteristics

The mean±SD age of the women included in the study was 27.31±0.41 years (range: 18-42 years), 35.1% of the women were secondary school graduates, 16.5% of the women were university graduates and 79.4% of the women were housewives. Sixty-eight-point six percent of the women had an income equal to their expenses (Table 1).

Table 1. *Socio-Demographic Features of the Pregnant Women (n=194)*

Socio-demographic features	X±SD	Range
Age	27.31±0.41	18-42
	n	**(%)**
Education		
Primary school	38	(19.6)
Secondary school	68	(35.1)
High school	56	(28.8)
University	32	(16.5)
Occupation		
Housewife	154	(79.4)
Worker	12	(6.2)
Government officials	8	(10.3)
Having one's own business	20	(4.1)
Income		
Income lower than expenses	17	(8.8)
Income equal to expenses	133	(68.5)
Income higher than expenses	44	(22.7)
Total	194	(100)

X±SD: *mean±standard deviation*

3.2. Risk Factors for Breast Cancer

Age at the first pregnancy was 29 years and lower in 95.5% of the women. Eighty-four-point three percent of the women had 1-3 deliveries. Age at menarche was 12 years or higher in 94.3% of the women. Ninety-six-point seven percent of the women breastfed their babies after their previous deliveries, 76.1% of the women fed their babies exclusively with breastmilk for six months and 51.3% of the women breastfed their babies until their babies were at least two years old. Seventeen-point five percent of the women had night shifts at their workplaces, 10.8% of the women were smokers and 8.8% of the women had a family history of breast cancer (mother and aunt etc.). Seven-point seven percent of the women had breast problems before pregnancy and 53.4% of these women had fibroadenoma (Table 2).

Table 2. *Risk Factors for Breast Cancer*

Risk factors	n	(%)
Age at the first pregnancy		
29 years old or younger	185	(95.5)
30 years old or older	8	(4.5)
Number of deliveries (n=140)		
0	19	(13,6)
1-3	118	(84,3)
4 or more	3	(2,1)
Age at menarche		
12 years old or older	183	(94.3)
11 years old or younger	11	(5.7)
Breastfeeding status after previous deliveries (n=121)		
Yes	117	(96.7)
No	4	(3.3)
Duration of exclusive breastfeeding (n=117)		
Minimum six months	86	(76.1)
Shorter than six months	28	(23.9)
Total duration of breastfeeding (n=117)		
2 years or longer	60	(51.3)
Shorter than 2 years	57	(48.7)
Familial history of breast cancer (mother, aunt, etc.)		
Yes	17	(8.8)
No	177	(91.2)
Smoking		
Yes	21	(10.8)
No	173	(89.2)
Alcohol intake		
Yes	0	(0)
No	194	(100)
Night shifts at work		
Yes	7	(17.5)
No	33	(82.5)
Benign breast diseases before pregnancy		
Yes	15	(7.7)
No	179	(92.3)
Diagnosis of the breast conditions (n=15)		
Lipoma	2	(13.3)
Hormonal changes	2	(13.3)
Fibroadenoma	8	(53.4)
Mastitis	3	(20.0)
Total	194	(100)

3.3. Knowledge and Behavior of Women Regarding Early Diagnosis Methods of Breast Cancer

Sixty-seven-point five percent of the women had heard about BSE before. Thirty-eight point seven percent of the women performed BSE before pregnancy and 26.7% of these women performed the examination regularly. The most frequent three signs of breast cancer evaluated during BSE were masses (97.7%), depression in the nipples (19.8%) and blood and clear fluid oozing from the nipples (19.8%). Twelve-point nine percent and 3.6% of the women had breast ultrasonography and mammography before pregnancy respectively (Table 3).

Table 3. *Knowledge and Behavior of Women Regarding Early Diagnosis Methods of Breast Cancer Before Pregnancy-1*

Features	n	%
Hearing about BSE		
Yes	131	(67.5)
No	63	(32.5)
Performing BSE before pregnancy		
Yes	75	(38.7)
No	119	(61.3)
Frequency of performing BSE before pregnancy (n=75)		
Regularly every month	20	(26.7)
Not regularly	55	(73.3)
Signs of breast cancer evaluated during BSE (n=86)*		
Mass	84	(97.7)
Depression in the nipples	17	(19.8)
Pits in the breasts	11	(12.8)
Blood and clear fluid oozing from the nipples	17	(19.8)
Wounds on the breasts	15	(17.4)
Orange peel appearance	14	(16.3)
Pain	9	(10.5)
Having breast ultrasonography before pregnancy		
Yes	25	(12.9)
No	169	(87.1)
Having mammography before pregnancy		
Yes	7	(3.6)
No	187	(96.4)
Total	**194**	(**100**)

Women signed multiple options;* ***BSE:* *Breast Self-Examination*

Twenty-five-point three percent of the women performed BSE during pregnancy and 32.7% of these women performed it regularly every month. Four-point one percent of the women had a health professional (doctors, midwives and nurses) perform their breast examination during pregnancy. All the women had this examination in their first trimester. Seven-point seven percent of the women felt anxious about changes in their breasts and 60% of these women were referred to a health professional due to these changes. All the women who were referred to a health professional due to breast problems thought that these were normal conditions resulting from pregnancy (Table 4).

Table 4. *Knowledge and Behavior of Women Regarding Early Diagnosis Methods of Breast Cancer During Pregnancy-2*

Features	n	%
Performing BSE during pregnancy		
Yes	49	(25.3)
No	145	(74.7)
Frequency of performing BSE during pregnancy (n=49)		
Regularly every month	16	(32.7)
Not regularly	33	(67.3)
Having a clinical breast examination by health professionals (doctors, midwives and nurses)	8	(4.1)
Yes	186	(95.9)
No		
Feeling anxious about changes in breasts during pregnancy	15	(7.7)
Yes	179	(92.3)
No		
Breast conditions causing anxiety (n=15)		
Excess enlargement of one or two breasts	5	(33.4)
Mass and discharge in the breasts	5	(33.4)
Changes in color of the breast skin	2	(13.2)
Pain	3	(20.0)
Seeing a health professional for breast problems (n=15)	9	(60.0)
Yes	6	(40.0)
No		0.00
Having breast ultrasonography during pregnancy		194
Yes		(100)
No		**(100)**
Total		

BSE: *Breast Self-Examination*

4. DISCUSSION

4.1. Risk Factors

Knowledge about risk factors for breast cancer appearing in pregnancy or the postpartum period is limited (16). In the present study, the pregnant women were evaluated in terms of unchangeable risk factors (family history, benign breast diseases and early menarche). Women having menarche before the age of 12 years have a higher risk of breast cancer since they are exposed to more estrogen and progesterone during their life. Breast cancer in one first-degree relative (mother, aunt or sister) increases the risk of breast cancer by about twofold, breast cancer in two first-degree relatives increases the risk by about threefold and both typical and atypical proliferative lesions also raise the risk (4,17). In the current study, 8.8% of the women had a familial history of breast cancer, 7.7% of the women had benign breast diseases and 5.7% of the women had menarche at or before the age of 11 years. These findings suggest that the women were at a low risk of breast cancer. Hou et al. reported that of all the women having breast cancer in pregnancy or postpartum two years, 5.9% had a familial history of breast cancer, 7.2% had benign breast diseases and 29.7% had menarche at the ages of 11-14 years (16).

In the current study, the women were also evaluated in terms of lifestyle-related risk factors (age at the first pregnancy, number of deliveries, duration of breastfeeding and alcohol intake) (4). In women giving birth after the age of 30 years or not giving birth at all, the risk of breast cancer rises to some extent (4). Accumulated evidence shows that prolonged breastfeeding reduces the risk of breast cancer. In a review of 47 epidemiological studies from 30 countries, breastfeeding was reported to cause a considerable decrease in the risk of breast cancer (18). In a meta-analysis, the breast cancer risk was found to decrease in breastfeeding women compared to those not breastfeeding at all and in women with prolonged breastfeeding compared to those breastfeeding for a short period. It has been emphasized that prolonged breastfeeding is necessary to reduce the breast cancer risk (17). The risk has been reported to increase slightly in women taking one glass of alcohol every day as compared with those not taking alcohol at all. Additionally, it increases by 20% in women taking 2-3 glasses of alcohol daily in comparison to those not taking alcohol (4).

In the present study, 4.5% of the women were aged 30 years or older, 13.6% of the women did not give birth before, 3.3% of the women did not breastfeed and 51.3% of the women breastfed until their children were two years old or older and did not have the habit of alcohol intake. These findings may suggest that the women had a low lifestyle-related risk. Başaran et al. found in their study on 20 women with pregnancy-associated breast cancer that the mean age of the women was 36 years and ranged between 28 and 43 years (19). In the current study, the women were aged between 18 and 42 years and 4.5% of the women had their first pregnancy at or over the age of 30 years, which are consistent with the literature. In a study by Hou et al., 6% of the women with breast cancer appearing in pregnancy or postpartum two years had the habit of alcohol intake (16). The fact that the women included in the present study did not take alcohol before their pregnancy can be considered as a factor reducing their breast cancer risk.

In the current study, in addition to unchangeable and lifestyle-related risk factors, uncertain risk factors (smoking and working at night) were evaluated. It has been stated in a meta-analysis that the breast cancer risk rises in smoking women and is much higher in women starting to smoke before their first pregnancy (20). Several studies have shown that women working in night shifts including nurses have an increased risk of breast cancer. In a review of 21 studies and five meta-analyses, the breast cancer risk could not be clearly shown in women working in night shifts for less than 20 years. In a meta-analysis of 15 studies, a weak relation was found between the risk of breast cancer and working in night shifts (4,21,22). In the current study, 10.8% of the women said that they smoked before and during pregnancy and only 17.5% of the employed women had night shifts. It can be suggested that the women had a low risk of uncertain factors.

4.2. Knowledge and Behavior Regarding Early Diagnosis Methods of Breast Cancer

Even though ACS does not recommend performing BSE every month, it suggests that all women should get to know how their breasts look and feel and refer to a doctor when they recognize a change (23). There have been studies showing that most of the breast cancer cases are detected by women themselves and that breast cancer is diagnosed in its early stages in women performing BSE (24-26). In the present study, 32.5% of the women heard about BSE and 38.7% of the women performed BSE before their pregnancies and 26.7% of the women performing BSE before pregnancy did it regularly. These findings demonstrated that the women had insufficient knowledge about BSE and did not have very positive attitudes towards it, which is congruent with the literature (27-29). Bellgam and Buowari found that 16.06% of the women heard about BSE and that 28.94% of the women performed it. It is stated in the literature that BSE is effective in early diagnosis of breast cancer (30). However, when socio-economic conditions in developing countries with low-moderate incomes including Turkey are taken into account, it becomes clear that BSE is a mandatory and inevitable practice (31). In view of the results of the present study, it can be recommended that health professionals should offer education about BSE individually or in groups in the antenatal period. In addition, people should be provided information about and made aware of it through traditional media and social media.

Even if BSE does not help to make a differential diagnosis, it is important for women to recognize changes in their breasts earlier and to see a doctor (10,11). Women should be informed about performing BSE in pregnancy and lactation (32). The present study revealed that 74.7% of the women did not perform BSE in pregnancy and that 95.9% of the women did not have a CBE by a health professional. Pregnancy-associated breast cancer is usually diagnosed as a painless mass while pregnant women perform a BSE or while health professionals perform a CBE (12). Therefore, it is recommended to perform breast examinations in the first step to detect breast cancer (14). It has been stated in case studies that pregnancy-associated breast cancer is diagnosed when patients detect a mass and refer to a doctor (11,15). For this reason, it is important to carry out BSE in pregnancy and lactation.

It is suggested that every pregnant woman should have a CBE and breast ultrasonography to decrease the frequency of pregnancy-associated breast cancer. In addition, it is necessary to increase women's awareness about performing BSE in pregnancy and lactation (15). In the current study, 4.1% of the women had a CBE in the first trimester. This low percentage suggested that health professionals and pregnant women should be informed about the issue. There may be considerable delays in the diagnosis of breast lumps in pregnant women just like in nonpregnant women. Pregnant women may consider changes in their breasts as benign changes likely to appear in pregnancy (12). Labidi et al. in their study with pregnant and nonpregnant women found a 7-month delay in the diagnosis of pregnancy-associated breast cancer (33). Raising awareness of pregnancy-associated breast cancer can facilitate the diagnosis of this disease and contribute to improvement of breast health outcomes (12).

5. CONCLUSION

Although breast cancer rarely appears in pregnancy, the number of pregnancy-associated breast cancer cases is expected to rise in the future since women prefer to become pregnant in later years of their lives due to their personal and occupational choices. When this is taken into consideration, women's knowledge and awareness about performing BSE in pregnancy and lactation should be raised. They should know how their breasts look and feel and see a doctor if they observe a change, and they should be recommended to perform BSE. It can also be suggested that all pregnant women should have a CBE and breast ultrasound in their first prenatal follow-up. This can help diagnose breast cancer earlier and prevent delays in its treatment.

Nurses should offer education about BSE in the first prenatal follow-up so that pregnant women can be informed and so that their awareness about breast cancer can be increased. Inservice training programs should be provided for obstetricians so that they can become more sensitive to encourage pregnant women to have a CBE and breast ultrasound in the first trimester.

Limitations of the Study

As the study was conducted in a state hospital in a city in Middle Anatolia in Turkey, its results cannot be generalized to the whole population. The study is also restricted with the dates when it was performed, the data collection tool developed in accordance with the aim of the study and the responses given by the participants to the questions in the data collection tool.

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Clinical and Experimental Health Sciences

The Effect of Health Belief Model-Based Training on Cervical Cancer Screening Behaviors

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ABSTRACT

Objective: Cervical cancer remain a major public health problem, ranking as the fourth most common cause of cancer incidence and mortality in women worldwide in 2019. Prevention and early detection of cancer are considered as critical factors in controlling the disease and increasing the survival of patients. Therefore, we aimed to investigate the effect of Health Belief Model (HBM)-based education on cervical cancer screening behaviors.

Methods: This is a descriptive type of study. The participants attended training workshops based on HBM. Data were collected by the questionnaire developed by the researchers and the Health Belief Model Scale Turkish version.

Results: Twelve women (13%) from the participants had already taken the test before training. After training, the majority (n=56) of women had the Pap smear test (70%). Reasons for not getting a Pap-Smear Test (n=24) were determined as no need / no risk (n=13), no opportunity (n=7) and shyness (n=4) respectively.

Conclusion: Our findings showed that the health education programs designed based on HBM could positively affect cervical cancer preventive behaviors.

Keywords: Health Belief Model, Pap smear, training program, cervical cancer

1. INTRODUCTION

Cervical cancer is the fourth most common cancer in women, with over 570,000 new cases diagnosed worldwide in 2019. The annual number of deaths due to cervical cancer is around 312,000 (1). According to 2018 data, the incidence of cervical cancer in Turkey has been determined as 4.5 in 100 thousand (2). In Turkey, 1005 new cases of cervical cancer were diagnosed and the total number is 4238. Cervical cancer is one of the nine cancers with the highest mortality rate. However, only 20% of women had Pap smear tests performed in 5 years (3).

World Health Organization (WHO) states that cervical cancer-related mortality has been increasing in developing countries (4). Risk factors such as increased rates of smoking in women, polygamy, decreased coitus age due to early marriage, multiparity, and low education/socio-economic levels are common in women in Turkey.

WHO reports cervical cancer as preventable cancer. Despite all risk factors, mortality and incidence of cervical cancer can be reduced by screening (5). The effectiveness of treatment is low since the symptoms of cervical cancer are absent until cancer progresses. An analysis by WHO stated that even by a Pap smear scan at 10-year intervals, the incidence of cervical cancer can be reduced by 64% (4). Pap smear and HPV tests have been initiated since 2014 within the scope of the National Cancer Screening Program in Turkey (2,3). However, many factors affect women's having Pap smear tests. These factors include sociocultural factors, cancer awareness, lack of courage, and attitudes and advice from healthcare providers (6).

WHO defines "health education" as information sharing on the benefits of available resources and access to services and how to prevent diseases. This information sharing is frequently used by healthcare providers to change behavior-based Health Belief Model (HBM), Transtheoretical Model, Social Cognitive Theory, Precede-Proceed Model, Protection Motivation Theory (PMT), combined model and health education models (4-6).

HBM has been widely used to measure the health beliefs and behaviors about cancer screening (6-11). HBM is a cognitive model that tries to identify patterns of health behavior. The perceived susceptibility, perceived seriousness, perceived

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benefits, perceived barriers, and perceived motivation are five main components of the HBM.

Perceived susceptibility refers to beliefs about the probability of obtaining a disease or condition. Perceived seriousness is about feelings concerning the seriousness of acquiring a sickness or of leaving it untreated. Perceived benefits focus on the effectiveness of healthy behavior in reducing the threat of the condition. Perceived barriers are the potential negative aspects of a particular health behavior, a kind of unconscious, cost-benefit analysis occurring when the individuals know the perceived barriers are more costly than the perceived benefits. Health motivation refers to a generalized state of intent that results in behaviors to maintain or improve health. The concept of health motivation used in combination with the original five HBM concepts has evidence of significant predictive ability (7-8). Therefore, in this study we used HBM focusing on prevention as a reference framework. We aimed to investigate the effect of HBM-based education within the context of cervical cancer screening behaviors.

2. METHODS

2.1. Objective

The aim of this study was to investigate the effect of HBM-based education on cervical cancer screening behaviors.

2.2. Study Design

This is a quasi-experimental study.

2.3. Setting and Participants

The inclusion criteria were as follows: sexual active women, able to read and speak Turkish who accepted to be included in the study. Women with a history of cancer or mental illness were excluded from the study. The study was carried out at the public education center.

2.4. Data Collection

The data were collected by the probable sampling method. Information Form and Health Belief Model Scale were used as data collection tools. At the first stage, all women who volunteered to participate in the study were trained about Pap smear test/cervical cancer for approximately 30 minutes. Theory-based educational interventions (Health Belief Model) were considered. These educational interventions included videos and power point presentations. After three weeks, the training was repeated by the same educator and the presentation was completed with an emphasis on the question-answer method. After three weeks, the research was completed by the same educator by filling in the Pap smear test uptake status and Health Belief Model Scale (9) and information form (Fig 1).

2.5. Ethical Consideration

Ethics approval with 01-2018/03 number was obtained from Karamanoglu Mehmetbey University on 31.01.2018.

2.6. Statistical Analysis

Data analysis was conducted using SPSS trial version 24.0. The mean and percentage values were calculated, and the Student's t test was used.

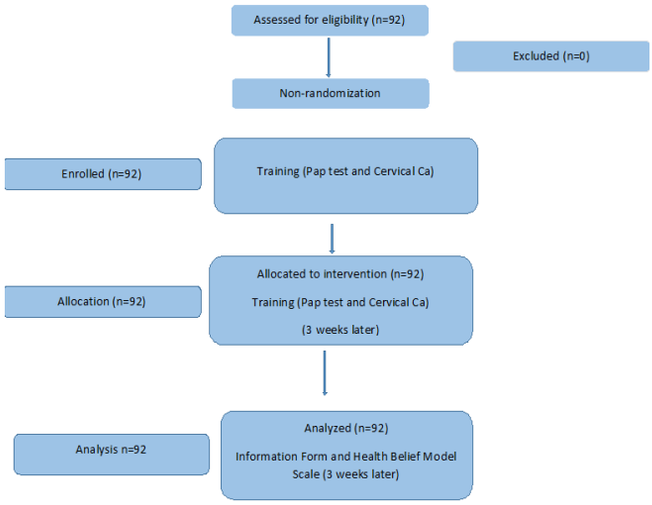

Figure 1. *Research Flow TREND Diagram*

3. RESULTS

The participants' characteristics are given in Table 1. A family history of gynecologic cancer was reported by 9.8% of the women while 94.2% did not smoke and 79.8% did not perform exercise. Parity-related features were as follows: 3.18 ± 1.84 pregnancy, 2.48 ± 1.15 labor, 1.29 ± 0.6 curettage, and 22.8% reported to be menopausal. Almost half of the women (43.5%) had never heard of the Pap smear test. Twelve women (13%) had already taken the test before training.

Table 1. *Participants Characteristics (n=92)*

		n	%
Year	20-38 years	30	32.6
	39-57 years	62	67.4
Marital Duration	1-5 years	8	8.7
	6-10 years	10	10.9
	≥11 years	74	80.4
Marital Status	Married	86	93.5
	Unmarried	6	6.5
Educational Level	≤ 8 years	45	48.9
	≥9 years	47	51.1
Working Status	Yes	14	15.2
	No	78	84.8
Family Type	Nuclear	84	91.3
	Joint	8	8.7

A relationship was found between the perceived seriousness of the scale and the family type (nuclear family) (Table 2) ($p<0.05$).

Table 2. *Participants' Characteristics and Comparison of the Health Belief Model Scale (n=92)*

Characteristics		Perceived Susceptibility	Perceived Seriousness	Perceived Benefits	Perceived Motivation	Perceived Barriers
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Marital Status	Married	7.63 ±2.18	24.51±0.72	30.25±1.76	8.83±1.18	37.01±1.16
	Unmarried	8.16 ±1.72	24.33±1.18	26.16±1.68	9.16±1.28	35.83±1.19
	p	0.116	0.21	0.19	0.55	0.61
Family Type	Nuclear	7.77±2.28	24.67±1.26	30.27±1.48	9.01±1.77	36.79±2.05
	Joint	6.62 ±1.96	21.87±2.08	27.00±1.98	7.25±2.07	38.37±2.72
	P*	0.41	**0.034**	0.15	0.82	0.58
Education Level	≤8 years	7.85 ±2.58	25.00±1.17	27.42±0.78	6.87±2.22	43.57±3.18
	≥9 years	8.33±1.96	25.33±2.18	30.16±1.16	8.50±1.96	63.50±2.96
	P*	0.22	0.43	0.16	0.49	0.98
Menopause	Yes	7.95±1.78	24.52±1.76	29.42±1.18	8.42±0.78	39.23±1.58
	No	7.59±2.17	24.40±2.58	30.15±1.26	8.98±0.96	36.25±2.04
	P*	0.62	0.32	0.09	0.08	0.45
Pap Smear Test Application After Education	Yes	7.66±3.01	24.62±2.28	30.39±1.78	8.92±2.36	36.76±2.18
	No	7.69±2.96	24.13±2.36	29.36±1.45	8.75±1.98	37.19±2.77
	P*	0.94	0.63	0.40	0.74	0.79

*SD: Standard Deviation; *Student's t-test*

After training, very few women (n=24; 26%) had no Pap smear test. Among the women who did not have the test, 12 women had a test in the past year (Fig 2). Reasons for not having Pap smear test are given in Fig 3.

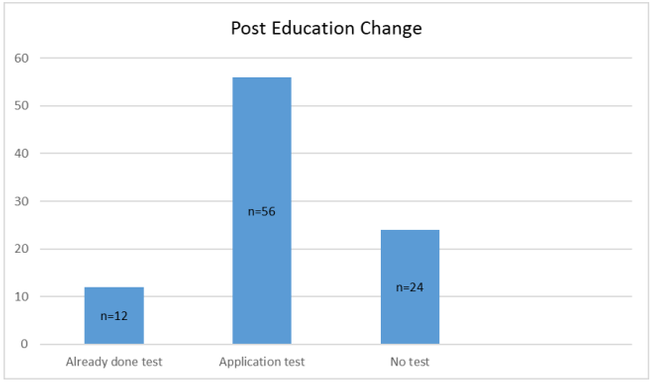

Figure 2. *Post Education Pap Smear Test Application*

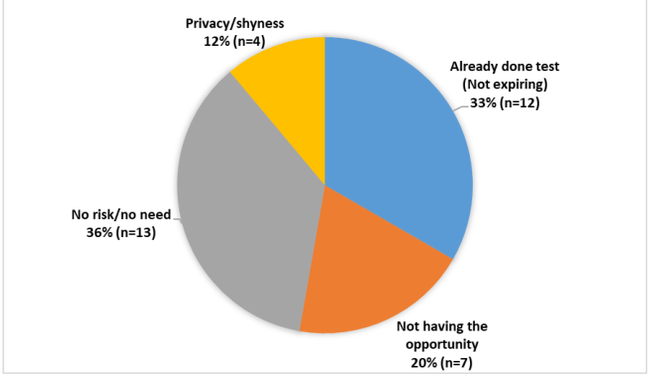

Figure 3. *Reasons for Not Having a Pap Smear Test*

4. DISCUSSION

The majority (87%) of the respondents reported not having gone through previous Pap smear testing; only 13% reported a regular Pap smear test. In the study of Temel conducted on 625 women aged 35 and over living in Sivas, women had Pap smear tests done at least once in a lifetime (12.3%), at least once in the last three years (10.5%) and at least once in the last five years (11%) (10). In a cross-sectional study on the protection of breast and cervical cancer on 1007 women aged 15–65 in Diyarbakır, it was reported that 10% of women had a Pap smear test in the past year (11). The regular use of the Pap test was also low in other developing countries (12,13). For example, only 7% of the nursing staff in a tertiary-level teaching institution in rural India had been screened by the Pap test (13). In a population-based study covering 57 countries, the country has estimated at least one Pap smear test rate of 40% for all countries, with an approach that takes into account the population. In that research it reports that the rate of having a Pap smear test at least once in the past three years was above 80% in Austria and Luxembourg, and below 1% in Bengalia, Ethiopia and Myanmar (14). In a study conducted among South Asian women living in New York, it was reported that 67% of women had a Pap smear test and 13% had this test in the last year (15). In this study, it is seen how important the health system of the country where women live more than their ethnic characteristics. In developed countries, where there is proper access to effective cervical cancer screening, the regular use of the Pap test is higher than in developing countries (16,17), resulting in a lower rate of death from cervical cancer in these countries (18).

In Turkey, cervical cancer screening is free of charge and are widely available in the public health sector. An understanding

of factors that predict cervical cancer screening behavior may contribute to the development of more effective screening. The present study used the Health Belief Model (HBM) theoretical frameworks for understanding the predictors of the behavior of Turkish women with regard to cervical cancer screening. According to our results, a relationship was found between the perceived seriousness of the scale and the family type (joint family). The perception of seriousness is a very important factor as it enables to act. People living in a large family may be affected by the emotions, thoughts and experiences of previous generations. Therefore, it is necessary to be aware that these women are risk groups (18).

It is underlined that the necessity of model-based education in an existing meta-analyses in undeveloped and developing countries like our country. Because it is claimed that education given without knowing the personal features and obstacles will only increase the knowledge of cervical cancer (19). In this study, training was provided using HBM. It was determined that only 30% of the women did not have Pap smear test after education. Considering that a single negative Pap smear test reduces the risk of developing cervical cancer by 45% and 9 smear tests are taken throughout life, it reduces cervical cancer risk by 99%; the importance of gaining regular screening habits in preventing cervical cancer is undeniable (20).

This study focused on possible reasons for not having Pap smear tests. In this research 12% of the women reported that they did not test because they could not overcome their sense of embarrassment. In a similar study conducted in our country, the rate of embarrassment was found to be 8% (21). Olaza-Maguiña determined the feeling of embarrassment as an obstacle in the 4-6 year follow-up study (22). In a similar study conducted in Thailand, it was determined that the most important obstacle in the Pap smear test was the sense of shame of women. They proposed using the Kato technique to overcome the embarrassment barrier in this study. In this technique, the woman can take the smear preparation on her own using the Kato device (21). In a study with medical school students in Malaysia, embarrassment has been identified as an important barrier. In this study, one out of every two women stated that she did not have a smear test due to embarrassment. In a similar studies the feeling of shame is underlined (23-24). In Liebermann's Latin America and the Caribbean region shame has been identified as an important barrier (25). The embarrassment barrier is universal, but it is relatively heavier in Muslim countries. Alternative ways to overcome this barrier, such as psychological support or using a Kato instrument, can be produced.

Many women think that there is no risk of cervical cancer in their lives and therefore no need for testing. This reason was underlined by several studies (26-30). In a similar study conducted in Sri Lanka, 47% of women reported that the test was unnecessary for them (31). In the studies of Guo et al. (32), no positive relationship was found between ob / gyn counseling and having a Pap smear. Similarly, in this study, some women answered "no need / no risk" (36%) even after training. Therefore, it may be effective, persuasive and cost-effective for healthcare professionals to use mass media as well as educate individual or local groups.

In this study, it was determined that 20% of women did not have Pap smear tests on the grounds of not having an opportunity. This allegation can be dealt more easily than "shyness" and "no risk" reasons. As a matter of fact, although women know that they should get it done, they are not able to get the Pap test due to the problem of time management. Perhaps, if women were followed in the following months, they might have been be found to have Pap tests.

There are many studies evaluating the effectiveness of training aimed at promoting the Pap smear test. Often these studies evaluated the impact of attempts such as reminder calls and messages, invitation letters, reminder letters and appointment requests over the phone. All training programs have performed the function of increasing the Pap smear test. In a recent meta-analysis, it was found that the training programs increased the test rate by 2.5 times (19). In our this study, 60% of women had a test after training.

As a result, HBM-based training significantly increases smear rate. After training, very few women had no Pap smear test. When using the model in future studies, it may be more effective to include solution suggestions for privacy, /no need risk, and lack of opportunity. Our findings showed that the health education programs designed based on HBM could positively affect cervical cancer preventive behaviors.

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Clinical and Experimental Health Sciences

Alterations in Enzyme Activity of Carbonic Anhydrase, 6-phosphogluconate Dehydrogenase and Thioredoxin Reductase in Rats Exposed to Doxorubicin and Morin

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ABSTRACT

Objectives: Carbonic anhydrase (CA), 6-phosphogluconate dehydrogenase (6PGD) and thioredoxin reductase (TrxR) enzymes are the essential biological molecules needed for metabolic processes in all living cells. This study was designed to investigate the activities of CA, 6PGD and TrxR enzymes in the brain, kidney, liver, heart and testis tissues of the rats exposed to doxorubicin (DOX) and morin.

Methods: Male Wistar albino rats were randomly divided into three groups as control, morin and DOX, each of them containing 7 rats. At the end of the experimental procedure, CA, 6PGD, and TrxR enzyme activities in tissues of rats were determined spectrophotometrically.

Results: In our study, we observed that DOX activated CA enzyme in liver and kidney tissues while inhibiting CA enzyme in the other tissues, activated 6PGD enzyme in the kidney, liver and heart tissues, and inhibited the TrxR enzyme in all the tissues. In addition, morin activated CA enzyme in the liver tissue while inhibiting CA enzyme in the brain, heart and testis tissues. Morin activated 6PGD enzyme activity while it inhibited TrxR enzyme in all the tissues.

Conclusion: The findings showed that doxorubicin and morin had similar properties in the tissues as to their effect on enzyme activities.

Keywords: Doxorubicin; Morin; Carbonic Anhydrase; 6-phosphogluconate Dehydrogenase; Thioredoxin Reductase

1. INTRODUCTION

Enzymes, the largest and most diverse group of proteins, play an important role in the metabolism of all organisms. All chemical reactions and metabolic pathways in the living cells are catalyzed and regulated by enzymes (1). Carbonic anhydrase (CA) is a zinc-containing monomeric metalloenzyme which catalyzes the reversible reaction of CO_2 to bicarbonate (2,3). This enzyme found in all living species is very important in terms of the reactions which it catalyzes (4). This reaction plays a critical role in various metabolic biosynthetic pathways such as metabolism of CO_2 and its transport between tissues that allow its excretion, secretion of electrolytes in various tissues and organs, pH regulation and homeostasis, gluconeogenesis, lipogenesis, ureogenesis, osteoporosis, tumorigenesis, and epilepsy (5,6). The pentose phosphate pathway is one of the most important components of cellular metabolism and the enzyme, 6-phosphogluconate dehydrogenase (6PGD), catalyzes the third reaction of this pathway (7). As a result of oxidative decarboxylation of 6-phosphogluconate, ribulose 5-phosphate and NADPH are the products of this reaction (8). It has been reported that the 6PGD enzyme increases in transcriptional and translational levels in various cancer types, and this is accompanied by an increase in activity. it has been stated that this causes resistance to chemotherapy and increased metastasis of cancer cells (9). While ribose 5-phosphate, one produce of the pentose phosphate pathway, serves as the building block for nucleic acid synthesis, NADPH is an antioxidant which is suppressed production of reactive oxygen species and contributes to the maintenance of the cell redox homeostasis as well as its role in lipid biosynthesis (10). Thioredoxin reductase enzyme (TrxR) forms the thioredoxin system together with thioredoxin and NADPH (11,12). Thioredoxin system, combined with the glutaredoxin system, performs some important biological functions such as protection against oxidative stress, DNA synthesis, regulation of receptor and transcription factors (13). Thioredoxin system is a broad-spectrum thiol reduction system and plays an important role in maintaining intracellular redox balance. This system is very active in the lung, liver, colorectal and gastric cancer types and it is of great importance in proliferation and survival of abnormal cells in tumor formation (11,14). Doxorubicin (DOX), an anthracycline antibiotic, is one of the most effective chemotherapeutic drugs used in the treatment of solid tumors and hematological malignancies such as leukemia, lymphomas, and breast cancer (15). DOX shows its effect by breaking into among DNA bases, by inhibiting the topoisomerase II enzyme and by increasing the formation of free radicals that damage DNA and membranes (16,17). Although it is a very effective chemotherapeutic agent,

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the use of DOX is restricted due to toxicity caused by the use process and its aftermath (18). Another limitation arises from the resistance to the DOX. These two conditions limit the use of DOX in treatment (19). However, the protective effects of some natural products against DOX-induced cardiotoxicity have been reported (20). Phenolic compounds form an important class of plant-based secondary metabolites. Flavonoids which are polyphenolic phytochemicals are biologically active and have antioxidant, antidepressant, anti-inflammatory effects and enzyme inhibition proterties (21-23). It has been reported morin (3, 5, 7, 2', 4'-pentahydroxyflavone), a bioflavonoid found in many plants and fruits, can regulate some metabolic enzyme activities and protect metabolism against oxidative stress due to its antioxidant properties (24, 25). Within the scope of the study, changes in CA, G6PD and TrxR activities in the liver, heart, kidney, and brain tissues of rats exposed to morin, which is polyphenolic phytochemical, and DOX, used as a cancer drug were investigated.

2. METHODS

2.1. Drugs and chemicals

DOX was obtained as Adrimisin® (50 mg/25 mL injectable solution) from Saba İlaç San. ve Tic. A.Ş, Turkey. Morin hydrate and the other chemicals were purchased from Sigma-Aldrich (St Louis, MO) and Merck (EMD Millipore Corporation, USA). The administration dose of DOX in rats was determined according to the literature (26).

2.2. Animals

The Wistar albino male rats used in this study were provided by Ataturk University Medical Experimental Application and Research Center. 10-week-old rats with a body weight between 200-250 g were kept in special cages in a controlled breeding room (24±1°C), relative humidity of 45±5% under a regular 12 h on/off sight cycle throughout the experiment. The rats were fed (pellet diet and water) *ad libitum.* This study was designed conforming to ethical norms approved by the Animal Experimentation Ethics Committee of Ataturk University.

2.3. Experimental design

The animals were randomly categorized into three groups consisting of 7 rats in each group.

Group C (Control group): Healthy control rats were administered normal saline daily using oral gavage for 10 days.

Grup M (Morin Hydrate 100 mg/kg): The rats were administered morin hydrate at a dose of 100 mg/kg b.wt daily using oral gavage for 10 days.

Group D (DOX 40 mg/kg): The rats were injected intraperitoneally with a single dose of Dox (40 mg/kg b.wt, i.p.) on the 8th day.

At the end of the study period (10th day), the rats were decapitated under mild sevoflurane anesthesia (Sevorane liquid 100%; Abbott Laboratory, Istanbul, Turkey). The liver, testis, heart, kidney, and brain tissues were evaluated from rats for biochemical analysis.

2.4. Preparation of homogenate

The rat tissues were washed with 0.9% NaCl solution and then cut into small pieces by means of a scalpel. The pieces were powdered by grinding in a mortar in the presence of liquid nitrogen. The powder was homogenized with 20 mM Tris-HCl (pH 7.5) solution and taken into Eppendorf tubes. After that, the homogenate was centrifuged at 12,900 rpm for 30 min at +4°C. The supernatant was removed and stored on ice.

2.5. Measurement of carbonic anhydrase activity

The CA activity was measured by the method of esterase activity. CA activity was assayed according to the method of Verpoorte et al (27). This method is based on the hydrolysis of p-nitrophenyl acetate to p-nitrophenol by CA. p-nitrophenol has a maximum absorbance at 348 nm. Thus, the enzyme activity was measured spectrophotometrically at 348 nm.

2.6. Measurement of 6-phosphogluconate dehydrogenase activity

The 6PGD activity was measured according to the method of Beutler (28). The basis of the method is the formation of NADP by reducing $NADP^+$ in the presence of 6-phosphogluconate. The resulting NADPH shows a maximum absorbance at 340 nm. Thus, the activity was measured spectrophotometrically at 340 nm.

2.7. Measurement of thioredoxin reductase activity

The TrxR activity was measured according to the method of Holmgren and Bjornstedt (29). This method is based on the reduction of 5,5'-Dithiobis- (2-Nitrobenzoic Acid) which is the artificial substrate of the enzyme in the presence of NADPH and the maximum absorbance of the resulting 5-thio-2-nitrobenzoic acid at 412 nm.

2.8. Statistical analysis

SPSS 16.0 program was used for statistical evaluation. Kruskal–Wallis test was used to determine the difference between the groups obtained semi-quantitative in the histopathological examination. Detection of different groups was determined by Mann–Whitney U-test. Statistical significance was considered $p < 0.05$. One-way ANOVA (Tukey) SPSS (version 12.0; SPSS, Chicago, IL) statistical program was used for biochemical analysis. All values were given as mean ± standard error (±S.E.M.), while the results at $p < 0.05$ were considered as significant.

3. RESULTS

The wistar albino male rats used in this study were randomly categorized into three groups consisting of 7 rats in each group. In group C, the rats were administered normal saline daily by oral gavage for 10 days. In group M, the rats were administered morin hydrate at a dose of 100 mg/kg b.wt daily by oral gavage

for 10 days. In group D, the rats were injected a single dose of DOX (40 mg/kg b.wt, i.p.) on the 8th day of the experiment. At the end of the 10th day, the rats were decapitated under mild sevoflurane anesthesia. After that, the liver, testis, heart, kidney, and brain tissues from the rats were evaluated for biochemical analysis. The spectrophotometer was used to measure the enzyme activities in all the tissues.

In accordance with our findings, CA activities was significantly decreased in the brain, heart, and testis tissues of the rats in the groups M and D when compared with that of the rats in the group C ($p<0.05$). CA activities in the brain and testis tissues of the rats were inhibited in the presence of DOX and morin, but the inhibitory effect of DOX was higher than that of morin. In contrast, the inhibitory effect of morin was higher than that of DOX in the heart tissue ($p<0.05$). In the kidney tissue, CA enzyme was activated in presence of DOX, but morin did not show any effect on CA activity when compared with that of the rats in the group C ($p<0.05$). In the liver tissue, CA enzyme activity was increased in presence of both morin and DOX when compared with the group C, but this increased CA enzyme activity was higher in the group M than the group D ($p<0.05$, Figure 1).

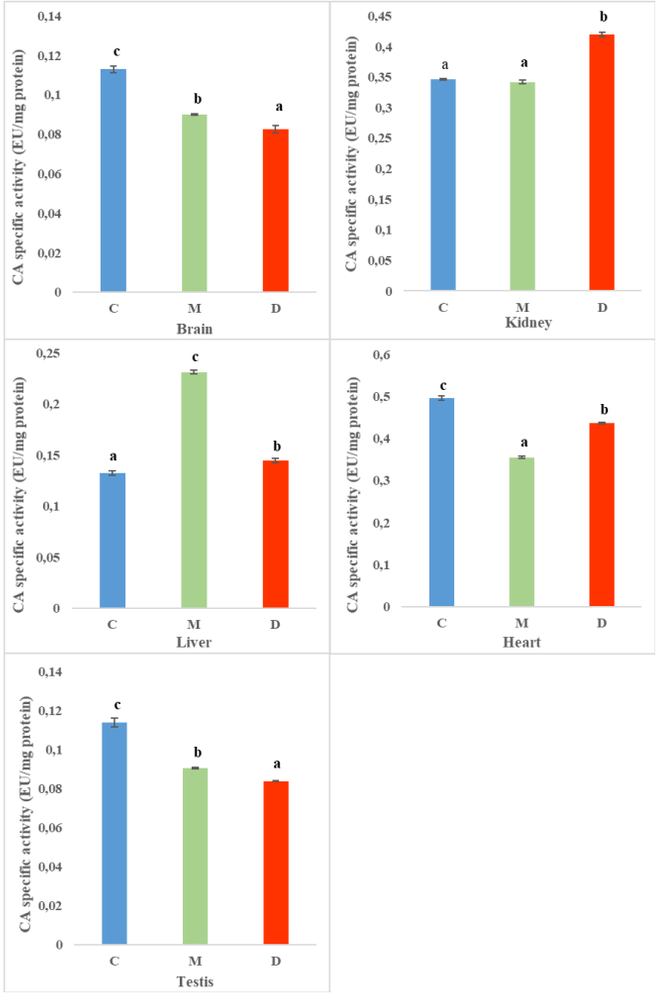

Figure 1. *The effects of morin and DOX on CA enzyme activity in different tissues (C: Control, M: Morin, D: Doxorubicin). Data represent the means ±S.E.M. of seven rats in each group. Results were derived from one-way ANOVA followed by Tukey's post hoc test. Graphs (a–c) show significant (P<0.05) differences among groups for each tissue.*

Within the scope of the study, the effects of morin and DOX on 6PGD enzyme activity were investigated. According to the measurement results, morin activated statistically the enzyme in all the tissues compared with that of the rats in the group C ($p<0.05$). DOX did not show any effect on 6PGD activities in the brain and testis tissues of the rats in the group D, but it activated 6PGD enzyme in the kidney, liver and heart tissues of the rats in the group D. However, 6PGD activities in the kidney, liver and heart tissues of the rats in the group D were lower than those of the rats in the group M ($p<0.05$, Figure 2).

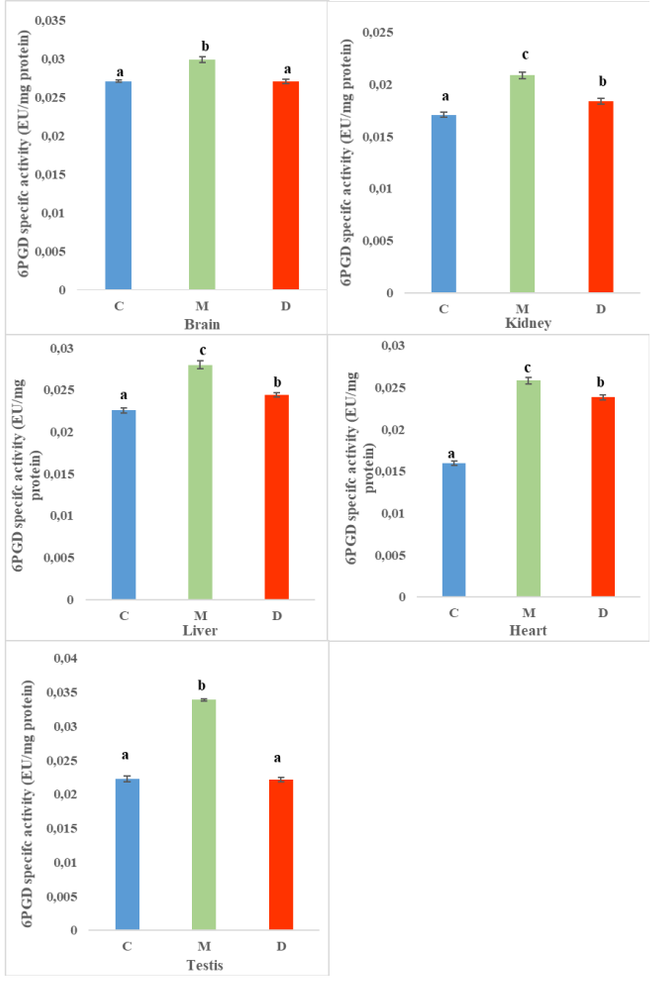

Figure 2. *The effects of morin and DOX on 6PGD enzyme activity in different tissues (C: Control, M: Morin, D: Doxorubicin). Data represent the means ±S.E.M. of seven rats in each group. Results were derived from one-way ANOVA followed by Tukey's post hoc test. Graphs (a–c) show significant (P<0.05) differences among groups for each tissue.*

In this study, the effects of morin and DOX on TrxR enzyme activity were investigated. It was determined that morin and DOX inhibited the enzyme in all the tissues when compared with that of the rats in the group C. The inhibitory effects of

DOX and morin on TrxR enzyme were statistically significant in all the tissues ($p<0.05$). Moreover, the inhibitory effect of DOX was higher than that of morin in the other tissues except for the brain tissue ($p<0.05$). In the brain tissue, there were no significant differences between the group M and D in terms of the inhibition effect of DOX and morin on TrxR enzyme ($p<0.05$, Figure 3).

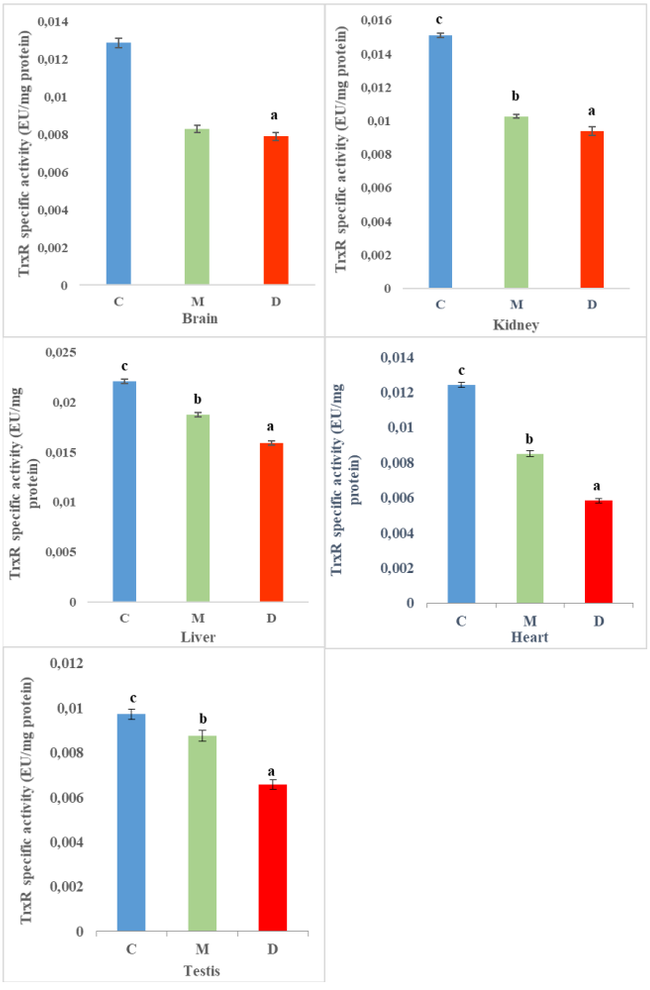

Figure 3. *The effects of morin and DOX on TrxR enzyme activity in different tissues (C: Control, M: Morin, D: Doxorubicin). Data represent the means ±S.E.M. of seven rats in each group. Results were derived from one-way ANOVA followed by Tukey's post hoc test. Graphs (a–c) show significant (P<0.05) differences among groups for each tissue.*

4. DISCUSSION

Morin (3,5,7,2',4'-pentahydroxyflavone), a member of the flavanol group, is a flavonoid isolated from members of the Moraceae family and has been reported to have many biological activities (30). In several studies, anti-epileptic (31), neuroprotective (32), anti-inflammatory (33), antioxidaznt (34), anti-fibrotic (35), anti-diabetic (36), anti-arthritic (37) and anti-mutagenesis (38) effects of morin have been reported. The anti-tumor effects of morin have been discovered in a variety of cancers, especially in breast cancer and leukemia. In addition, it has been shown that morin is capable of promoting the apoptosis and inhibiting the proliferation in prostate cancer cell line LNCaP (39). DOX, an antibiotic of anthracycline group, is one of the most potent broad-spectrum antitumor agents. DOX is widely used in the treatment of cancer types such as solid tumor, leukemia, and lymphoma (40,41). However, its clinical use is limited due to its serious toxicity. In previous studies, it has been reported that DOX can cause cardiotoxicity (42), hepatotoxicity (43), pulmonary toxicity (44) and nephrotoxicity (45). The present study was designed to investigate the effects of DOX, a drug that is frequently used in chemotherapy, and morin, a flavanol that is determined its biological activities by many researchers, on the activities of CA, 6PGD and TrxR enzymes which are important in the events of metabolism.

CA is an enzyme found in almost all cell types and subcellular organelles, from unicellular cyanobacteria to mammals (46). Carbonic anhydrase isoenzymes with 16 isoforms in mammals are involved in numerous pathological and physiological processes such as gluconeogenesis, lipogenesis, tumor formation, and virulence of some pathogens (47). In addition, it has recently been reported that CA inhibitors may have potential to use as anti-obesity (48), anticancer (49) and anti-infective (50) drugs as well as their roles as diuretic and antiglaucoma drugs (47). In some studies, it has been reported that the activation of CA enzyme may offer new approaches to the treatment of Alzheimer's disease (51).

According to the results of our study, the activity of CA enzyme in the brain, heart and testis tissues of the rats was significantly inhibited while CA enzyme in the liver tissues of the rats was activated in the present of morin. As for the kidney tissue, morin did not show any effect on CA enzyme. In previous studies, the effects of morin on CA isoforms were investigated under *in vitro* conditions and it was reported that morin inhibited the enzyme isoforms I, II, III and IV (52). Some studies also indicated that taxifolin and naringenin, which are some natural flavonoids, may inhibit CA-I and II isoenzymes under *in vitro* or *in vivo* conditions (53,54). As will be understood, our conclusions are in agreement with most literary data obtained. Additionally, the liver tissue contains a monooxygenase enzyme system in which endogenous and exogenous compounds are metabolized (55). Thus, it can be said that the metabolites have different effects on the enzyme activity as a result of the metabolism of morin. When the effects of DOX on the enzyme activity were examined, the inhibition effect of DOX was similar to that of morin. It was observed that DOX inhibited the enzyme activity in the other tissues with the exception of the liver and kidney tissues. In liver and kidney tissues, DOX increased CA enzyme activity. Katzenmeyer et al. reported that 7-deoxydoxorubicinolone and 7-deoxydoxorubicinone metabolites were found in the liver tissues of the rats while doxorubicinol was the major DOX metabolite in the plasma samples of the rats treated with doxorubicin (56). Therefore, the effect of DOX on CA enzyme activity may be different because of the different metabolite concentrations in the different tissues. The results from our research show that morin and DOX may

have similar effects on CA enzyme activity in the brain, liver, heart, and testis tissues.

6PGD is the third enzyme in the oxidative pentose phosphate pathway. This pathway is involved in the redox balance and rapid proliferation of cancer cells by connecting glycolysis to the anabolic biosynthesis (57). 6PGD activation leads to redox homeostasis, glycolysis, and anabolic biosynthesis, which are advantageous for the survival and proliferation of tumor cells (10,58). In addition, 6PGD activity has been reported to increase in many cancers including colon, breast, cervical, and thyroid cancers (59). The effects of some drugs on certain enzymes of carbohydrate metabolism in MCF-7 cells in culture have been investigated and it has been reported that DOX activates the enzyme (60). Moreover, it has been reported that 6PGD activity increases in anaplastic thyroid carcinoma cell in response to doxorubicin which is the most commonly used chemotherapeutic agent in patients with anaplastic thyroid carcinoma, and cancer cells have been reported to cause resistance to the drug. 6PGD inhibition has been found to sensitize effectively the cells to doxorubicin by eliminating this resistance (8). According to the results of our study, it was observed that DOX activates the enzyme in the kidney, liver, and heart tissues. Thus, it can be seen that the cells are resistant to the effects of DOX through 6PGD enzyme activation. Similarly, morin increased 6PGD enzyme activity in all the tissues. Therefore, morin can help in the development of resistance by increasing the activity of 6PGD enzyme in DOX-exposed cells.

The cellular control of the thiol redox state is mainly exerted by the thioredoxin and glutathione systems (61). The thioredoxin system, which comprises TrxR, thioredoxin, and NADPH, regulates crucial cell functions such as proliferation and viability (62). It has been reported that TrxR and thioredoxin expression have increased in some types of cancer (63), indicating that the thioredoxin system may play an important role in tumor formation and progression (64). Thus, it has been reported that TrxR enzyme may serve as a therapeutic target in the treatment of cancer. Furthermore, it has been reported that mouse lung carcinoma cells return to normal morphology as a result of reducing the expression of TrxR enzyme. Besides, there have been a decrease in the rate of proliferation and a decrease in the expression of some proteins related to cancer (65). In our study, it was determined that morin and DOX inhibited significantly TrxR enzyme in all the tissues. In several studies, some researchers have investigated the effects of DOX on TrxR enzyme in rats and they have found that DOX can decrease the enzyme activity in the skin (66). To the best of our knowledge, the effect of morin on TrxR enzyme activity was determined for the first time in this study. In conclusion, the present study has demonstrated that morin might have anticancer properties and one of the effective ways of DOX used in cancer treatment might be TrxR inhibition.

5. CONCLUSION

Within the scope of the study, doxorubicin which is widely used in the treatment of some cancers and morin, a bioflavonoid, have been applied to rats. Changes in CA, 6PGD and TrxR activities, which are metabolically important enzymes in brain, kidney, liver, heart, and testis tissues of rats, were examined. The findings showed that doxorubicin and morin had similar properties in the tissues as to their effect on enzyme activities.

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Clinical and Experimental Health Sciences

Knowledge, Beliefs and Practices of University Students Regarding Testicular Cancer and Testicular Self-Examination

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ABSTRACT

Objective: Although seen rarely, testicular cancer remains to be a public health problem as it is the most common cancer type in males aged between 15 and 35 years. The aim of this study is to evaluate knowledge, beliefs and practices of male university students regarding testicular cancer and testicular self-examination.

Methods: This descriptive study was conducted with 681 first-year university students between November and December 2016. The data were collected using a Descriptive Form, Testicular Cancer Questionnaire and the Champion's Health Belief Model Scale (HBMS).

Results: It was found that 91% of the students had no previous knowledge on testicular cancer, 88.3% had never heard of Testicular Self-Examination (TSE). Most of the students stated that they obtained information on testicular cancer and TSE from the internet. Students were found to have moderate level of perceived benefit, susceptibility, barrier, motivation/seriousness and self-efficacy with respect to testicular cancer and TSE.

Conclusion: As a result of this research, it was found that university students lacked sufficient knowledge on testicular cancer and TSE. In order to improve the level of knowledge on testicular cancer and TSE, health professionals may provide trainings at universities, awareness may be raised among university students through elective courses and information may be disseminated via mass media.

Keywords: Testicular neoplasms, men's health, students

1. INTRODUCTION

Testicular cancer is the second most common cancer after leukemia in males aged between 15 and 19 and remains to be a public health problem for its high prevalence in males aged 15-35 years (1). The worldwide prevalence of testicular cancer varies depending on geographic, racial and ethnic factors. While testicular cancer is seen in less than 1 out of 100,000 people in Africa and a large part of Asia, and at rates as low as 1.2 out of 100,000 among the black people in the United States, rates up to 9.4 out of 100,000 can be seen in Denmark and 9.9 out of 100,000 in Norway. Testicular cancer is becoming increasingly more common in the world and particularly among the white race with a prevalence of 6-11 in 100,000 and the annual increase is reported to be 3-6% (1-3).

According to the data issued by American Cancer Society, testicular cancer is rarely seen before adolescence but its prevalence increases after adolescence. Although it is more common in young and middle-aged males, it can occur in any period of life. In the cases seen so far, 6% involved children and adolescents and 8% individuals aged 55 and over. Testicular cancer cases have increased in the last 40 years without an apparent reason. It is estimated that 410 deaths due to testicular cancer will occur in 2019 (4). An age-specific rate distribution in Turkey showed that the prevalence of testicular cancer is 3.7 in 100,000 and it is the leading cancer type seen in males in the 15-24 age group (5).

Despite increasing prevalence of testicular cancer, testicular cancer screening behaviors of males are not at a desired level due to lack of knowledge (6). Such insufficient knowledge and behavior lead to delayed recognition of testicular cancer symptoms, decreased efficacy of the treatment and increased mortality (7). Early diagnosis of testicular cancer allows employment of less toxic and simpler treatment options (8). Testicular self-examination (TSE) is a screening method used for early detection of testicular cancer. With this very easily exercised method, physical abnormalities in the testicles can be noticed at an early stage (9). TSE is an easy, fast and costless method as compared to those that

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involve intense treatment and heavy costs (10). TSE can also prevent delays due to hesitance of the individual to seek healthcare assistance (9).

The epidemiological studies in the literature show that people in the world and in our country have limited knowledge on testicular cancer and TSE. For this reason, public health campaigns are being organized in many developed countries to raise awareness for testicular cancer and to promote TSE (11). Many studies on the subject have also recommended that the society's awareness for testicular cancer should be increased and people should be informed and educated on TSE (11-15).

The cancer-related knowledge, attitudes and behaviors of people are highly associated with personal factors such as age, gender, education and cancer-related experiences (16). Considering the ages during which testicular cancer is frequently encountered, research for identifying knowledge, attitudes and behaviors of university students regarding testicular cancer and TSE becomes extremely important in providing guidance for the trainings on testicular cancer and TSE to be given to the males in this age group and in raising awareness about this issue. For this reason, this study was planned thinking that it will provide guidance for the trainings to be organized for this group of male university students by way of identifying their knowledge, health beliefs and practices in relation to testicular cancer and TSE.

Research Questions:

- At what level is the knowledge of male university students on testicular cancer and TSE?
- What are the factors influencing students to exercise TSE?
- What are the health beliefs of students about TSE?

2. METHODS

2.1. Design and Sample

This descriptive study was conducted with the 681 first-year university students between November and December 2016. The study population consisted of the entire first-year male university students (n=1094). No sampling method was used and 681 students who met the inclusion criteria and agreed to take part in the study comprised the sample of the study. The study was conducted with the first-year male undergraduate students who were receiving education in 9 faculties and 1 occupational college of the foundation university. A total of 413 students who suspended their education, who refused to take part in the study, who were absent on the day of administering the questionnaires and who filled out inaccurate or incomplete questionnaires were excluded. The study was completed with a 62% participation.

2.2. Measurements

The study data were collected on a self-report basis using a Descriptive Form, Testicular Cancer Questionnaire prepared by the researchers, and the Champion's Health Belief Model Scale.

Descriptive Form: This form consisted of 13 questions about the socio-demographic characteristics of the students such as age, department of study and place of living.

Testicular Cancer Questionnaire: Prepared by the investigators, this questionnaire also consisted of 13 questions, which were designed to measure the students' level of knowledge on testicular cancer and TSE.

Champion's Health Belief Model Scale: By modifying Champion's Health Belief Model (CHBM), a CHBM Scale (CHBMS) was developed for testicular cancer screenings to identify beliefs and practices regarding TSE (17). The Turkish version of the scale that was adapted by Pınar et al. (18) was used in this study.

Champion's Health Belief Model Scale consists of 5 subdomains, 'susceptibility', 'motivation/seriousness', 'barriers of TSE', 'benefits of TSE' and 'TSE self-efficacy'. The scale is a 5-point Likert-type measurement tool. The response 'strongly disagree' receives 1 point, 'disagree' 2 points, 'neutral' 3 points, 'agree' 4 points and 'strongly agree' 5 points in the scale. The minimum and maximum points obtainable are 5 and 25 for susceptibility, 7 and 35 for motivation, 3 and 15 for benefits of TSE, 5 and 25 for barriers of TSE and 6 and 30 for TSE self-efficacy. Higher scores indicate increased perception of the respective subdomains (18). The Alpha reliability coefficients of the scale were found to be 0.93 for susceptibility, 0.89 for motivation/seriousness, 0.86 for benefits, 0.84 for barriers and 0.87 for self-efficacy in this study.

2.3. Data Collection Procedures

Appointments were made with the heads of the departments before going to the academic units to collect data. The study data were collected on a self-report basis while the students were in their classrooms. Before initiating the data collection procedure, detailed information on the study was provided to the students and informed consent forms were distributed to those who agreed to participate. Data were collected by administering the Descriptive Form, Testicular Cancer Questionnaire and Champion's Health Belief Model Scale to the students who read and signed the Informed Consent Form.

2.4. Data Analysis

The data were analyzed using the The SPSS 11.5 program (SPSS Inc., Chicago, IL, USA). While evaluating the study data, the parameters were checked for normal distribution using the Kolmogorov Smirnov test and they were found

to be normally distributed. Descriptive statistical methods, medians and frequencies were used to assess the study data.

2.5. Ethical Considerations

An ethics committee approval (letter numbered 87517843 and dated 25/10/2016) was obtained from the Ethics Committee of the Maltepe University. An informed consent form was given to each of the participants to be read and signed.

3. RESULTS

The mean ± standard deviation age of the male students who participated in the study was 20.11±3.27. Of these students, 98.8% were single, 69.8% lived with their families, 16.2% with their friends and 14.1% in dormitories. A large majority of the students (91.9%) did not have any health problems. It was found that 1.5% of the students had a family member diagnosed with testicular cancer and 4.8% experienced a health problem related to their testicles in a period of their life (Table 1).

Table 1. *Descriptive Characteristics of Participants (n=681)*

		n	%
Marital status	Single	673	98.8
	Married	8	1.2
Place where you lived longest	City	420	61.7
	Town	233	34.2
	Village	18	2.6
	Abroad	10	1.5
Your present place of living	With family	474	69.6
	With friends	110	16.2
	In a dormitory	97	14.2
Do you have any health problems?	Yes	55	8.1
	No	626	91.9
Is there anyone in your family who was diagnosed with testicular cancer?	Yes	10	1.5
	No	671	98.5
Have you experienced any health problems with your testicles?	Yes	33	4.8
	No	648	95.2
Age [mean (standard deviation)] = 20.11 (3.27) years			

Of the participating students, 91% reported that they had not been informed about testicular cancer previously. When the students who had some knowledge of testicular cancer were asked about their source of information, 4.7% stated that it was the Internet and 2.5% the media. While 88.3% of the students reported that they had never heard of TSE before, 3.1% of those who had heard about it stated that they had received the information from the internet. It was found that 96.2% of the students did not know how to do TSE and 73.3% of those who did not practice TSE stated that it was because of lack of knowledge and 10.9% because of negligence (Table 2).

Table 2. *Past Experiences and Opinions of Participants about Testicular Cancer and TSE (n=681)*

		n	%
Have you received any information on testicular cancer before?	Yes	61	9.0
	No	620	91.0
From where did you receive information on testicular cancer?	Internet	32	4.7
	Media	17	2.5
	Physician	15	2.2
	Instructor	13	1.9
	Family	9	1.3
	Friends	7	1.0
Have you heard of TSE before?	Yes	80	11.7
	No	601	88.3
Have you received information on TSE before?	Yes	55	8.1
	No	626	91.9
From where did you receive information on TSE?	Internet	21	3.1
	Physician	17	2.5
	Media	14	2.1
	Family	13	1.9
	Friends	10	1.5
	Instructor	6	0.9
Do you know how to do TSE?	Yes	26	3.8
	No	655	96.2
What is your reason to avoid TSE?	I do not know how to do TSE	499	73.3
	I have no motivation for the examination	74	10.9
	I am too young for having cancer, I postpone it	33	4.8
	I am afraid that something bad will arise as a result of the examination	18	2.6
	I find it sinful to do the examination	12	1.8
	I feel guilty due to the examination	10	1.5
Would you like to receive information on testicular cancer and TSE?	Yes	368	54
	No	313	46
If yes, from whom?	Health professionals	244	35.8
	The internet	104	15.3
	Books/journals	102	14.9
	Elective sex education lessons	78	11.5
	Television	40	5.9

TSE: testicular self-examination

Of the students who participated in the study, 54% wished to receive information on testicular cancer and TSE. While 35.8% of those who wished to receive information on testicular cancer and TSE preferred to receive such information from health professionals, 15.3% preferred to receive it from health sites on the Internet, 14.9% from books/journals, 11.5% from elective sex education lessons, and 5.9% from television programs (Table 2).

A review of the knowledge levels of the students regarding testicular cancer and TSE showed that 83.7% of the students did not know that a history of an undescended testicle was a risk factor for testicular cancer and only 4.8% of them

thought that testicular cancer is a curable disease. It was also found that 70.5% of the students did not know that TSE was an early screening method used for testicular cancer (Table 3).

Table 3. *Knowledge Status of Students on Testicular Cancer and TSE (n=681)*

	Right	Wrong	Do not know
	n (%)	n (%)	n (%)
Testicular cancer is most common in males aged between 15 and 35 years.	105 (15.4)	58 (8.5)	518 (76.1)
The largest group at risk of testicular cancer is those who have undescended testicles.	79 (11.6)	32 (4.7)	570 (83.7)
Testicular cancer can never be treated fully.	33 (4.8)	163 (25.9)	485 (71.2)
Males who have a family member with testicular cancer are at greater risk of having this disease.	176 (25.8)	25 (3.7)	480 (70.5)
Males who have testicular cancer are usually at my age.	41 (6)	85 (12.5)	555 (81.5)
With early diagnosis, the chances of recovery from testicular cancer increases by 80-90%.	191 (28)	25 (3.7)	465 (68.3)
The method for the earliest diagnosis of testicular cancer is self-examination.	164 (24.1)	37 (5.4)	480 (70.5)
A testicular examination should be done in shower or immediately after shower.	65 (9.5)	31 (4.6)	585 (85.9)
Testicular self-examination should be done regularly every month.	127 (18.6)	22 (3.2)	532 (78.1)

TSE: testicular self-examination

It was found that the students did not know that a mass felt by hand on a testicle (76.9%) and swelling of a testicle (75%) were symptoms of testicular cancer (Table 4).

Table 4. *Testicular Cancer Symptom Recognition Status of Students (n=681)*

Symptoms	Can recognise n (%)	Cannot recognise n (%)
Lump-mass felt by hand on a testicle	157 (23.1)	524 (76.9)
Generalized swelling of a testicle	170 (25)	511 (75)
Pain in a testicle	215 (31.6)	466 (68.4)
Pain or feeling of heaviness in the groin	194 (28.5)	487 (71.5)

The students' mean health belief scores with respect to testicular cancer and TSE are shown in Table 5. The students obtained 20.21±6.30 from the seriousness subdomain of the Health Belief Model Scale and 9.01±2.78 from the benefit subdomain, and the barrier to practicing TSE as perceived by the students was 12.94±3.99 (Table 5).

Table 5. *Mean Scores Obtained by Students from the Subdomains of the Health Belief Model Scale (n=681)*

	Min-Max Score	Median	1st Quarter - 3rd Quarter	Mean±SD	Number of Items
Perceived Susceptibility	5-25	13	10-15	12.19±4.23	5
Perceived Motivation/ Seriousness	7-35	21	16-24	20.21±6.30	7
Perceived Benefit	3-15	9	8-11	9.01±2.78	3
Perceived Barrier	5-25	14	10-15	12.94±3.99	5
Self-Efficacy	6-30	18	14-18	16.14±4.97	6

SD: standard deviation

4. DISCUSSION

Although there is an increase in the prevalence of testicular cancer almost all over the world, the knowledge of males on testicular cancer and TSE is still not at a desired level. Testicular cancer is one of the cancer types that have a good chance of recovery if detected at an early stage. For early diagnosis, individuals need to be aware of the issue and to practice TSE (18-19).

It was found in this study that a large majority of the students were not knowledgeable about testicular cancer and did not know how to do TSE. In their study with health college students, Yilmaz et al. (20) found that 60.9% of the students had previously heard about testicular cancer and 45.2% had received this information within the scope of their undergraduate lessons. In the same study, 92.7% of the students were found to be willing to receive further information on testicular cancer and TSE (20). Altinel and Avci (21) reported in their study that 57.6% of the students had heard about testicular cancer. In another study made by Pour and Cam (22), 72.4% of the male subjects had not heard about TSE previously, 89.4% did not know how to do it and 90.6% had not received any training on it. These results revealed the fact that young males had insufficient knowledge on testicular cancer and TSE, and almost all of them showed a need for information on the issue when their attention was attracted through study questions. This result, which is similar to the results of the present study, shows that trainings on testicular cancer and TSE should be included in the educational subjects on health development and protection to be provided to young males.

This study revealed that most of the students did not know that a history of undescended testicle was a risk factor for testicular cancer and a mass felt by hand and swelling of a testicle were symptoms of testicular cancer, and very few of them thought that testicular cancer was a treatable disease. In a study made by Altinel and Avci (21), 82.9% of the students did not know that the greatest risk factor for testicular cancer was an undescended testicle and 63.8% that early diagnosis increased the chance of recovering from

testicular cancer by 80-90%. The same study showed that only 9.2% of the students were aware of the testicular cancer symptoms (21). This lack of knowledge about testicular cancer and TSE can be explained by the fact that the health of males is overlooked both in the world and in Turkey and males behave indifferently towards their own health due to the social roles attributed them.

The reasons for the students to avoid TSE were explored in this study and it was found that most of the students chose to not practice TSE because they were unaware of it, some neglected it, and a few thought either they were too young to have cancer, were afraid that the outcome would be bad, or considered it to be sinful and felt guilty. In the study of Yilmaz et al. (20), 94% of the students did not practice TSE because they did not know how to do it, 2.4% because they felt guilty, 6% because they neglected it, 2.4% because they were afraid something bad would turn out during examination, 3.6% because they thought they were too young to have cancer, and 2.4% because they feared it and became stressed. In the study of Pour and Cam (22), again a large majority of the students (89.4%) stated that they chose to not practice self-examination because they did not know about TSE, 2.2% because they thought it was sinful, and 10.4% because they were afraid something bad would come up. Most of the studies on the subject found high rates of unawareness and lack of knowledge as the leading reasons for not doing TSE. However low their rates may be, the other barriers to practicing self-examination (guilt, fear, negligence, etc.) are actually the barriers/problems arising from lack of knowledge. The results of a qualitative study made by Carbone et al. (23) on patients diagnosed with testicular cancer demonstrated that lack of knowledge on cancer was one of the factors that delayed seeking health assistance even when the problem emerged. Ugwumba et al. (24) found in their study that the low level of knowledge and awareness of the students of medicine in their final year before a training provided to them about testicular cancer and TSE increased significantly after the training. These results suggest that if trainings on the subject are planned and effectively implemented, feelings of fear, sinfulness, guiltiness etc. may be reduced and such trainings may prevent in the future delays in seeking assistance when a problem arises.

While most of the students in this study wished to receive information on testicular cancer and TSE from health professionals, other students showed the internet and elective sex education lessons as their potential source of information. In the study of Gocgeldi and Kocak (25), 49.2% of the students wanted to receive this information from health professionals and 47.7% from conferences. When the sources of information on TSE were explored in the same study, 24.1% of the students were found to obtain this information from health professionals, 20.7% on their own, and 10.3% from television and newspapers (25). In the study of Ugwumba et al. (24), 73.1% of the students obtained the information from a hospital/clinic, 11.9% from conversations on health, 7.9% from the media, and 5.9% from their friends. Similarly, it was also found in the present study that the students obtained the information on testicular cancer and TSE mostly from the Internet. These results show that young people cannot adequately benefit from health professionals and health developing/protecting programs and widely use the internet as their source of information. These results suggest that health professionals should increase health developing/protecting programs for the youth and should reach those who use the Internet by also using the same medium.

The Champion's Health Belief Model Scale is not only a valid and reliable scale for testicular cancer and TSE, but it also shows that perceptions related to TSE affect the behavior (18, 21). The students in the present study obtained 20.21 points out of 35 from the seriousness subdomain of the Health Belief Model Scale and 9.01 points out of 15 from the benefit subdomain, and the barrier to practicing TSE as perceived by the students was 12.94 points out of 25 (Table 5). Similar to our study, a study made by Pinar et al. (18) also reported that their students obtained 9.36 points out of 15 from the benefit subdomain of the Champion's Health Belief Model Scale. A study reported that the likelihood of exercising TSE increased with perceived susceptibility and seriousness and as perceived benefit increased and perceived barriers decreased, the rate of doing TSE increased (26). When demonstrating a health behavior, the belief of individuals that such health behavior is beneficial for their health plays a great role in actualizing that behavior. Therefore, if the focal point of the trainings to be given on the subject is to increase the magnitude of benefit perceived by individuals, the likelihood of achieving a behavioral change will be greater.

5. CONCLUSION

This study has revealed that university students do not have adequate knowledge on testicular cancer and TSE and they wish to obtain more information on the subject. The students were found to have moderate level of perceived benefit, susceptibility, barrier, motivation/seriousness and self-efficacy with respect to testicular cancer and TSE. To improve knowledge on testicular cancer, which is extremely important for male health, and to promote the habit of TSE, health professionals may provide trainings at universities, awareness may be raised among university students through electives on the subject and information may be disseminated via mass media.

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Clinical and Experimental Health Sciences

What Do Turkish Nursing Students Think About Palliative Care?

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ABSTRACT

Objective: The purpose of this study was to identify Turkish nursing students' knowledge, thoughts and attitudes about palliative care.

Methods: This is a descriptive survey study that was conducted in Ankara, Turkey on nursing students between 2015 and 2016. In order to obtain the broadest viewpoint, we decided to survey 163 nursing students, including 77 juniors and 86 seniors. The data was collected using an 'Opinion Form on Palliative Care' as well as an Introductory information form.

Results: 136 out of a total of 163 junior and senior students were included in this study. Most of the students practiced general medicine, surgery and ICU, and described palliative care as a multidisciplinary health service for terminally ill patients with cancer. Although 89.7% of students had received some degree of palliative care training as part of their education, most of them (66.2%) had felt that it was insufficient. Palliative care topics that students wished were included as part of the curriculum included the basic concepts of palliative care (94.9%), communication (91.9%), legal and ethical issues (89.7%), loss and bereavement (89%), and symptom management (86%). Most students (77.9-76.5%) stated that palliative care training ought to be a mandatory training program.

Conclusion: Nursing students who have heard about the concept of palliative care during their education thought that the content of the palliative care education was lacking within their nursing program. Also, most had felt that this specialized content ought to be expanded upon as part of specialization training.

Keywords: Palliative care, nursing education, undergraduate, student experience.

1. INTRODUCTION

The World Health Organization (WHO) defines palliative care as "an approach that aims to improve the quality of life of patients and their families facing the problem associated with life-threatening illness, by means of early identification and impeccable assessment of pain and other problems; prevention and relief of suffering by meeting physical, psychosocial and spiritual needs" (1). One important premise of palliative care is that it involves an interdisciplinary team approach because there is no specific discipline that is able to meet the holistic needs of patients and their families. Therefore, a palliative care team may consist of a combination of physicians, nurses, pharmacists, social workers, physiotherapists, and spiritual advisors. Each team member plays a unique role that contributes towards achieving successful patient and family outcomes (2).

The nursing role on the palliative care team includes the assessment of the individual's culture, values, beliefs, preferences, direct care, and often care coordination (3). Specialized training is required in order to properly carry out this role. Thus, the degree of education depends on whether the nurse aims to provide care in primary care or at a specialist level (4). Primary palliative care is a level of care that would be expected of any general nurse who encounters a terminally ill patient, and would include the basic principles of pain and symptom management, communication skills, and awareness of resources such as hospice care. On the other hand, specialty-level care requires advanced education that would allow for the assessment of much more complex symptoms, alongside communication and supportive decisions.

Despite standards both for undergraduate primary palliative nursing education and continuing education programs such as End of Life Nursing Education Nursing Consortium (ELNEC), many nurses throughout Turkey still do not possess a solid foundation or awareness of the broad spectrum meaning of palliative care concept, often confusing it with hospice and

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end of life care (5,6). Even though palliative care content has been introduced as part of the basic nursing program, nurses often report that the amount of education that they received as being inadequate (7,8). Hence, it will take time for nursing curricula to accurately reflect current developments in the field, meaning that practicing clinicians will be deprived of gaining the necessary knowledge, skills, and awareness until they reach the point to provide symptom management and palliative care (3,7). Therefore, it is important to integrate palliative care into basic nursing education (9).

Although there is no specific, standard core curriculum for palliative care, progress in terms of incorporating palliative care content is being made in other parts of Europe. For example, palliative care is included as a chapter in the European Oncology Nursing Association (EONS) Cancer Nursing Education Framework (10). Being a special area of expertise, palliative care needs to be a part of basic nursing education, and all nurses need to be completely aware of the fundamentals of palliative care (6).

There are few studies investigating the knowledge and attitudes of nursing students about palliative care (11,12). There is very limited research about palliative care in general, with regards to nursing in Turkey (13,14). Moreover, there are no known studies on palliative care conducted on nursing students in Turkey. Therefore, we decided to conduct a study assessing the knowledge and attitudes of Turkish nursing students regarding palliative care. It is important to depict the status of palliative care education in basic nursing education in order to properly integrate palliative care education into the nursing curriculum and design a continuing education program that reinforcing knowledge and skills of the nurses after graduation. As part of an initial needs assessment for future palliative care training in Turkey, the aim of this study is to determine current knowledge and views of nursing students about palliative care.

2. METHODS

2.1. Sample and Setting

This study is a descriptive study carried out with junior and senior nursing students studying at a nursing school in Ankara between 2015 and 2016. The nursing school where the study was conducted currently provides a four-year nursing undergraduate education. In the first three years, both clinical practice and theoretical courses are carried out simultaneously; in the final year, students do clinical practice in hospital settings four days a week. Junior and senior students were chosen for the study; given that both have taken courses that consist of subjects about palliative care as well as have had clinical practice in related settings. Currently, there is no specific palliative care course in the nursing curriculum. We decided to conduct the study of 163 nursing students (77 junior and 86 senior), in order to obtain the widest perspective. 136 out of a total of 163 (72 junior and 64 senior) students were included in the study.

2.2. Data Collection Tool

The data on demographic features of the students were collected by the introductory information form and data on knowledge and views of the students were collected by the 'Opinion Form on Palliative Care'. The 'Opinion Form on Palliative Care' was developed by Turgay and Kav in order to determine the views of nurses on the philosophy of palliative care and the provision of care services (15). This form consists of sixteen questions and is based on the opinions provided by a group of specialists consisting of health professionals (nurses, physicians, social workers) working in palliative care settings (15). The introductory information form consists of eight questions that define the socio-demographic characteristics of students, such as age, study year (class) and gender.

2.2.1 Data collection

Considering that the nursing school where this study was conducted was also a boarding school, all students were expected to attend study hours during weekday evenings. In one of these study periods, all students were informed about the study, and those who agreed to participate were asked to fill out the questionnaires. Students filled out the data collection forms by themselves using paper and pencil. As students filled out the questionnaires, a researcher (ES) accompanied these students in order to answer possible questions. It took between 15 and 20 minutes to fill out the questionnaires.

2.3 Ethical Dimension of the Study

Prior to the study initiation, ethical approval was obtained from the ethical board of the university where the study was conducted. The research protocol was approved by the Gulhane Military Medical Academy research ethics committee before the study (Ethics Committee Approval Number: 03/2015). Students were verbally informed about the aim of study and were told that their personal data would not be disclosed. Students who agreed to participate in this study signed written informed consent forms.

2.4 Data Analysis

The data were analysed using SPSS 21.0 software package. The findings were shown in terms of numbers and percentages for categorical variables, and mean ± standard deviation (SD) for continuous variables.

3. RESULTS

Table 1 shows students' characteristics. The mean ± SD age of students was 21±0.61 years. More than half of the students were juniors (52.9%), and all of them were female. Most students (72.1%) had practiced in general medicine wards as part of their clinical training.

Table 1. *Nursing Students' Characteristics (n=136)*

	n (%)
Study year (class)	
Third year	72 (52.9)
Fourth year	64 (47.1)
Units where the clinical practice performed	
Internal units*	98 (72.1)
Surgical units**	88 (64.7)

**Internal medicine, gastroenterology, respiratory disease service, cardiology, nephrology, endocrinology, hematology, oncology, neurology, rheumatology.*

***Neurosurgery, general surgery, respiratory surgery, cardiovascular surgery, orthopedics, urology.*

Table 2 shows 'student' experiences and thoughts about palliative care training'. Most of the students (90%) stated that they had heard about palliative care content during their education. They had never taken any specific palliative care courses. They heard about palliative care in their oncology lectures during their theoretical education as well as during their clinical practice in oncology clinics. 55% of the students who had some degree of palliative care training had thought that that training was not sufficient. The most frequent topics given by students for the question 'what topics should be in the palliative care courses' including 'fundamental principles and concept of palliative care' (94.9%), 'communication' (91.9%), and 'legal and ethical issues in palliative care' (89.7%). Majority of the students thought that nurses (97.1%), physicians (93.4%), psychologists (91.9%), and social workers (85.3%) should be on a palliative care team.

Table 2. *Nursing Students' Experiences and Thoughts About Palliative Care Training*

	n (%)
Have you heard of palliative care in nursing education?	
Is palliative care mentioned in your education? Yes*	123 (90.4)
In your opinion, is palliative care training sufficient?**	
Yes	47 (34.5)
No	76 (55.9)
What topics should be included in the palliative care courses? ***	129 (94.9)
Fundamental principles and concept of palliative care	125 (91.9)
Communication	122 (89.7)
Legal and ethical issues in palliative care	121 (89.0)
Loss and grief	117 (86.0)
Symptom management	
Who should be in palliative care team? ***	
Nurses	132 (97.1)
Physicians	127 (93.4)
Psychologists	125 (91.9)
Social workers	116 (85.3)
Physical therapists	98 (72.1)
Spiritual advisors	94 (69.1)
Dieticians	89 (65.4)
Volunteers	65 (47.9)
Pharmacists	61 (44.9)

**In the topics of oncology nursing and end of life.*

***If they said they had palliative care training.*

****More than one answer.*

Table 3 shows students' views of palliative care. Most of the students are of the opinion that 'palliative care should be provided by a multidisciplinary team (91.2%)', 'working in palliative care requires being able to control one's own feelings (81.6%)', 'patient and family caregivers should collectively make decisions (78.7%)', 'the government should assure individuals' reaching out to palliative care when needed (78.7%)', 'palliative care should be a specific field specialty exclusive of oncology and other fields (77.9%)', and 'palliative care courses should be a mandatory component of health science programs (76.5%)'. The least agreed upon thoughts by students included that 'palliative care should consist of only pain management (12.5%)', 'physicians should lead palliative care teams (15.4%)', 'emotionally empowering programs should cover only patients and their families (19.1%)', 'palliative care should be hospital based (25.7%)', and 'palliative care should focus on symptom management without focusing on reason behind the symptoms (36.8%)'.

Table 3. *Nursing Students' Views About Palliative Care*

	Agree n (%)	Neutral n (%)	Disagree n (%)
Palliative care should be served by a multidisciplinary team	124 (91.2)	10 (7.4)	2 (1.5)
Working in palliative care requires one to be able to control their emotions	111 (81.6)	23 (16.9)	2 (1.5)
Patient and family caregivers are collective decision makers	107 (78.7)	23 (16.9)	6 (4.4)
Being able to reach palliative care when needed should be a government guarantee	107 (78.7)	26 (19.1)	3 (2.2)
Palliative care should be a specific specialty exclusive oncology and other branches	106 (77.9)	26 (19.1)	4 (2.9)
Palliative care courses should be mandatory in all health science programs	104 (76.5)	28 (20.6)	4 (2.9)
Patients and caregivers should be able to reach palliative care teams 24/7	94 (69.1)	39 (28.7)	3 (2.2)
Palliative care comprises of health services for terminal cancer patients	85 (62.5)	30 (22.1)	21(15.4)
Palliative care should start when curative treatment is not possible or at the terminal stage of disease	82 (60.3)	44 (32.4)	10 (7.4)
Exhaustion is inevitable for those who work in palliative care since they continuously face loss	72 (52.9)	51 (37.5)	13 (9.6)
Patients should have right to not be resuscitated (DNR). Legislative regulations about DNR should be made	66 (48.5)	61 (44.9)	9 (6.6)
Palliative care focuses on symptom management without focusing on the reason of symptoms	53 (39.0)	48 (35.3)	35 (25.7)
Palliative care needs to be hospital based	35 (25.7)	50 (36.8)	51 (37.5)
Emotionally empowering programs should only serve patients and their families	26 (19.1)	56 (41.2)	54 (39.7)
Physicians should lead palliative care teams	21 (15.4)	60 (44.1)	55 (40.4)
Palliative care consists only of pain management	17 (12.5)	37 (27.2)	82 (60.3)

4. DISCUSSION

This is the first study to be carried out in Turkey that assesses nursing students' views about palliative care. In this study, most of the students had received palliative care education, but more than half of the students who received that education had reported that they found their education inadequate. Similarly, a literature review also shows that nursing students have inadequate training on palliative care (16-18). In addition, Karkada et al. reported that only 43.4% of nursing students were aware of palliative care, and that they gained such awareness during their training on cancer management (16). In a study conducted by Glover et al., 85% of nursing students reported that they did not receive adequate palliative care training (17). Khraisat et al. reported that 70% of nursing students did not receive any training on palliative care during their nursing education (18). Recent studies on palliative care have recommended that undergraduate nursing programs should include a significant amount of theoretical and clinical practice on palliative care (19-21). Many other studies also report that attitudes towards palliative care can be improved through palliative care courses (22-24). Moreover, the World Health Organization (WHO) recommends compulsory palliative care courses for a basic professional qualification (1). In this study, although most of the students thought that they have inadequate palliative care education, their views on palliative care education for health professionals were positive.

In this study, most of the students stated with regard to palliative care that the following topics should be included in the curriculum: 'fundamental principles and concept of palliative care', 'communication', 'legal and ethical issues in palliative care', 'loss and grief', and 'symptom management'. This shows the tendency towards the basic concepts of palliative care with regards to the concept and scope of palliative care education among students. WHO emphasizes the importance of symptom management in the content of palliative care; it also highlights that symptom management is not the only component of palliative care in the curriculum (1). The fact is that students think that topics such as communication and legal regulations hold a very important place in both palliative care philosophy and symptom management, as well as think that they ought to be included in palliative care education. Considering the legal regulations on practice are extremely important in palliative care, it is important to address legal regulations and ethical issues related to the subject in palliative care education.

Most of the students stated that palliative care should be given by a multidisciplinary team. They also stated that the palliative care team should include nurses, physicians, psychologists, and social workers. In order to provide effective palliative care, the members of a multidisciplinary team need to have understanding of their role and contributions in order to achieve their desired goals (2). In this study, the necessity of multidisciplinary team was correctly identified by nursing students. Consistent with the results of current study, a study conducted by Sujatha et al. had also revealed that nursing students had identified the occupational groups that are included in palliative care (2). In addition, students believe that 'patient and family caregivers are important decision makers, and communication is important for palliative care'. Palliative care aims to improve the quality of life of the patient and their family by involvement of patient, family members, and all healthcare professionals. Seeing patient and family as the members of a team is a key factor in terms of setting goals and thus achieving those goals. Communication is crucial for successful care. Improving communication means more patient and family participation both in decision-making and in advanced care planning. A recent integrative review had reported that communication is a key component of palliative care, and moreover that it is important to evaluate educational outcomes related to self-efficacy, comfort level, and knowledge related to therapeutic communication with the patient, with caregivers, and among the multidisciplinary team (7).

More than half of the students in the present study also stated that palliative care should start when medical treatment is no longer possible or at the terminal stage of disease, as well as indicated that palliative care practices are necessary for cancer patients in terminal period. Given the general principles of palliative care, it was observed that some of the students' opinions were not compatible with the understanding that palliative care practices should start at the stage of diagnosis. It is thought that this result is related to the provision of available palliative care services in our country, whereby there are mostly hospital-based services and inpatient units, and whereby terminally ill patients are generally treated in these units. This result also may be related to the fact that students learn palliative care only in intensive care and oncology courses.

In this study, most students, think that all palliative care patients should be able to reach palliative care services and the team that provides those services 24/7. These results were also supported by the results of the study conducted by Sujatha et al. (2). Many studies indicate that hospital-based palliative care teams can be helpful in order to relieve cancer patients' physical discomfort and to provide both psychological and social support (25,26). The hospital-based palliative care teams are to provide active care to patients whose disease is not responsive to medical treatment, to offer consultation service to acute care clinicians, and to embed the principles of hospice and palliative care within acute hospital settings (27). In our country, palliative care services are provided by the state in public hospitals; however, the concept of palliative care is commonly understood as 'end-of-life care' and is presented in a hospital-based structure (14). In this study, a small number of students believe that palliative care services should be provided as a community-based service in hospitals. Although there are home health services that function in cooperation with palliative care in our country, it is known that these services are not enough for all patients needing to palliative care in their living areas.

Although the opinions of students about working in this field with palliative care are positive, most of the participants mentioned the importance of controlling emotions while working in this care field. Slightly more than half of the students stated that exhaustion is inevitable for people who work in palliative care due to frequently facing loss. This result shows that almost half of the nursing students provide palliative care for terminally ill patients who are emotionally stressed. This reality, which teaches to provide care only for survival rather than death, can create distress and difficulty in health professionals (28). Attitudes towards death and dying may be related to healthcare professionals' personal spiritual and/or scientific beliefs (29), and may also be related to the belief systems of the population within which nurses are giving healthcare to. When the healthcare professions are not well prepared to deal with all this reality, facing death and giving care to dying people can be distressing and exhausting (28). These findings show the importance of equipping nurses and nursing students with improved self-efficacy in order to deal with death, as well as in order to care for patients with life-threatening diseases.

Limitations of the Study

There are some limitations identified in this study. The study was conducted in only one nursing school with a small sample size. This prevents us from generalising the study results; there is a need for future research with a larger sample size. Despite these limitations, the results of this study provide valuable insight about Turkish nursing students' viewpoints towards palliative care.

5. CONCLUSION

Our results show that although most nursing students receive palliative care training, this training remains inadequate and unspecific. It is highly recommended that topics such as fundamental principles and concepts of palliative care, communication, legal and ethical subjects in palliative care, loss and grief, and symptom management be included as part of the curriculum. No other health care professionals spend more time with seriously ill patients than the nurses do. Therefore, prior to entering professional career, nursing students should be taught about basic palliative care practice and its importance during the course of diagnosis and treatment of any serious illness, as well as be taught about the nurses' responsibilities within the scope of delivering basic palliative care. Education is the key for improving palliative and end of life care. Nursing faculties also play an important role in terms of improving quality of life of patients and their families through providing students with palliative care training.

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Clinical and Experimental Health Sciences

Examining Sleep Quality and Job Satisfaction of Critical Care Nurses

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ABSTRACT

Objective: Ensuring patient safety in intensive care units, which constitute the key area of nursing profession, depends on protecting the safety of nurses. Intensive care units providing nonstop treatment in challenging conditions change the sleep quality, psychological and physiological rhythm of nurses. In this units nurses need to work very carefully always. Based on this thought, we aimed to examine the sleep quality and job satisfaction of intensive care nurses.

Methods: 234 nurses from different intensive care units in five different hospitals participated in this descriptive cross-sectional study. Personal information form, Pittsburgh Sleep Quality Index (PSQI) and Minnesota Satisfaction Questionnaire (MSQ) were used in data analysis.

Results: No differences were found among Pittsburgh Sleep Quality Index scores of nurses with respect to intensive care units, whereas Minnesota Satisfaction Questionnaire scores were found to be different ($p<0.005$). The average Pittsburgh Sleep Quality Index score was found to be 8.99±3.45. The highest Minnesota Satisfaction Questionnaire scores were found at level 3 general surgery, anesthesia intensive care units and neonatal intensive care units. A significant relationship was found between the job satisfaction of nurses and the duration of work in intensive care units ($p<0.001$).

Conclusions: Although nurses were happy to work in intensive care units, their sleep quality was found to be 'poor' and their job satisfaction was found to be at 'medium level'. In order to improve sleep quality of intensive care nurses, working hours should be revised. It is recommended that managers make improvements that increase motivation and take into account the feedback of employees.

1. INTRODUCTION

Sleep is the temporary, partial, periodic and rather reversible interruption in the organism's interaction with its environment (1,2). Sleep can also be defined as a functional and developmental 'process' where tired neural system is repaired, and data is sorted. Sleep which is an important physiological need considerably affects an individual's life and well-being. The sequence in which humans sleep at night and pursue their activities during the day is called the 'circadian rhythm'. Working in shifts results in circadian rhythm disorders and changes sleep quality (2-5). Sleep quality means feeling energetic and ready for the new day (6). It may be affected by occupational factors, working conditions, age, gender, exercise, acquired habits and stress (7). Working at night shifts requires a daytime sleep affecting sleep qualitatively and quantitatively. More than 60% of those who work in shifts were observed to have trouble in terms of adaptation to working hours, general unwillingness and inclination to sleep and higher rates of occupational accidents (6,8,9). In addition, a relationship between nurses' sleep quality and level of burnout was identified (10).

Job satisfaction is the positive perception of an individual's job or job-related life. As a consequence of employees' physical and mental health as well as emotions, job satisfaction of nurses and their organizational or professional commitment are closely related to their decision to continue their profession. Studies have revealed that job satisfaction of nurses is the determinant of their intention to quit their job. Moreover, offering a good patient care is also an important factor in the job satisfaction of nurses (11). Various variables including personal traits, intensive working conditions, wrong hospital policies and attitudes of colleagues and team members affect the job satisfaction of nurses. The studies have revealed that job satisfaction level of nurses was found to be at medium level (12,13).

Intensive care units where the treatment and care services of risky patients are carried out are the most critical and equipped units of the hospitals. Intensive Care Unit (ICU) patients need careful care (14). Treatment in these units is provided so intensely that it could case changes in the social, psychological, physiological and sleep rhythm of an

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employee. If working shifts have long hours, lack of attention may cause patients to get harmed (15).

The ability of intensive care nurses to provide the expected level of treatment and care depends on their physical and mental health. Working in shifts and under difficult conditions changes the physiological, psychological and social rhythm of the nurses and even adversely affects their sleep quality and job satisfaction. Sleep problems and low motivation of nurses who connect intensive care patients to life with devotion and holistic approach can threaten both employee and patient safety (14,15).

Ensuring patient safety requires ensuring physical and mental safety of healthcare professionals. In addition, occupational safety is closely related to whether appropriate working conditions are provided. The burden of treatment and care is acknowledged by the hospital administration, other healthcare professionals and the society.

Effective nursing care and continuous patient safety depend on the occupational health and safety and the prevalence of relevant applicable principals. Considering the direct impact of low performance of intensive care nurses, the aim of the study was to examine the 'sleep quality' and levels of 'job satisfaction' of intensive care nurses.

2. METHODS

2.1. Study Design and Sample

This descriptive cross-sectional study was conducted at five hospitals located in the European side of Istanbul, Turkey, between the 10th July and 6th September 2018. The study included 3 educational and research hospitals and 2 state hospitals. Institution permissions were obtained from the hospitals where the research was conducted. 462 nurses working in the intensive care units constituted the population of the study. Reaching all intensive care nurses was aimed before sample selection. 234 nurses who were not in parental or annual leave, actively working in ICUs, who have been working in intensive care for at least one month and agreed to participate in the study constituted the sample group. The questionnaires were distributed and received back within the same day after the nurses were explained about the study and their consent was received. Questionnaires of the nurses who were non-available in the study day were collected a few days later.

2.2. Ethical Considerations

Permit was obtained from the Ethical Committee at Beykent University (08. 05.2018; ethical no: 2018/02). The institutional permits pertaining to the study were obtained from the Public Hospitals Association and the respective hospitals. Written informed consents of nurses were received. The study was conducted between July and September 2018.

2.3. Data Collection and Tools

'Personal Information Form', structured by reviewing the literature was used to collect socio- demographic data of nurses. 'Pittsburgh Sleep Quality Index (PSQI)' and 'Minnesota Satisfaction Questionnaire (MSQ)' were used in data collection.

Pittsburgh Sleep Quality Index was developed by Buysee et al. in 1938 and examined for validity and reliability in Turkish and its Cronbach's alpha value was found to be 0.80 (16). It is a self-report scale of 24 questions assessing sleep quality and disorder within one-month period. 19 of them are self-reported questions and the remaining 5 are to be answered by the spouse or a roommate. It has a range between 0-21. When the total score is lower than 5, it is considered as 'good' sleep quality whereas when the total score is higher than 5, it is considered as 'poor' sleep quality or 'a high level of sleep disorder' clinically (16,17).

Minnesota Satisfaction Questionnaire was developed by Weiss, Dawis, England and Lofquist in 1967, translated into Turkish and examined for validity and reliability by Baycan in 1985 (Cronbach's alpha = 0.77). MSQ is a 5-point Likert scale with 20 questions. The highest and lowest scores from this scale can be 100 and 20 respectively, 60 at the mid-range indicates neutral satisfaction. Scores approximating 20 mean decreased satisfaction while those approximating 100 show increase in satisfaction. The intrinsic satisfaction score consists of factors related to the 'intrinsic nature of the job' including success, being recognized or appreciated, the job itself, job-related responsibility, and change of position due to advancement or getting promoted. On the other hand, extrinsic satisfaction score includes external factors such as organizational policy and administration, audit method, relationships with the administrator, colleagues and top management, working conditions and the wages. Scores below 25 mean 'lower job satisfaction', between 26-74 mean 'normal job satisfaction' and above 75 mean 'higher job satisfaction' (12,18). In this study, Cronbach's alpha values for intrinsic satisfaction, extrinsic satisfaction and total satisfaction were found to be 0.869, 0.833 and 0.907, respectively.

2.4. Statistical Analysis

Mean value, Chi-squared test, Mann Whitney U, Kruskal-Wallis, correlation and Spearman correlation analyses were used in data analysis. Statistical analysis was performed using SPSS.23 software.

3. RESULTS

3.1. Demographics

Of the participants, 78.4% were female, 58.8% were single and 67.8% had associate or bachelor's degree. Among the participating nurses; 80.2 % worked for more than 40 hours a week, 47.3% performed on-call duties 10-13 times a week,

79.7 % worked both day and night shifts, 85.7 % working contentedness, 83.9 % preferred working at ICUs on their own will, 80.2% had various health problems, (68.5% of these health problems were sleep disorders and 62.5 % these health problems were physical health problems), 65.2% did not attend any congresses in the recent year and 71.2% sometimes followed professional publications. In addition, the average number of on-call duties per month was found to be 9.85 and the nurses slept after the shifts for 5.41 hours on average (Table 1).

Table 1. *Socio-Demographical Characteristics and Mode of Work of the Nurses (n=234)*

Characteristics	Median	
Age	28.71	
Duration of the intensive care work/years	3.96	
Number of on-call duties per month	9.85	
Number of patients per nurse	3.35	
Hours of sleep after the shift	5.41	
	Number (n)	**Percentage (%)**
Gender		
Women	181	78.4
Men	50	21.6
Marital status		
Married	96	41.2
Unmarried	137	58.8
Level of education		
High school	22	9.4
Associate degree	28	12.0
Bachelor's degree	158	67.8
Graduate	25	10.7
Working preference		
On my own will	194	83.98
Administration's request	37	16.02
Intensive Care Unit of Work		
Level 2 ICU (General Surgery and Anesthesia ICU)	7	3.0
Level 3 ICU (General Surgery and Anesthesia ICU)	38	16.2
Level 2 and 3 ICU (Mixed ICU)	52	22.2
Cardiovascular ICU	30	12.8
Coronary ICU	34	14.5
Neurology ICU	5	2.1
Palliative ICU	10	4.3
Pediatric ICU	8	3.4
Neonatal ICU	50	21.4
Mode of work		
Continuous/Daytime: (08-16) (08-20)	24	10.34
Continuous/Night: (16-08) (20-08)	23	9.91
Day and Night, variable shifts: 08-16, 16-08, 08-20, 20-08.	185	79.74
Working contentedness		
Yes	198	85.71
No	33	14.29
Weekly working hours		
Less than 40 hours	4	1.72
40 hours	42	18.03
More than 40 hours	187	80.26
Number of on-call duties per month		
0-9	82	35.4
10-13	109	47.3
14+	40	17.3
Health problems		
Yes	187	80.26
No	46	19.74
If yes / Type of health problem		
Physical health problem	115	62.5
Psychological health problem	68	37.0
Physical and psychological health problem	55	29.9
Sleep disorder	126	68.5
Congress attendance in the recent year		
Yes	81	34.8
No	152	65.2
Following professional publications		
Regularly	15	6.4
Sometimes	166	71.2
Never	52	22.3

ICU: Intensive Care Unit

3.2. Pittsburgh Sleep Quality Index (PSQI)

There was no difference in average PSQI scores with respect to the ICUs (p>0.05). The lowest PSQI score (7.14±2.41) was obtained from the level 2 general surgery and anesthesia ICUs while the highest (12.94±17.64) was from neonatal ICUs (Table 2). The average of PSQI scores was found to be 8.99±3.45 (med: 9, min: 1.00, max: 20.00) PSQI scores for sleep quality between 0-5 were considered 'good' (Table 2).

Table 2. *MSQ and PSQI Scores Across Intensive Care Units (n=234)*

Intensive Care Unit of Work	Intrinsic Satisfaction mean+SD Med. (Min. Max.)	Extrinsic Satisfaction mean+SD Med. (Min.-Max.)	Total Satisfaction Score mean+SD Med. (Min.-Max.)	Total PSQI Score mean+SD Med. (Min.-Max.)
Level 2 General Surgery and Anesthesia ICU	42.14±7.65 41 (31-53)	23.86±5.52 26 (16-31)	66±12.3 67 (50-81)	**7.14±2.41** **6- (5-11)**
Level 3 General Surgery and Anesthesia ICU	**43.05±8.67** **45 (12-60)**	**26.11±5.94** **27 (8-40)**	**69.16±13.76** **72.5 (20-100)**	8.16±3.45 8 (1-16)
Levels 2 and 3 Mixed ICU	37.48±6.45 38 (22-51)	21.38±5.11 20.5 (13-32)	58.87±10.2 58.5 (37-80)	9.22±3.51 9 (3-20)
Cardiovascular Surgery ICU	40.57±8.12 41.5 (16-59)	22.47±6.35 23 (6-40)	63.03±13.53 65 (22-99)	8.61±3.92 8 (3-17)
Coronary ICU	36.88±8.61 39 (15-49)	21.09±6.02 21 (10-32)	57.97±13.48 59.5 (25-80)	9.41±3.36 9 (2-17)
Neurology ICU	39.6±6.23 42 (32-46)	22.8±3.03 21 (21-28)	62.4±8.65 63 (53-74)	8.4±3.29 10 (4-12)
Palliative ICU	37.4±7.89 39.5 (24-46)	**19.2±6.48** **19 (10-28)**	56.6±13.38 56 (34-74)	9.4±2.72 9.5 (4-13)
Pediatric ICU	38.75±9.18 38 (26-53)	19.63±7.05 20 (8-28)	58.38±15.56 55 (34-81)	9.63±3.66 8 (6-17)
Neonatal ICU	41.35±7.84 43 (16-60)	23.29±7.62 25 (8-39)	**64.63±14.61** **66 (30-99)**	**12.94±17.64** **10 (3-95,62)**
p	**0.003**	**0.007**	**0.002**	**0.503**
x^2	23.187	21.163	24.964	7.311
SD ***Kruskal-Wallis H Test***	8	8	8	8

MSQ and PSQI for All Intensive Care Nurses - Assessment of Results						
	n	$\overline{X}$	$S.S.$	Median	Minimum	Maximum
Intrinsic Satisfaction	233	39.74	8.06	41.00	12.00	60.00
Extrinsic Satisfaction	233	22.60	6.42	23.00	6.00	40.00
Total Job Satisfaction	233	**62.34**	13.49	63.00	20.00	100.00
Total PSQI	229	**8.99**	3.45	9.00	1.00	20.00

Scale	Assessment	Number (n)	Percentage %
MSQ	60 Neutral	5	2.15
	Below 60	92	39.48
	Above 60	136	**58.37**
PSQI	Good	22	9.61
	Poor	207	**90.39**

MSQ: *Minnesota Satisfaction Questionnaire*
PSQI: *Pittsburgh Sleep Quality Index*
ICU: *Intensive Care Unit*

3.3. Minnesota Satisfaction Questionnaire (MSQ)

The study revealed that MSQ, intrinsic satisfaction, extrinsic satisfaction and total satisfaction score averages of nurses were found to be different with respect to ICUs (p<0.005) (Table 2). The highest scores for intrinsic satisfaction, extrinsic satisfaction and total satisfaction subgroups of the MSQ (mean+SD: 69.16±13.76) were obtained from level 3 general surgery and anesthesia ICUs. Neonatal ICUs ranked the second among those with the highest total satisfaction score (mean+SD: 64.63±14.61) (Table 2). MSQ score average was found to be 62.34±13.49 (med: 63, min: 20, max: 100) (Table 2).

The distribution of the job satisfaction score averages of nurses with respect to their attendance in professional events was presented in Table 3. Accordingly, there was a significant relationship between nurses' attendance in the congress in the recent year and their intrinsic satisfaction, and between their professional publication following level and intrinsic, extrinsic and total job satisfactions ($p<0.05$). Nurses who followed publications regularly were found to have higher total job satisfaction score averages than those who never did (Table 3).

Table 3. *MSQ Mean Scores Based on Nurses' Professional Activities (n=234)*

Mann Whitney U Test	**Congress Attendance in the Recent Year**				
Job Satisfaction Sub-group	**Yes** Mean+SD Med. (Min. Max.)	**No** Mean+SD Med. (Min. Max.)	**u**	**z**	**p**
Intrinsic Satisfaction	**40.7±8.15** 43 (12-60)	39.2±8.01 40 (15-60)	5111.500	-1.995	**0.046**
Extrinsic Satisfaction	22.81±6.87 24.5 (6-40)	22.42±6.16 21 (8-40)	5672.000	-0.841	0.400
Total Satisfaction	63.51±13.89 67 (20-100)	61.62±13.26 62 (25-99)	5292.000	-1.622	0.105

Kruskal-Wallis H Test	**Level of Following Professional Publications**					
Job Satisfaction Sub-group	**Regularly** Mean+SD Med. (Min.-Max.) (n=15)	**Sometimes** Mean+SD Med. (Min.-Max.) (n=165)	**Never** Mean+SD Med. (Min.-Max.) (n=52)	x^2	**sd**	**p**
Intrinsic Satisfaction	**42.07±7.54** 45 (23-54)	40.64±7.48 41 (15-60)	36.12±9.07 36 (12-55)	12.229	2	**0.002**
Extrinsic Satisfaction	22.8±6.98 26 (10-34)	**23.16±6.33** 23 (8-40)	20.58±6.17 20 (6-32)	6.318	2	0.042
Total Satisfaction	**64.87±13.77** 71 (33-82)	63.79±12.79 65 (25-100)	56.69±14.28 56,5 (20-86)	10.822	2	0.004

MSQ: Minnesota Satisfaction Questionnaire

It has been seen that the satisfaction of nurses working in an ICU was found to affect both their job satisfaction ($p<0.001$) and PSQI scores ($p<0.05$) (Table 4). Accordingly, the total job satisfaction scores of nurses who were satisfied with working in an ICU were higher (63.63±13.24) whereas their total PSQI scores were found to be lower (8.84±3.51) (Table 4). The study also revealed a negative significant relationship between the duration of intensive care work and job satisfaction. Accordingly, when the duration of working increases in ICUs, the intrinsic satisfaction, extrinsic satisfaction and total satisfaction scores of nurses' decrease ($p<0.001$) (Table 4). As a result of the study, the nurses working in the ICUs on their own will had a high total job satisfaction score and a low PSQI score. No statistical significance was found between the mode of preference and total job satisfaction and PSQI scores ($p>0.05$) (Table 4).

Table 4. *MSQ and PSQI Mean Scores of Nurses Based on Intensive Care Work Related Matters (n=234)*

Mann Whitney U Test	To Be Pleasure To Work In Intensive Care Unit				
Job Satisfaction Sub-Group	**Yes** (n=195) Mean+SD Med. (Min.-Max.)	**No** (n=31) Mean+SD Med. (Min.-Max.)	**u**	**z**	**p**
Intrinsic Satisfaction	40.39±7.88 42 (12-60)	35.55±8.64 36 (15-48)	2030.500	-2.937	**0.003**
Extrinsic Satisfaction	23.23±6.28 23 (6-40)	18.94±6.19 18 (8-32)	1877.000	-3.393	**0.001**
Total Satisfaction	**63.63±13.24** 65 (20-100)	54.48±13.2 57 (25-77)	1888.000	-3.356	**0.001**
Total PSQI Score	n= 189 **8.84±3.51** 9 (1-20)	n=37 10.19±2.8 11 (6-17)			**0.029**

Mann Whitney U Test	Intensive Care Work Preference				
	On My Own Will Mean+SD Med. (Min.-Max.) (n=193)	**Administration's Request** Mean+SD Med. (Min.-Max.) (n=37)	**u**	**z**	**p**
Intrinsic Satisfaction	39.6±8.32 41 (12-60)	40.7±6.65 41 (26-60)	3457.500	-0.305	0.760
Extrinsic Satisfaction	22.65±6.43 23 (6-40)	22.19±6.38 23 (8-39)	3370.500	-0.540	0.589
Total Satisfaction	**62.24±13.79** **64 (20-100)**	62.89±11.95 60 (34-99)	3556.000	-0.039	0.969
Total PSQI Score	**8.96±3.44** **9 (1-20)**	9.24±3.51 9 (3-170)	3365.000	-0.363	0.717

Pearson Correlation		Duration of Intensive Care Work (year)				
**Significant for 0.01		How many years?	Intrinsic Satisfaction	Extrinsic Satisfaction	Total Satisfaction	Total PSQI Score
How many years?	rho	1	-.228**	-.253**	**-.256****	-0.030
	p	.	0.001	0	0	0.653
	n	228	227	227	227	223

MSQ: Minnesota Satisfaction Questionnaire
PSQI : Pittsburgh Sleep Quality Index

4. DISCUSSION

Intensive care units are the most challenging and risky occupational areas for nursing, and it is also a profession with the largest variety of working areas with other healthcare professionals. Fatigue, lack of sleep, circadian arrhythmia, administrative mistakes, job dissatisfaction, insufficient team work and burden of care for the patients at high risk affect the performance of intensive care nurses. Professional underperformance prevents providing desired level of healthcare services in the ICUs preferred individually. According to Baj (19) there is a complete relationship between intensive care quality and a healthy working environment. The low performance of healthcare professionals causes crucial risks for the safety of patients and employees due to all kinds of occupational accidents and malpractices.

While there are many studies addressing sleep quality of nurses in the literature, only a limited number of them focuses on intensive care nurses. In the studies examining sleep quality Karagözoğlu and Bingöl (20) found the PSQI score average to be 7.28±3.56, Yang et al. (21) found 7.31±3.45, Karataş et al. (7) as 8.64±4.19 and Çetinol and Özvurmaz (17) found 2.69±6.8 and they all identified sleep quality as 'poor'. Shcao et al. (22) showed that sleep quality of 57% of the nurses in Taiwan was poor. Günaydın (23) has revealed that the majority of nurses have poor sleep quality. According to Zverev and Misiri (24) working night shifts affects sleep quality negatively and causes fatigue the following day. It has been noted by Şentürk (10) that, sleep quality of ICU nurses is 'poor' and also associated with the level of their burn-out. Another study suggests that working under intensive care conditions affects sleep quality of nurses and causes sleep disorders (25). In this study, the highest PSQI score was found in neonatal ICUs and the lowest was found in the level 2 general surgery and anesthesia ICUs. On the other hand,

sleep quality of 90.39% of the ICU nurses was found to be 'poor' due to the fact that all the scores were 5 and above. The result of the study was in consistency with the results of the other researches. In another study, it was found that the sleep quality of nurses was good since the majority of the nurses were performing as ward nurses (6). This conclusion proves that the burden of intensive care nurses is far more than that of ward nurses.

In this study, no difference was found among PSQI score averages with respect to the ICUs and sleep quality was found to be poor in all of them. This has been showed that, the working conditions are similar in intensive care units. Sleeping for 5.41 hours on average after their shifts, working more than 40 hours a week, frequent and variable shifts, night shift 10-13 times in per month, affect the sleep quality of nurses. Since sleep disorder of healthcare professionals threatens the safety of the employee and the patient, it is an important cause of malpractice. Aldem et al. (26) have stated that employee safety violations are caused by fatigue, working overload and inadequate number of personnel at the rates of 91.3%, 79.8% and 70.2%, respectively.

Job satisfaction, one of the most important requirements for successful, happy and productive individuals, can be defined as an individual's general attitude towards his/her job as well as the harmony between what he/she expects of and gets from the institution (27). Intensive care nurses have a key role in providing patients a reliable treatment and care. Thus, nurses need to be pleased and satisfied with their work, happy and productive to provide effective healthcare services (12). Studies have revealed that job satisfaction of nurses compared to that of other health professionals was found to be either medium or less than medium (13,27,28). Some studies have shown that the job satisfaction of nurses was low compared to that of other healthcare professionals (29-31). On the other hand, studies only focusing on intensive care nurses indicated that their job satisfaction level was 'medium' (12,32,33) or 'low' was detected (34-38). Since MSQ scores between 26-74 indicated a normal/medium-level job satisfaction, (18) job satisfaction of nurses in this study was found to be 'medium-level' (62.34±13.49; med:63).

Job satisfaction is affected by personal and organizational factors. Personal factors include age, gender, marital status, education level, personal features and socio-cultural environment whereas organizational factors denote working environment and conditions, wage, administration and opportunities of development and promotion (27). According to a study (39) conducted in China, it has been seen that job satisfaction of intensive care nurses depends on administration. Dilig-Ruiz A et al. (40) could not find any evidence supporting the relationship between the personal (socio-demographical) factors and job satisfaction of intensive care nurses. However, they found evidence supporting the relationship between several employment and organizational factors and job satisfaction. In the same study, four factors showed significant positive relationships: shift work/rotating for 8 to 12 hours and doing call-on duty in the daytime, evening or night; autonomy; personnel resources and staffing and; teamwork and cohesion. On the other hand, two factors showed significant negative relationships: job stress and burnout/emotional exhaustion. In our study, personal factors such as socio-demographical characteristics and preference of working in the ICUs were not found to affect job satisfaction. However, employment and organizational factors such as type of the ICU, satisfaction with the ICU, duration (year) of intensive care work, being able to attend the congress and to follow publications were found to be influential in job satisfaction. Nurses also verbally expressed that duration of on-call duties needed to be improved. It has been noted that the job satisfaction of nurses decreases due to performing on-call duties for 9.85 times a week, taking care of 3.35 patients per nurse and having weekly working hours over 40 hours. The study also revealed that when the working duration in ICUs increased, job satisfaction of nurses decreased. Challenging working conditions were considered to be the reason. Job satisfaction of intensive care nurses was found to be affected by their level of education, years of work, mode of work (12) as well as the unit of work (35). Our study is different in terms of examining job satisfaction with respect to the ICUs. The highest level of job satisfaction was observed in level 3 general surgery and anesthesia intensive care nurses and neonatal intensive care nurses. It has been noted that caring a patient who was fully dependent on the nurse had a positive impact on job satisfaction. This created a unique working and nursing area where the nurse was offered their own autonomy.

5. CONCLUSION

As a conclusion, nurses were found to be pleased to work in intensive care units. On the other hand, it has been found that sleep quality of the nurses was 'poor' and working in different ICUs did not make any difference in terms of the effect on sleep quality. It has been concluded that the type of the ICU and the duration of working in the unit affected job satisfaction, the job satisfaction was at 'medium level' and participating in professional events positively affected job satisfaction.

It was observed that nurses worked more than forty hours a week, had minimum ten-night shifts per month and after the shift they can sleep for five hours. In this case, it is understood that the main cause of sleep quality disorder is working conditions. In the study, job satisfaction levels of nurses were found to be different according to the type of intensive care unit, and the highest job satisfaction was determined in the level 3 general surgery intensive care units. Also it was seen that job satisfaction decreased as the duration of intensive care unit work increased.

It should be noted that safety of patients depends on the safety of nurses in intensive care units where nonstop treatment and care services are provided. Therefore, working conditions, duration of on-call duties and other organizational matters should be reevaluated and improved,

employee feedbacks should be considered for sleep quality and job satisfaction of nurses. It was determined that new action plans are needed to improve the quality of health services and employee satisfaction. The primary objective in terms of ensuring patient safety should be forming healthier and safer working conditions for intensive care nurses. In addition, in order to increase job satisfaction, professional development of intensive care nurses should be supported and rewarding system should be prevalent.

Limitations of this study

The participants were from only at five hospitals which are located in the European Side of Istanbul, Turkey. Data were collected only from the intensive care nurses of these hospitals. Therefore, the findings from the study are limited to these hospitals.

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Clinical and Experimental Health Sciences

The Effect of Group Case Studies on NANDA-I Nursing Diagnosis Identification and Perception of Nursing Students

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ABSTRACT

Objective: The study aimed to identify the effect of group case studies on the accuracy of nursing diagnoses made by student nurses and their perception of NANDA International nursing diagnoses.

Methods: The study was conducted as an interventional and descriptive research with a sample that comprised 164 nursing students studying at the Department of Nursing of a university. Study data were collected using Descriptive Characteristics Form, Perception of Nursing Diagnoses Scale, Students' Opinion about Nursing Diagnoses Form, and Care Plan Assessment Form. A researcher offered four hours of theoretical training about the nursing process and NANDA International nursing diagnoses, after which the students filled out the Descriptive Characteristics Form and the Perception of Nursing Diagnoses Scale. Thereafter, the students were randomly divided into five groups, and each group performed two case studies with a researcher every week. After the completion of the case studies, the students were asked to fill out the forms again. The data were analyzed using the number, percentage, mean, standard deviation values, and Wilcoxon test.

Results: The results emerging from the case studies indicate that the majority (89.6%) of the students were able to make accurate diagnoses in compliance with the criteria set in NANDA International. The total score of the students in the perception of nursing diagnoses was not statistically significant ($p > 0.05$). Statistically significant differences were found, on the other hand, in the negative, and positive way in the subscales of 'clear representation of the patient situation' and that of 'the ease of use', respectively ($p < 0.05$).

Conclusion: Almost all the students were able to define the accurate diagnoses complying with the criteria set in NANDA International, and while this did not affect the students' perception of nursing diagnoses, the subscale of 'the ease of use' had a positive effect in this respect.

Keywords: Group study, nursing diagnosis, nursing students, group study.

1. INTRODUCTION

The nursing process which brings a professional identity to the nursing profession contributes to distinguishing nursing care and measuring the quality of care (1,2). Nursing diagnoses that are considered to be the most important component of nursing care process are based on a synthesis of all data collected from patients covering the patient's problems, risk conditions, preservation, and improvement of health (3-5). Besides, nursing diagnoses define conditions that might be treated by nurses, help to identify the scope of nursing practice and create, in return, a consistent and universally readable terminology among nurses (6).

Nursing diagnoses are an essential part of the nursing process which contributes to developing nursing knowledge and practice (7-9). Negative or positive perceptions of nursing diagnoses by nurses do influence the use and implementation of diagnoses (8). While a positive perception of nursing diagnoses improves the quality of patient care (6,10), a negative perception would affect the use of the common diagnostic terminology and the methods universally accepted in planning patient care (5,7,8). Today, instructors in undergraduate nursing education focus on implementing various training methods to improve students' active learning and critical thinking skills concerning the nursing process and nursing diagnoses (11-13). In case-based education, which is one of these methods, students are allowed to efficiently and actively learn by combining critical thinking skills, nursing knowledge, and practice based on scenario/patient-based cases they plot themselves (13,14). Although case-based teaching is actively used in education, it alone does not suffice to provide target-oriented information and fails to yield desired and expected results concerning efficiency (15). In addition to these learning methods, in group learning, which is a student-centered

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teaching method frequently preferred in higher education, group members are allowed to actively participate in the learning-teaching process by cooperating among themselves rather than memorizing and studying the information, and they structure knowledge by themselves by creating an association with their own experiences and knowledge (16). In this process, students create group interactions and communication among the individuals in the group, thereby creating an environment where they can develop information and exchange views (16,17). Therefore, students easily express their opinions, recognize different perspectives, and create common wisdom with other students (18). In this respect, group learning creates an efficient learning environment, thus contributing to students' active participation in the classroom setting and increasing their success.

In nursing education, commitment, understanding, and competency perception should be vested in nursing students, particularly to identify nursing diagnoses. Educational programs designed to teach nursing diagnoses should accordingly include efficient teaching methods that can create and reinforce a positive perception of diagnoses. Developing such a perception during their studies will enable students to acquire and adopt the habit of using nursing diagnoses after graduation. Previous research reports that nursing students could identify the proper nursing diagnoses from NANDA-International (I) taxonomy list, but that their level of skills to correctly name the diagnoses according to the relevant terminology was not satisfactory (19). Past research also reported that nursing students had a correct perception and held positive attitudes of the rules set in NANDA-I (20). Reviewing the current literature, we see that several studies are available that addressed the care plans designed by nursing students in our country (1,19,21-25). Besides, while there has been only one study that aimed to identify the nursing diagnoses and interventions of student nurses in case scenarios developed for probable patients with traumas using 'Scenario-based Case Definition Form' (15), no study is available that has investigated the effects of group case studies on the students' perception of nursing diagnoses. The results of this study are believed to offer statistical data concerning the knowledge level and perception of students who recently started and continue with the nursing undergraduate education as well as how the group case study (GCS) affects this perception and contribute to developing training programs. A clear understanding of the knowledge level and perception of students pursuing a graduate degree in nursing concerning nursing diagnoses and determining the effect of GCSs on such perception is of great importance concerning a realistic and purposeful designing of curricula intended for nursing education and training. In this context, the present study aimed at identifying the effect of GCSs on the accuracy of nursing diagnoses made by student nurses and their perception of nursing diagnoses.

2. METHODS

The study was conducted between March and May 2016 as an interventional and descriptive research.

2.1 Participants

The population of the study consisted of 180 students who enrolled in the course 'Fundamentals of Nursing-II' in the 2015-2016 academic year at the Department of Nursing, Faculty of Health Sciences, University, Turkey. The sample of the study comprised 164 of the students in the sample population who agreed to participate in the study and were included in the sample criteria. We reached 91% of the students in the study.

The criteria for being included in the study are enrolling in the course 'Fundamentals of Nursing-II' for the first time and voluntary taking part in the study. The criteria for exclusion from the study are having been trained in nursing before, not voluntary taking part in the study, and not attending group studies.

2.2 Data Collection and Instruments

Data was collected with the Descriptive Characteristics Form, Students' Opinion About Nursing Diagnoses Form, Care Plan Assessment Form, and Perception of Nursing Diagnoses Scale prepared by the researchers based on the literature (5,8,26,27).

2.2.1 Descriptive Characteristics Form (DCF)

In the form, there are four questions in total: 'students' gender, graduated school, the reason for preferring the profession, and if they heard of NANDA-I nursing diagnoses before'.

2.2.2 Students' Opinion About Nursing Diagnoses Form (SOANDF)

In the form, there are six questions in total, namely, the students' ability to identify the diagnosis and to make an accurate diagnosis, the necessity of identifying nursing diagnosis, whether making a diagnosis is useful, the hardest part while preparing the nursing care plan, and their opinions about the care plan (7,15,28).

2.2.3 Care Plan Assessment Form (CPAF)

In the form, there are eight sections which are comprised of yes/no questions to evaluate the students' competency in collecting data, recognizing the accurate diagnosis, prioritizing diagnoses, identifying symptoms and etiology, planning, identifying nursing interventions and evaluating care results. These sections were evaluated by the researcher in charge of every group.

2.2.4 Perception of Nursing Diagnoses Scale (PNDS)

In the form, there are 26 items which were filled out by the students. The scale was developed by Olsen, Frost, and Orth (29) and Turkish validity and reliability was verified by Akin-Korhan et al. (9). The Cronbach's alpha value of the scale is 0.82. The scale is a five-point Likert type and is made up of 26 items. The scale has subscales of definition and introduction

of the nursing profession (13 items), clearly defining the patient's condition (5 items), ease of use (4 items), and conceptual direction (4 items). The total score varies between one and five. Lower total scores in the scale point to a positive perception of nursing diagnoses. In this study, the Cronbach's alpha value was 0.74.

2.3 Intervention and Data Collection

A researcher offered four hours of theoretical training about the nursing process and NANDA-I nursing diagnoses (NANDA International Nursing Diagnoses Definitions and Classifications 2015-2017) in the Fundamentals of Nursing-II course. The researcher presented the theoretical training and first case study by use of PowerPoint, explanation, and questions and she discussed the case with questions and answers. After the first case study, the students who volunteered filled out the DCF, SOANDF, and PNDS. Then the students in five different groups in total were divided into groups randomly by drawing lots by the researcher with 33 students in four groups and 32 students in one group. Each week, every group was given a sample of two cases created as realistic as possible by the researchers. Expert opinion was taken for each case before they were handled to the students and were put in order by researchers according to expert opinion. The sample cases included elements of nursing history, patient explanations, physical examination, and medical treatment. The same cases were given to every group. A week duration was respited to the students for preparation. The cases were prepared according to Gordon's Functional Health Patterns Model and NANDA-I. The researchers worked simultaneously with their groups every week, in a total of five weeks and 10 case studies. The students presented their care plans in PowerPoint to their peers in the group and the researcher. The case was discussed with questions and answers at the end of the presentation. After the last case study, the students filled out the SOANDF and PNDS again. After completion of the case studies, the students obtained data from patients individually and created a care plan in duration clinical practice. Care plans were evaluated in charge of the group and were recorded in the CPAF by the researchers (Fig 1).

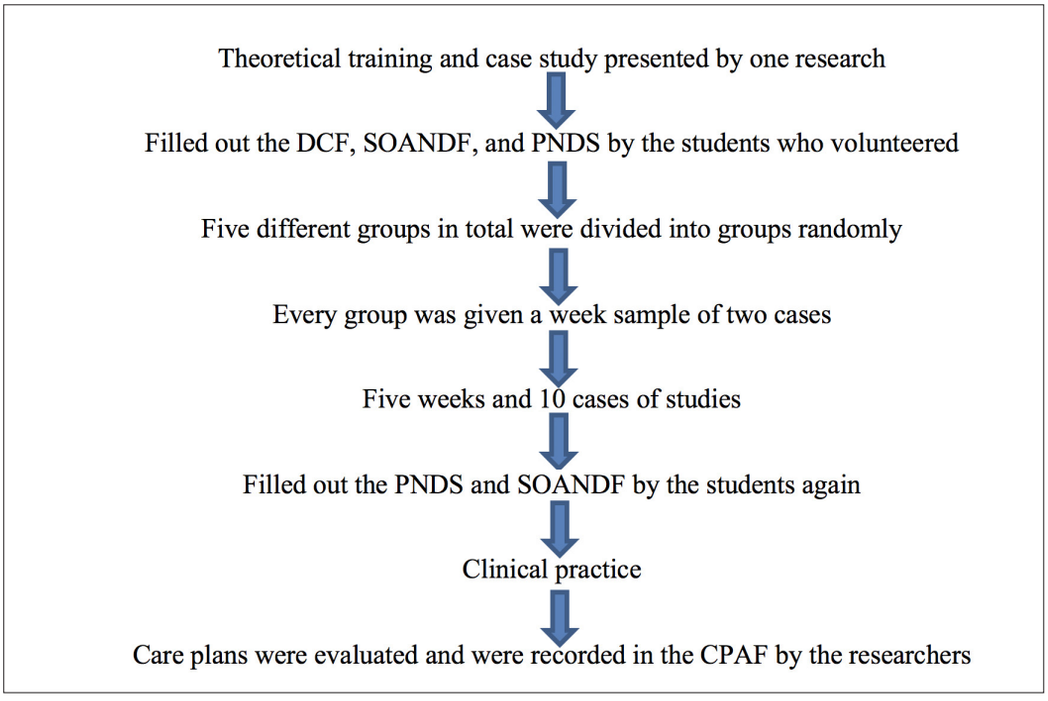

Figure 1. *Flow Chart of Research*

2.4 Ethical Consideration

Written permission was obtained from Karadeniz Technical University, Faculty of Health Sciences (decision no.:63582098/200, dated 11.03.2016) to carry out the study. The students who were included in the study were informed about the study, and their verbal permissions were obtained. Consent was obtained from Korhan et al. who verified Turkish validity and reliability of the scale, the organization where the study took place, and the students who took part in the study.

2.5 Data Analysis

The data obtained from the study were analyzed using SPSS (Statistical Package for Social Sciences) for Windows version 22.0. Number, percentage, mean, and standard deviation values were used as descriptive statistical methods in the evaluation of the data. The Wilcoxon test was used for the matched groups to determine the differences between the first and last scores. The findings were evaluated in 95% confidence interval and on $p < 0.05$ significance level.

3. RESULTS

The mean (standard deviation [SD]) age of the students participating in the study was 18.83±0.96years, and 78% of the students were female, mean±SD academic degree is 3.09±1.32 (over 4), 77.4% are graduates of Science, and Anatolian High School. 61.2% of the students preferred nursing for easy employment, 82.3% reported they never heard of nursing diagnosis.

Students' opinions about NANDA-I nursing diagnoses and care plans of the students before and after GCS. It was found that 70.9% of the students before GCS could determine their NANDA-I diagnoses and 92.7% could diagnose according to the data, 91.5% could determine the diagnosis after the GCS and 90.9% could make the diagnosis according to the data. Make a diagnosis of nursing was stated that 90.6% of students were necessary before GCS and 96.3% stated that the care plan was beneficial. After GCS, 87.8% of the students stated that it is necessary to make a diagnosis of nursing and 89% of them stated that the nursing care plan is beneficial. Before GCS, it has been identified that 35.9% of the students had difficulty in finding a diagnosis name and 29.9% of them had difficulty writing PES (Problem, Etiology, Symptom), and after GCS, only 2.5% of the students had a diagnosis name, and 47.9% had difficulty writing the PES section of the care plan (Table 1).

Table 1. *Students' Opinions about NANDA-I Diagnoses and Care Plan (n=164)*

Students' opinions	Before GCS		After GCS	
	n	%	n	%
Determined the nursing diagnosis				
Yes	117	70.9	150	91.5
No	47	28.7	14	8.5
Made an accurate diagnosis based on the data				
Yes	152	92.7	158	96.3
No	12	7.3	6	3.7
Is diagnosis necessary?				
Yes	149	90.9	144	87.8
No	15	9.1	20	12.2
Is the nursing care plan useful?				
Yes	158	96.3	146	89.0
No	6	3.7	18	11.0
Problems in identifying nursing diagnoses				
Categorization	31	18.9	29	17.8
Name of diagnosis	26	35.9	4	2.5
PES	49	29.9	78	47.9
Order of priority	30	18.3	26	16.0
Not challenged	8	4.9	21	12.9
The entire stage is challenging	20	12.2	5	3.1
Opinions about the care plan				
Offers accurate, planned care	148	90.2	147	89.6
Offers well being	109	66.5	99	60.4
Waste of time	5	3.0	10	6.1
Not necessary for the nurse	10	6.1	5	3.0
Offers a holistic approach	94	57.3	122	74.4
Offers patient specific, quality and qualified care	115	70.1	121	73.8
Not useful in practice	8	4.9	3	1.8

GCS: group case study, PES: problem, etiology, symptom

PNDS total scale score was 2.39±0.39 before GCS, it was 2.34±0.35 after GCS, but there was no statistically significant difference between the scores. However, there was a statistically significant difference between the scores before and after GCS in the subscale of F2 and F3. It was determined that F1 (definition and introduction of the nursing profession) sub-dimension score of the scale was 2.02 ± 0.53 before GCS, then 1.93 ± 0.57 and there was no statistically significant difference between the scores (p=0.097). F2 (clearly defining the patient's status) sub-dimension score of the scale was 2.85 ± 0.53 before GCS, then 2.99 ± 0.53 and there was a statistically significant difference between the scores (p=0.011). F3 (ease of use) subscale score of the scale was 2.63 ± 0.54 before GCS, then 2.41 ± 0.54 and there was a statistically significant difference between the scores (p=0.000). No statistically significant difference was found in the F1 (definition of the nursing profession) and F4 (conceptual aspect) subscales of the scale (Table 2).

Table 2. *Comparisons of the Scores in the Students' Perception of Nursing Diagnoses Scale Before and After GCS (n=164)*

Scale subscale	Before GCS	After GCS	p*
	Mean ± SD	Mean ± SD	
F1**	2.02 ± 0.53	1.93 ± 0.57	0.097
F2***	2.85 ± 0.53	2.99 ± 0.53	**0.011**
F3****	2.63 ± 0.54	2.41 ± 0.54	**0.000**
F4*****	2.77 ± 0.54	2.79 ± 0.53	0.531
Total Score	2.39 ± 0.39	2.34 ± 0.35	0.281

*GCS: group case study, SD: standard deviation, *Wilcoxon test was made, **F1: Delineation and promotion of the nursing profession, ***F2: Clear representation of the patient situation, ****F3: Ease of use, *****F4: Conceptual orientation*

The results of the evaluation of the care plans prepared by the students after clinical practice are given in Table 3. When the care plans prepared by the students are evaluated by the researchers, 80.5% of the students are sufficient in collecting data, 89.6% of them can determine the correct nursing diagnosis, 50% of them are diagnosed following the order of priority, 79.9% of them are diagnosing the symptoms correctly. It has been determined that 77.4% can determine the etiology related to diagnosis, 73.8% can make nursing care planning correctly, 64.0% can correctly determine nursing interventions and 62.2% are sufficient to evaluate the care applied (Table 3).

The students identified 9 different nursing diagnoses in the care plan. The most frequently identified three nursing diagnoses were 'Risk for falls (00155)', 'Risk for infection (00004)' and 'Acute pain (00132)'. The students' planned interventions were suitable for the diagnosis of 'Risk for falls (62.2%)', 'Risk for infection (56.1%)', and 'Acute Pain (42.7%)' (Table 4).

Table 3. *Evaluation of the Results of Care Plans Prepared by the Students After Clinical Practice (n=164)*

Care Plan Assessment Criteria	n	%
Sufficient in data collection		
Yes	132	80.5
No	32	19.5
Identified the accurate diagnosis		
Yes	147	89.6
No	17	10.4
Determined the order of priority of diagnoses		
Yes	82	50.0
No	82	50.0
Accurately identified the symptom		
Yes	131	79.9
No	33	20.1
Accurately identified the etiology		
Yes	127	77.4
No	37	22.6
Made accurate planning		
Yes	121	73.8
No	43	26.2
Accurately identified nursing interventions		
Yes	105	64.0
No	59	36.0
Sufficient in evaluation		
Yes	102	62.2
No	62	37.8

Table 4. *The Most Frequently Identified Nursing Diagnoses in the Care Plan and Number of Students Who Planned Suitable Interventions for the Diagnosis (n=164)*

NANDA-I diagnoses	Identified nursing diagnosis	Nursing interventions planned for the diagnosis
	n (%)	n (%)
Risk for falls	123 (75.0)	102 (62.2)
Risk for infection	119 (72.6)	92 (56.1)
Acute pain	93 (56.7)	70 (42.7)
Risk of bleeding	76 (46.3)	51 (31.1)
Disturbed sleep pattern	73 (44.5)	59 (36.0)
Self-care deficit	63 (38.4)	49 (29.9)
Activity intolerance	51 (31.1)	34 (20.7)
Impaired skin integrity	49 (29.9)	39 (23.8)
Anxiety	41 (25.0)	34 (20.7)

4. DISCUSSION

The teaching of the nursing process starts with the principles of nursing in the first year and continues after graduation. As the students need to implement the nursing process in their entire education and professional life, the benefits of the process are discussed in the classes and its necessity is emphasized. In this study, before and after GCS, almost all of the students found nursing diagnoses necessary and preparing the care plan useful. In parallel to the results of our study, in the studies that were found that most of the student nurses found the nursing process necessary and almost half of them explained all steps in the process on the desired level (30,31). Although its significance and usefulness are acknowledged, students and nurses can also be seen as having troubles in the stages of the nursing process (27,30,31). In our study, almost all of the students were able to identify the diagnosis suitable for NANDA-I Taxonomy II, and the data after GCS. In parallel to the results of our study, Uysal et al. (2016) concluded in their study that 80.2% of the students accurately identified diagnoses according to NANDA-I taxonomy, and success was achieved using the nursing process scenario offered by using the problem-based learning method (32). In other studies in the field in the literature, it was determined that most of the students had troubles with identifying NANDA-I nursing diagnoses (30), they had the most problems with nursing diagnosis and data collection (31,33), the nurses made wrong diagnoses outside NANDA-I Taxonomy (2) and 'sometimes' had challenges in the diagnosis identification stage (27).

In our study, the number of students who thought nursing diagnoses were necessarily reduced after GCS. It might be an outcome of the students noticing they were challenged in some stages while creating the nursing process. The students reported having the most problems while identifying the nursing diagnosis suitable for NANDA-I and identifying PES format. Similar to our study result, Tambağ and Can (7) determined that the PES format of 71.5% of the students was not identified. On the other hand, Müller et al. (3) reported in their systematic review on the evaluation of nursing diagnoses that the nurses were the most inadequate in identifying symptom/finding and etiology. However, when the students' care plans were reviewed by the researchers in our study, they concluded most of the students were able to collect data and identify for nursing diagnosis. According to this result, it can be concluded that the case discussions held in groups proved to be effective. Case studies were the least effective while the students were prioritizing nursing diagnoses. The reason is suggested to be the lack of information about the disease as they were freshmen.

It is of essential importance that students provide individual care to patients they care for and make proper use of the nursing process so that the scientific identity of the nursing profession can be maintained, but it is of equal importance that they are aware of the benefits and necessity of nursing process in care. The study findings show, based on the results regards the benefits of care provided based on a nursing care plan, that almost all the students expressed that a care plan led to proper and systematic care, and more than half of them held the opinion that such plans made a holistic, patient-specific and quality care possible and thus contributed to the general well-being of patients. In the study performed by Seval and Çiftçi (34), when asked about the benefits of the nursing process, the students expressed that the use of nursing diagnoses was easy and informative and that it was supportive of professional autonomy, facilitating them to focus on nursing-specific fields and thus providing an improved professional image of nursing. Past

research also reports that classification systems used in the field of professional nursing provide reliable, systematic, and efficient interventions concerning knowledge organization, nursing care planning, and satisfaction of patients' needs (20,35,36). Research also suggests that a patient-centered approach is necessary for inpatient care (37). These findings of previous studies are inconsistent with those we observed in the present study.

One of the purposes of nursing education is to create a positive perception of students towards nursing diagnoses. Considering the definition of perception, it means paying attention to something, being aware of, understanding, and comprehending plain information obtained through the senses. In our study, although the perception of nursing diagnoses by the students before and after GCS was in the positive direction, the difference between them was not statistically significant. The fact that there was no difference in the scale score between and after GCS in our study might an outcome of the fact that the student had knowledge about the diagnosis but did not have clinical experience. Because students need to have clinical experience starting from meeting the patient or non-patient individual/family/group to understand and comprehend diagnoses (38). Therefore, after meeting the patient, obtaining and analyzing patient data, in other words, learning by experience, students can create a perception about diagnoses. Although the students in the study did not have clinical experience, their perception scores for nursing diagnoses increased after GCS. It can be explained by the outcome that the method had a positive impact on perception. In the study conducted by Rahman et al. (20) evaluating the university students' attitudes towards and perception of NANDA-I nursing diagnoses and the study conducted by Karaca and Aslan (26), the students who took training on nursing classification and diagnosis had a positive perception of nursing diagnoses compared to those who did not take the class. As in these studies, students having a positive perception about nursing diagnoses can facilitate identifying the patient's problems, positively affect the planning of patient care, and improve the quality of care (10). Although students have a positive perception of diagnoses in our study and others in the literature (20,26), the negative perception of the nurses towards nursing diagnoses in the study conducted on nurses by Olsen (29), Halverson (8) and Akin-Korhan et al. (9) is noteworthy. The difference in perception among the nurses might be a result of educational differences (39). Moreover, it demonstrates the students who had a positive perception of nursing diagnoses but changed to have negative perceptions after having started to work in the clinic and it is also important after graduation. Therefore, training professional nurses who have a high positive perception about nursing diagnosis and have adopted specificity as a guideline in care is important for care standardization.

Considering the subscales of scale in our study, the perception in the subscale of 'clear representation of patient situation' significantly diminishes after GCS. As students do not have clinical experience, they cannot understand the patient's findings and notice problems enough before the case and they can think that they can define the patient's condition. On the other hand, as the students discussed cases and noticed it was not easy to understand data from the patient to use the nursing diagnoses while identifying the patient's problems, their perception in this subscale might have diminished. Similar to the findings of the study, Halverson demonstrated as the reason for negative perception in this subscale that nurses were not able to comprehend the real definition and use of nursing diagnosis (8). In our study, the perception of the students in the 'ease of use' subscale significantly increases after GCS. It helped the students analyze the case and identify the accurate nursing diagnosis. Moreover, it shows GCS helped the students use the diagnoses. Similar to the results of this study, Ogunfowokan et al. (28) reported diagnoses were useful in practice and Karaca and Aslan (26) reported the perception was positive in 'the ease of use' based on the results emerging from the evaluations of the care plans schemed by the students after practical work subscale (26,28).

The study showed, in a clinic, that while the majority of the students were efficient in collecting data, could establish accurate diagnoses based on correct symptoms and etiology and classify the diagnoses by order of priority, more than 50% were able to define the nursing interventions based on a self-schemed plan and successfully perform what was necessary at the step of evaluation. These results show that nursing students were more successful at the nursing process steps of assessment and nursing diagnosis than the steps of planning, implementation, and evaluation. This success may be attributed to the fact that the steps of diagnosing and nursing diagnoses are based rather on theoretical knowledge, giving the students the possibility to draw on several written reference sources in a more efficient way in scheming their care plans. The study also demonstrated that the students were less successful in the steps of planning and implementing nursing care. Due to several reasons such as lack of theoretical knowledge and clinical experience as well as fear of making errors, these steps cannot be sufficiently understood by the students and are accepted, as a result, as difficult practices (40). The study suggests that students need more clinical experience in the steps of planning, implementation, and evaluation to define, implement and evaluate the interventions specific to an individual in a given case because these steps of the process require the students to properly evaluate the individual's problems and choose the right intervention to this end. Previous research also reports that the majority of students expressed having difficulty in these steps (1,2,41). The results of the present study are in agreement with those observed in past research. Besides, the findings of the study reveal that the students had higher success when compared with previous studies, which may be attributed to the positive effects of GCS on the learning process. Previous research reports that group learning, which is a student-centered learning method, is one that facilitates the cooperation (16), and knowledge and opinion sharing among students, thus creating an

environment marked with active learning (16,17). In this context, group learning may have contributed to the active participation of students in classroom activities by creating an effective learning environment, which, in turn, may have increased their success.

The study showed that the students most frequently used the following 9 nursing diagnosis in their care plans: 'risk for falls', 'rick for infection', 'acute pain', 'risk for bleeding', 'disturbed sleep pattern', 'self-care deficit', 'activity intolerance', 'impaired skin integrity' and 'anxiety'. These results regarding the diagnoses most frequently used by students are inconsistent with past research. In agreement with our findings, previous studies report the following diagnoses as the ones most frequently mentioned by students: 'Risk for infection' (1,19,22,25,32,42-44), 'Acute pain' (19,22,25,32, 2-45), 'Bleeding risk' (32), 'Disturbed sleep pattern' (1,22,25,42,43) 'Self-care deficit' (1,22,32,42,44), 'Activity intolerance' (19,22,25,32,43), 'Impaired skin integrity' (22,45) and 'Anxiety' (19,22,32,42-44). Other diagnoses reported in the literature are 'Imbalanced nutrition: less than body requirements' (19) and 'Constipation' (19,22,25,43,45), 'Risk for impaired skin integrity' (1,25), 'Impaired physical mobility' (1,43), 'Risk for physical trauma' (32,43), 'Ineffective airway clearance', 'Ineffective respiratory pattern' (44), 'Hyperthermia' (45), 'Deficient knowledge' (32,45). The results of our study reveal, in consistence with previous research, that the diagnoses defined by the students were the ones of concrete structure that rather concern physiological dimension, with the 'Risk for falls' being the diagnosis most frequently defined by the majority of the participating nursing students in this study. It is remarkable that in the abovementioned studies the 'Risk for falls' was the diagnosis that was not at all defined or in some less frequently defined (22,32) by the participating students. That the 'Risk for falls' ranked first as the most frequently defined diagnosis in our study unlike past research may be attributed either to the specific case handled in our study or to the effect of the activities/projects integrated into the relevant study programs.

Another result emerging from our study is that relatively fewer students could plan the proper interventions following the nursing diagnoses they had defined in their care plans. The fact that fewer students could plan proper interventions for each diagnosis concerning the diagnoses defined by them in the planning phase may be attributed to the ineffective use of time in clinical work by some students and lack of knowledge typical for first-year students. Similar to the results of our study, Sendir et al. (46) report that the students had difficulty in the step of defining nursing interventions in the planning stage. The study suggests that lack of motivation in students, their inability to collect data, the influence of the role models available to them, or insufficient inclusion of nursing interventions into schemes of clinical practical work were the factors that may have influenced their failure to define proper nursing interventions.

Limitations and Strengths of the Study

The study covers freshmen enrolled in the Faculty of Health Sciences, Department of Nursing. Group and case studies were conducted as the teaching method. Possibility to participate in group discussions within the scope of the study course of the nursing process based on the case studies constitutes the strength of the present study.

5. CONCLUSION

In the study, it was found out that the students cared about the nursing process practice and group studies and sample case discussions made a difference in the perception of the nursing process. Although the students reported they were the most challenged while making a nursing diagnosis and creating PES, in the care plans they prepared after the group study, most of them were able to identify the diagnosis and prepare PES. It was also determined that the students were able to define NANDA-I nursing diagnoses according to the case but they had shortcomings in determining the order of priority. As can be seen from the results of our study, long term studies with the students in groups and based on sample cases improved the students' perception of the nursing process and diminished the challenges they faced while preparing the care plan.

The student uses the nursing process, which is taught for the first time in the fundamentals of nursing class, throughout his/her educational and professional life. Therefore, it is important that using various techniques such as group and case studies to make nursing process education effective and permanent. It is recommended to study in groups and with sample cases in the nursing process education to develop the students' ability to use the nursing process to the desired extent and positively increase the level of perception of nursing diagnoses. It can be suggested to make group case studies with more sample cases in the educational environment and to frequently repeat them after graduation. Students to move together, have a team spirit, and gain experience. It can be suggested to implement group case studies in every class to the extent possible in nursing education to allow students to acquire skills for working in teams. Organizing competitions where students prepare their cases and discuss it in the group can allow them to refresh their knowledge about the process and their perception of diagnosis in a positive way.

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Clinical and Experimental Health Sciences

Opinions and Experiences of Patients Receiving Oral Chemotherapy: A Qualitative Study

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ABSTRACT

Objective: This study is a qualitative study aimed to determine the opinions and experiences of patients receiving oral chemotherapy.

Methods: The study was conducted in 2018 with 18 volunteers who admitted to the outpatient oncology clinic of a university hospital and received oral chemotherapy. In data collection, a description form and a semi-structured interview form were used. In data analysis process, transcription after each interview and evaluation steps through thematic content analysis method were employed. COREQ-Consolidated Criteria for Reporting Qualitative research was used in the structuring and reporting phase of the qualitative study.

Results: The themes obtained in the study were access to drugs, use of medicine, side effects and control of treatment, approach of health professionals and support of family and relatives. There is a need to ensure the continuity of the treatment follow-up period in order to improve treatment compliance of patients, to monitor side effects of treatment and to ensure safe use of medicines.

Conclusion: In this process, it is crucial that nurses take an active role in patient education and counseling among health professionals so that weaknesses in the patient's drug use process and opportunities for improvement could be identified.

Keywords: Oral chemotherapy agents, patient, qualitative study, experience

1. INTRODUCTION

Oral chemotherapy drugs provide patients and caregivers with a great number of benefits socially and economically. The advantages include the feeling of control over treatment and ease of application for patients and caregivers, the ability to maintain daily life activities, the reduction in costs associated with transportation and treatment services, and the reduction in burden due to treatment in terms of health services (1-4). While oral chemotherapy drugs sometimes become complicated for patients considering their narrow therapeutic indices, they can also cause patients and caregivers to get confused due to the intermittent nature of treatment regimens. In addition, there are different factors that affect the compliance of a patient with oral chemotherapy drugs. Amongst these factors can be listed as the accessibility to drugs, costs - a barrier for all age groups, complexity in dosing, obliviousness, side effects and misinterpretation of instructions (4-8).

In the application of oral chemotherapy, the responsibility of the patient in self-management has increased as some traditional responsibilities of healthcare professionals were transferred to patients (9). Moreover, continuous increase in the application of especially oral chemotherapy and self-application instructions on medicines carried on the agenda the compliance of the patient with treatment. This challenge helps patients to be in compliance with treatment (10). Ruddy et al. (11) emphasize that the indication of patients' optimal compliance with oral chemotherapy could be as 'no dose skipping, no extra doses, no wrong doses or no doses taken at the wrong time'. Özkaraman et al. (1) determined that those who were motivated by patients in the rheumatology and oncology group, who were confident and believed that the treatment would be beneficial, had a high level of compliance.

Recent studies have shown that patients and healthcare workers considered oral chemotherapy as safer than intravenous chemotherapy (12,13). Several people believe that oral chemotherapy drugs are less toxic than IV chemotherapy drugs. Chemotherapy drugs, whether oral or IV, typically have a narrow therapeutic index that places patients at greater risk against harmful effects (14,15). All available drugs should be reviewed with patients or their family members in order to intervene in potential

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drug interactions or dietary requirement, and clear dosing instructions should be provided including the instructions to be carried out when a dose is skipped or a dose of vomit occurs. Patients should be trained on the requirements for medication storage such as temperature or light resistance. Patients, their families and caregivers should also be informed of oral chemotherapy, dose adjustments, or safe return of drugs to pharmacies or oncology clinics (16). Side effects experienced by patients could reduce the adherence to treatment, or patients could be generally reluctant to report side effects because of fear of treatment interruption and reduction of doses (17). The involvement of a family member or caregiver in the treatment can help cement the educational knowledge at home and motivate patients to comply with the treatment. This situation is of great importance for especially elderly population whose cognitive challenges are likely to affect the compliance with treatment and for children and adolescents for whom the relationship between parental participation and compliance are critical (18).

There is no single standard intervention to overcome obstacles in patient compliance (19). However, multidisciplinary and multimodal strategies are considered to be effective. Patient education is primarily emphasized in the literature (10,20). Patient education is a significant component for the enhancement of adherence to treatment. It should be tailored for each patient individually and patients should prefer direct interaction in general with health care providers. Healthcare professionals should consistently keep a tab on patients in order to answer their questions, and remind them about counseling and treatment regimens. Primarily oncology nurses, who are constantly interacting with patients, play a crucial role in providing the patients with education on many aspects of the treatment regimen of oral chemotherapy treatment. Training for oral chemotherapy is not a one-off event but should be continued throughout the treatment. The follow-up of patients provides the opportunity for nurses to reinforce previously discussed concepts, educate them on symptom management, and assess the compliance with treatment. Oncology nurses have a unique position to step into new roles that emphasize patient and family education and support. Individualized patient support will be of vital importance in quality patient care and family, and management of side effects during oral chemotherapy. Patient education, communication, symptom management and proactive follow-up are among the primary roles of oncology nurses (9,10,20,21). In conclusion, it is stressed that the safe administration of oral chemotherapy would have a positive impact on the clinical and care outcomes of patients.

The related literature examined reveals that the studies, conducted with patients who received oral chemotherapy, are quantitative studies and that these studies mostly evaluate the knowledge and application related to drug use and compliance (1,13). However, very little is known about the experience of patients with such medication. In the study designed to better understand how these gaps could be filled and how patients manage oral chemotherapy, it is aimed to identify the weak points and recovery opportunities of patients during oral chemotherapy. Therefore, multidisciplinary approaches are needed to safely use oral chemotherapeutics and to reduce medical errors. This study was carried out with a phenomenological research pattern within the scope of qualitative research model in order to determine the opinions and experiences of cancer patients receiving oral chemotherapy.

2. METHODS

2.1. Study Design

The study was carried out on 18 people in total, 10 women and 8 men, who agreed to participate in the study and submitted their informed consent out of the patients who stayed in the oncology clinic at a university hospital, were diagnosed to have cancer, have been taking oral chemotherapy after being admitted to the oncology clinic of a university hospital with diagnosis of cancer. In qualitative research, there is no set rule for sample size. The size of the sample can vary according to the status of getting sufficient information from the participant and the diversity of the sample chosen In qualitative research, the sample size is generally determined based on the information needs. One of the principles guiding this issue is data saturation. In other words, the sampling reaches a point where no new information is obtained and reaches the surplus (22). COREQ-Consolidated Criteria for Reporting Qualitative research was used in the structuring and reporting phase of the qualitative study.

2.2. Procedure

The study aimed to investigate in-depth the issue through semi-structured interview guide examining the problems which patients face due to the use of oral chemotherapy, the ways they cope with and the problems related to the access to drugs, how they paid attention to security measurement related to the preparation and storing of medication and whether they receive support. The questions in the interview guide were determined following the experiences of researchers and review of the related literature. The interviews were held in the interview rooms, which were quiet, well-lit and comfortable enough for individuals to express themselves without interruption. Interviews and analyzes were carried out by researchers who participated in qualitative research course and had previous qualitative study experience. The purpose of the study was explained to the patients who came to the outpatient appointment. After receiving verbal and written consent from the patients, a semi-structured interview was conducted using face-to-face interview technique. A voice recorder was employed to record the data obtained during the interview.

2.3. Measures

An identification form and interview guide were used to obtain the data in this study. Identification form includes details

about the age, sex, marital status, educational background, diagnosis, drugs used in oral chemotherapy and whether information has been provided on oral chemotherapy drugs. The semi-structured questionnaire was created from the researchers' experiences and academic literature.

The questions in the interview guide were as follows:

1. Please tell us your name and introduce yourself briefly.

2. Can you share with us your life experiences since you started taking oral chemotherapy?

- What are the convenience/difficulty, advantage/ disadvantage you have experienced with the use of oral chemotherapy? How are their effects on your daily life?

-Are there problems with access to drug? What kind of problems?

- Have you experienced any of the side effects? What kind of applications do you do to reduce the side effects?

- What are the difficulties you have regarding the storing, preparing and using your drugs?

3. What should be the approach of health professionals from the moment you start taking oral chemotherapy? What are the issues you are in need of support?

4. What do you think of the support you receive during the course of your treatment from your family members and immediate circle?

2.4. Data Analysis

The data obtained from face-to-face interviews were analyzed using thematic analysis method. Voice recordings were documented immediately after the interview. Qualitative content analysis method was used in data analysis. According to Braun and Clarke (23), the content analysis method includes the following steps: (1) practice with data; (2) generation of initial codes; (3) searching for themes; (4) reviewing themes; (5) defining and naming themes and (6) generating the report. Researchers with qualitative research knowledge and experience applied content analysis and identified themes independently of each other, based on the specified steps. Each transcription process took about 30-45 minutes. The researchers debated until they agreed on the data and identified the themes that were thought to best describe the findings. In the research, codes such as H1, H2, H3… were used for the interviewers.

2.5. Ethical Considerations

Before the study was conducted, researchers obtained the necessary approvals from the institution, where the study would take place and the Ethics Commission at Gazi University (77082166-604.01.02-). Patients were informed that the decision on whether they wanted to participate in the study or not was entirely up to them, that the data to be collected through the study would only be used for purposes of this research and that their privacy would be protected.

3. RESULTS

3.1. Description of the Participants

The patients, taking oral chemotherapy, are in the age range of 36-58 years, dominantly female (n=10), married (n=15), unemployed (n=12) and have middle income. A majority of patients (n=10) have a chronic disease such as diabetes and hypertension, have admission medical records (n=16), are mostly diagnosed with colon cancer (n=8) and are using capecitabine for oral chemotherapy (n=9).The findings of the study were categorized under such topics as access to drugs, drug use, approach of health professionals, support of family members and close relatives, and side effects and control of the treatment (Figure 1).

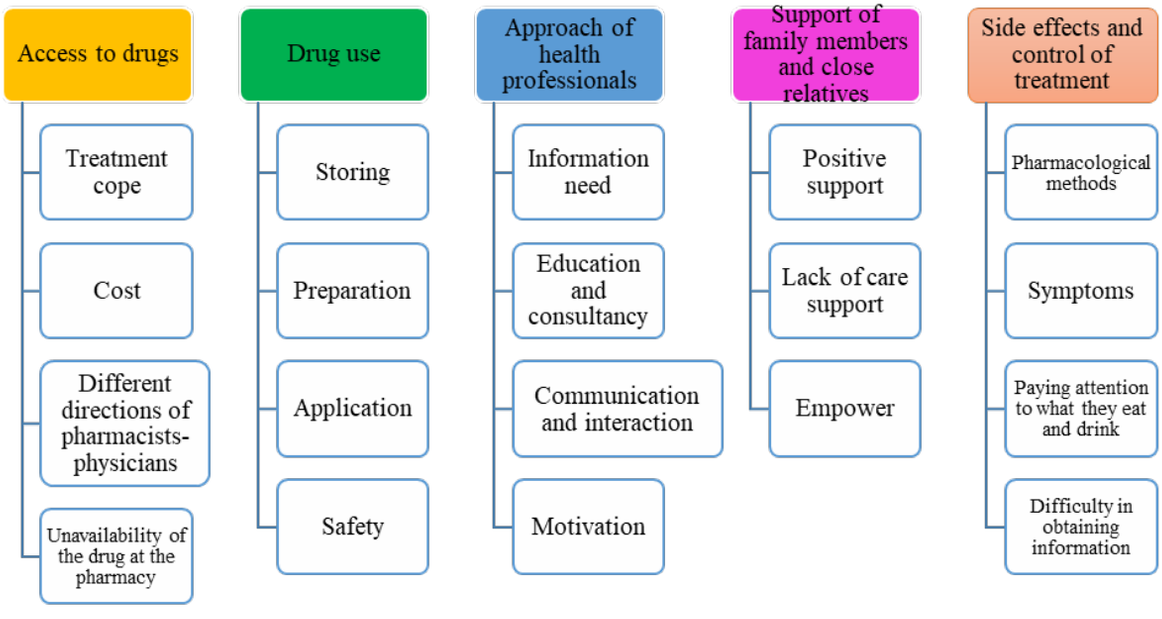

Figure 1. *Themes and sub-themes*

3.2. Theme 1: Access to Drugs

While most of the patients stated that they did not have any problems with access to drugs, they encountered problems with the costs, scope, different directives of pharmacists-physicians and unavailability of the drug at the pharmacy. A few statements of the patients are shown as in the following:

"There are times when I could not find the drug at the pharmacy…" (H1)

"We could not find the drug in any pharmacy'. Later, we found it in a drug warehouse. They were so nice and helped us. Otherwise, we had to keep searching for the drug…" (H4)

"The statements of our pharmacist about the use of the drug contradicted with the statement of our doctors. Why is there such a dilemma?..." (H5)

3.3. Theme 2: Drug Use (Storing, Preparation, Application)

Majority of the patients stated that they did not have any difficulties using oral chemotherapy drugs. However, according to the statements of the patients, they did not have enough knowledge on the safety measures on such issues as the preparation and preservation of the drug. Elderly patients are supported by their family members due to the loss in their motor skills and cognitive level. They receive help in such activities as remembering the drug, calculating the dose, and opening the drug lid. Some of the statements of the patients are illustrated as follows:

"My daughter often reminds me to take my medication on time and keeps a note of the time I take the dose of the drug. We keep the drugs in their own packages. She makes cleaning twice after I go to toilet…" (H6)

"I take the pill in my hand and swallow it. I am able to fulfil my own needs. I take the drug in my house so there is no need to come and go between the house and hospital. It is easier this way…" (H12)

"I keep it in the cupboards to which my child cannot reach. I do the same for all medication. Apart from that… (thinking), there is nothing I pay attention to, indeed..." (H13)

"We searched on the web and learned that there was a lot to pay attention to… I am worried that I am also putting my family at risk…" (H18)

3.4. Theme 3: Approach of Health Professionals

A few patients in our study reported that their doctors informed them of the intake method and dose of the drug in the first clinical encounter in which oral chemotherapy was prescribed so that they could meet their needs. The statements of the patients with regards to the approach of health professionals showed that no communication and interaction on education and consultation regarding the management of oral chemotherapy treatment. The statements of the patients are listed below:

"We receive information on the drug from our doctor. We go to Emergency Department when we face a problem. We sometimes consult with a pharmacist…" (H2)

"Healthcare professionals are good at giving moral support and motivation. They are friendly. I also have difficulty in hearing. They speak loudly, for instance…" (H7)

"When I started to receive treatment through the vein, they gave me a paper. I read the information about the treatment and gave my approval for it. I also read the booklet they gave me…" (H11)

"While taking medication from the vein, they informed me briefly about the possible side effects such as hair loss, anemia and nausea. However, I did not get any other education about the drug…" (H14)

3.5. Theme 4: Support of Family Members and Close Relatives

The patients interviewed for the study often expressed the positive support they received from their families. Only one patient stated that he was not taken care of by his wife and family. The statements of the related patients are listed below:

"My family has been there for me throughout the entire treatment process… They show their support in many ways. They help me come to the hospital and get my medication from the pharmacy. Even though I sometimes feel bad about it, it gives me strength to know that they are with me…" (H11)

"I need care. I have a husband…(hesitating). Since he is a man, he cannot help me much. In other words, I need care. My family does not support me. That is why the members of my family are very busy and my parents have many babies. They have 10 children and it is a lot to take care of. I ... (thinking) am the one who needs care more. It is so difficult to deal with this disease. It is so difficult…" (H15)

3.6. Theme 5: Side Effects and Control of the Treatment

Majority of the patients reported to have experienced one or more of the side effects that might be associated with oral chemotherapy. While controlling the side effects, they mentioned that they applied to pharmacological methods and paid attention to eating habits. Below are a few examples of the side effects experienced by the patients:

"It slightly lowers my resistance, also causing nausea and fatigue…" (H9)

"It shakes me up so much. My hands and feet become numb. It caused all my nails to fall off. My toenails also fell off. It makes me suffer from insomnia. I became an alarming person…" (H10)

"…it only causes constipation at an extreme level. That was the only side effect I had… I used a herbal medicine for constipation…"

"I had many sores in my mouth. I was not even able to drink water. I could not eat. I ate less. But later they started to heal little by little…"

"I was suffering for 2-3 days when I used this drug. I mean, I did not suffer, but rather drinking water tasted different for instance…" (H14)

"I am extra careful when it comes to my food I eat yogurt every day. I drink as much water as I can. I drink two jugs of water. There are times I drink one jug of water..." (H15)

"It gets harder to consult when side effects occur as I am a patient at the outpatient clinic..." (H17)

"I do not think that the drug is that effective... Let me put it this way. I have been using this drug for nearly two years. However, I did not see any changes… It does not work on me…" (H18)

4. DISCUSSION

On the fundamental basis of application of oral chemotherapy in an efficient and successful way lies patient's compliance with the treatment. Noncompliance with treatment reduces the efficiency of oral chemotherapy treatment (21,24). The opinions and experiences of the patients who are using oral chemotherapy drugs were examined in this study in order to better understand how they would deal with oral chemotherapy. Findings obtained in line with the opinions of the patients were discussed according to the following titles.

4.1. Theme 1: Access to Drugs

It is important for patients to have easy access to health care and home care services. Oral chemotherapy is usually performed at home under the supervision of the patient (24,25). This is the most significant advantage of oral chemotherapy. Patients usually have the opportunity of choosing oral chemotherapy because of the easy method of using the drug in the comfort of their home. They think that it reduces the number of visits to the hospital, reduces anxiety, allows them to maintain their work, and enables them to avoid complications arising from intravenous injections. Liu et al. chose to use oral chemotherapy (89%) due to convenience of treatment (57%), issues related to IV administration and needles (55%) and environmental control (using drugs at home) in the study they conducted with 103 patients with terminal cancer. Factors such as the high cost of the drug and not being able to find it in drug stores affect the patients using oral chemotherapy and their relatives physically, financially and emotionally (26). However, the loss of dose and the delay of treatment slow down the process. Similarly, the patients often stated in our study that they had problems finding the drug in pharmacies.

4.2. Theme 2: Drug Use (Storing, Preparation, Application)

The correct and safe use of the drugs during the course of the treatment is important for the efficacy of the treatment (1). When the relevant literature on the accurate and safe use of oral chemotherapeutics is reviewed, safety measures such as washing hands before touching the drug, keeping antineoplastic drugs separate from other drugs, interaction of oral chemotherapy with other drugs and return of unused drugs should be taken. The drug that drips onto the table, sink, floor and clothes could enter the body through skin contact during the preparation and administration of the drug. Patients and caregivers are advised to immediately transfer the drug from its packaging to a drug container before taking it so that they will not touch the drug with their bare hands. In case the tablet breaks or crushes and the capsule opens or the drug falls onto the floor, the drug could enter the body via inhalation. If the patient is having difficulty swallowing solid oral dosage forms, the patient or caregiver needs to contact a pharmacist for information (14,25,27). Patients should be briefed on not chewing or crushing tablets or not opening capsules before getting in touch with a pharmacist to get information about how to make the process safer. Oral chemotherapeutics should be kept in safe places where children and animals cannot reach. In addition, improper disposal of drugs and drug disposal, which remain after the preparation and administration of drugs, cause environmental contamination (14,22,25,27,28). In our study, it was found out that a vast majority of the patients taking oral chemotherapy did not have adequate knowledge of the safe use of the drug. This result indicates that it influences adversely patients' coping with side effects related to compliance with treatment and treatment itself.

4.3. Theme 3: Approach of Health Professionals

The activities of healthcare providers and their attitudes towards patients strongly influence adherence to drugs. During the course of treatment, nurses are responsible for informing patients taking oral chemotherapy about drugs, potential side effects, and measures to mitigate the side effects (29,30). Patient's physical and psychosocial state, recognition state of diagnosis and scheduled treatment should be initially evaluated. Nurses should plan training sessions for patients and their families about approaches to the side effects of drugs, recognition and evaluation of the side effects and minimization of the side effects. They should make time for the questions of patients and their families and cooperate with other health professionals when needed. Health professionals need to be aware of possible problems and keep an eye on these issues in order to maintain the effectiveness of oral chemotherapeutics (14,24,25,29,30). It is necessary to determine the variable levels of absorption of oral chemotherapeutics, unexpected and incomplete bioavailability, and uncertainties in patient compliance. On the other hand, as suggested in the statements of the patients in our study, training materials and counseling on patients taking intravenous chemotherapy are of particular importance. The study proved that the patients were often in touch with their physicians during their appointments at the outpatient clinics. They stated that their physicians informed them about the use of drugs. The training and counseling

needs of patients should also be met by nurses who have a long-term association with patients in order to ensure the sustainability of treatment and to improve the quality of care (14,24,25,29-31).

4.4. Theme 4: Support of Family Members and Close Relatives

In addition to the support of health professionals, it is important for patients to get psychosocial and environmental support in order to cope with the issues that occurred over the course of their treatment. In our study, the patients reported that their families cared more about them after they were diagnosed with cancer, that they were pleased about it and that they supported them with the preparation of the drug, reminders for the medication time and management of the side effects. Families have the greatest responsibility in this regard (32). Nevertheless, some of the patients in the study stated that they were worried that they became a burden on their families and jeopardized the safety of their families who were living with them in the same house. Tuncay's study also supports the results of our study. According to this study, patients sometimes think that they burden their families and feel weak against their families who feel sorry and try hard for them (33).

4.5. Theme 5: Side Effects and Control of the Treatment

Like all types of chemotherapy, oral chemotherapy also has side effects. Each individual has a different response for treatment. Hence, the severity and type of side effects can vary from person to person. While the patients in our study stated that they faced some problems such as fatigue, constipation, numbness in hands and feet, loss of appetite and dry mouth, some of them said that they did not have any problems. It should be assessed whether the patients who had no problems were informed about the side effects of the treatment. That is why, in a qualitative study conducted for patients taking oral chemotherapy by Denois et al. (34), it was determined that patients could not identify and report significant signs of toxicity. It was noted that patients should be trained in identifying side effects and reporting.

Özkaraman et al (1) stated that in a quantitative study with patients taking oral anticancer drugs, 41.4% of oncology patients experienced side effects. Nausea-vomiting and malaise are among the first symptoms. In the same study, it was determined that more than half of rheumatology patients experience fear due to side effects that may occur due to oral chemotherapies. It was also found that individuals who do not have the fear of developing side effects due to drugs have better drug compliance (1). Similarly, Joplin et al (35) determined that individuals with rheumatoid arthritis have anxiety about the necessity of treatment against side effects. It can be said that the planning of nursing interventions for questioning the beliefs of patients about the side effects of oral chemotherapeutic agents, understanding their fears and coping will increase the compliance of the treatment.

4.6. Implications for Research and Practice

The use of oral chemotherapy drugs for cancer treatment has an increasing trend. The desired impact in oral chemotherapy treatment can be achieved with patient adherence. Oncology nurses need to take an active role in patient education and counseling and to support patients and their families in increasing their compliance to treatment. It is believed that the drug compliance of individuals using oral chemotherapeutic drugs could be improved through education and support programs, and that the beliefs of patients about treatment regimens could affect drug compliance and treatment process. However, an interdisciplinary approach is considered to be inevitable for the monitoring of the side effects of treatment and the safe use of drugs.

Oncology patients can make use of preliminary and/or periodic training, follow-up by phone, monitoring of self-managing drugs and participation of family members or caregivers in order to improve the compliance of patients to oral treatment regimens. As the therapeutic paradigm for cancer continues to evolve, oncology nurses will play an important role in engaging with patients and providing them with individualized education.

Having one-on-one interviews with the patients in qualitative studies and trying to figure out the management of oral chemotherapy treatment are the strong points of this study. Working with a heterogeneous group is the weak point of the study. Even though the side effects of drugs are generally similar, some drugs have more side effects. Despite the fact that there is a significant increase in the availability and use of oral chemotherapeutic agents, guidelines for safe use are still being developed.

5. CONCLUSION

Patients reported that they were greatly satisfied with oral chemotherapy. However, it was observed that patients need information about access to drugs, use of drugs, education and counseling on symptom management, and social support, that lack of knowledge leads to anxiety, and that patients get information about treatment mostly from doctors. Further research is required to determine the best practice in patient education, monitoring and safety management and to determine the gaps that may appear between the roles and practices of nurses.

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Clinical and Experimental Health Sciences

Acute Progressive Dyspnea in a Patient With Chronic Obstructive Pulmonary Disease

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ABSTRACT

The cause of acute dyspnea in chronic obstructive pulmonary disease is challenging. A 69-year old man complained of dyspnea, and cough. He had a diagnosis of chronic obstructive pulmonary disease for 6 years. Oxygen therapy started for respiratory failure. At the 6th day of hospitalization, dyspnea progressively increased. Chest X-ray showed a right-sided radiolucency. The preliminary diagnosis was spontaneous pneumothorax but chest computerized tomography-scan demonstrated a space-occupying lesion in left main bronchus. The latter diagnosis was mucoid impaction but bronchoscopy revealed a malignant lesion. In conclusions, lung cancer can be a rare cause of acute dyspnea.

Keywords: Dyspnea, respiratory insufficiency, pulmonary disease, chronic obstructive

1. INTRODUCTION

The many causes of dyspnea make it a diagnostic challenge. Rapid evaluation and diagnosis are crucial for reducing mortality especially in the patients with respiratory failure. The most common underlying causes for the exacerbation of chronic obstructive pulmonary disease are pneumonia, pneumothorax, pulmonary embolism, cardiac diseases such as cardiac arrhythmia, ischemia, and heart failure (1). In this educative case, we present another cause of acute progressive dyspnea in a patient with chronic obstructive pulmonary disease. The clinical picture was associated with right-sided radiolucency and left hilar enlargement.

2. CASE PRESENTATION

A 69-year old man was hospitalized for chronic obstructive pulmonary disease. Pulmonary function tests showed a forced vital capacity (FVC) of 1680 mL (48%), forced expiratory volume in 1 second (FEV1) of 1060 mL (39%), and FEV1/FVC of 63%. Arterial blood gas analyses were as follows: PaCO2: 44 mmHg, PaO2: 53 mmHg, pH: 7.48, bicarbonate: 31.8 mmol/L. Chest x-ray showed left hilar enlargement, a small cardiothoracic ratio, and prominent bronchovascular markings in the right lung (Figure 1). Oxygen therapy (2-3 L/min) was given by a nasal cannula. The patient was treated with ampicillin+sulbactam, nebulized salbutamol and ipratropium, methylprednisolone (40 mg/day), and ranitidine.

On the 6th day of hospitalization, the patient suffered from acute dyspnea under oxygen therapy. Pulse was 124/min, blood pressure was 100/70 mmHg, and SpO2 decreased to 85%. A chest x-ray demonstrated total radiolucency in the right hemithorax (Figure 2). This picture was strongly suggestive of pneumothorax. However, the *tracheal air column* was seen to have shifted to the left and elevated the hemidiaphragm in the left-side. These findings indicated a volume loss due to atelectasis. On the other hand, a progression in the left hilar region was also observed. A chest computerized tomography (CT) was performed for the underlying mechanisms. Chest CT -scan revealed an obstructive lesion in the left main bronchus, left upper lobe collapse, minimal pneumothorax on the left-side, and compensatory herniation of the right lung (Figure 3). This picture strongly suggested mucoid impaction but fiberoptic bronchoscopy showed a malignant lesion (Figure 4). Bronchoscopic biopsy demonstrated malignant epithelial tumor. The patient underwent external beam radiotherapy for total atelectasis of the left lung.

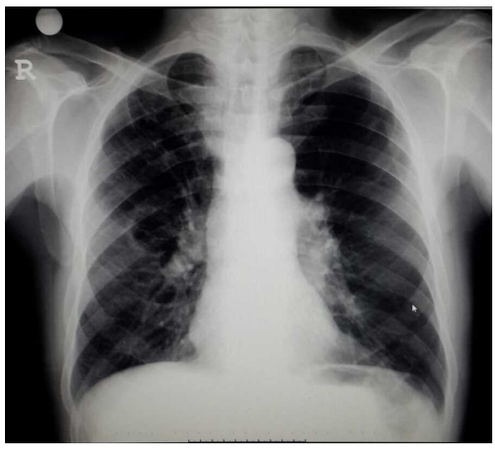

Figure 1. *Chest x-ray showing a non-homogenous opacity in the right middle zone, and the left hilar enlargement*

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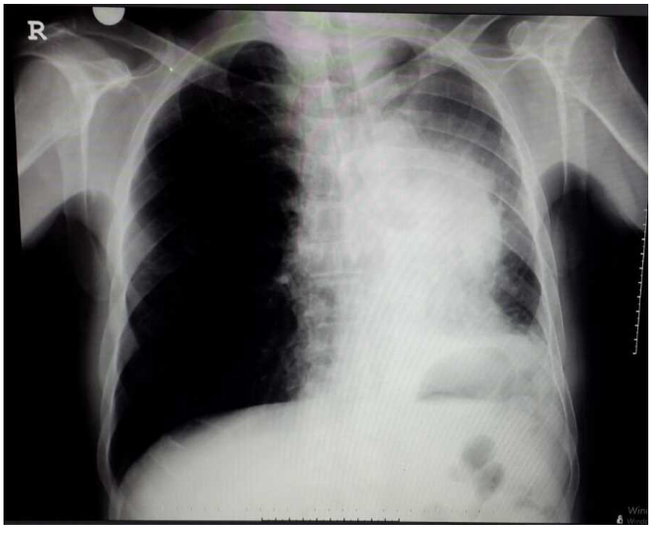

Figure 2. *A total radiolucency in the right hemithorax, progression of the left hilar opacity, and volume loss in the left hemithorax*

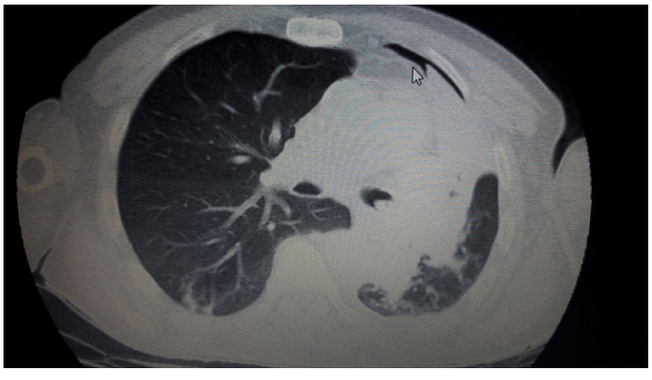

Figure 3. *A space occupying lesion in the left main bronchus, and a minimal partial pneumothorax in the left side*

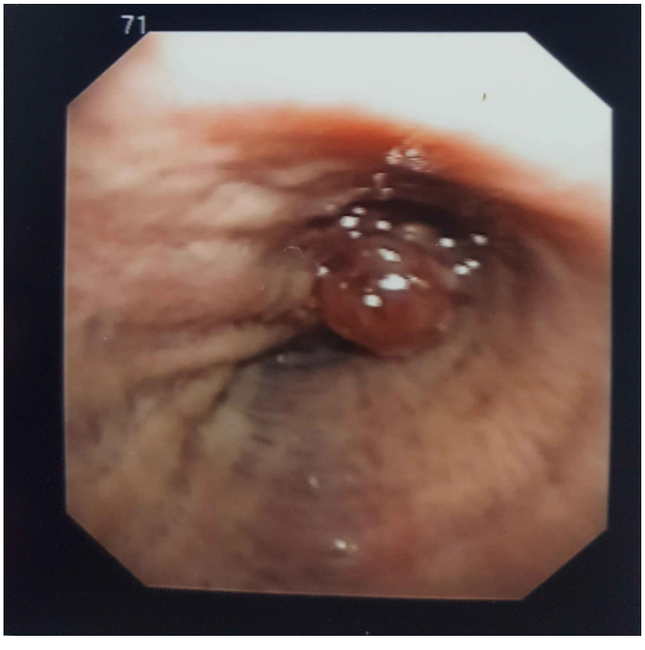

Figure 4. *A malignant endobronchial tumor in the left main bronchus*

3. DISCUSSION AND CONCLUSION

Acute dyspnea in patients with known chronic obstructive pulmonary disease (COPD) can be a clinical challenge due to the nonspecific nature of atypical presentations. Acute progressive dyspnea, right-sided radiolucency, mediastinal shift, and left-sided volume loss displayed by our patient might have been due to tension pneumothorax but t*he patient was not hypotensive* and it was necessary to explain the left hilar mass. The frequency of pneumothorax in COPD was reported as 8.5% (2). There were only two case reports about tension pneumothorax in COPD (3,4). In our patient, volume loss in the left lung was caused by compensatory over-inflation and hyperlucency in the right hemithorax. Minimal pneumothorax in the left side may be due to acute check valve bronchial obstruction.

Radiolucency and acute progressive dyspnea in a patient with COPD may be due to ruptured aortic or pulmonary aneurysm (5), late-onset atelectasis after abdominal surgery (6), diaphragmatic hernia with sepsis (7), congenital lobar emphysema (8). Also, aspergillus tracheobronchitis (9), pulmonary capillary hemangiomatosis (10), hemophagocytic syndrome (11), and hepatocellular carcinoma (12) were reported as other causes of acute dyspnea in patients with COPD but radiolucency did not accompany with these diseases.

In conclusion, dyspnea has a broad differential diagnosis even in patients with known pre-existing COPD. Our patient was unique because there was not another report of acute progressive dyspnea presenting with left hilar tumor and right-sided radiolucency. Because hyperlucency can be primary or secondary, it is necessary to look counter side before correct diagnosis. It should be kept in mind that lung cancer can be a rare cause of acute progressive dyspnea in the intensive care setting.

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Clinical and Experimental Health Sciences

Should We Remove the Alkaline Batteries, Which Are Got Caught in Upper Gastrointestinal Segment Immediately?

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ABSTRACT

There have been many publications indicating that especially alkaline batteries are used in adults who attempt suicide by ingesting foreign objects. In our study, a convict patient of 45-years of age has been taken to the emergency service of our hospital after ingesting 12 alkaline batteries of AA type for suicide. The patient had chronic depression and epilepsy. After identification of foreign objects in patient's stomach corpus by means of performed examination, direct graphy and tomography, the patient has been hospitalized in the general surgery service for follow up. After a follow up of 6 hours, the patient has been taken under upper gastrointestinal endoscopy and 10 alkaline batteries of AA type have been taken out from abdomen with the help of endoscope, although 2 alkaline batteries have passed the Treitz ligament. In the upper gastrointestinal endoscopy, generalized erythema and ulcerations have been determined in stomach corpus. The remaining 2 alkaline batteries have been taken out from the abdomen by natural ways. While studies have advised us to wait for 48 hours in cases of battery swallowing, the literature information which has changed in 2018, advises us not to wait for 48 hours, if possible, to remove it immediately. We wanted to present you that removing the alkaline batteries out of the abdomen by endoscopy in the centres having upper gastrointestinal endoscopy, is healthier for the patient and supports the literature, as the pathology, which may occur in stomach within this 48-hour period, may progress.

Keywords: Alkaline battery, foreign object, ingestion.

1. INTRODUCTION

Ingesting foreign objects constitutes a significant part of admissions to emergency services, particularly for children. Ingestion of alkaline batteries and similar foreign objects by adults is particularly for the purpose of suicide (1). Close follow up is necessary in patients applying to the emergency service due to ingestion of alkaline battery, as the batteries contain alkaline solutions, and they may cause severe complications such as perforation and fistula, as a result of liquefaction necrosis (2). Response regarding patients' interaction periods with alkaline battery, symptoms, localizations, and complications, must be carried out without delay. Treatment approach for ingested alkaline batteries vary depending on the placement of the battery. Batteries remaining in oesophagus must immediately be removed endoscopically; because mucosal damage may occur within one hour, and full-thickness injury may occur within four hours (3). The approach of 'if the battery is in the stomach, you may wait' has changed in the recent literature (4). In this study, we demonstrated the importance of presence of endoscopy units at health care centres for patients who have ingested alkaline batteries.

2. CASE PRESENTATION

A 45-year old male patient was taken to our emergency service from the prison, because he has committed suicide by ingesting foreign object (12 pieces of AA type alkaline battery). There was tenderness in abdomen epigastric region during the examination of the patient. Defence and rebound findings did not exist. Any features were not determined in rectal touch. The patient had chronic depression and epilepsy. The patient was using olanzapin 10 mg 1x1 and venlafaksin 75 mg 1x1 regularly. The patient did not have nausea and vomiting. The laboratory findings were as follows: White Blood Cell (WBC):6.47x10^3/µL; Hemoglobin (HGB):13.9 g/dL; Hematocrit (HCT):40.2 %; Platelet (PLT):226x10^3/µL; C-Reactive Protein (CRP):0,7 mg/dL; Glucose:88 mg/dL; Ure:29 mg/dL; Creatinine:0.79 mg/dL; Blood Urea Nitrogen (BUN):14 mg/dL; Lactate dehydrogenase (LDH):32 U/L; Aspartate transaminase (AST):9 U/L; Alanine transaminase (ALT):19 U/L; Direct Bilirubin:0.27 mg/dL; Creatine kinase:43 U/L; Amylase:49 U/L; Sodium (Na):138 mmol/L; Potassium (K):4.3 mmol/L; Chlorine (Cl):99 mmol/L. Electrical heart radiography of the patient was determined as ordinary. No features were determined in PA chest radiography. There

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were foreign bodies displaying extensive calcification within the abdomen in patient's ADBG (ambulant direct abdomen radiography) (Figure 1). Patient's intravenous contrast-enhanced abdomen tomography was carried out. In the tomography, multiple metallic linear, identical appearances approximately in 1.5 cm thickness were observed in stomach fundus; and they were interpreted as in favour of foreign bodies (Figure 2). The patient was hospitalized in general surgery service after inserting naso-gastric catheter, stopping oral intake, and starting proton-pump inhibitor and fluid, due to the possibility of gastric bleeding in patients who have ingested alkaline battery. Endoscopy preparation of the patient was performed while he was hospitalized in the service. Then, the patient underwent upper gastrointestinal endoscopy. Any pathologies in oesophagus were not determined during the upper gastrointestinal endoscopy, which was carried out under sedation anaesthesia. 10 pieces of AA type alkaline batteries were determined in stomach corpus and fundus (Figure 3). 10 pieces of AA type alkaline batteries were removed out of abdomen with the help of endoscopic snare (Figure 4). Extensive hyperaemia and ulcers were determined in cardia, corpus and fundus in the patient's endoscopy (Figure 5). Any other pathologies were not determined in the examination which was carried out up to the second part of the duodenum. Control ADBG was performed on the patient after the treatment. Two alkaline batteries were determined in intestines during ADBG, there was not free air beneath the diaphragm, which could have been assessed in favour of perforation. There were not any developing complications, so oral intake was initiated. In ADBG, it was seen that alkaline batteries were moving. After 72 hours, the alkaline batteries came out with spontaneous stool. The patient, whose psychiatric consultation was carried out and treatment was adjusted was prescribed a proton-pump inhibitor in tablet form. The patient was called for control endoscopy 2 weeks later. In the upper gastrointestinal endoscopy control, which was performed 2 weeks later, it was observed that the ulcers in the stomach were getting better, hyperaemic areas have recovered.

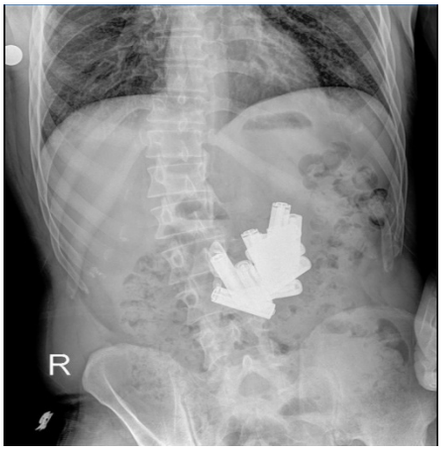

Figure 1. *Foreign bodies displaying extensive calcification within the abdomen in ambulant direct abdomen radiography*

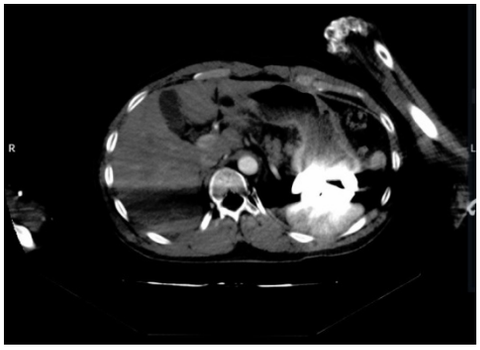

Figure 2. *Foreign bodies observed as multiple metallic linear, identical appearances approximately in 1.5 cm thickness in stomach fundus during abdomen computed tomography*

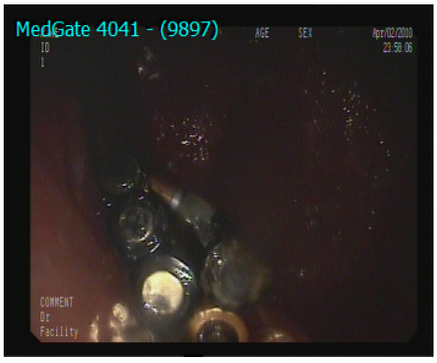

Figure 3. *10 pieces of AA type alkaline batteries in stomach corpus and fundus during upper gastrointestinal endoscopy procedure*

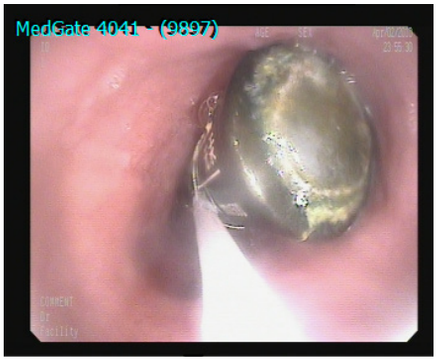

Figure 4. *10 pieces of AA type alkaline batteries were removed out of abdomen with the help of endoscopic snare.*

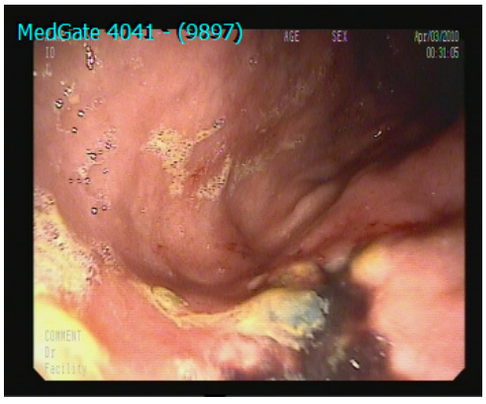

Figure 5. *Extensive hyperaemia and ulcers were determined in cardia, corpus, and fundus during endoscopy.*

3. DISCUSSION AND CONCLUSION

Ingestion of foreign body is a situation faced in adults, mostly in mentally handicapped persons, by mistake, or in those having psychiatric disorders intended for committing suicide. In our case, as the patient was a convict, ingestion of foreign body was intended for committing suicide. In the study conducted by Velitchkov et al., psychosis was determined in 22.9% of 542 adult patients. In the study conducted by Misdrahi et al., it was determined that suicide decision was taken as a result of negative effects of antipsychotic medication incompatibility, ranging between 11-80% (1). As there was an advanced level of depression in our patient, there was combined use of antipsychotic and antidepressant medications.

Approach to the handling of foreign bodies determined in gastrointestinal system may vary depending on the location, type, form of the foreign body, age of the patient, and other findings. Oesophagus constitutes the narrowest part of the digestive tract. Most of the ingested objects are got caught by oesophagus. Most of the foreign bodies reaching to the stomach passes the digestive tract easily. Rarely, they cause complete obstruction or perforation, in such cases surgical intervention is needed. It must be monitored whether they got caught in the anatomically narrowing or angled regions of gastrointestinal system such as the 'C' loop of duodenum, Treitz ligament, and ileocecal valve, or not. While most of the ingested foreign bodies are removed from the gastrointestinal system spontaneously without causing any problems, it is needed to remove 10-20% of them with endoscopy. Surgical intervention is needed only in 1% of the cases, due to the development of obstruction, fistula, and perforation. The important thing in foreign body ingestion is the type of the ingested foreign body. Hard, non-soluble objects, which are eaten or ingested, may cause bleeding, obstruction, and enteric fistulas in the gastrointestinal system (5). In our case, the ingested foreign body was AA type alkaline batteries, and 2 of them passed Treitz ligament, and 10 of them caused ulcers in stomach corpus. The batteries, which passed Treitz ligament, have come out of abdomen spontaneously within 72 hours. 10 pieces of alkaline batteries, which were located in stomach corpus, generated ulcers and erosions. Litovitz et al. noted that there are four types of alkaline batteries which are mercury oxide, silver oxide, manganese oxide, and lithium. There is potassium or sodium hydroxide at levels of 20-45% in all four types. Mercury oxide is one of the most common and dangerous ingredients. Elemental mercury is released by the reduction of mercury oxide in the acidic environment of the stomach, and it may cause hydrargyriasis. When alkaline batteries contact with saline human tissue, sodium hydroxide and chlorine gases are freed, and cause denaturation and necrosis. An alkaline battery cause tissue damage with four mechanisms: 1. Cellular damage due to the dissemination of heavy metals; 2. If the battery faces with bidirectional diffusion of the fluids, in the surrounding fluids; 3. Low-voltage burns which is caused by the exterior electricity production in the battery due to the potential electricity between cathode and anode; 4. Presence of necrosis with the effects of local pressure (6).

One of the most important questions in battery ingestion cases is the timing of endoscopic intervention. In the study conducted by Anderson et al., daily radiography monitoring was recommended. In addition, they suggested that batteries passed the gastrointestinal tract in 85% of the cases, and recommended endoscopic removal procedure after remaining in stomach for 36-48 hours (2). In the study conducted by Çobanoğlu et al., it was determined that ingestion of alkaline battery caused permanent damage in oesophagus, and extensive ulcers were generated, and therefore we support the approach of 'immediate removal of the battery' in order to prevent these complications (7). In the study conducted by Kayıpmaz et al., 3 pieces of AAA type alkaline batteries within the stomach were removed with endoscopic method successfully (8). According to the decision taken by the European Gastrointestinal Endoscopy Community in 2016, carrying out endoscopy within the first 4 hours was found appropriate (9). Litovitz, the author, who reported the largest series in battery ingestion investigated 8648 patients, who have ingested batteries, suggested that it is difficult for the batteries having a diameter over 20 mm to pass from the regions, which are the natural narrowness regions of the body (pylorus, duodenum C loop, ileocecal valve, etc.), so the batteries in such sizes must be removed with endoscope (6). As a result of the study conducted by Anfang et al., Jatana et al., and Litovitz et al., a treatment algorithm was produced by the National Poison Information Centre, regarding the things to be done for the patients admitting for battery ingestion (4,10,11).

According to this algorithm, in case of AA type alkaline battery ingestion, immediate removal out of abdomen by upper gastrointestinal endoscopy is recommended. The former approach of waiting for 36-48 hours generates various ulcers in organs such as stomach and oesophagus, and causes various morbidity and mortality risks due to its complications; therefore, immediate endoscopy is recommended in the updated literature demonstrating the necessity of endoscopy centres.

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Clinical and Experimental Health Sciences

The Effect of Algan Hemostatic Agent (AHA) on Wound Healing

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ABSTRACT

Objective: The Algan Hemostatic Agent (AHA) is a novel herbal originated blood stopper. The aim of this study is to investigate the effect of AHA on wound healing on excisional wound model in rats.

Methods: In this study, 54 adult Wistar albino rats were used. Rats were divided into 3 groups (saline, Madecassol® and AHA). Each group was then divided into 3 subgroups as the 3rd, 7th and 14th days. Two wounds were created in the dorsal thoracic region of the rats. One of the lesions was used for histopathological examinations and the other for hydroxyproline measurement. In order to evaluate the wound healing, wound area were measured during the whole treatment period and animals were sacrificed at the end of the 3rd, 7th and 14th days and tissue samples were taken for the determination of hydroxyproline levels.

Results: AHA treatment did not cause significant difference in hydroxyproline level on days 3, 7, 14. The contraction percentage of wound area was higher in the AHA group on day 7 than that of the control group. However, the difference was not statistically significant ($p>0.05$). On days 3 and 14, no significant difference was detected in the contraction percentage of wound area between the control and the AHA groups. AHA and Madecassol® results of epidermis regeneration on the 14th day, neutrophil infiltration on the 7th day and edema on the 3rd, 7th and 14th days were different in terms of histopathological parameters compared to the control group.

Conclusion: Despite good histological findings, AHA did not significantly accelerate wound healing, but did not adversely affect wound healing as well.

Keywords: Algan hemostatic agent, Madecassol®, wound healing, hydroxyproline

1. INTRODUCTION

Wound healing is a complex physiological process involving soluble mediators, blood cells, and extracellular matrix in damaged tissue. It consists of four programmed phases as hemostasis, inflammation, proliferation, and tissue remodeling (1). The presence of certain factors such as diabetes, obesity, infection, aging, stress, defiency of steroids such as observed in postmenopausal period, and poor nutrition impair wound healing processes (2). It is important for proper wound healing to be rapid without infection and not leave a scar tissue. If the contraction of the scar tissue formed in the final stage of wound healing is excessive, it may also causes deformities in the internal organs (3). Different local and systemic therapies are being investigated to accelerate wound healing. Some extracts obtained from species such as *Aesculus hippocastanum*, *Cotinus coggygria* and bioactive molecules have been reported to accelerate the wound healing process by promoting collagen deposition, increasing fibroblasts, preventing infection or suppressing oxidative stress (4-7).

The Algan hemostatic agent (AHA, Algan Group Health Services Import and Export Industry and Trade Ltd., Co., Istanbul, Turkey) is a novel polysaccharide based hemostatic agent and has been patented (Patent application no: a2015 / 00018, application publication no. TR2015 0018 A2). It is in the form of powder, and liquid and produced for bleeding control. It consists of a standardized mixture of 6 different plants (*Achillea millefolium*, *Juglans regia*, *Lycopodium clavatum*, *Rubus caesius*, *Viscum album*, *Vitis vinifera*). Preclinical studies of AHA have been completed and clinical studies are continuing. It has been shown to be

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effective in some bleeding models like experimental partial splenoctomy (8), renal venous bleeding (9), liver laceration (10) and hepatectomy bleeding (11). According to the hemodynamic test, AHA primarily provides hemostasis by forming a mechanical barrier in the area where it is applied (9). It has been reported that the coagulation mechanism forms by confining and gelling blood elements into the polymeric network (12). AHA has a similar mechanism of action with polymer-based hemostatic products (8,9). Local hemostatic (antihemorrhagic) agents promote hemostasis in postoperative and posttraumatic bleeding. An ideal hemostatic agent should be safe. A hemostatic agent is expected to have a positive effect on wound healing or at least not to have a negative effect. Besides their favourable hemostatic effects, some hemostatic agents are shown to accelarete wound healing (13-15).

In this study, we aimed to investigate time-dependent effects of AHA on wound healing on the excisional wound model created in rats. The effects of AHA on wound healing is important because it is a product that will be used as a local hemostatic agent in internal and external bleedings.

2. MATERIALS AND METHODS

2.1. Animals

In this study, female Wistar albino rats, weighing 250-300 g and 16 weeks old, were used. Rats were obtained from Marmara University Experimental Animal Research and Application Center. The animals were kept in individual wire-bottomed cages, in a room at a constant temperature (22° ± 2°C) with 12-h light and dark periods, and fed with standard rat chow. All procedures for the experimental protocols of this study were approved by the Marmara School of Medicine Animal Care and Use Ethics Committee (protocol number: 15.2020.mar). During the experiments, all animals were subjected to the same stress under the same conditions.

2.2. Treatments

Test agent, Algan Hemostatic Agent, was obtained from Algan Group Health Services Import and Export Industry and Trade Ltd., Co., Istanbul, Turkey. The rats were divided into the following three groups of eighteen rats each: Control group, AHA treatment group, Madecassol® (Bayer, as reference oinment) treatment group. Each group was then divided into 3 subgroups (as the 3rd, 7th and 14th days), each consisting of six rats.

2.3. Excisional Wound Model

This model is a convenient method to monitor wound contraction. The study was conducted as described in the literature (6). Under anesthesia, the dorsal region hairs of rats were removed with shaving machine and the wound area was cleaned with 0.2% chlorhexidine solution. Two circular wounds were excised from the skin with a 6 mm biopsy punch to create wounds in the dorsal thoracic region of each animal. The wounds were left open throughout the study. The progressive changes in wound area of all groups were monitored.

Saline was administered topically once daily to the control group. AHA solution (liquid form) and Madecassol® (Bayer) were also applied topically once daily. These applications were continued until the end of the 3rd, 7th and 14th days. Tissue samples were taken at the end of the experiment. One of the two wound tissues created in each animal was used for the determination of hydroxyproline content and the other for histopathological examinations.

2.4. The contraction Percentage of Wound Area

In order to evaluate the contraction percentage of the wound areas, sizes of wounds were measured on the end of days 3, 7, and 14. The shape of each wound was drawn on transparent paper. Then, wound shapes were transferred on a 1 mm chart paper. The contraction percentage of wound area was calculated by using the following formula (16). The contraction percentage of wound area = (initial wound size – specific day wound size)/initial wound size) × 100.

2.5. Determination of Hydroxyproline Content

Tissue samples were taken at the end of the 3rd, 7th and 14th days. Hydroxyproline content was measured using the method by Reddy and Enwemeka (17). Briefly, tissue samples were mixed with 2 N NaOH and incubated for 20 minutes at 120 °C. The samples were oxidized with chloramine T and Ehrlich reagent was added. Then, the absorbance of the coloured product formed at 65 °C was read at 550 nm. The concentration of hydroxyproline was calculated using the standard curve. The results were presented as mg/g tissue.

2.6. Histological Scoring

For histological examination, at the end of the 3rd, 7th and 14th days wounded skin specimens were collected from the experimental groups and fixed in 10% neutral buffered formalin solution. After fixation, tissue samples were dehydrated in graded ethanol series (70%, 90%, 96% and 100%), cleared in toluene and mounted in paraffin. Sections were cut into 4-μm-thick sections by rotary microtome from paraffin-embedded blocks and stained with hematoxylin and eosin (H&E). Finally, the sections were viewed under a light microscope (Olympus BX51, Tokyo, Japan) for epidermal and dermal regeneration, fibroblast density, angiogenesis, neutrophil infiltration and edema scored semiquantitatively by two blinded histologists according to the scoring system outlined in Table 1.

Table 1. *System for Scoring the Histological Features of Wound Tissue Samples.*

Score	Epidermal and dermal regeneration	Fibroblast density	Angiogenesis	Neutrophil infiltration	Edema
1	Little epidermal and dermal organization	Mild fibroblast density	Altered angiogenesis (1-2 vessels per site)	Mild	Mild
2	Moderate epidermal and dermal organization	Moderate fibroblast density	Few newly formed capillary vessels (3-6 vessels per site)	Moderate	Moderate
3	Complete remodeling of epidermis and dermis	Increased fibroblast density	Newly formed capillary vessels (7-10 vessels per site)	Severe	Severe
4			Newly formed and well-structured capillary vessels (>10 vessels per site)		

2.7. Statistical Analysis

Data were analyzed using GraphPad Prism 4.0 (La Jolla, CA). Statistical differences between groups were evaluated by one-way ANOVA and Tukey's post-hoc test. Data were expressed as mean ± standard deviation (SD). A *p* value <0.05 was considered as statistical significance.

3. RESULTS

The wound closure values and hydroxyproline levels of control, AHA and Madecassol® treated groups were as shown in Table 2. The contraction percentages of wound areas were observed as 43.83%, 74.34%, and 86.57% at the end of the 3rd, 7th and 14th days in the AHA group, respectively. The contraction percentages of wound areas of the AHA group were close to those of the control group on the 3rd and the 14th days, whereas the contraction percentages of wound areas of the AHA group on the 7th day was 16% higher than those of the control group. However, this difference was not statistically significant. In the reference drug Madecassol® group, the contraction percentages of wound areas were higher on the 7th day compared to those of the control group, whereas they were higher on the 14th day compared to those of the control and the AHA groups (p<0.05, p<0.01 respectively). Figure 1 shows the wound images of rats randomly selected from each group.

Table 2*. Wound Tissue Hydroxyproline Levels and Contraction Rates of Wound Areas (%) of the Control and the Treatment Groups.*

	Hydroxyproline (mg/g tissue), mean (SD)	Contraction rate of wound area (%), mean (SD)
3rd day		
Control	24.74±4.88	47.33± 14.45
AHA	24.16±1.53	43.83± 4.50
Madecassol®	32.14±5.81*,≠	48.67±3.30
7th day		
Control	26.50±4.50	64.00± 2.76
AHA	29.65±2.37	74.34± 9.00
Madecassol®	31.21±4.85	78.86±3.24*
14th day		
Control	24.80±1.21	88.00± 4.78
AHA	22.97±2.40	86.57± 6.03
Madecassol®	24.89±1.66	95.00±1.44*,≠≠

**p<0.05, versus control group; ≠ p<0.05, ≠≠ p<0.01 versus AHA group; SD: standard deviation; AHA: Algan Hemostatic Agent*

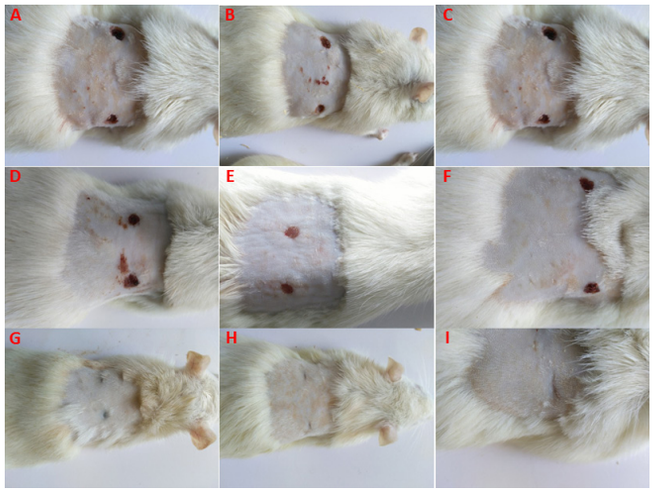

Figure 1*. The wound images of randomly selected rats from each group. A, B, C: 3rd day; D, E, F: 7th day; G, H, I: 14th day. On day 3, the wound closure was similar in all groups.*

Hydroxyproline levels of the AHA group on days 3 and 14 were close to those of the control group. There was no difference between hyroxyproline levels of the control and the AHA groups on day 7. However, hyroxyproline level of the AHA group was found to be higher than that of the control group. Hydroxyproline levels of the Madecassol® group on day 3 were significantly higher than those of the control and the AHA groups (p<0.05). No significant difference was observed in the hydroxyproline levels of the Madecassol® group on days 7 and 14 compared to those of the AHA and control groups.

There was no difference between the groups regarding the histopathological parameters evaluated in terms of epidermis regeneration, on the 3rd and 7th days. However, on the 14th day, histopathological parameters of the Madecassol® and the AHA groups were found to be better than those of the

control group. Similarly, there was no difference in fibroblast density between the groups on the 3rd and the 7th days. On day 14, fibroblast density was found to be less in the Madecassol® and the AHA groups compared to that of the control group. Angiogenesis was found to be less in the AHA and Madecassol® groups on the 3rd and 14th days compared to that of the control group. Neutrophil infiltration was found to be less in the AHA and Madecassol® groups on the 7th day than that in the control group. Edema was found to be less in the AHA and the Madecassol® groups on the 3rd, 7th and 14th days compared to the control group (Figure 2). The results are shown in Table 3.

***Table 3**. Comparison of Histological Parameters Between Groups.*

	Epidermis regeneration	Fibroblast density	Angiogenesis	Neutrophil infiltration	Edema
3rd day, mean (min.-max.)					
Control (Group 1)	0	0	2.80 (2-3)	2.83 (2-3)	2.66 (2-3)
Madecassol® (Group 2)	0.33 (0-1)	0	3.66 (3-4)	2.33 (1-3)	1.33 (0-2)
AHA (Group 3)	0.16 (0-1)	0	3.50 (2-4)	2.50 (1-3)	1.50 (1-2)
P	1&2&3 > 0.05	1&2&3 >0.05	*1&2 < 0.05 *1&3 < 0.05 2&3 > 0.05	1&2&3 > 0.05	*1&2 < 0.05 *1&3 < 0.05 2&3 > 0.05
7th day, mean (min.-max.)					
Control (Group 1)	0.5 (0-1)	2.33 (1-3)	3.16 (2-4)	2.16 (1-3)	2.33 (1-3)
Madecassol® (Group 2)	1.16 (1-2)	2.66 (2-3)	3.50 (3-4)	1.33 (0-3)	1.50 (1-2)
AHA (Group 3)	1.00 (1-2)	2.66 (2-3)	3.50 (3-4)	1.83 (1-3)	1.66 (1-2)
P	1&2&3 >0.05	1&2&3 >0.05	1&2&3 >0.05	*1&2 < 0.05 *1&3 < 0.05 2&3 > 0.05	*1&2 < 0.05 *1&3 < 0.05 2&3 > 0.05
14th day, mean (min.-max.)					
Control (Group 1)	1.66 (1-2)	2 (1-3)	2.16 (1-2)	0.5 (0-1)	0.83 (0-2)
Madecassol® (Group 2)	2.66 (2-3)	1.5 (1-2)	1.66 (1-2)	0	0
AHA (Group 3)	2.33 (2-3)	1.66 (1-2)	1.66 (1-2)	0	0.16 (0-1)
P	*1&2 < 0.05 *1&3 < 0.05 2&3 > 0.05	*1&2 < 0.05 *1&3 < 0.05 2&3 > 0.05	*1&2 < 0.05 *1&3 < 0.05 2&3 > 0.05	1&2&3> 0.05	*1&2 < 0.05 *1&3 < 0.05 2&3 > 0.05

Min.: minimum; max.: maximum; AHA: Algan Hemostatic Agent

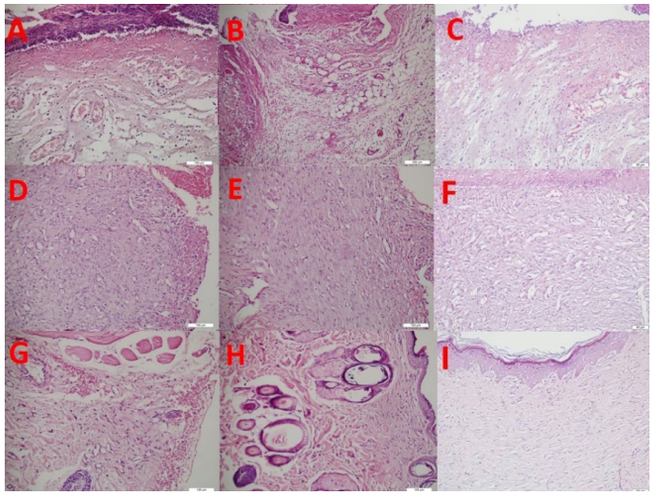

***Figure 2.** Histopathological view of the wound in the control, AHA and Madecassol® groups, respectively. A, B, C: 3rd day; D, E, F: 7th day; G, H, I: 14th days. On the 3rd day, histopathologic examination revealed acute inflammatory cell infiltration with intense bleeding in all groups. Intense edema is noted in the control group (A, B, C). On the 7th day, granulation tissue was rich in cells in the control group and the other two groups had less cellularity. On day 14, the connective tissue rich in healing area was observed in the Madecassol® and the AHA groups compared to the control group. In the control group, vascular granulation tissue is still present.*

4. DISCUSSION

Many studies have clinically and histologically investigated the healing potential of natural products on incisional and excisional wound models. It has been shown that plants and extracts with pro-collagen syhthesis, antioxidant, antiinflammatory and antimicrobial activities have accelerated wound healing (4). AHA is a new local hemostatic agent and a herbal biopolymer. Its effectiveness on experimental bleeding models is being investigated (8-11). However, its effects on wound healing have not been investigated. Therefore, this study was planned to evaluate the potential effect of AHA with biochemical and histological parameters in the excisional wound model.

Based on the ethnobotanical use of plants, making scientific research in this direction is very important in revealing the products or medicines that will be used as primitive raw materials in the treatment (if the active compound is to be isolated). Proof of this effect of these plants, which are used by the public for wounding purposes, is of great importance when evaluated in this respect. As AHA will be used in bleeding areas, it will be directly related to wound healing. Therefore, its effect on wound healing was tested. There are many studies conducted in the literature about the effect of many other haemostatic agents on wound healing. In these studies, it has been shown that many hemostatic products have a positive effect on wound healing (13-15). In a study conducted by Akalin et al. (18) with a herbal hemostatic agent, it was reported that the hemostatic agent was superior to the control group in terms of wound contraction

rates, type I / type III collagen ratio and inflammatory scoring in the dermal wound model.

AHA is an effective product in preclinical studies (8-11). Furthermore, the ability to produce AHA in powder and liquid forms is important in terms of ease of use in clinical practice. In splenectomy hemorrhage model with AHA, it is shown that it does not have intra-abdominal adhesion (8). Biopolymers are naturally occurring biomolecules synthesized by bacteria, plants and animals. Their bioactive properties such as antimicrobial, immune modulator and cell proliferative can create a micro-environment suitable for wound healing process (19).

AHA consists of a standardized mixture of 6 different plants. Several studies were carried out on this standard mixture of wound healing. *Achillea millefolium* has been shown to accelerate wound healing with its effect on collagen production, wound proliferation phase (20) and edema (21). *Juglans regia* has antioxidant, antimicrobial and wound healing activity (22,23), *Lycopodium clavatum* has been shown to have potential effects on inhibition of ROS production and tissue repair associated signaling pathways during wound healing (24,25). *Viscum album* accelerates cell migration (26). *Vitis vinifera* accelerates wound healing by acting on collagenation (27). *Rubus caesius* has high antioxidant potential (28). Wound healing activities of different *Rubus* species such as *Rubus niveus* (29)*, Rubus ellipticus* (30)*, Rubus fairholmianus* (31)*, Rubus sanctus* (32) have also been reported.

In our study, the effect of liquid form of AHA on wound healing was examined histopathologically as well as by measurement of wound areas and hydroxyproline levels. Wound area measurement is a good indicator for monitoring wound healing (7). Collagen is synthesis by the healing tissue and the level of hydroxyproline in the tissue is an indicator of collagen concentration. As the concentration of hydroxyproline increases, wound healing accelerates (28). In our study, no significant difference was observed in hydroxyproline levels in the wound tissue of the AHA groups compared to the control group. Parallel to hydroxyproline levels, AHA did not cause a significant difference in wound closure compared to the control group. The effect of AHA on wound healing in terms of histopathological parameters was close to that of the Madecassol® group. However, positive histopathological findings were not reflected in macroscopic and biochemical findings.

As a result, when applied to wounds, AHA did not adversely affect wound healing and had a low positive effect compared to Madecassol®. AHA may be used safely on excision wounds as a herbal product.

5. CONCLUSION

The present study affirms that AHA does not delay wound healing and has a low positive effect on wound healing in an excsion wound model. Further studies are needed to demonstrate the possible effects of AHA on different wound models such as burn and diabetic wounds.

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Clinical and Experimental Health Sciences

The Association of *ABCC5* and *ABCC11* Polymorphisms with The Pharmacokinetics of 5-FU in Advanced Gastric Cancer Patients

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ABSTRACT

Objective: Gastric cancer is the second leading cause of cancer-related deaths worldwide. 5-Fluorouracil (5-FU) is one of the most commonly used drugs to treat cancer, but 5-FU and its forms are characterized by wide inter-individual pharmacokinetic variability. *ABCC5* and *ABCC11* are members of the ABC transporter superfamily and play a role in the efflux of antineoplastic drugs like 5-FU.

Methods: The influence of two SNPs in *ABCC5* (rs562, *T>C*) and *ABCC11* (rs17822931, *G>A*) was evaluated based on the pharmacokinetics and toxicity of 5-FU in HER2-negative advanced gastric cancer patients treated with cisplatin and 5-FU (n=18). The genetic variants and plasma 5-FU concentrations were detected by RT-PCR and HPLC, respectively.

Results: There was no statistically significant difference between 5-FU $AUC_{0\text{-}96\ h}$ values and *ABCC5* (rs562; *T>C*), 21.04 ±3.46 vs 16.65 µg.h/mL, *p*=0.261 and *ABCC11* (rs17822931; *G>A*), 17.04 ±4.39 vs 54 ±3.79 µg.h/mL, *p*=0.564 variants. Similarly, there were no statistically significant differences between the variants and the most frequently observed side effects of diarrhea and mucositis.

Conclusion: We recommend investigating the noted SNPs more precisely in a larger study population with more comprehensive evaluation.

Keywords: *ABCC5*, *ABCC11*, 5-FU, pharmacogenetics, gastric cancer

1. INTRODUCTION

Gastric cancer is the fourth most commonly diagnosed type of cancer and the second leading cause of cancer-related deaths worldwide. Surgery is usually the only curative therapy, but most patients are diagnosed with unrespectable, locally advanced, or metastatic disease. Unfortunately, the most common treatment is palliative chemotherapy. Although there is no standard therapy regimen; 5-Fluorouracil (5-FU) and its forms are the backbone of chemotherapy. To improve therapy outcomes, 5-FU is usually given in combination with other antineoplastic agents such as cisplatin, oxaliplatin, and irinotecan. 5-FU is a fluoropyrimidine and antimetabolite drug. It inhibits essential biosynthetic processes and is incorporated into DNA and RNA and thus inhibits their normal function (1–5).

5-FU is generally administered based on the traditional body surface area (BSA) dosing. In the treatment of metastatic or locally advanced gastric cancers, it is commonly given in a dose of 1000 mg/m^2 for 1-4 days using a continuous infusion in combination with cisplatin 75-100 mg/m^2 (6,7).

Like most chemotherapeutics, 5-FU is generally characterized by a narrow therapeutic index and a large inter-individual pharmacokinetic variability that directly affects the efficacy and toxicity. Studies have shown that many patients who are treated with 5-FU are not receiving the appropriate doses to achieve optimal plasma concentrations. Indeed, only 20-30% of patients are treated in the appropriate dose range with approximately 40-60% of patients underdosed and 10-20% of patients overdosed. These findings indicate considerable variability in plasma 5-FU levels resulting in inter-patient pharmacokinetic variability. This in turn leads to differences in the drug-response relationship and contributes to toxicity and treatment failure (8–11).

ABCC5 (MRP5) and *ABCC11* (MRP8) from the MRP class of the ATP-binding cassette (ABC) transporter superfamily are expressed in most human tissues. Whereas ABCC5 is localized on the basolateral membrane, *ABCC11* is located on both the basolateral and apical membranes in polarized cells. They can extrude various exogenous and endogenous compounds in an ATP-dependent manner from the cell. Several studies

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have shown that 5-FU and its active metabolite, 5-FdUMP, are potential substrates for *ABCC5* and *ABCC11* (12,13). Genetic alterations in genes encoding ABC transporters are an important pharmacokinetic-based source for differences in response to antineoplastic drugs including 5-FU. Therefore, genetic differences in genes encoding *ABCC5* and *ABCC11* might be identified as cyclic nucleotide transporters that mediate the cellular efflux of cytotoxic monophosphorylated metabolites of 5-FU .These differences have been associated with 5-FU resistance and may affect the pharmacokinetic behavior of 5-FU and partially account for the differences between individuals (14,15).

This study examined the influence of two single nucleotide polymorphisms (SNPs) in *ABCC5* (rs562; *T>C*) and *ABCC11* (rs17822931; *G>A*) on the pharmacokinetics and toxicity of 5-FU in HER2-negative advanced gastric cancer patients treated with cisplatin and 5-FU. These results can lead to individualized chemotherapy with 5-FU in patients with (HER2-negative) advanced gastric/gastroesophageal junction cancer.

2. METHODS

The study was performed in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines. The study protocol was approved by the institutional review board (Local Clinical Ethics Committee of Istanbul University Cerrahpaşa Medical Faculty, No. 2012-05/A-28).

2.1. Patients

The study group existed of a total 18 male and female patients with HER2-negative advanced gastric – or gastroesophageal junction cancer.

2.1.1. Inclusion criteria

We enrolled patients with recurrent (HER2-negative) gastric cancer with impossible curative surgical resection and who had received no previous chemotherapy other than (neo) adjuvant regimens in the last six months after curative surgical resection. They age range was between 18 and 75 years and performance status between 0-2 according to Eastern Cooperative Oncology Group (ECOG) with no cardiac problems in their history and normal kidney, liver, and bone marrow functions.

2.2.2. Exclusion criteria

We excluded those with the following: other malignancies except *in situ* cervical cancer and basal cell carcinoma; HER2-positive subjects; subjects with active gastrointestinal bleeding, malabsorption, and jejunostomy; patients who previously received (neo)adjuvant treatment and had toxicity events above grade 2.0 according to Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 (National Cancer Institute, USA 2017); creatinine clearance above 60 mL/min (calculated with Cockcroft-Gault formula); neutrophil count above $1.5x10^9$/L and thrombocyte count above $100x10^9$/L; serum bilirubin concentrations 1.5 times higher than upper limit of normal; AST and ALT concentrations 2.5 times higher than upper limit of normal; alkaline phosphatase concentrations 2.5 times more than upper limit of normal; serum albumin concentrations above 2.5 g/dL; clinically significant hearing impairment; subjects with known dihydropyrimidine dehydrogenase (DPYD) deficiency; subjects with known or symptomatic brain metastases; serious systemic illnesses (uncontrolled diabetes, congestive heart failure etc.); subjects who have had a surgical procedure in a period of shorter than 4 weeks prior to study entry; subjects who have had radiotherapy for a period less than 4 weeks prior to study entry; and subjects who have had allergy against 5-FU or cisplatin. Pregnant women and those likely to become pregnant were also excluded.

2.2. Treatment and Sample Collection

The patients who met the inclusion criteria and voluntarily participated in the study were treated with a standard therapy plan at Istanbul Cerraspasa University Medical Faculty, Department of Medical Oncology. The therapy plan consisted of the combined administration of cisplatin and 5-FU. Cisplatin (75 mg/m^2) was supplied as intravenous infusion for 2 hr on day 1 of every cycle after hydration and premedication was administered. 5-FU (750-1000 mg/m^2) was supplied as continuous intravenous infusion (CIV) via a port-a-cath on day 1-4 of every cycle after cisplatin administration was completed. The steps were repeated every three weeks for 6 cycles.

The blood samples (5 mL) for genotype analysis were collected in ethylene diamine tetra acetic acid (EDTA) tubes on day 1 of the first cycle. Genomic DNA was isolated from whole blood for genotyping analysis of the *ABCC5* (rs562; *T>C*) and *ABCC11* (rs17822931; *G>A*) variants at the same time. Before analysis, the DNA samples were stored at +4 °C. The blood samples (6 mL) for quantitative analysis of 5-FU were collected at 24th hr of iv infusion of the first cycle (C_{ss}-steady state concentration) in heparinized tubes and were placed on ice. The samples were centrifuged immediately for 10 min at 3000 rpm and the plasma was separated. The plasma samples were stored at –70 °C until analysis.

2.3. Genotyping Analysis

Genomic DNA was isolated from whole blood using High Pure PCR Template preparation kit (Roche Diagnostics GmbH, Mannheim, Germany). Genotyping of *ABCC5* (rs562; *T>C*) and *ABCC11* (rs17822931; *G>A*) was performed on real-time PCR platform using a 96-well LightCyler® 480 instrument II system (Roche Diagnostics GmbH, Mannheim, Germany) using hybridization probes and master mix according to the manufacturer's instructions. The features of custom-designed LightSNiP assay probes are summarized in Table 1.

Table 1. *Features of custom-designed LightSNiP assay probes.*

Gene	Position	Alleles	Reference Sequence	Melting Temperature
ABCC5 **(rs562)**	chr3:183920057	*T>C*	CACgACATgCAACgCTgACCATTCAA[C/T] TgATgACAgCAgTgACCACgCCCAC	57.62 °C for *T* 65.35 °C for *C*
ABCC11 **(rs17822931)**	chr16:48224287	*G>A*	AgTggTTCAgACggTgAATgACCg[g/A] CTCATgTgACCgTTACgTCTTCgTC	55.54 °C for *A* 65.11 °C for *G*

rs: reference SNP number; alleles in the square brackets indicates the polymorphisms.

The 1X FastStart DNA Master Mix, 2 mM $MgCl_2$, 0.2 mM LightSNP HybProbe, PCR-grade water, and 500 ng DNA sample was added to each sample at a final volume of 20 µL reaction mix. The run was repeated in a different day with three randomly selected DNA samples for assay control, and PCR-grade water was used for negative control. Each genotype was determined according to the melting curve analysis of the related allele by the Carousel Based System PCR program (16).

2.4. Pharmacokinetic Analysis

A validated High Performance Liquid Chromatography – Ultraviolet and Visible light (HPLC-UV/VIS) method by Casale et al. was used for the determination of 5-FU in plasma (17).

2.4.1. Chemicals

5-FU 1000 mg/20 mL solution for injection (Batch 1919601) was supplied by Kocak Farma Company (Turkey). The 5-bromo-5,6-dihydrouracil (5-BrH_2) was obtained from Sigma-Aldrich (Munich, Germany). Isopropanol and acetonitrile were supplied from Riedel-de Haën (Hanover, Germany). Potassium phosphate tribasic was purchased from Sigma-Aldrich (Munich, Germany). Ammonium sulphate (powder), diethyl ether and other reagents and solvents were supplied from Merck KGaA (Darmstadt, Germany).

2.4.2. Instruments and chromatographic conditions

5-FU and the internal standard (IS) 5-bromo-5,6-dihydrouracil were separated on a 5 µm C18 110 Å, reversed phase column 250 x 4.6 mm (Phenomenex® Gemini®, USA) with a SecurityGuard™ C18 column 4 x 3.0 mm (Phenomenex® Gemini®, USA) operating at a temperature of 35 °C. The mobile phase was a solution consisting of 1.5 mM K_3PO_4 buffer and acetonitrile (99.5:0.5, v:v). The solution was adjusted to pH 4.5 with ortho-phosphoric acid (1 M). The flow rate was 1.0 mL/min, and the eluate was detected at 210 nm wavelength by a Waters 2487® dual λ absorbance detector (USA). Sample injection (50 µL) was performed with an integrated autosampler separations module (Waters® 2695 Alliance, USA). Data were recorded with Empower (Waters, USA), and further calculations used Microsoft Excel (USA).

First, 100 µg/mL of IS was added to 1000 µL of plasma. After brief vortexing, 1000 µL of saturated ammonium sulphate was added to precipitate the proteins. This mixture was again vortexed, and then 4 mL of isopropanol:diethyl ether (80:20, v:v) was added. After 3 minutes of vortex-mixing, the samples were centrifuged for 10 minutes at 4000 rpm. Subsequently, the organic phase was transferred into a clean tube and evaporated to dryness in a 45 °C block heater with sample concentrator (Stuart®, UK), under a nitrogen stream; 500 µL of saturated ammonium sulphate was added again to this residue. After briefly vortexing, 2 mL of isopropanol:diethyl ether (80:20, v:v) was added. Hereafter, the sample is vortex-mixed for 3 minutes and then centrifuged at 4000 rpm for 10 minutes. Subsequently, the organic phase is separated and filtered through a Sartorius PTFE (0.20 µM) filter (Germany) into a clean tube. The content of clean tube was evaporated again to dryness. After evaporating to dryness, the residue was dissolved in 250 µL of mobile phase and vortex-mixed for 3 minutes. The sample was then centrifuged for 5 minutes at 4000 rpm.

2.4.3. Calculation of Pharmacokinetic Parameters

The main 5-FU pharmacokinetic parameters were selected for steady state concentration (C_{ss}) and area under the curve (AUC) analyses. We assumed that samples taken at t=24 resemble the C_{ss} of 5-FU. Subsequently, the AUC was calculated as C_{ss} multiplied by the duration of the infusion (TCI) as follows: AUC = 'C_{ss} x TCI' (18).

2.5. Statistical Analysis

The Hardy-Weinberg equilibrium reports whether the studied population was biased or not using the chi-square (χ^2) test. Mann-Whitney U and Kruskal Wallis tests were used for testing the significance of this relationship between genotype and pharmacokinetics parameters. A *P* value of <0.05 was considered statistically significant.

3. RESULTS

A total of 18 patients with HER2-negative advanced gastric – or gastroesophageal junction cancer were enrolled in the study at Istanbul Cerrahpaşa University, Medical Oncology Department in Istanbul, Turkey. All patients received 5-FU 750-1000 mg/m^2 via continuous intravenous infusion on day 1-4 after intravenous cisplatin 75 mg/m^2 administration for 2 hr on day 1. An overview of the patients' characteristics is shown in Table 2.

Table 2. *Characteristics of the study population.*

Patient Characteristics	
***Age* (years, mean ±SEM)**	59 ±10
***Body surface area* (m^2, mean ±SD)**	1.75 ±0.24
	Number of patients (%)
Gender	
Female	6 (33.33)
Male	12 (66.67)
ECOG performance status	
Grade 1	18 (100)
Body surface area ($m^{2)}$	
Mean ± SD	1.75 ± 0.24 m^2
TNM stage	
Stage 3	1 (5.55)
Stage 4	17 (94.45)
Histopathological diagnosis	
Signet ring cell carcinoma (SRCC)	5 (27.78)
Adenocarcinoma	13 (72.22)

SEM: Standart Error Mean; SD: Standart Deviation; ECOG: Eastern Cooperative Oncology Group

3.1. Genotyping Results

ABCC5 (rs562; *T>C*) and *ABCC11* (rs17822931; *G>A*) variants were evaluated in patients with HER2-negative advanced gastric – or gastroesophageal junction cancer. All samples (n=18) were genotyped with 100% success rate and concordance. The genotype distributions and features of studied population are summarized in Table 3. The genotype distribution was found to be consistent with the Hardy-Weinberg equilibrium (HWE) model suggesting that the studied population was unbiased. The allele frequencies were found and were notably similar to Europeans as stated in 1000 Genomes Project phase3 release V3+ (ID: 257713) in the NCBI (National Center for Biotechnology Information) SNP database (dbSNP, https://www.ncbi.nlm.nih.gov/snp).

Table 3. *Variant alleles, genotype distribution, minor allele frequencies, and HWE of the studied SNPs.*

SNP	Variant allele	Geno-type	n (%)	Minor allele frequencies	HWE	
					X^2	*p value*
***ABCC5* (rs562)**	*C*	*TT*	2 (11.11)	0.56	2.205	0.562
		TC	12 (66.67)			
		CC	4 (22.22)			
***ABCC11* (rs17822931)**	*A*	*GG*	14 (77.77)	0.11	0.281	0.882
		GA	4 (22.22)			
		AA	0 (0)			

SNP: Single nucleotide polymorphism; rs: reference SNP number; n (%): number (percentage) of patients; HWE: Hardy-Weinberg equilibrium; χ^2: chi-square; p<0.05 indicates statistical significance.

3.2. Pharmacokinetic Results

The pharmacokinetics results were evaluated by examining 5-FU exposure expressed as $AUC_{0\text{-}96h}$ for 18 patients. There was a nearly 10-fold inter-individual variation of 5-FU exposure among patients (4.48 to 49.19 mg.h/L; mean: 20.55 mg.h/L; SD: 13.10 mg.h/L; CV%: 63.74%) (Fig. 1). According to therapeutic window of 5-FU infusion (9), only 23% of patients had an AUC within the therapeutic range (20–30 mg.h/L); 16% of patients had an AUC>30 mg.h/L, and 61% of patients had an AUC<20 mg.h/L. All patients were dosed according to the BSA standard but AUC levels of 5-FU were not correlated with BSA (Pearson's correlation efficiency: 0.2860; *p*=0.250).

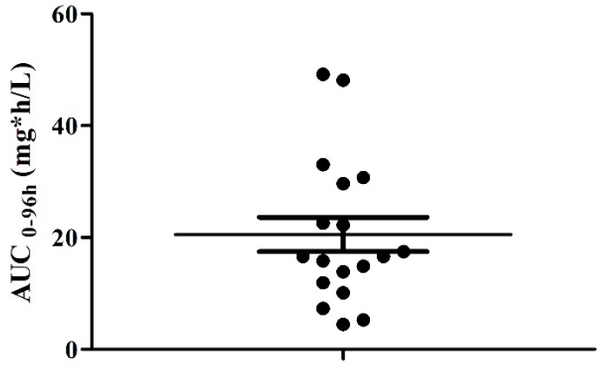

Fig 1. *Comparison of $AUC_{0\text{-}96h}$ patient values of each subject individually in the study group.*

Stomatitis, diarrhea, mucositis, and hand–foot syndrome were considered toxicity events highly-related to 5-FU within the 5-FU and cisplatin regimens. Hand-foot syndrome and stomatitis were not observed in this study group at the end of the 1st cycle of the therapy. Five patients had mucositis and mean 5-FU $AUC_{0\text{-}96h}$ values (28.37 ±19.53 mg.h/L vs 17.54 ±8.98 mg.h/L; *p*=0.119) were higher in patients with mucositis. Only three patients had grade 2 diarrhea. Mean 5-FU AUC values (33.22 ±15.86 mg.h/L vs 18.02 ±11.06 mg.h/L; *p*=0,058) were higher in patients with grade 2 diarrhea. The relationship between side effects and genetic mutations was analyzed using chi-square test. No statistically significant differences between genotypes and the most frequently observed side effects of diarrhea (*p*=1.00) and mucositis (*p*=0.490) were observed.

Homozygosity for the *ABCC11* (rs17822931) variant (*A*) was not observed in the studied population, and thus the pharmacokinetic parameters were evaluated based on the dominant model. The mean 5-FU $AUC_{0\text{-}96h}$ values and heterozygous and mutant allele carries distributions are shown in Table 4. No statistically significant differences between 5-FU $AUC_{0\text{-}96h}$ values and *ABCC5* (rs562; *T>C*) and *ABCC11 (*rs17822931; *G>A*) gene mutations were observed in this cohort (Table 4, Fig. 2A, Fig. 2B).

Table 4. *Statistical analysis for the relationship between genotypes and 5-FU $AUC_{0\text{-}96h}$ levels.*

Genotype		n	AUC (mg.h/L)	SE	*p* value
ABCC5	*T/C: C/C*	16	21.04	3.46	
(rs562)					0.261
	T/T	2	16.65	0.0	
ABCC11	*G/A:A/A*	4	17.08	4.39	
(rs17822931)					0.564
	G/G	14	21.54	3.79	

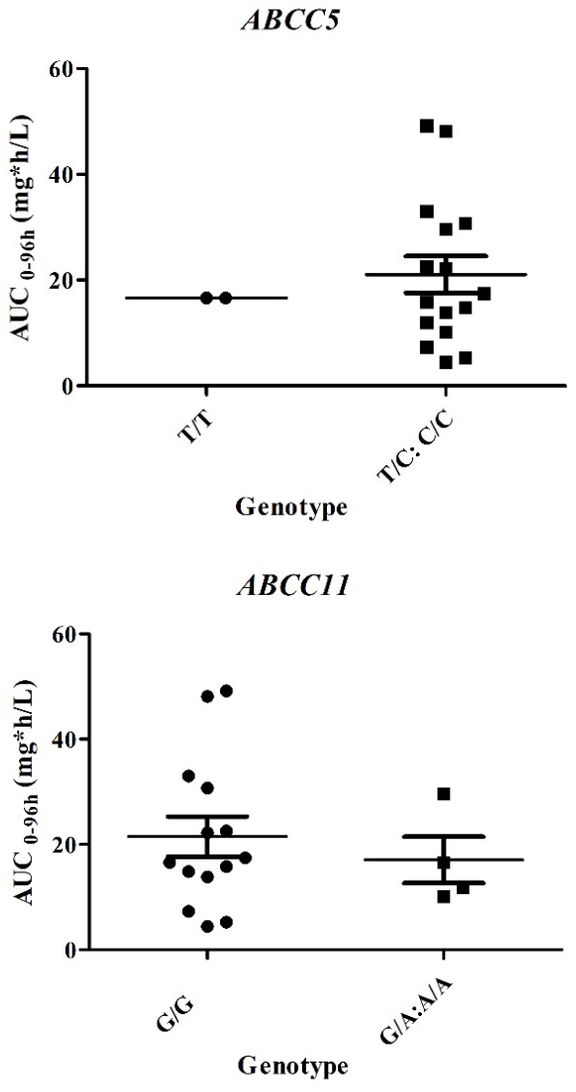

Fig 2. *Box plot showing $AUC_{0\text{-}96h}$ values of each subject individually for variants of (A) ABCC5 (rs562, T>C) and (B) ABCC11 (rs17822931, G>A).*

4. DISCUSSION

The aim of this study was to examine whether specific SNPs in genes encoding *ABCC5* (rs562; *T>C*) and *ABCC11* (rs17822931; *G>A*) affect the pharmacokinetics of 5-FU in patients with advanced gastric or gastro-esophageal junction cancer. This is the first study to examine the effect of these SNPs in *ABCC5* and *ABCC11* on exposure of 5-FU regimens in advanced gastric cancer patient. The blood samples for pharmacokinetics analysis were collected on day 1 of the first cycle, 24 hr after 5-FU administration which indicates the C_{ss} of 5-FU. Because of the short half-life of 5-FU (10–15 minutes), the C_{ss} of the samples are suggested to be taken at least 18 hr after the start of 5-FU infusion (9,10). 5-FU infusion started at the same time for all patient and plasma samples were collected near the same time of the day after starting infusion to eliminate circadian changes in 5-FU metabolism (19–21).

The AUC levels of this study showed high inter-patient 5-FU $AUC_{0\text{-}96h}$ variability, which is consistent with previous reports showing more than 40% coefficient of variation (CV). There were no significant correlations between BSA and 5-FU exposure in this study. According to therapeutic window of 5-FU infusion (AUC 20-30 mg.h/L), 23% of patients had an AUC within the therapeutic range, 16% of patients were overdosed, and 61% were underdosed. Our results are in the same range as previous studies reporting that 20-30% of patients were treated in the appropriate dose range, approximately 40-60% of patients were underdosed, and 10-20% of patients were overdosed (8–10).

We observed a nearly two-fold higher $AUC_{0\text{-}96h}$ levels in patients with mucositis (n=5; mean $AUC_{0\text{-}96h}$: 28.37±19.53 mg.h/L) and diarrhea (n=3; mean $AUC_{0\text{-}96h}$: 33.22±15.86 mg.h/L) versus patients without these toxicities. The mean 5-FU $AUC_{0\text{-}96h}$ values were around the toxicity levels (AUC>30 mg.h/L) according to previous studies (9). There was no statistically significant correlation likely due to the small sample size.

No statistically significant differences were seen between 5-FU $AUC_{0\text{-}96h}$ values and *ABCC5* (rs562, *T>C*) and *ABCC11* (rs17822931, *G>A*) gene mutations in this cohort. Importantly, this is the first study to examine the effect of these SNPs in *ABCC5* and *ABCC11* on pharmacokinetics of 5-FU. This is related to findings with different outcomes of previous studies even if one cannot directly compare the current findings with former reports. The *ABCC5* (rs562, *T>C*) heterozygous patients (*T/C:C/C*) had slightly higher mean 5-FU $AUC_{0\text{-}96h}$ levels versus wild type patients (*T/T*): 21.04 ±3.46 µg.h/ml and 16.65 µg.h/ml, respectively. However, the statistical analysis showed no significant differences in our findings (p=0.261). This is in contrast to the results of Teft et al. (2015) who noted significantly reduced irinotecan and metabolite (SN-38G and APC) levels in *ABCC5* (rs562) '*C*' allele carriers (*CC* and *TC* genotypes) versus wild type (*TT*) patients (22). On the other hand, Lal et al. (2017) found no significant impact of *ABCC5* (rs562, *T>C*) polymorphism on doxorubicin pharmacokinetic parameters (23).

The *ABCC11* (rs17822931, *G>A*) heterozygous patients (*G/A : A/A*) had a mean 5-FU $AUC_{0\text{-}96h}$ of 17.04 ±4.39 mg.h/L, which was slightly lower than *G/G* carriers 21.54 ±3.79 mg.h/L. In addition, due to previous findings about rs17822921, we expected an alteration in 5-FU $AUC_{0\text{-}96h}$ levels. Particularly lower 5-FU $AUC_{0\text{-}96h}$ values were expected in patients carrying the mutant type because the mutant form of *ABCC11* lacks an N-linked glycosylation (24,25).

Dose limiting toxicities of 5-FU include diarrhea, abdominal pain, nausea, stomatitis, mucositis, and hand-foot syndrome (26,27). At the end of the first cycle, the most frequently observed side effects were diarrhea and mucositis (related to 5-FU); however, we did not note any hand-foot syndrome or stomatitis. No statistically significant differences between genotypes and the most frequently reported side effects were observed.

5. CONCLUSION

There was no significant impact of *ABCC5* (rs562; *T>C*) and *ABCC11* (rs17822931; *G>A*) found on the pharmacokinetics of 5-FU. Therefore, we recommend studying these SNPs in *ABCC5* and *ABCC11* more precisely in a larger cohort. This could be an important determinant for 5-FU-based treatment sensitivity and might contribute to personalized therapy for patients receiving 5-FU.

Conflict of Interest: The authors declare that they have no conflict of interest.

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Clinical and Experimental Health Sciences

A Description of Oral and Swallowing Characteristics in Pediatric Patients with Neuromuscular Diseases

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ABSTRACT

Objective: The aim of our study was to put forth the existing problems about oral structure/function and swallowing function in patients with different pediatric neuromuscular diseases.

Methods: Forty-five pediatric patients with NMD's aged 10.22±3.32 years were included in the study. Oral intake levels of patients were determined with Functional Oral Intake Scale. Oral structural and functional examination, assessment of complaints related to swallowing problems, and three-ounce water swallow test were performed to all patients.

Results: In oral assessment, structural problems were determined at varying rates between 13.3% and 46.6%, problems related to oral functions between %4.4 and 26.6%, and complaints related to swallowing problems between 11.1% to 33.3%. Five patients (%11) failed the three-ounce water swallow test.

Conclusion: The findings related to oral structure/function and swallowing function indicate a risk of swallowing dysfunction with different symptoms and problems in neuromuscular diseases. These symptoms may result in life-threatening complications added to their current neuromuscular problems.

Keywords: Deglutition, oral examination, aspiration pneumonia

1. INTRODUCTION

Swallowing function consists of a series of sequential movements which provide food passage from oral cavity to stomach. Swallowing problems may occur in any part of oral, pharyngeal and esophageal phases. Swallowing problems which occur in oral phase are related to insufficient bolus control in mouth, inability to turn the food into bolus and, and transport the bolus from mouth to pharyngeal area. The most common problem in pharyngeal phase is inadequate airway protection during swallowing which may lead to life-threatening problems such as aspiration, pneumonia, and airway obstruction. The problems encountered in esophageal phase are difficulty in transporting the bolus through the esophagus and gastroesophageal reflux (1).

Dysphagia symptoms were reported in 34.9%-80% of patients with pediatric neuromuscular diseases (NMDs) (2-4). In addition, incidence of aspiration pneumonia increases up to 75% in this group (5,6).

Piecemeal deglutition, residues in vallecula and pyriform sinuses after semi-solid food swallowing, difficulty in swallowing and mastication problems were seen in patients with spinal muscular atrophy (SMA) (7). Both oral (mastication problems) and pharyngeal phase problems (coughing during feeding, difficulty in swallowing, need to clear throat) were determined in patients with Duchenne muscular dystrophy (DMD) (8,9).

Published studies about swallowing problems in pediatric neuromuscular diseases were found to be performed on subgroups of NMDs. However no consensus on oro-motor and swallowing problems exists in pediatric NMDs (10-12). Thus the aim of our study was to put forth the descriptive characteristics of oral and swallowing problems in a group of pediatric NMD patients with different diagnosis.

2. METHODS

2.1. Participants

This study was approved by the Non-Invasive Clinical Research Ethics Committee of Hacettepe University (No: GO 16/125-28). Written consent forms were signed by all patients and caregivers included in the study.

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Forty-five patients who were diagnosed with one of the neuromuscular disorder by a neurologist were included in the study. Inclusion criteria were as follows: (i) age between 5-18 years, (ii) no additional disease/oral appliance/medicine which may effect oral structures and swallowing functions, (iii) adequate cognitive level to understand and follow instructions for assessments, (iii) full oral intake without restriction.

2.2. Measures

Demographic data including age (years), weight (kg), height (cm), and diagnosis were recorded. Oral intake levels of patients were determined with Functional Oral Intake Scale (FOIS) which has adequate reliability, validity, and sensitivity to changes in functional oral intake. FOIS is a scoring system ranging from 1 to 7 for oral intake level of patients (1= nothing by mouth; 7= total oral diet with no restrictions) (13). The following assessments were performed to the patients who scored 7 points from the FOIS;

2.2.1. Oral structural examination

Oral structural examination was performed with a tongue depressor and an illuminator. Structural characteristics of lip, tongue, jaw, and palate and oral hygiene were assessed during this examination.

The existence of asymmetrical nasolabial folds and/or any disintegrity around labial region at rest were considered as an impairment in lip structure. Abnormally large (macroglossia) or small (microglossia) tongue, short frenulum and disintegrity in tongue were considered as an impairment in tongue structure (14). In the assessment of jaw structure, the alignment of teeth on mandible and maxillary bone with each other was evaluated in anterior and lateral aspects. In anterior aspect, first incisor teeth on maxilla must cover 1/2 portion of first incisor teeth on mandible. The increased rate for this covering was considered closed bite, while decreased rate was considered open bite. In lateral aspect, the first molar teeth on mandible must be placed a little bit anterior to the first molar teeth on maxilla for normal alignment. The patient was determined to have a protruded jaw if the position of first molar teeth on maxilla was placed highly anterior to the first molar teeth on mandible, while he/she was determined to have a retruded jaw when the first molar teeth on maxilla was placed posterior to the first molar teeth on mandible. These problems were considered as malocclusion (15). In palatal structure evaluation, unusually high and narrow palate is defined as high arched palate by observation and palpation.

2.2.2. Assessment of oral functions

The movements intended to be tested were initially performed by the swallowing therapist, then patients were asked to follow these commands.

The commands for the evaluation of tongue functions were to round lips, draw corners back, close lips, puff cheeks and bite lower lip while tip up, tip down, tip right, tip left and tip drawn back along hard palate for lip functions (14). Patients who succeeded these comments were considered to have 'no problem', and those who failed were considered to have 'problem'.

In the supine position the patient was also asked to open their mouths at a maximum level and the distance between the upper and lower teeth incisor teeth was measured with a caliper. Thus, a distance between the upper and lower incisors below 35 mm was considered as restricted opening of mouth (16).

In addition, the presence of packing food in mouth, leaking food from mouth during feeding, fatigue in the muscles of mastication, fasciculations in tongue, and uncontrolled saliva were asked to patients and/or their caregivers. The answers were recorded as yes or no.

2.2.3. Assessment of complaints related to swallowing problems

Presence of coughing, difficulty in swallowing, food getting stuck in throat during feeding, and change in voice quality after meals were assessed by asking to patients and/or caregivers. The frequency of recurrent pulmonary infections in the previous year, persistent wheezing and secretion were also asked.

2.2.4. Three-ounce water swallow test

The three-ounce water swallow test is a widely used method of screening individuals who are at risk for oropharyngeal dysphagia and aspiration. Criteria for referral for further assessment of swallowing include inability to complete the task, coughing, choking, or a wet-hoarse vocal quality exhibited either during or within 1 minute of test completion. A swallowing therapist administered the three-ounce water swallow test while the child was placed in a sitting position on a chair with the head upright and midline position and the arms and legs supported. Each patient was given three ounces of water and asked to drink from a cup or straw without interruption, and results were noted (17). Three-ounce water swallow test can be used in children between the ages of 2-18 (18).

All evaluations were performed by the same therapist.

2.3. Data Analysis

Statistical analysis was performed by using IBM-SPSS for Windows version 20. Descriptive statistics were presented as number/percent (n/%) for qualitative data and mean (X) and standard deviation (SD) for quantitative variables.

3. RESULTS

The demographic characteristics of the 45 patients included in the study were as given in Table 1. All patients were in FOIS level 7 which means total oral diet with no restrictions (n=45, 100%).

Table 1. *Demographic Characteristics of the Patients.*

Demographic characteristics	X±SD
Age (years)	10.22±3.32
Height (cm)	125.42±16.48
Weight(kg)	29.83±12.42

X: Mean, SD: Standard Deviation

The diagnostic distribution of the patients was as shown in Table 2.

Table 2. *Diagnosis of the Patients.*

Diagnosis	n	%
Duchenne muscular dystrophy	25	55.6
Becker muscular dystrophy	1	2.2
Spinal muscular atrophy type 2	8	17.8
Spinal muscular atrophy type 3	2	4.4
Congenital muscular dystrophy	5	11.1
Guillain Barre syndrome	1	2.2
Mitochondrial myopathy	2	4.4
Dermatomyositis	1	2.2
Total	45	100

3.1. Results Related to Oral Structural Examination

No structural abnormality in lips was detected. None of the patients were determined to have foul breath, infection, or tooth decay related to bad oral hygiene. The frequencies of encountered problems in oral structures were as given in Table 3.

Table 3. *Oral Structural Abnormalities and Their Frequencies.*

Oral Structural Abnormalities	n	%
Macroglossia (tongue)	6	13.3
Malocclusion (jaw)	21	46.6
High arched palate	6	13.3

3.2. Results Related to Assessment of Oral Functions

All of the patients had normal lip and tongue functions. Packing food in mouth, leaking food from mouth during feeding, and uncontrolled saliva were not seen in oral function assessment. The existing abnormalities in the assessment of oral functions were as given in Table 4.

Table 4. *Impairments in Oral Functions.*

Oral Functions	n	%
Restricted opening of mouth	2	4.4
Fatigue during mastication	12	26.6
Fasciculations in tongue	4	8.8

3.3. Results of the Assessment of Swallowing Problems

Complaints related to swallowing problems were as given in Table 5.

Table 5. *Swallowing Problems in the Study Population.*

Complaints related to swallowing problems	n	%
Coughing during feeding	9	20
Difficulty in swallowing	9	20
Food getting stuck in throat during feeding	8	17.7
Change in voice quality after feeding	5	11.1
Recurrent pulmonary infections	5	11.1
Persistent wheezing and secretion	15	33.3

3.4. Results of the Three-Ounce Water Swallow Test

Five (11.1%) of the patients (2 SMA type 2, 2 CMD, and 1 mitochondrial myopathy) failed the three-ounce water swallow test (Table 6).

Table 6. *Three-Ounce Water Swallowing Test.*

Diagnosis	n	%
Duchenne muscular dystrophy	-	-
Becker muscular dystrophy	-	-
Spinal muscular atrophy type 2	2	4.4
Spinal muscular atrophy type 3	-	-
Congenital muscular dystrophy	2	4.4
Guillain Barre syndrome	-	-
Mitochondrial myopathy	1	2.2
Dermatomyositis	-	-
Total	5	11.1

3.5. The Most Frequent Problems Seen in NMD Subgroups

Malocclusion (n=16, 64.0%) was found to be the most frequent problem in DMD, while recurrent pulmonary infection and persistent wheezing and secretion (n=5, 62.5%) was the most common one in SMA type 2. Besides, the patients (n=2) who had restriction in opening of the mouth and the majority (n=3, 75.0%) of patients who had fasciculation in tongue were diagnosed as SMA type 2. Persistent wheezing and secretion, coughing during feeding, and change in voice quality after feeding were encountered frequently in CMD (3 of the 5 patients, 60.0%).

4. DISCUSSION

The oral and swallowing characteristics of a group of pediatric patients with different NMD diagnosis were assessed in this study. The intraoral structural impairments in NMDs were macroglossia, malocclusion and high arched palate. Restricted opening of mouth, fatigue during mastication and fasciculations in tongue were found as oral functioning problems. The complaints of patients related to pharyngeal swallowing problems were persistent wheezing and secretion, coughing during feeding, difficulty in swallowing, food getting stuck in throat, change in voice quality after feeding and recurrent pulmonary infection. Also five of the patients failed the three-ounce water swallow test.

Intact oral structures play a crucial role in oral preparation phase in NMDs. Occlusal impairments may lead to problems

in oral preparatory phase of swallowing function especially in chewing process. Occlusal problems such as open bite may cause problems like leaking food from mouth during feeding and uncontrolled saliva. However, the most important handicap of occlusal impairments is the failure in mechanical breakdown of food for forming appropriate bolus ready to swallow. If foods could not have been converted into a bolus ready for swallowing, appropriate pharyngeal triggering would not occur. As a result of this, all phases of the swallowing would be affected (19). In our study, malocclusion was reported to be the most common oral structural impairment especially in DMD which may lead insufficient chewing function, and a limited number of study reported this problem as one of the predisposing factors for this insufficiency (20).

The tongue should be capable of manipulating the foods sufficiently to convert into bolus. When the tongue becomes extremely hypertrophic (macroglossia) as seen in our study population, it covers a lot of space in the mouth. Hence, this problem does not allow enough mobility of tongue in the mouth. It is emphasized that macroglossia which can be seen in pediatric NMDs may lead to swallowing problems (4). In a videofluroscopic study with 102 DMD patients, reduced tongue mobilization due to macroglossia was reported to lead inefficient bolus transport to the pharyngeal region (21). Tongue fasciculation which is an another impairment seen in our SMA type 2 patients was identified as a common problem in diseases with bulbar weakness previously (22). Basically, fasciculation indicates weak tongue muscles. Thus, loss of muscular strength of the tongue affects swallowing function negatively, rather than fasciculations. Weakened tongue leads to inefficient bolus formation and bolus transporting from base of the tongue to pharyngeal region (23).

High arched palate was commonly reported to be seen in patients with cerebral palsy, and partially in pediatric neuromuscular diseases (24). High arched palate was found to be a problem in our study similar to previous studies. This finding is important for it may cause a disadvantage in transportation viscous and solid foods to the pharynx due to the excessive space between the tongue and the palate (25). This may be the reason of why we had patients with problems with difficulty in swallowing in our study.

Restriction in the opening of the mouth occurs based on different problems such as stiffness in masticator muscles, temporomandibular joint problems, weakness in masticator muscles etc. In our study, restriction in the opening of the mouth in our SMA type 2 patients was thought to be originated from weak masticator muscles. This problem may affect oro-motor functions such as chewing, speaking, and swallowing.

Fatigue during mastication seen in our patients may be associated with the loss of strength and endurance of the masticator muscles. In patients who have fatigue during mastication may have a tendency to swallow bolus without adequate chewing. As a result, insufficiently fragmented and softened foods may cause difficulty in swallowing and residue on vallecula, pharyngeal wall, and pyriform sinuses in pharyngeal phase of the swallowing, and also extended feeding time.

The findings such as coughing during feeding, change in voice quality after feeding, difficulty in swallowing, food getting stuck in throat, persistent wheezing and secretion, and recurrent pulmonary infections were thought to be the signs of pharyngeal swallowing disorders in our pediatric patients with NMDs. Coughing during feeding and change in voice quality after feeding indicate food penetration into the vocal cords which are impairments of airway protection mechanism (26). Problems related to the protection of the airway may be seen in a certain extent in patients with pediatric neuromuscular diseases (23). Difficulty in swallowing depends on inadequate tongue base pressure and deficiency in pharyngeal constructor muscle strength (27). Likewise, food getting stuck in throat is related to insufficient squeezing function in the pharyngeal phase of the swallowing (28). Difficulty in swallowing as well as food getting stuck in throat may lead to residue on pharyngeal wall, vallecula and pyriform sinuses. After swallowing remaining residue in these structures constitute a serious risk factor for aspiration. Moreover, these problems may cause a more dramatic result such as airway obstruction. In recent studies, problems due to insufficient pharyngeal muscle strength have been reported especially in SMA type 2 patients (8,29). The risk of aspiration mentioned above may be the cause of persistent wheezing and secretion, and recurrent pulmonary infections except for problems such as immobilization and weak cough force in NMDs.

Three-ounce water swallow test is an important indicator for detecting insufficient airway protection mechanism supporting our previous findings related to complaints of swallowing disorder. This clinical swallowing evaluation of our patient population showed that the percentage of pharyngeal swallowing disorders was 11.1%. However, approximately 40% of food aspirations are silent (without coughing). Therefore, the consideration to have adequate airway protection mechanism in patients who successfully performed this test may be misleading. The rate as 11.1% is also an important rate for this problem may result in life-threatening conditions.

Our limitations are, not using an advanced assessment method such as videofluoroscopy to detect aspiration symptoms and no following period of time. Studies with larger sample size are needed in the future.

5. CONCLUSION

As a conclusion, a large variety of problems related to oral structure, function, and swallowing were observed in pediatric NMDs. The severity of these problems had also a wide spectrum. These patients may present with more destructive manifestations when swallowing problems are added to their current neuromuscular problems. Therefore, swallowing problems should not be ignored in these patients

due to their life-threatening results. Information obtained for the possible swallowing disorders in patients with NMDs with the use of cheap and easily performed evaluation methods play a key role in terms of consulting to advanced tests such as Videofluoroscopy, Fiberoptic Endoscopic Evaluation of Swallowing (FEES) and appropriate treatment options.

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Clinical and Experimental Health Sciences

Management of Deep Gingival Recessions by Modified Coronally Advanced Tunnel Technique with Titanium Platelet Rich Fibrin Membrane or Connective Tissue Graft: 36 Months Follow-up Clinical Study

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ABSTRACT

Objective: The goal of the study is to evaluate the results of Titanium-Platelet Rich Fibrin (T-PRF) membrane and Connective Tissue Graft (CTG) with modified coronally advanced tunnel technique (MCATT) in treatment of deep gingival recession defects.

Methods: Twenty-one systemically healthy patients displaying 62 Miller Class I/II gingival recession defects ≥ 3.0 mm in depth, treated either with MCATT with CTG or with T-PRF membrane were included in this retrospective study. The periodontal parameters were assessed at baseline, and at 6 and 36 months after surgery. The percentages of the mean root coverage (MRC) and complete root coverage (CRC) were calculated.

Results: The probing depth values were decreased at 36 months according to baseline values for both groups *(p<0.05)*. Keratinized tissue (KT) was increased at 6 months according to baseline for both groups (from 1.69±0.74 mm to 3.61±0.67 mm for T-PRF; and 3.40±1.60 mm to 4.52±2.33 for CTG). The 36th month measurement of KT showed an increase in the T-PRF group compared to the 6th month measurement, while the CTG group showed a significant decrease (3.86±0.76 mm and 2.76±1.45 mm, respectively). The CRC ratios were 80% and 56% at 6 and 36 months, respectively for the CTG group. However, this ratio remained the same (64.86%) for the T-PRF group. There was statistically significant difference between CRC ratios of both groups at 36 months *(p<0.05)*.

Conclusion: T-PRF membrane with MCATT procedure is as predictable as CTG with MCATT for management of deep gingival recessions. However, future prospective studies about this topic with a split-mouth design are needed.

Keywords: Gingival recession; titanium-platelet rich fibrin; root coverage; periodontal plastic surgery

1. INTRODUCTION

Gingival recession (GR) is defined as exposure of the root surface due to apical migration of the gingival margin beyond the cemento-enamel junction (CEJ) (1). The exposed root surfaces are commonly incorporated with root hypersensitivity, esthetic problems, and difficulties to obtain optimal oral hygiene level (2). The main goal of plastic periodontal surgical procedures is to ensure an optimal esthetic appearance with complete root coverage (CRC), and gingival recessions have been treated by various techniques (1-3). For this purpose, the laterally advanced flap (LAF), double papilla flap (DPF) or the coronally advanced flap (CAF) was used alone or with a connective tissue graft (CTG) (4). Complete root coverage can predictably be obtained when using CAF or CAF with CTG biomaterials in single Miller Classes I and II GR (5). Additionally, a recent systematic review indicated that CAF and modifications of this technique solely or combined with CTG may give the most predictable results and improve the long-term stability in multiple adjacent gingival recessions (MAGR) (2). On the other hand, CTG harvesting is frequently associated with a prolonged surgical time, limited donor tissue, increased post-operative discomfort and the possibility of post-surgical bleeding (6,7). Many attempts have been made to develop alternative materials that aim to replace CTG to improve patient acceptance and reduce patient discomfort associated with root coverage (3). Autogenous and non-autogenous materials, acellular dermal matrix, collagen matrix, platelet-rich plasma, and platelet-rich fibrin (L-PRF) in combination with CAF, were used for these purposes (3,8-12).

L-PRF was developed by Choukroun et al., in France in 2001 and described as an autologous leukocyte and platelet rich biomaterial (13,14). The advantages of the L – PRF

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technique over the other platelet-rich products include shorter and simplified preparation, low expense and lack of a requirement for an anticoagulant or any gelling agent (14). Previous studies have demonstrated that autologous growth factors, such as Platelet Derived Growth Factor (PDGF-AB), Transforming Growth Factor (TGF), Vascular Endothelial Growth Factor (VEGF), cytokines, and healing proteins (fibrinogen, fibronectin, etc.), were released in the L-PRF (14,15). L-PRF consists of a dense fibrin matrix that can be easily trimmed, adapted and sutured. In recent years, successful clinical results have been achieved with this autogenous material in GR treatment (11,12). However, in the original protocol, glass or glass-coated plastic tubes are used, and the unavoidable silica contact was described as follows: The silica particles in the tube are sufficiently small for a fraction to remain pendant colloidally in parts of the L-PRF (fibrin, buffy coat, and platelet poor layers of plasma) and might reach the patient (16). The initial L-PRF method was modified by our study group in changing the structure of the tubes and used a more biocompatible material (titanium), which was named titanium-prepared platelet-rich fibrin (T-PRF) (17). The fibrin in the T-PRF samples seemed thicker and better organized than L-PRF samples. As a result, there is degradation of fibrin and release of growth factors for a longer time period (18). The results of the first clinical cases demonstrated that T-PRF combined with CAF is a safe, effective choice for treating gingival recessions (GR) without complication or significant morbidity. Therefore, the aim of this clinical study was to evaluate the effectiveness of a T-PRF combined with MCATT in treating multiple adjacent Miller Class I/II GR ≥ 3.0 mm in 36 months.

2. METHODS

The present study protocol was reviewed and approved by the Ethical Committee of the Istanbul Aydin University, Faculty of Dentistry (Reg. No: 196). The study was conducted between 2012 and 2016 in accordance with the Helsinki Declaration in 2000. Written informed consent was obtained from all patients after detailed information on the risks and benefits of the clinical investigation and associated procedures was provided.

This multicenter study was designed as a retrospective trial to evaluate the results of titanium-platelet rich fibrin membrane with modified coronally advanced tunnel technique in treatment of deep gingival recession defects. The T-PRF subjects were treated at the Periodontology Department of Istanbul Aydin University, the CTG subjects were treated at the Periodontology Department of Karadeniz Technical University. At the end of the phase I treatment (oral hygiene instructions + full mouth scaling + polishing), patient selection was based on the following criteria: maxillary/mandibular, multiple adjacent Miller Class I/II GR ≥ 3.0 mm in depth; complaint of esthetic problems or dental hypersensitivity; vital teeth; no caries or prosthetic restoration on the selected teeth with partially fixable enamel-cement border; no bleeding on probing at the surgical sites; and no previous surgical treatment in the recession site. These criteria were met by twenty-one systemically healthy patients. The exclusion criteria were as follows: current smoking; fixed orthodontic or removable prosthesis; root surface restorations on surgical sites; any systemic disease that might be a contraindication for periodontal surgery; and lactation or pregnancy.

Sample size calculation was provided with G Power 3.1. software (Statistical Power Analyses for Windows and Mac, Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany). The sample size calculation was confirmed according to the previous study (19). The alpha error=0.05, beta error=0.80 and effect size=0.35 were selected for the required sample size. Final analysis showed that 55 samples could be adequate for the study design. However; it was averaged 60 for the possible dropouts. Since we have 62 samples for the-study design (in our data base); we used 62 (37/25) teeth which is acceptable and above than the 60.

Three patients not involved in the present study were used to test the reproducibility of the clinician for two sessions 24 hours apart. The intra-examiner calibrations within 1 mm for gingival recession and probing depth (PD) measurements ≥ were 90%. A computer-generated randomization scheme was used by one of the authors. To hide the allocation, number-labeled opaque envelopes including the name of the assigned surgical method were used. The plaque index (PI) and gingival index (GI) were scored and the following periodontal parameters were recorded at baseline, and at 6 and 36 months after surgery using a standard periodontal probe (PCP-UNC 15, Hu Friedy, Chicago, IL, USA): GR, distance from CEJ to the gingival margin; PD, distance from the gingival margin to the base of the gingival sulcus; clinical attachment level (CAL), distance from CEJ to the bottom of the gingival sulcus; and keratinized tissue (KT), distance from the gingival margin to the mucogingival junction (MGJ). The Lugol's iodine solution was used for staining the mucogingival complex and KT could be assessed visually. The measurements were recorded on the mid-buccal point of teeth and rounded up to the nearest millimeter. The percentages of the mean root coverage (MRC) and complete root coverage (CRC) were calculated according to the following standard formulas:

Percentage of RC = ([preoperative GR – post-operative GR]/ pre-operative GR) x 100.

Percentage of CRC = (teeth with complete root coverage/all treated teeth)]x 100.

The present study's primary outcome variable was the percentage of RC. The secondary outcome variables were KT and AL.

The T-PRF was prepared according to the L-PRF protocol. 10 mL of blood was quickly drawn from the antecubital vein of each patient's right/left arm and was transferred to the specially designed grade IV titanium sterile tubes without

any anticoagulant. The tubes were immediately centrifuged at 2,700 rpm for 12 minutes using a table centrifuge (Hettich Universal 320, Hettich Zentrifugen, Germany) at room temperature (20). After centrifugation, the T-PRF clot (in the middle part of the tube) was removed with sterile tweezers, separated from the red blood cell base without using scissors, and placed on sterile woven gauze to release the serum slowly over 10 minutes. Then, the clot was compressed to create a constant thickness of the T-PRF membrane.

The graft width was measured to include 1 mm beyond the root surface defects in the recipient area. The borders of the start and finish incisions were marked after palatal anesthesia. Subepithelial connective tissue graft excluded the periosteum was removed and maintained in physiological saline. The palate was sutured with 4-0 absorbable poly (glycolide-co-lactide) synthetic sutures (Pegalak, Doğsan, Turkey) and covered with non-eugenol periodontal dressing (Voco Pac, Cuxhaven, Germany). Before placing the connective tissue in the recipient area, the fat and glandular tissues and the band-shaped epithelium on the connective tissue were removed using scissors.

After local anesthesia (Ultracain D-S forte, Hoechst Roussel, Frankfurt, Germany), root planning (using Gracey curettes: Hu-Friedy Inc., Chicago, IL, USA) of exposed root surfaces was performed to reduce convexity, and no root conditioning was used. The operation sites were prepared without any vertical releasing incisions as previously described (21). The intrasulcular incisions were made with a 15-C blade involving affected teeth and were extended to one tooth adjacent on both sides. The mucoperiosteal flaps were raised using specially designed tunnel knifes (Helmut Zepf Medizintechnik GMBH, Eitingen-Oberflacht, Germany). The partial-thickness buccal flap was raised beyond the mucogingival junction for tension-free mobilization for coronal positioning. This part of the surgery was performed with great care to avoid flap perforation. Afterwards, T-PRF membrane or CTG was placed and gently pushed into the tunneled area with an instrument and sutured. In the final step, the flaps were positioned slightly coronally to the CEJ with vertical mattress sutures. The 5-0 absorbable poly (glycolide-co-lactide) synthetic sutures (Pegalak, Doğsan, Turkey) were used.

The patients were given a cold compress extra-orally for the first 24 hours to minimize bleeding and swelling. All patients were given an appropriate non-steroidal anti-inflammatory drug (2x550 mg naproxen sodium for the first 3 days Abdi İbrahim, Apranax Fort, Istanbul, Turkey) and 0.2% chlorhexidine mouthwash (3x1, for 1 minute, 2 weeks; Klorhex, Drogsan, Ankara, Turkey). Advice was given to patients to brush all teeth except those in the operated region. Sutures were removed, and the surgical wounds were gently cleansed with 0.12% chlorhexidine digluconate solution two weeks after treatment. The patients were informed to use a post-operative surgical toothbrush (Curaprox CS Surgical MegaSoft Brush) only in the surgical area for four weeks. Afterwards, plaque control was reinforced, and the modified Stillman technique was taught with an ultrasoft toothbrush (Curaprox Ultrasoft Brush CS 5460, CURADEN International, Ankara, Turkey). The patients were invited to control visits 1, 3, 6, 12, 18, 24, 30, and 36 months after surgery. In the present study, the post-operative clinical measurements of the 6. and 36. months are presented.

The statistical analysis was performed using a commercially available software program (IBM Corp. Released 2013, Version 22.0. Armonk, NY, USA). The results were averaged (mean ± standard deviation [SD]) for all clinical parameters at baseline, and at the 6^{th} and 36^{th} months. The data were not normally distributed, and non-parametric tests were performed. Friedman test was used to perform the statistical analysis. The differences between examination times were evaluated by the Wilcoxon Signed Ranks Test. Mann-Whitney U test was used for the MRC differences in Miller Classes I and II. A p value <0.05 was considered as a statistically significant difference.

3. RESULTS

Sixty-two recession defects in 21 patients (12 females and 9 males; mean age of the patients: 43.4) were treated, and all patients completed examinations for 36 months. There were no significant differences between the groups in terms of gender and age distribution ($p > 0.05$). Wound healing was uneventful at all operated sites (there were no postoperative complications, severe pain, post-operative bleeding or edema). No complaints of esthetic problems or dental hypersensitivity were recorded after operations and in the evaluation periods. The statistical analyses for the clinical parameters are summarized in Table 1.

The post-operative PI and GI scores at 6 and 36 months were significantly lower than the baseline records for both groups ($p<0.05$). The probing depths values were significantly

Table 1. *Descriptive statistics of the periodontal parameters at baseline and post-surgical measurements.*

Periodontal Parameters	n	Baseline (mean ± SD)	6th month (mean ± SD)	36th month (mean ± SD)	p
PD (mm)					
T-PRF	37	1.19±0.29	1.10±0.26	1.08±0.20[b,c]	0.141 (0-1) 0.024 (1-2) 0.007 (0-2)
CTG	25	2.01±0.59	2.29±0.47	1.48±0.54[b,c]	0.130 (0-1) 0.000 (1-2) 0.006 (0-2)
GR (mm)					
T-PRF	37	4.12±0.88	0.30±0.45[a]	0.27±0.40[b]	0.000 (0-1) 0.157 (1-2) 0.000 (0-2)
CTG	25	3.60±0.80	0.16±0.37[a]	0.56±0.71[b,c]	0.000 (0-1) 0.021 (1-2) 0.000 (0-2)
KT (mm)					
T-PRF	37	1.69±0.75	3.61±0.68[a]	3.86±0.76[b,c]	0.000 (0-1) 0.003 (1-2) 0.000 (0-2)
CTG	25	3.40±1.60	4.52±2.33[a]	2.76±1.45[c]	0.034 (0-1) 0.000 (1-2) 0.078 (0-2)

*T-PRF: Titanium Platelet Rich Fibrin; CTG: connective tissue graft; PD: probing pocket depth; GR: gingival recession; KT: keratinized tissue; SD: standard deviation; *statistically significant difference (*Friedman test; p<0.05), Wilcoxon Signed Ranks Test: [a]Significant difference between baseline and the 6th month (p<0.05). [b] Significant difference between baseline and the 36th month (p<0.05). [c]Significant difference between the 6th and the 36th month (p<0.05). 0-1: Baseline – 6th month; 1-2: 6th month – 36th month; 0-2: Baseline – 36th month.*

lower at 36th month according to baseline values for both groups *(p<0.05).* The baseline PD value of the T-PRF group was 1.19±0.29 mm; the 6th and the 36th month values were 1.10±0.26 mm and 1.08±0.20 mm, respectively. This value was 2.01±0.59 mm at baseline, 2.29±0.47 mm at the 6th month, and 1.48±0.54 mm at the 36th month for the CTG group.

The baseline mean GR value of the T-PRF group was reduced from 4.12±0.88 mm to 0.30±0.45 at the 6th month (*p<0.05*) and to 0.27±0.40 mm at the 36th month (*p<0.05*). CTG group baseline GR value (3.60± 0.80) was also, significantly decreased to 0.16±0.37 mm and to 0.56±0.71 mm at the 6th and the 36th months, respectively (*p<0.05*).

The KT showed a significant increase from baseline to the 6th month (average from 1.69±0.74 mm to 3.61±0.67 mm, (*p<0.05*)) T-PRF group. At the 36th month, the KT continued to increase, but this was not significant (Table 1). The baseline KT showed an increase in the CTG group at the 6th month (average from 3.40±1.60 mm to 4.52±2.33 mm (*p<0.05)).* However, this value was significantly reduced to 2.76±1.45 mm at the 36th month (*p<0.05).*

The root coverage (RC) values of the groups were presented at Table 2. At the 6th month, the RC values were 3.82±0.89 mm, and they were 3.85±0.87 mm at the 36th month for the T-PRF group (*p>0.05)*. However, there was a significant reduction of RC values in the CTG group at the 36th month according to the baseline (3.44±0.97 mm at the 6th month; 3.02±1.05 mm at the 36th month (*p<0.05)).* There was a significant difference between the groups at the 36th month by mean of RC (*p<0.05).* The MRC percentages were 93.01% and 93.68% following CAF combined with T-PRF treatment at the 6th and the 36th months, respectively. This percentage was reduced at the CTG group at the 36th month. The MRC percentages were 94.45% and 84.31% at the 6th and the 36th months, respectively. The percentage of CRC was stable for the T-PRF group (64.86% at the 6th and the 36th months). However, a significant reduction of CRC was observed at the CTG group (80% at the 6th month and 56% at the 36th month). The baseline and post-operative 6th and 36th month results of the T-PRF and the CTG treated multiple recession-type defects were as shown in Figure 1 and 2.

Table 2. *Descriptive statistics of the root coverage at the 6th and the 36th months.*

RC	n	6th month (mean ± SD)	36th month (mean ± SD)	p
T-PRF	37	3.82±0.90	3.85±0.87	0.157
CTG	25	3.44±0.97	3.02±1,05	0.005*
p		0.093	0.001	

*T-PRF: Titanium Platelet Rich Fibrin; CTG: connective tissue graft; RC: root coverage; SD: standard deviation; *Wilcoxon signed rank test (p<0.05);*

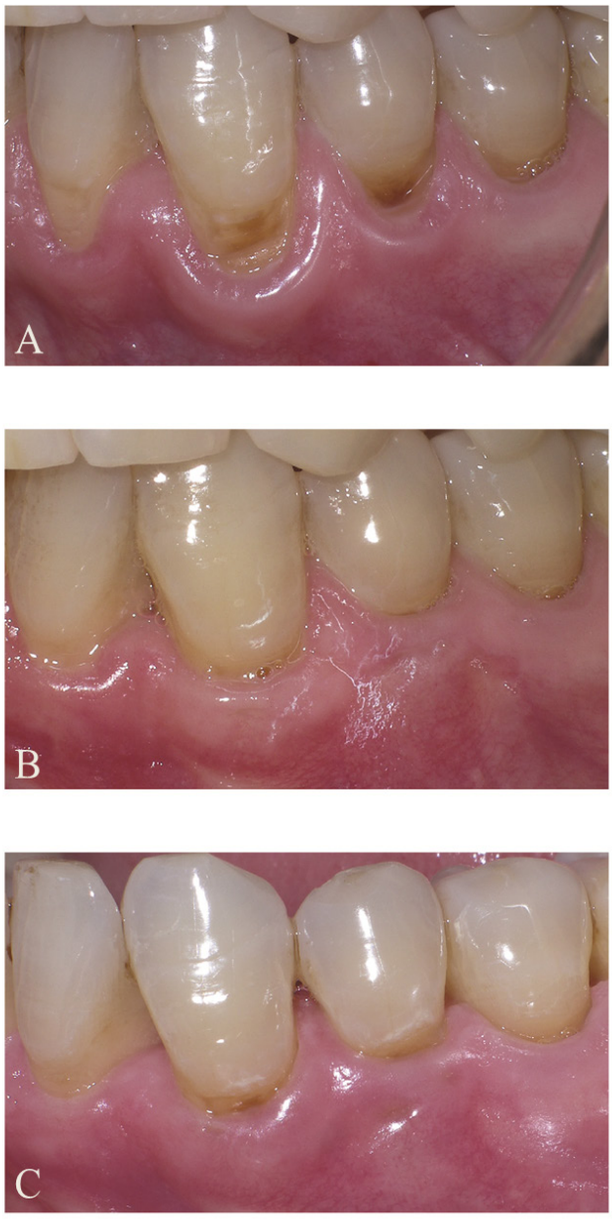

Figure 1: *Results of treatment of multiple recession-type defects with Titanium Platelet Rich Fibrin on the left mandibular site of the patient. A: Preoperative view of gingival recessions; B: 6 months after surgery; C: 36 months after surgery.*

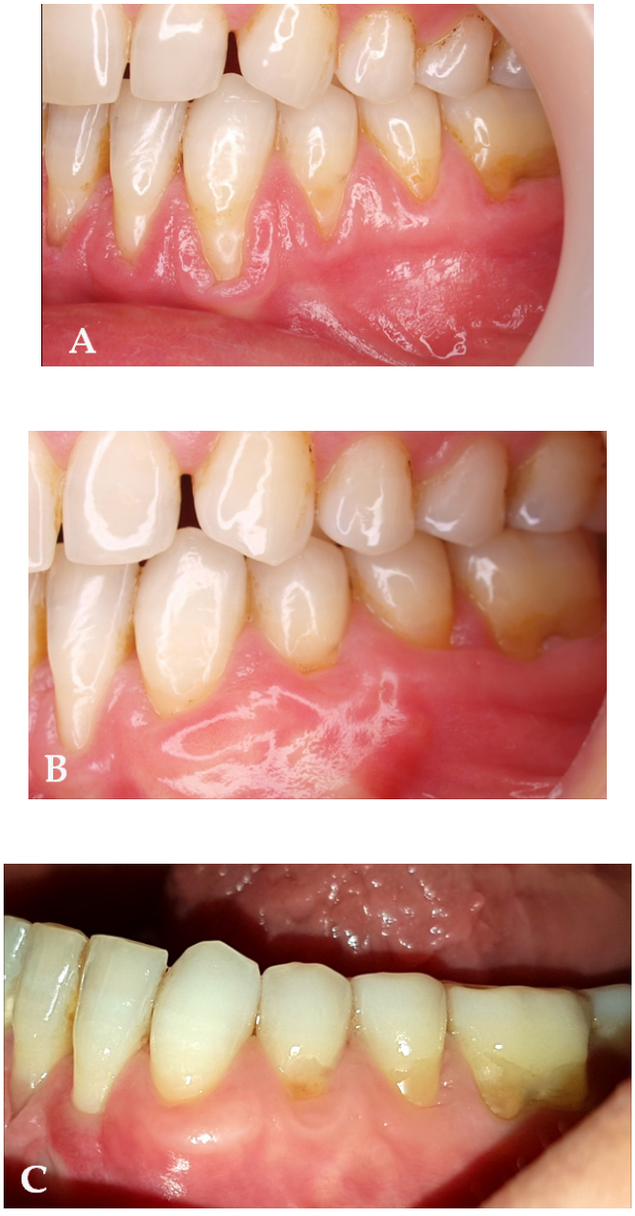

Figure 2*: Results of treatment of multiple recession-type defects with connective tissue graft on the left mandibular site of the patient. A: Preoperative view of gingival recessions; B: 6 months after surgery; C: 36 months after surgery.*

4. DISCUSSION

To the best of our knowledge, the present retrospective clinical study is the first to evaluate the effectiveness of T-PRF (as compared with CTG) in treating gingival recessions in 36 months. The present data demonstrated that this autogenous material is an effective method to cover recession defects.

Aroca et al., compared the modified coronally advanced flap (MCAF) alone with MCAF+L-PRF, and the MRC levels were 91.5±11.4% and 80.7±14.7%, respectively, at 6 months. They reported no additional benefits of L-PRF combined with CAF in terms of periodontal parameters. In addition, the difference between the groups was statistically significant, which favored modified CAF alone (3). This result could be explained by dehydration of the L-PRF membrane due to the inappropriate conditions (refrigerator storage) in an unidentified time period. Additionally, researchers applied only one layer of the membrane, while they must use at least two layers of membranes in gingival recession sites (22). Recent studies reported similar root coverage results with L-PRF to CTG (11,12,23). Uraz et al., evaluated the CAF combined with CTG or L-PRF in multiple adjacent gingival recessions (MAGR), and similar MRC percentages were obtained (23). Tunali et al., demonstrated that the use of both

CTG and L-PRF options were effective treatment methods in MAGR according to the 12-month results (77.36% and 76.63%, respectively) (12). Similarly, Eren and Atilla found no statistically significant difference between CAF + L-PRF and CAF + CTG in terms of MRC in localized gingival recessions (92.7% and 94.2%, respectively) (11). However, in a recent randomized clinical trial, the combination of MCAF and L-PRF or CTG was compared. The percentage of MRC was 77.12% in the test group and 84% in the control control (24).

L-PRF is a second-generation platelet concentrate, and T-PRF is a modification of the initial method by changing the structure of the tubes with a more biocompatible titanium material (17). In this study, although 6-month follow-up period, similar results were obtained with previous studies, it was shown that T-PRF was better in RC than CTG after 36 months (11,12,23). In a recent study, the clinical effects of T-PRF on human palatal mucosal wound healing were evaluated. Because of the positive results, the researchers concluded that T-PRF is a promising autogenous material for histoconduction, and it may also be preferred as an alternative to CTG in the treatment of gingival recessions (25).

The CRC values differed between studies with L-PRF (52.2% to 75.85%) (3,11,22,25). Aroca et al., obtained a CRC of 52.2% of the sites treated with MCAF + L-PRF and 74.6% for those treated with MCAF alone (3). Similarly, in a recent study, CRC obtained in the MCAF + L-PRF and MCAF + CTG groups had values of 50% and 60% (24). However, Uraz et al., achieved superior results in the CAF + L-PRF group (73.3%) and the CAF + CTG group (80%) (23). The similar groups were compared in another study, and there were no statistically significant differences between the groups for the CRC percentages (72.7% and 77.3%, respectively) (11). In the present study, slightly lower CRC values were obtained. It may be related to baseline recession depth values (RC≥3 mm).

It is known that CTG obtained from the palatinal region provided keratinized tissue gain (KTG) because of the ability to induce keratinization of the epithelium (26). Chambrone et al., reported that CTG seems to be more adequate, and both KT and root coverage are expected (5). The KT in cases treated with L-PRF may be explained by the positive effect of proliferation of gingival and periodontal fibroblasts as a result of the influence of growth factors released from thrombocytes in the natural fibrin matrix (11,27). In the present study, a statistically significant increase in KT was observed at 6 months after surgery compared to baseline in both groups ($p<0.05$). Surprisingly, the 36th month measurement of KT showed an increase in the T-PRF group compared to the 6th month measurement, while the CTG group showed a significant decrease. However, Aroca et al., compared the MCAF and MCAF + L-PRF, and a significant decrease in KT values was reported at 6 months in both groups (3). In contrast to the first published L-PRF study for the treatment of gingival recession, the other studies described that a significant increase in KT was detected in L-PRF groups at different examination times after surgery (11,12,23).

The significance of gingival thickness (GT) in periodontal plastic surgeries for gingival recessions was reported in a previous review (28). It should be noted that initial GT has more influence than KT for the root coverage (29). Prior studies have evaluated the effect of L-PRF on the gingival thickness (3,11). According to Aroca et al., the application of MCAF alone did not positively or negatively affect the gingival thickness at the 6th month (3). The addition of L-PRF formed a statistically significant increase in GT (0.3 mm on average). The reason for the soft tissue thickness increase was speculated to be due to the proliferative effect of growth factors in L-PRF on gingival and periodontal ligament fibroblasts or to a spacing effect of the L-PRF as a membrane. In a recent study, the application of CAF with L-PRF or CTG in localized gingival recessions was evaluated, and GT increased up to 1.5 mm in both groups (11). The results of this study were much better than those reported by Aroca et al. (3). The initial or post-operative GT was not measured, and this was one of the limitations of the present study. Additionally, the smaller sample size was another limitation of the study.

5. CONCLUSION

Within the limits of this study, the results demonstrated that T-PRF with the MCATT procedure is a safe, effective method in treating class I and II Miller gingival recessions. In addition, this procedure can be recommended to treat localized or multiple-adjacent gingival recessions without additional surgery. However, future randomized clinical trials with a split-mouth design and larger sample size are essential for evaluating the T-PRF efficiency in gingival recession treatment modalities.

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Clinical and Experimental Health Sciences

Evaluation of the Biological and Wound Healing Activities of *Centaurea virgata* Lam.

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ABSTRACT

Objective: Plants have significant potential effects in treating wounds. Some plants are also used in traditional medicine for their wound healing properties. The aim of this study was to investigate the antioxidant and *in vitro* wound healing activities of *Centaurea virgata*.

Methods: The antioxidant activities of extracts were examined by 2,2-diphenyl-1-picryl-hydrazyl (DPPH) and 2,2-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid (ABTS) methods. The total phenolic content was determined using the Folin-Ciocalteu reagent (FCR) method. In addition, the antiproliferative activity of different extracts on fibroblast cells was performed by MTT method and *in vitro* wound healing activity of ethanol extract of *C. virgata* was determined by scratch assay.

Results: Ethyl acetate fraction of ethanol extract of *C. virgata* (CVEA) had the highest DPPH radical scavenging activity with an IC_{50} value of 138.7 µg/mL. According to ABTS results, it was determined that the CVEA had a stronger radical scavenging activity than the other extracts. Hexane (CVH) and aqueous ethanol fractions (CVAE) revealed poor antioxidant activity with IC_{50} values of 824.8 µg/mL and 610.3 µg/mL, respectively. On the other hand, among all of the extracts analysed, it was found that phenolic content of CVEA was higher than other extracts. Except for CVAE, other extracts showed antiproliferative activity depending on the dose.

Conclusion: According to the obtained results, our finding suggests that CVAE has a migratory effect on fibroblasts and that CVAE might be a potential therapeutic agent for wound healing.

Keywords: *Centaurea virgata*, antioxidant activity, wound healing

1. INTRODUCTION

Wound healing is a collection of highly coordinated biological events consisting of three intertwining phases such as inflammation, proliferation and remodeling (1). Arachidonic acid metabolites released from thrombocytes collected in the wound area, proteases, and various growth factors contribute to the formation of the inflammation process. At the end of this phase, the number of immunologic cells begins to decrease; endothelial cells and fibroblasts emerged in the wound area and they initiate the proliferative phase (2). Within 2-3 days of injury, angiogenesis is indicated as the beginning of the proliferative phase. In angiogenesis, new blood vessels are formed from the epithelial cells to provide nutrients and oxygen for new cells. Epithelial cells proliferate into the wound areas. On the other hand, fibroblasts proliferate and differentiate into myofibroblasts. At the same time, fibroblasts secrete collagen and fibronectin to form new connective tissue (3). In the course of wound healing, keratinocytes differentiate into dermis so that the remodeling phase begins. The most important feature of this phase is the increase of collagen accumulation in the wound area (4). Despite the great success achieved through research on the mechanism of wound healing, the development of effective therapeutics for wound healing remains a complex issue for scientists.

Plants have significant potential effects wound treatment. Also, there are reports showing that some plant species have been traditionally used by the public to treat wounds (5-8). Secondary metabolites found in plants such as phenolic acids, tannins, flavonoids and triterpenes are known to have wound healing activities (5,9). These natural products or compounds accelerate the regeneration and healing of tissue loss through complex mechanisms. Today, many phyto-pharmacology laboratories are working extensively to isolate the active components of various medical plants and to determine their mechanism of action. The screening of herbal extracts is of great interest to scientists for the discovery of new effective drugs in the treatment of wounds. For this purpose, *in vitro* wound healing activity of *C. virgata*, which is used by the people for wound healing purposes, was investigated in our current study.

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The genus *Centaurea*, the fourth largest genus of the Asteraceae family, has about 400-700 species on the earth. They usually spread around the Middle East and east of the Northern hemisphere (10). *Centaurea* genus is widely distributed in Turkey. It has many medicinal properties including anti-inflammatory, antidiabetic, digestive, antipyretic, cytotoxic, anti-dandruff and antibacterial (11-13). *Centaurea virgata* Lam. is a species used in the traditional medicine in Turkey for the treatment of stomach pain, jaundice, headache, kidney stone and also as a diuretic and wound healing. (14). As far as we know, there is no report on the wound healing activity of *C. virgata*. Therefore, we aimed to determine *in vitro* antioxidant and wound healing activities of *C. virgata* in this study.

2. MATERIAL AND METHODS

2.1. Plant Material Collection and Extraction

Plant samples were collected in the flowering periods from the Çatalça district of Istanbul province of Turkey in 2010 and were identified by Dr. İsmail Şenkardeş, a botanist of the Faculty of Pharmacy, University of Marmara. Voucher specimens were deposited in the Herbarium of the Faculty of Pharmacy, Marmara University (Mare No: 22438)

Dried and ground aerial part of *C. virgata* (16.18 g) were extracted with (6 × 200 mL) EtOH, using an ultrasonic bath. After filtration and evaporation, the ethanol extract (CVE) was dissolved in 30 mL 60% aqueous ethanol, and subjected to solvent-solvent partition between n-hexane (5 × 50 mL), chloroform (3 × 50 mL), and ethyl acetate (2 x 50 mL). The n-hexane, chloroform, ethyl acetate and aqueous ethanol fractions of ethanol extracts of *C. virgata* obtained by this method were coded as CVH, CVC, CVEA and CVAE, respectively. All extracts were stored under refrigeration for further analysis.

2.2. 2,2-diphenyl-1-picryl-hydrazyl (DPPH) Radical Scavenging Activity

Free radical scavenging capacity of *C. virgata* extracts were evaluated according to the previously reported procedure using the stable DPPH (15). Briefly, 10 µL of extracts in DMSO at different concentrations (250-0,048 µg/mL) were added to 190 µL methanol solution of DPPH (0,1 mM) in a well of 96-well plate. The mixture was shaken vigorously and allowed to stand in the dark at room temperature for 30 min. Absorbance readings were taken at 517 nm. The percent radical scavenging activity of extracts and standard against DPPH were calculated according to the following:

DPPH radical-scavenging activity (%) = $[(A_0–A_1)/A_0]\times100$

where A_0 is the absorbance of the control (containing all reagents except the test compounds), and A_1 is the absorbance of the extracts/standard. Extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotting inhibition percentage against extracts concentration. Tests were carried out in triplicate. Butylated hydroxyanisole (BHA), ascorbic acid and trolox were used as positive control.

2.3. 2,2-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid (ABTS) Radical Scavenging Activity

Free radical scavenging capacity of *C. virgata* extracts were evaluated according to the previously reported procedure (15). ABTS radical cations were prepared by mixing equal volume of ABTS (7 mM in H_2O) and potassium persulfate (4.9 mM in H_2O), allowing them to react for 12-16 h at room temperature in the dark. Then, ABTS radical solution was diluted with 96% ethanol to an absorbance of about 0.7 at 734 nm. 10 µL of extracts in DMSO at different concentrations (250-0.048 µg/mL) were added to 190 µL of ABTS radical solution in a well of 96-well plate. The mixture was shaken vigorously and allowed to stand in the dark at room temperature for 30 min. Absorbance readings were taken at 734 nm. The percent radical scavenging activity of extracts and standard against ABTS were calculated according to the following:

ABTS radical-scavenging activity (%) = $[(A_0–A_1)/A_0]\times100$

where A_0 is the absorbance of the control (containing all reagents except the test compounds), and A_1 is the absorbance of the extracts/standard. Extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotting inhibition percentage against extracts concentration. Tests were carried out in triplicate. BHA, ascorbic acid and trolox were used as positive control.

2.4. Determination of Total Phenolic Content (TPC)

Total phenolic content of *C. virgata* extracts was measured using Folin–Ciocalteau reagent (16). The assay was adapted to the 96 well microplate format. 10 µL of extracts in various concentrations (151.52-18.94 µg/mL) were mixed with 20 µL Folin-Ciocalteu reagent (Sigma), 200 µL of H_2O, and 100 µL of 15% Na_2CO_3, and the absorbance was measured at 765 nm after 2 h incubation at room temperature. Gallic acid was used as a standard and the total phenolic content was expressed as 'mg gallic acid equivalent (GAE)/g extract'.

2.5. In-vitro Fibroblast Cell Proliferation Assay

The effects of plant extracts on fibroblast cell proliferation were determined by MTT method. The test samples were prepared by dissolving extracts in DMSO and followed by dilution with supplemented DMEM medium to obtain the final concentration of the extracts of *C. virgata* (10, 25, 50, 100 and 200 µg/mL, respectively). Briefly, NIH3T3 fibroblasts were seeded at $1x10^4$ cells/well into 96-well plate in DMEM containing 10% FBS. The final DMSO concentration in the assay was 0.1%. After 48h,cells were exposed to five different concentrations (10-200 µg/mL) of plant extracts and were then incubated for 48h at 37°C in a humidified atmosphere containing 5% CO_2. The MTT solution (5 mg/mL) was added directly to the each well and the plate was then incubated at 37°C for 4h. At the end of the incubation, 100 µL SDS was added to each well and then incubated for another 12 h solubilize the formazan formed in the viable cells. The absorbance of the samples was measured at 570 nm using

a microplate reader The percentage of cell proliferation was calculated and compared to a negative control.

2.6. Scratch Assay

Briefly, NIH3T3 fibroblasts were seeded at $5x10^4$ cells/well in DMEM containing 10% FBS were seeded into each well of 24 well plate and incubated at 37°C with 5% CO_2. After the confluent monolayer of NIH3T3 fibroblasts was formed, two horizontal scratches were generated using a sterile pipette tip of 100 µL in each well. Any cellular debris was removed by washing with PBS and replaced with 1 mL of fresh medium in the absence or presence of plant extracts. Photographs were taken as two views on the left and right of each well at 10x magnification using a microphotograph (Olympus CK2, Japan) on day 0; then, plates were incubated at 37° C with 5% CO_2 and photographs were taken at 24 h.

3. RESULTS

The DPPH and ABTS radical scavenging activity results are shown in Table 1 as comparable with known antioxidants such as ascorbic acid, trolox and BHA. CVEA had the highest DPPH radical scavenging activity with an IC_{50} value of 138.7 µg/mL. On the other hand, CVH and CVAE revealed a poor antioxidant activity with IC_{50} values of 827.8 µg/mL and 610.3 µg/mL, respectively. When the results obtained from ABTS radical scavenging activity were evaluated, it was determined that the CVEA had stronger radical scavenging activity than the other extracts. Similarly, CVH had the lowest radical scavenging activities.

Table 1. *Antioxidant Activities of Various Extracts Obtained From Different Parts of Centaurea virgata (n=3).*

Extracts*/ Standards	DPPH radical scavenging activity IC_{50} (µg.mL^{-1}) Mean ± SD	ABTS radical scavenging activity IC_{50} (µg.mL^{-1}), Mean ± SD
CVE	200.3 ± 1.49	240.5 ± 1.70
CVH	827.8 ± 13.72	487.4 ± 3.11
CVC	293.2 ± 1.74	234.6 ± 0.57
CVEA	138.7 ± 2.48	72.89 ± 0.44
CVAE	610.3 ± 12.8	288.8 ± 2.12
Ascorbic acid	17.6 ± 0.37	14.5 ± 0.32
Trolox	14.54 ± 0.18	13.00 ± 0.21
BHA	57.15 ± 0.09	17.06 ± 0.58

**SD: standard deviation; CVE, CVH, CHC, CVEA, CVAE show the ethanol extract and its n-hexane, chloroform, ethyl acetate, and aqueous ethanol fractions of the leaves of Centaurea virgata, respectively. BHA: Butylated hydroxyanisole.*

Table 2 presents total phenolic contents obtained for all the *C. virgata* extracts. Among all of the extracts analysed, it was found that phenolic content of CVEA was higher than that of the other extracts. While the content for CVH could not be determined, it was revealed that the CVAE extract had very low phenolic content.

Table 2. *Total Phenolic Contents of Various Extracts Obtained From C. virgata.*

Extracts*	TPC (mg GAE/g extract)**
CVE	23.83
CVH	-
CVC	24.05
CVEA	51.30
CVAE	2.88

**CVE, CVH, CHC, CVEA, CVAE show the ethanol extracts and its n-hexane, chloroform, ethyl acetate, and aqueous ethanol fractions of the leaves of Centaurea virgata, respectively. ** Results were expressed as gallic acid equivalent (GAE). –: could not be determined.*

MTT results revealed that CVC had the highest cytotoxic effect on NIH3T3 cells with an IC_{50} value of 23.07 µg/mL (Table 3). Except for CVAE, other extracts also showed cytotoxic effects depending on the dose.

Table 3. *The Proliferative Effect of Extracts on NIH3T3 Cells.*

Extracts	200 µg	100 µg	50 µg	25 µg	10 µg	IC50 (µg)
	% Proliferation					
CVEA	33.41	43.9	61.04	80.91	110.91	96.8
CVE	20.67	31.4	49.57	73.05	114.94	65.31
CVAE	95.06	95.79	97.4	104.8	107.8	4517.25
CVC	18.96	19.15	37.93	53.29	72.68	23.07
CVH	53.9	66.1	77.68	88.7	89.09	393.8

**CVE, CVH, CHC, CVEA, CVAE show the ethanol extract and its n-hexane, chloroform, ethyl acetate, and aqueous ethanol fractions of the leaves of Centaurea virgata, respectively.*

As a result of MTT analysis, wound healing experiments were studied with this extract since it was determined that only CVAE had no cytotoxic effect on the NIH3T3 cells. Depending on the dose, it was determined that CVAE increased the migration of fibroblasts in the scratch assay (Figure 1).

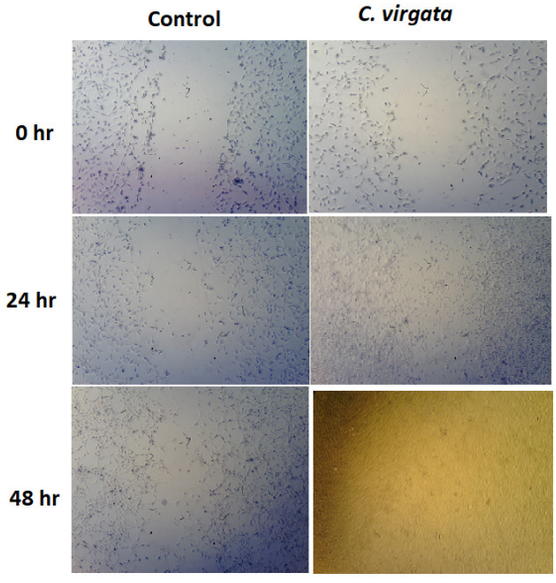

Figure 1. *CVAE promoted the migration and wound closure of NIH3T3 cells in a wound scratch assay.*

4. DISCUSSION

Plants have rich medical value and sources of molecules with potential wound-healing activity due to the phytochemical compounds they contain. Today, plant extracts are seen as an effective alternative for wound healing due to their widespread availability (8,17,18). In the current study, *in vitro* wound healing activity of *C. virgata* was revealed for the first time.

Wound healing is a rather complicated repair mechanism that begins after injury. Inflammation during the healing process leads to reactive oxygen species (ROS) formation, which is known for its harmful effects. The wound healing improving effects of various plant extracts can be attributed to the antioxidant and antimicrobial properties of the phytocomponents (17). Studies have shown a positive role between the free radical scavenging effect and the wound healing process.

Dermal fibroblasts are defense cells that respond to injury. Proliferation and migration of fibroblasts to the wound site are important events in the wound healing process. It is a useful model for stimulating fibroblast cell growth and studying wound healing activity *in vitro*. Therefore, isolation of active compounds found in natural products that affect migration of fibroblasts can help improve cutaneous wound healing. It has been reported that flavonoids, tannins, saponins and terpenoids in plants show wound healing activity by stimulating the growth of fibroblasts (19,20).

Interestingly, CVC exhibited the highest antiproliferative effect on fibroblast cells depending on the dose. Studies have shown that *C. virgata* predominantly contained flavonoids such as apigenin and eupatorin (21). In activity studies with apigenin, it has been found that it inhibited osteoblastogenesis and osteoclastogenesis (22). In addition, apigenin and its derivatives are known to have antiproliferative activity (23). Similarly, various studies have shown that eupatorin has an antiproliferative effect (24). Tüzün et al (2017) reported that the chloroform extract of *C. virgata* predominantly contained apigenin and eupatorin flavonoids. In line with this study, in our study CVC showed the highest antiproliferative effect (25).

CVAE, in particular, promoted the growth of fibroblasts at concentrations of 10 and 25 µg/mL. This mitogenic effect is a positive event for the wound healing process because fibroblasts are important cells involved in wound contraction and ECM production.

In our study, we determined that none of the extracts had significant antioxidant activity. Morever, the obtained results for DPPH are in agreement with the phenol contents determined for each sample. The low antioxidant activities of these extracts might be attributable to their low phenolic contents.

In a study performed by Keser et al. (2020), it was demostrated that *C. virgata* ethanol extract at concentration of 500 µg/mL had antioxidant activity with percent inhibition values of 70.15% and 57.24% against ABTS and DPPH radicals, respectively. (21). When we compared these results with our current study, CVE with IC_{50} values of 200.3 µg/mL and 240.5 µg/mL showed a better antioxidant activity. These differences in the antioxidant activity of *C. virgata* may be due to the fact that different parts of the plant have been studied and/or different extraction methods have been used.

5. CONCLUSION

According to the obtained results, our finding suggests that CVAE has a migratory effect on fibroblasts and that CVAE might be a potential therapeutic agent for wound healing. It is also reported in the literature that a *Centaurea* species, *C. lycopifolia*, is used by public for wound healing purposes (26). This information supports the wound healing activity of the aqueous ethanol extract of *C. virgata* which have found in our current study. However, it is necessary to isolate the active compounds responsible for the activity in order to understand the mechanism of action. Also, our results corroborate the use of *C. virgata* in traditional medicine as an wound healing.

Conflict of Interest

The author declares no conflict of interest.

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Clinical and Experimental Health Sciences

Evaluation of Knowledge and Awareness of Dentists and Dental Students About Human Papillomavirus Vaccination and Oropharyngeal Cancer Relationship

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ABSTRACT

Objective: As the incidence of oropharyngeal cancer associated with human papillomavirus has increased rapidly in recent years, the role of dentists on this issue is also increasing. This study aims to determine the knowledge and awareness of dentists and dental students about human papillomavirus vaccination and the relationship of oropharyngeal cancer.

Methods: A total of 209 participants, consisting of 82 dentists and 127 dental students filled out the questionnaires about the general human papillomavirus knowledge, human papillomavirus – oropharyngeal cancer relationship, and human papillomavirus vaccination. The Fisher Exact and the Mann Whitney U tests were used for determining the difference between the two groups.

Results: The majority of the respondents answered the general questions about human papillomavirus correctly. Almost all of the participants in our study were found to have high awareness about the human papillomavirus – oropharyngeal cancer relationship, most of them did not have enough information about the human papillomavirus – related oropharyngeal cancers have a better prognosis than other oropharyngeal cancers. Both dentists and dental students stated that they did not think they had enough information about human papillomavirus vaccination. Dental students were more conscious and more motivated than dentists in recommending human papillomavirus vaccination to their patients ($p < 0.05$).

Conclusion: Our study showed that dentists and dentistry students had a high level of knowledge in general subjects related to human papillomavirus. It was determined that the knowledge of both groups was insufficient regarding the human papillomavirus – oropharyngeal cancer relationship and human papillomavirus vaccination, and the participants were eager to overcome these shortcomings.

Keywords: Dental education, dental student, human papillomavirus, human papillomavirus vaccines, oropharyngeal cancer

1. INTRODUCTION

Human papillomavirus (HPV) is a small, enveloped, double-stranded, and circular DNA virus (1). HPV, the most common sexually transmitted infection in the world, is known for causing cervical cancer, and its relationship with oropharyngeal cancer (OPC) has been emphasized in recent years (2). The virus can be transmitted to the oral cavity through sexual contact or auto-infection, rarely at birth perinatally (1,3,4). HPV contamination to the oral region is more related to the number of previous oral sex and oral kissing partners than to the number of vaginal sex partners (1,4,5). The prevalence of this virus, which can cause both HPV infections and HPV-related head and neck cancers, in the oral mucosa, ranged from 0.6 to 81% (1,4,5).

Head and neck squamous cell carcinomas are the sixth most common cancers in the world (1). Although there have been serious advances in the diagnosis and treatment process in recent years, the mortality rate is still high and causes an average of 200 thousand deaths per year (6). The five-year survival rate is about 50%, and in the case of metastasis, the rate drops to 26% (6). Tobacco and alcohol use are among the most common risk factors for OPCs (1). Besides, some HPV types have recently been shown to be an important risk factor for OPC (3). While the type 6 and type 11 of HPV in the low-risk group cause benign papillomatous lesions in the oral mucosa, the type 16 and type 18 groups of the virus are at high risk and may cause OPCs (1).

HPV prevalence in patients with whole OPCs has been reported to range from 0% to 91% (1). It is stated that proportional changes vary according to geographical features, sample type, preparation method, and virus detection method (7).

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Ninety percent of OPC cases have been shown to have high-risk HPV type-16 (6). HPV-related OPC is more common in young male patients with high socioeconomic status and has a better prognosis than head and neck cancers not associated with HPV (3,8). HPV-related OPC usually occurs in the tongue base and posterior oropharynx (2).

The incidence of HPV-related OPC is increasing day by day. HPV Type 16 and Type 18, which are the cause of 90% of HPV-related OPCs, can be prevented by HPV vaccination. HPV vaccination not only reduces the incidence of cervical and anal cancer but also reduces the incidence of HPV-related OPC (9). Knowing the importance of HPV vaccination and directing patients to vaccination is important for the prevention of HPV-related OPCs (10). Although the HPV vaccine is included in the vaccination schedule in developed countries, it is not included in the routine vaccination schedule in many countries, including Turkey (11). In a previous study (12), it has been reported that the biggest obstacle in the spread of HPV vaccination is that healthcare professionals do not have enough awareness and knowledge in this regard.

Although there are studies investigating the knowledge of dentists about HPV abroad, to the best of our knowledge, there is no study investigating the knowledge levels and awareness of dentists or dental students about the relationship between HPV vaccination and HPV-related OPCs in our country. The aim of this study is to determine the knowledge level and awareness of dentists and dental students about HPV vaccination and OPC relationship.

2. METHODS

Before the study, ethical approval was obtained from the Ethics Committee of Gazi University (No: 91610558-604.01.01, Date of approval: 07.04.2020). The questionnaires were prepared on an online survey website (www.surveey.com) and the link was sent to the participants via e-mail and a mobile phone application (WhatsApp Inc, Menlo Park, CA, USA). The students participating in this study were selected from two dental schools. The participants were informed that their identity information (name, or ID number) would not be requested. It was stated at the beginning of the questionnaire that the study was based on volunteerism, and those who agreed to participate in the study started to answer the questionnaire.

2.1. Measuring Tools

A measurement tool (Appendix) consisting of five categories: (A) demographic features (four items), (B) general HPV knowledge (16 items), (C) HPV-OPC relationship (six items), (D) HPV vaccination information (11 items), and (E) HPV vaccination survey (13 items) was applied. B, C and D categories were prepared as "True-False" test type and E was 5-point Likert type scale between "I disagree at all" and "I totally agree". In preparing the true/false questions, Rutkoski et al.'s (13) and Patel et al.'s (10) studies were used, and in the preparation of the survey items, Arnell et al.'s (9) and Patel et al.'s (10) studies were used. In translating the items from English to Turkish, specialist dentists, and experts' opinions (expert of measurement and evaluation) were received. According to their feedback, the final version of the survey was obtained.

2.2. Data Analysis

The measurement tools used in the research were collected online; all of the items were responded by the participants. Therefore, there was no missing data in the data set. Since the questionnaires did not go through a test or scale development process, total scores were not obtained, and all analyzes were conducted on an item basis. The Fisher Exact test was used to test the difference between the dentists and the dental students in answering the True/False information questions correctly. The Mann Whitney U test was used to determine whether these two groups differed in answering other questionnaire items. The level of statistical significance was set at 0.05 for all analyzes performed in this study. Statistical analyzes were performed using R (R Core Team, Vienna, Austria).

3. RESULTS

A total of 209 participants, consisting of 82 dentists and 127 dental students responded to the survey. The mean age of the dentists was 31.8 ± 8.4 (ranged from 23 to 53) while the age of dental students was between 18 and 24, with a mean of 21.8 ± 1.3. The frequency of the demographic characteristics of the participants in the study is presented in Table 1.

Table 1. *Demographic Features of Dentists and Dental Students.*

Participants	Variables		Number (n)	Percent (%)
Dentists (n=82, 100%)	Gender	Female	65	79.3
		Male	17	20.7
	Specialty	Oral surgery	4	4.9
		Oral radiology	13	15.9
		Endodontics	3	3.7
		Oral Pathology	1	1.2
		Orthodontics	14	17.1
		Pediatric dentistry	15	18.3
		Periodontology	12	14.6
		Prosthodontics	4	4.9
		Restorative dentistry	2	2.4
		General dentist	14	17.1
	Experience (years)	1-4 years	39	47.6
		5-9 years	37	45.1
		10 years and over	6	7.3
Dental students (n=127, 100%)	Gender	Female	92	72.4
		Male	35	27.6
	Education level	Third years of school	44	34.6
		Fourth years of school	52	40.9
		Fifth years of school	31	24.4

Regarding Category B, the number of dentists who responded the items correctly was higher than the number of dental students for almost all items. There were statistically significant differences ($p < 0.05$) between two groups for the items 6, 7, 9, 13, 15, and 16. Responses to the items 11, 14 and 18 were statistically similar (Table 2, Figure 1). On the subject of Category C, there was no statistical difference between the groups in the results of these items (Table 2, Figure 1). Regarding Category D, dentists responded more accurately than dental students ($p < 0.05$) for the items 28 and 32, while dental students gave a statistically significantly higher correct response compared to dentists ($p < 0.05$) in the items of 30, 33, and 34 (Table 2, Figure 1).

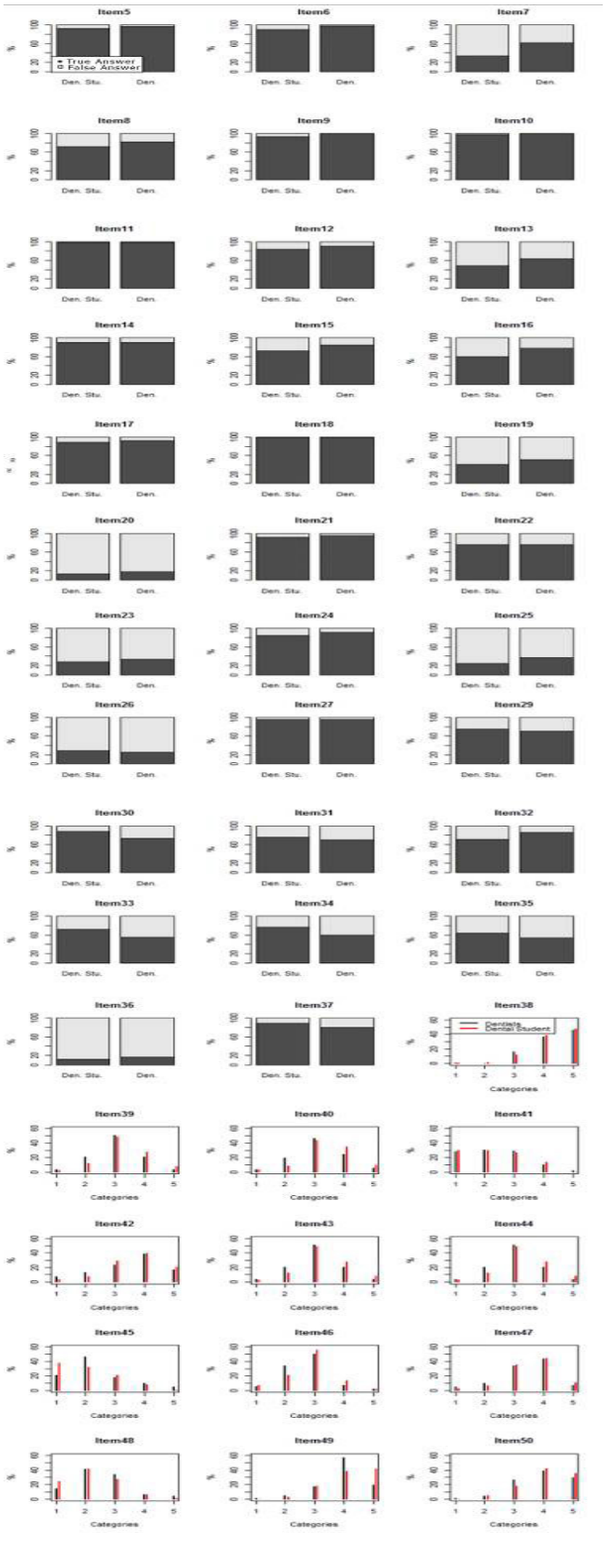

Figure 1. *Graphical Distribution of the Items*

Table 2. *Fisher Exact Test Results on General HPV Knowledge Items, HPV-OPC Knowledge Items, and HPV Vaccination Knowledge Items.*

Items	Number and rates of the correct answerers (n, %)		*p* value
	Dentists (n=82)	Dental students (n=127)	
5	80 (97.6%)	117 (92.1%)	0.132
6	81 (98.8%)	115 (90.6%)	0.018*
7	51 (62.2%)	43 (33.9%)	0.000*
8	67 (81.7%)	91 (71.7%)	0.103
9	82 (100.0%)	119 (93.7%)	0.024*
10	82 (100.0%)	125 (98.4%)	0.521
11	81 (98.8%)	125 (98.4%)	1
12	74 (90.2%)	106 (83.5%)	0.219
13	52 (63.4%)	62 (48.8%)	0.047*
14	74 (90.2%)	115 (90.6%)	1
15	69 (84.1%)	91 (71.7%)	0.045*
16	64 (78.0%)	76 (59.8%)	0.007*
17	76 (92.7%)	113 (89.0%)	0.473
18	82 (100.0%)	127 (100.0%)	1
19	42 (51.2%)	53 (41.7%)	0.202
20	15 (18.3%)	17 (13.4%)	0.431
21	78 (95.1%)	116 (91.3%)	0.413
22	62 (75.6%)	96 (75.6%)	1
23	28 (34.1%)	36 (28.3%)	0.443
24	75 (91.5%)	108 (85.0%)	0.202
25	30 (36.6%)	31 (24.4%)	0.063
26	21 (25.6%)	36 (28.3%)	0.751
27	78 (95.1%)	121 (95.3%)	1
28	77 (93.9%)	103 (81.1%)	0.013*
29	58 (70.7%)	96 (75.6%)	0.520
30	60 (73.2%)	112 (88.2%)	0.009*
31	58 (70.7%)	96 (75.6%)	0.520
32	71 (86.6%)	91 (71.7%)	0.012*
33	45 (54.9%)	91 (71.7%)	0.017*
34	49 (59.8%)	97 (76.4%)	0.013*
35	44 (53.7%)	82 (64.6%)	0.148
36	14 (17.1%)	16 (12.6%)	0.421
37	66 (80.5%)	113 (89.0%)	0.107

** p < 0.05*

Regarding Category E, statistically significant differences ($p < 0.05$) were found between two groups for the items 39, 40, 43, 44, 45, and 49 (Table 3, Figure 1).

Table 3. *Mann Whitney U Test Results Regarding the Survey For HPV Vaccination.*

Items	Dentists		Dental students		U	Z	*p value*
	Median	Mean rank	Median	Mean rank			
38	4.00	103.67	4.00	105.86	5098.0	-0.279	0.781
39	3.00	95.27	3.00	111.28	4409.5	-2.018	0.044*
40	3.00	93.86	3.00	112.19	4293.5	-2.280	0.023*
41	2.00	106.09	2.00	104.30	5118.0	-0.217	0.828
42	4.00	99.61	4.00	108.48	4765.0	-1.084	0.278
43	3.00	121.35	2.00	94.44	3866.5	-3.234	0.001*
44	3.00	123.35	2.00	93.15	3702.0	-3.627	0.000*
45	2.00	114.95	2.00	98.58	4391.5	-2.006	0.045*
46	3.00	96.72	3.00	110.35	4528.0	-1.750	0.080
47	4.00	100.26	4.00	108.06	4818.0	-0.977	0.329
48	2.00	113.79	2.00	99.33	4486.5	-1.785	0.074
49	4.00	92.70	4.00	112.94	4198.5	-2.543	0.011*
50	4.00	98.55	4.00	109.17	4678.0	-1.316	0.188

** $p < 0.05$*

4. DISCUSSION

Dentists have an important role in OPC screening, diagnosis, awareness, and patient education. Although the general risk factors of cancers in the oral region are well known, information about the role of viruses in oral cancer risk is unclear (10). The relationship between HPV and OPC has been revealed recently (3,9,10,13). HPV vaccines applied to prevent cervical cancers associated with HPV reduce the incidence of cervical, anal, penile cancers as well as OPCs (14). The most frequently referred physicians are dentists, and during the oral examination, they examine the face, neck, lips, gums, floor of the mouth, tongue, hard and soft palates in detail (14). During the dental examinations, in which patients are evaluated at short intervals, oral cancer screening is also performed. Dentists who have a great responsibility in the prevention of HPV-related oral cancers have sufficient knowledge about HPV vaccination and motivate their patients to vaccinate HPV, making them one of the key healthcare professionals in raising awareness about this issue (14,15). In the literature, limited number of studies have been carried out on this subject in the last few years (9,10,13). However, it has been reported that the knowledge and awareness of dentists on this issue is quite low (14,16). Our study aims to reduce this deficiency in the literature.

As HPV-related OPC rates are increasing, the importance of this issue and its place in dentistry are increasing (9,17). The important role of dentists in the prevention of tobacco-related OPCs is as important as the HPV-related OPCs, which can be prevented by HPV vaccination (9). HPV-related OPCs have become popular both in the literature and in the media, so patients can request information from dentists and expect them to direct themselves (14). Thus, the knowledge of dentists on this subject should increase and dentists should prepare themselves to talk about this with their patients (14).

Daley et al (14) conducted an HPV survey for dentists, nearly half of the dentists answered the general information sections about HPV correctly. Arnell et al (9) reported that dentists have moderate knowledge about HPV. In the research of Rutkoski et al (13), it was stated that most of the dental students are sufficient in general subjects related to HPV. In our study, the responses of the participants to the general knowledge questions about HPV were substantially correct. On the other hand, the rate of participants indicating that HPV will recover spontaneously was found to be extremely low in the present study. This result is also compatible with the results of Patel et al (10). Regarding the question about HPV's absence of obvious signs, the majority of dentists in Patel et al's study (10) responded correctly, but in our study, the correct response rate was lower for both dentists and students. In the question of increasing the HPV risk of having multiple sexual partners, the correct response rate was found to be high in both Patel et al's study (10) and the participants in our study.

In a study of Applebaum et al (16), only half of the dentists reported that HPV-related OPC information was up to date, which prevented physicians from talking freely to their patients. On the other hand, dental students are quite inadequate in this regard (13). Almost all of the participants in our study were found to have high awareness about the HPV-OPC relationship. Similarly, almost all dentists who participated in Patel et al's study (10) were reported to know that HPV could lead to OPC. However, the correct response rate for HPV-related OPC prognosis, which group of patients is more common and OPC localization in the oral region was found to be quite low in our study. These results are compatible with the study of Patel et al (10). It was stated that the reason for the inadequacy of dentists in these questions may be due to the missing information of dentists in the pathophysiology of HPV and HPV-related OPCs (10).

Regarding the safety or side effects of HPV vaccination, the level of knowledge of dentists participating in our study was found to be high in many questions, consistent with the previous studies (10,18). However, the correct response rate in our study for questions regarding the administration and pricing of HPV vaccines was low for both dentists and dental students. In the study of Rutkoski et al (13), less than half of the dental students were reported to have sufficient knowledge about HPV vaccination.

In the study of Daley et al (14), it was stated that only 9% of dentists talk with women patients about HPV vaccination and 81% of them have insufficient information about HPV vaccination. In the study of Arnell et al (9), it was reported that dentists do not think that they have sufficient information about HPV vaccination, therefore they refrained from discussing these issues with their patients. In our study, both dentists and dental students stated that they did not think they had enough information about HPV vaccination, in line with previous reports (9,10). In the present study, most of the participants answered most of the questions about HPV vaccination, but the majority answered the question

that HPV vaccines were more effective in individuals who have not previously had sexual intercourse. The results of our study also revealed that, if the participants had sufficient knowledge, they were eager to direct their patients to HPV vaccination and to participate in related training. Putting HPV vaccination on the agenda of training, guides, conferences, and publications of professional organizations can increase the awareness of dentists.

Arnell et al (9) determined that almost all of the dentists thought that they should play an active role in the general health of patients, only half of them believed that HPV vaccination was their responsibility. On the other hand, Daley et al (19) reported that dentists think that information about sexual health issues should be done by family physicians or gynecologists. The dentists in Patel et al's study (10) stated that they were neutral for their role in this matter. In our study, the vast majority of the participants think that they should play an active role in the general health of patients, however, the role of dentists in HPV vaccination was found to be moderate. Our results showed that dental students were more conscious and more motivated than dentists in recommending HPV vaccination to their patients, but dental students have less knowledge about when the vaccination is performed. The role of dentists in HPV vaccination and informative announcements of professional organizations on this topic was reported in previous studies (19). The American Dental Association also issued a declaration in late 2018 on promoting the HPV vaccination (20).

In the study of Arnell et al (9), most of the dentists thought that HPV vaccination could encourage young individuals to undergo earlier/risky sexual intercourse, while in our study the participants gave the opposite view. In the same study (9), it was stated that dentists were keen to have their children vaccinated with HPV, similar to our study. In Patel et al's study (10), the item's score on the point that dentists did not have enough time (and should be) to discuss HPV vaccination with their patients was average. While the score of this item was higher in our study, we think that this situation may be related with dentists treat too many patients. in our country.

According to the best of our knowledge, there is no published article on dentists about HPV and HPV vaccination in Turkey. However, it has been determined that there are many studies evaluating the knowledge levels of other healthcare professionals on subjects such as HPV, HPV vaccine, and cervical cancer (12,21,22). In the survey study conducted by Yuksel et al. among hospital staff (12), medical doctors and nurses were reported to have a higher level of correct knowledge about HPV vaccination compared to other healthcare professionals. In the survey conducted by Gorkem et al. with female healthcare workers other than doctors (21), 85.4% of the participants knew the HPV vaccine. In the same study, it was stated that the highest information rate was 95.7% for midwives and 86.4% for nurses (21). It was determined that the participants in our study were aware of the HPV vaccine but there was a lack of information about its application.

In the study of Yuksel et al. (12), 84.9% of the doctors who participated in the survey stated that they were considering getting their daughter vaccinated with HPV, this rate was reported as 64% in nurses and 52% in other healthcare personnel. In the same study, 58.4% of doctors and 46% of nurses and other healthcare personnel were reported to consider getting their children vaccinated with HPV (12). In the survey conducted by Gorkem et al. with female healthcare staff other than doctors (21), it was reported that three-quarters of the participants wanted to vaccinate their daughters. In the study conducted by Gudugu et al (22), approximately two-thirds of the medical students stated that they will get the HPV vaccination to their daughters (in the future), while only half of the nursing students and healthcare workers were positive about vaccination. Dentists and dental students in our study reported their willingness to get their children vaccinated with HPV.

In the study of Yuksel et al. (12), for the question of "Do you think that the widespread HPV vaccine may increase the frequency of sexual intercourse or polygamy?", it was determined that most of the doctors, nurses, and other health personnel answered no. The participants in our study also largely answered the question negatively. In the study of Ozakar-Akca et al among nurses (23), the rate of those who stated that HPV vaccine has high efficacy in the prevention of cervical cancer is 87.8%, The rate of those who reported that they prevented anal, vulvar and head and neck cancers was reported as 73%. In the same study, the rate of those who think that the HPV vaccine is a preventive feature of genital cancer and warts in both sexes is stated to be 86.9% (23). The rate of responses given by dentists and dental students to these items in our study was found similar to the study for nurses (23).

One of the limitations of our research is that the participants in our study were from only one country. However, the fact that dentists who filled out the questionnaire had lower professional experience in general, limits the measurement of knowledge of experienced dentists on the subject. In future studies, dentists from all experience levels can be reached with a higher number of participants, and the current status of dentists in different countries can be evaluated. Also, the change of knowledge levels can be examined after the inclusion of HPV topics in training programs.

5. CONCLUSION

According to the results of our research, it was determined that dentists and dental students have a high level of knowledge about general issues related to HPV. However, it was found that the knowledge of both groups was insufficient regarding the HPV-OPC relationship and HPV vaccination and the participants were eager to overcome these shortcomings. A larger place should be reserved for HPV-related OPC and HPV vaccination knowledge, after the graduation to dentists, and dentistry students during the education period.

Conflict of interest: The authors declare that there is no conflict of interest.

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Appendix. *The Items in the Survey and Correct Answers.*

Item no	A. Demographic features	
1	Gender	
2	Specialty (for dentists)	
3	Experience (for dentists)	
4	Education level (for dental students)	
	B. General HPV knowledge items	**Correct answers**
5	HPV is a bacterial infection.	False
6	Antibiotics can treat HPV.	False
7	HPV can cause herpes.	False
8	HPV can cause HIV/AIDS.	False
9	HPV can cause cervical cancer.	True
10	HPV can cause genital warts.	True
11	HPV can be transmitted through sexual contact.	True
12	An individual with HPV without a genital wart can infect HPV.	True
13	HPV usually has no obvious signs.	True
14	A person with HPV can live for many years without realizing this situation.	True
15	HPV rate is highest in women in their 30s.	True
16	Using a condom reduces the risk of HPV contamination.	True
17	Oral sex can cause transmission of HPV infection.	True
18	Having more than one sexual partner increases the risk of HPV.	True
19	Most sexually active people will encounter HPV once in their lives.	True
20	HPV usually resolves spontaneously without any treatment.	True
	C. HPV-OPC knowledge items	
21	HPV can lead to OPC.	True
22	The same HPV types cause genital warts and OPC.	False
23	OPCs caused by HPV have a worse prognosis than OPCs not associated with HPV.	False
24	The early stages of OPC associated with HPV are often asymptomatic.	True
25	HPV-related OPCs are more common in young, well-educated men.	True
26	The tongue is the most common site of HPV-related OPCs.	False
	D. HPV vaccination knowledge items	
27	There are vaccines that immunize against some types of HPV.	True
28	HPV vaccines can protect women against cervical cancer associated with HPV.	True
29	HPV vaccines can protect men and women against HPV-related anal cancer.	True
30	HPV vaccines can protect men and women against HPV-related genital warts.	True
31	HPV vaccines can protect men and women against HPV-related OPC.	True
32	HPV vaccines have serious side effects.	False
33	HPV vaccines are expensive.	True
34	HPV vaccines are covered by health insurance.	False
35	HPV vaccines are administered in one dose.	False
36	HPV vaccines are more effective only for people who have never had sex.	True
37	HPV vaccine is recommended to be applied to both men and women.	True
	E. HPV vaccination survey	
38	It is important that dentists take an active role in the general medical health of patients.	
39	The recommendation of HPV vaccination is the responsibility of dentists.	
40	The recommendation of the HPV vaccine should be within the professional scope and role of a dentist.	
41	HPV vaccination can encourage young individuals to have an earlier or more risky sexual relationship.	
42	I have/would have/plan to have HPV vaccination for my children.	
43	I am knowledgeable about when to apply the HPV vaccine ideally.	
44	I am knowledgeable about which ages are the most appropriate period for the HPV vaccine in women.	
45	I am knowledgeable about which ages are the most appropriate period for the HPV vaccine in men.	
46	If I recommend HPV vaccination to my patients, my patients will get the vaccination.	
47	There is not enough time to discuss HPV vaccination (and need for it) during appointments.	
48	I think I have enough information when talking to my patients about HPV vaccination.	
49	If I have enough information, I will be willing to educate my patients about the importance of the HPV vaccine to prevent OPCs.	
50	I would be willing to participate in a training program to promote and manage the HPV vaccine in dentistry.	

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Clinical and Experimental Health Sciences

Effect of Nebivolol on Isolated Rat Bladder Strips Precontracted with Carbachol or Potassium Chloride

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ABSTRACT

Objective: One of the proposed mechanism mediating the vasorelaxant effect of nebivolol is based on its agonistic activity on beta-2 and/or beta-3 adrenergic receptors. These receptors are also involved in the relaxation of urinary bladder. The aim of this study was to explore the ability of nebivolol to induce relaxation of the isolated rat bladder strip precontracted with cholinergic stimuli using carbachol or non-cholinergic stimuli using potassium chloride (KCl).

Methods: The isolated bladder strips were mounted in organ bath and contracted by KCl (40 μM) or carbachol (1mM) before the cumulative addition of nebivolol (0.0001-100 μM). To investigate the role of beta-adrenergic receptors in the nebivolol-induced relaxant response, some bladder strips were incubated with propranolol (1 μM) for 30 min. Statistical significance was tested by Student's *t*-test. $p<0.05$ was considered to be statistically significant.

Results: Nebivolol elicited concentration-dependent relaxant response in the bladder strips precontracted with KCl or carbachol. Although the relaxant response to nebivolol in the bladder strips precontracted with carbachol was significantly inhibited by propranolol ($p<0.05$), the nebivolol-induced relaxation failed to be inhibited by propranolol in the bladder strips precontracted with KCl. The maximum relaxation in response to nebivolol was found to be significantly higher in the bladder strips precontracted with carbachol compared to that of KCl ($p<0.05$).

Conclusion: The findings of the present study indicate that beta-adrenergic receptors play role in the relaxant response of nebivolol in the isolated rat bladder strip precontracted with carbachol.

Keywords: Nebivolol, relaxation, carbachol, potassium chloride, bladder, rat

1. INTRODUCTION

Nebivolol is a selective beta-1 adrenergic receptor (β_1-AR) blocker differently from conventional β-blockers because of its vasodilator and antioxidant properties (1,2). Although the precise mechanisms by which nebivolol induces relaxation are not completely understood, there is increasing evidence showing that nitric oxide (NO) production by endothelium-dependent mechanisms including β_2 – and/or β_3-ARs are thought to be primarily responsible for the vasorelaxant effect of nebivolol (3,4).

There is substantial evidence to indicate that β-ARs also involve in the relaxation of urinary bladder (5-7). Further evidence may come from the pharmacological studies which showed that the relaxant response to isoproterenol and other non-selective β-agonists is associated with β_2 – and/or β_3-ARs in the rat bladder (8, 9).

The physiological voiding function of urinary bladder is controlled by the contraction of urinary bladder smooth muscle (10). This contraction is primarily elicited by muscarinic receptor stimulation by acetylcholine released from parasympathetic nerve endings (10,11). However, under pathophysiological conditions, both cholinergic and non-cholinergic stimulus play role in the bladder contraction (12).

The present study was designed to investigate the effect of nebivolol on the isolated rat urinary bladder precontracted with either cholinergic or non-cholinergic stimulus induced by carbachol or potassium chloride (KCl), respectively.

2. METHODS

2.1. Drugs and Chemicals

Nebivolol hydrochloride, carbachol and propranolol hydrochloride were obtained from Sigma-Aldrich (USA). All compounds were dissolved in distilled water except nebivolol. Nebivolol was dissolved in dimethylsulphoxide and the final concentration of the solvent in the organ bath was less than 0.01% (v/v).

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2.2. Experimental Animals

Experimental protocols were approved by KOBAY DHL Inc. Ethical Committee for Experimental Research on Animals (Approval date and number: 14.01.2020/451). Male Sprague–Dawley rats (250-300g, n=6) were used in this study. The rats were housed in cages and were allowed ad libitum access to standard laboratory diet and tap water.

2.3. Experimental Design

The anesthetized rats were sacrificed by cervical dislocation. Thereafter, the urinary bladder was rapidly excised and placed in Krebs–Henseleit solution (composition in mM: NaCl, 118; KCl, 4.7; MgSO4•7H2O, 1.2; KH_2PO_4, 1.2; $CaCl_2$, 2.5; $NaHCO_3$, 25; and glucose, 11). The bladder was cleaned from surrounding adjacent adipose and soft connective tissue. Afterwards, the upper most dome and the lower trigone area were removed and the remaining body of the bladder was sliced longitudinally into approximately 2 × 10 mm strips. The isolated bladder strips were placed in organ bath chambers attached to force displacement that were connected to a computer for isometric force recording. The Krebs–Henseleit solution in the bath was continuously aerated by mixture of 95% O_2 and 5% CO_2, and maintained at 37°C. Resting tension of strips was set to 1 g and allowed to equilibrate for 60 minutes with replacing fresh Krebs–Henseleit solution every 15 min. To investigate whether the relaxant effect of nebivolol may differ in bladder strips due to distinct precontractile stimulus, carbachol for muscarinic receptor activation or KCl for membrane depolarization was used for this purpose. After the equilibration period, nebivolol (0.0001–100 µM) was cumulatively added to organ bath to obtain cumulative concentration response curves (CCRCs) of the bladder strips precontracted with KCl (40 mM, n=6) or carbachol (1 µM, n=5) and served as controls. To test the role of β-ARs in this response, some bladder strips were incubated for 30 min with propranolol (non-selective β-AR antagonist, 1 µM).

2.4. Statistical Analysis

Data are expressed as mean ± standard error of the mean (SEM). Relaxation is expressed as the percentage of the contraction caused by KCl or carbachol. Efficacy of nebivolol was expressed as maximum relaxation (E_{max}). The analysis was performed using the statistical software package (GraphPad Prism, USA). Statistical significance was tested by Student's t-test. Differences were considered to be statistically significant when $p<0.05$.

3. RESULTS

Nebivolol elicited a concentration dependent relaxation in the KCl-precontracted bladder strips (n=6). However, there is no significant inhibition in the nebivolol-induced relaxation by the presence of propranolol (Figure 1, $p>0.05$).

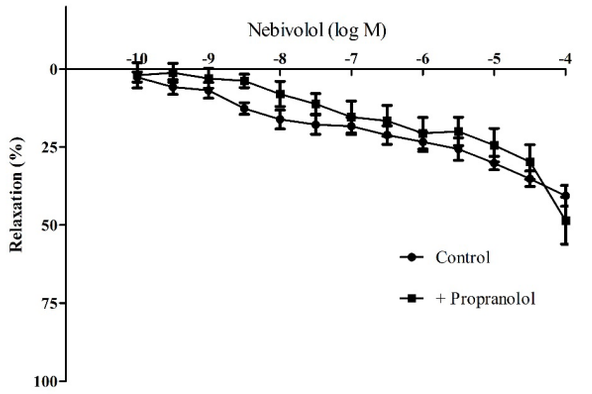

Figure 1. *Nebivolol-induced relaxation in the rat bladder strips precontracted with KCl (40 mM) in the absence (Control, n=6) or presence of propranolol (+Propranolol, 1 mM, n=5)*

Nebivolol also caused relaxation in the carbachol-precontracted bladder strips in a concentration-dependent manner (n=5). Additionally, the relaxant response to nebivolol was significantly inhibited by the presence of propranolol in the isolated bladder strips (Figure 2, $p<0.05$).

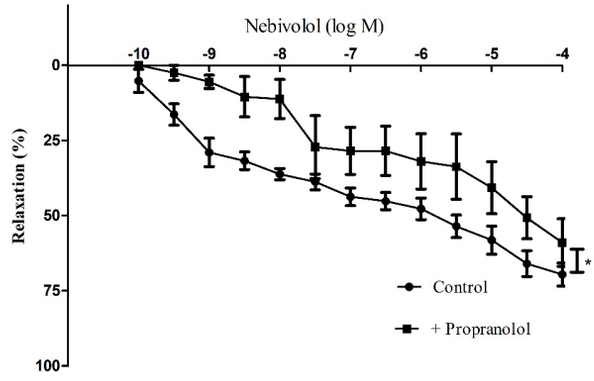

Figure 2. *Nebivolol-induced relaxation in the rat bladder strips precontracted with carbachol (1 µM) in the absence (Control, n=5) or presence of propranolol (+Propranolol, 1 mM, n=4). p<0.05 vs control*

The maximum relaxation (E_{max}) in response to nebivolol was significantly higher when bladder strips were precontracted with carbachol than that of KCl (Figure 3, $p<0.05$).

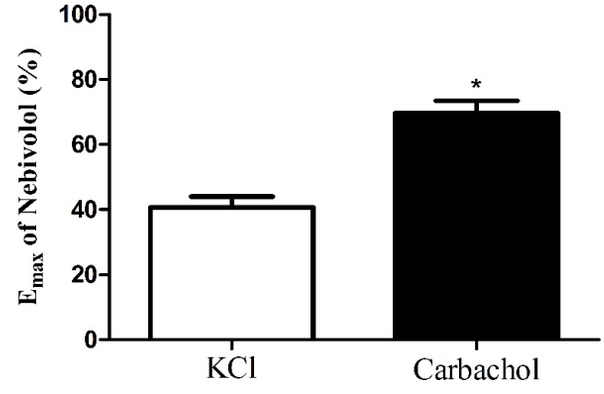

Figure 3. *E_{max} of nebivolol in the rat bladder strips precontracted with KCl (40 mM, n=6) or carbachol (1 µM, n=5). p<0.05 vs KCl*

4. DISCUSSION

In the present study, the relaxation induced by nebivolol was concentration-dependent both in the KCl-precontracted or carbachol-precontracted isolated bladder strips. However, this response to nebivolol was found to be more efficacious when the precontracted stimulus was carbachol instead of KCl. Additionally, the concentration-dependent relaxant effect of nebivolol was inhibited by propranolol in the bladder strips precontracted with carbachol.

As mentioned before, the contractions in the bladder evoked by carbachol or KCl indicate cholinergic and non-cholinergic stimulus, respectively. The present data showing a higher efficacy of nebivolol in the bladder strips precontracted with carbachol indicate that distinct mechanisms involve in the strength of precontraction contribute to differences in the ability of nebivolol to induce relaxation. The mechanisms responsible for the differences will be investigated in future studies, but one possible explanation of these findings is that different calcium sources play role in the contractile response to various stimulus in the bladder smooth muscle (13). Although the KCl-induced contraction mainly depends on entry of calcium from extracellular sources, both calcium from extracellular sources and release from sarcoplasmic reticulum mediate the contractile response to carbachol in the bladder smooth muscle (13). Nebivolol has been reported to possess an inhibitor action on the both calcium sources and this may partly explain the question why nebivolol is more efficacious in the bladder strips precontracted with carbachol (14,15).

The β-ARs are thought to be involved in the relaxation of the urinary bladder smooth muscle (16,17). For this reason, some bladder strips were incubated with propranolol before addition of nebivolol cumulatively to the organ bath in the present study. Interestingly, propranolol inhibited the relaxant response to nebivolol in the bladder strips precontracted with carbachol but failed to inhibit this response in the KCl-precontracted bladder strips. The present data support the hypothesis that β-ARs are involved in the nebivolol-mediated relaxation in the isolated urinary bladder strips precontracted with a cholinergic stimuli. However, the contribution of each subtype (especially β_2 – and β_3-ARs) in this action is needed to be elucidated by further studies.

The present data are in line with a previous study showing nebivolol-induced relaxation in the rat bladder strips precontracted with KCl (18). In the same study, it has also been shown that this response to nebivolol was insensitive to SR 59230A (β_3-AR antagonist) (18). However, to the best of knowledge, there is no study evaluating the effects of nebivolol on the bladder strips precontracted with carbachol.

As previously mentioned, NO has been shown to be involved in the nebivolol-mediated vasorelaxation through endothelial β_2 – and/or β_3-ARs (3, 4). Additionally, NO has also been reported to play a role in the relaxation of isolated trigonal and urethral preparations (19). However, in the human detrusor smooth muscle, it has been found that the relaxation induced by β-AR agonist might not relate with NO release (20). Although not addressed in the present study, the contribution of NO in the bladder relaxation through nebivolol is still question.

In conclusion, the present results show that nebivolol could produce more efficacious relaxation in the rat bladder strips precontracted with carbachol than that of KCl. Additionally, these findings suggest that relaxation elicited by nebivolol is involved in the β-ARs in the isolated rat urinary bladder strips precontracted with a cholinergic stimuli.

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Clinical and Experimental Health Sciences

Comparison of Fracture Resistance Between Two Monolithic and One Veneered Zirconia Materials on Molar Crowns After Thermomechanical Fatigue

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ABSTRACT

Objective: The purpose of this in-vitro study is to evaluate fracture resistance of two monolithic and one veneered zirconia crowns on human molar teeth fabricated after thermomechanical fatigue.

Methods: Seventy-two human molar teeth were prepared to receive zirconia crowns. The specimens were divided into three experimental groups (n=24) according to restoration design, monolithic or veneered. The crowns were fabricated from GC initial zirconia, Dentsply Sirona TZI and Dentsply Sirona ZI. The prepared teeth were scanned with Sirona inEos X5 and the restorations were milled using Cerec inLab MC X5. The crowns were cemented by resin cement. Twelve crowns of each experimental group underwent thermomechanical fatigue using chewing Simulator for 240 000 chewing cycles with load of (100 N) and thermocycling (5 °C/55 °C), the remaining 12 crowns in each group did not undergo any thermomechanical fatigue and were considered as control group. All specimens were loaded until fracture using universal testing machine. Forces were applied to occlusal surface with 90° angle. Loads of fracture were recorded. Collected data of fracture loads of all specimens were analyzed using SPSS 23.00 program.

Results: Although thermomechanical fatigue significantly decreased fracture loads of only monolithic groups, monolithic zirconia crowns had higher fracture loads than veneered one. Among all specimens, the highest fracture load was found in GC group (5001,81 N) and the lowest was found in ZI group (2117.37 N). Two fracture patterns were observed among monolithic zirconia groups; total and crack, while three fracture patterns were observed in veneered group; porcelain fracture, porcelain and core, porcelain and core with tooth fracture.

Conclusion: Thermomechanical fatigue has significant influence on monolithic zirconia, however, it showed higher fracture loads and can be alternative to veneered design.

Keywords: Zirconia, monolithic, fracture, thermomechanical fatigue.

1. INTRODUCTION

Esthetics has become a crucial issue in modern communities. Until recently, functional demands were the main focus of restorative dentistry, however, the decrease of caries prevalence shifted the focus gradually from functional to esthetic dentistry which promoted the commercialization of newly introduced products. As a result, all-ceramic restorations are replacing metal-based restorations with wide range of ceramic systems being introduced in the market (1).

All-ceramic crowns showed similar survival rates with metal-ceramic crowns when they are indicated in the anterior dentition. Chipping and law fracture resistance associated with all-ceramic multilayered restorations are still popular cause of failure, strongly related to the location of the restoration. Molars has shown significantly higher fracture values than restorations in premolars and anterior teeth, 21%, 7%, and 3%, respectively. Traditional ceramics such as glass, glass-reinforced, and feldspathic ceramics and AL_2O_3-reinforced ceramics exhibited some complications, especially in the posterior dentition where occlusal forces are generally higher. Hence, great attempts have been expended in the growth of more efficient all-ceramic systems (2,3).

Zirconia-based restorations emerged to be popular as they obtain high aesthetic potential, excellent biocompatibility with high mechanical and optical properties which let them to be used as a framework material. Studies on tooth-supported zirconia-based restorations rarely reported complete fracture failures while no study reported complete fracture in implant-supported ones.[2] On the other hand, most studies on bilayer restorations reported chip-off failure of the porcelain-veneer (3-6). These issues need to be taken

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in consideration although only few fractures caused the removal of restorations (7,8).

New processing techniques were developed to encounter the chipping problem within ceramic veneering layers. Elimination the porosity produced within the veneering layer and injection of veneering porcelain over the zirconia framework. Further, techniques of CAD-on and rapid layering has become popular recently in prosthetic dentistry. Consequently, advances in CAD-CAM technology have expanded the range of restorations' material for both zirconia framework and veneer resulting almost flawless components as the ceramic blanks are fabricated industrially (6,9,10).

As in other industries, production procedures are becoming automated more and more in dental technology. Many benefits are associated with CAD/CAM dental restorations such as: the accessibility to new, almost flawless, industrially produced and controlled materials; an enhancement in quality and reproducibility and data storage proportional with a standardized sequence of production; an advancement in precision and planning, as well as increased efficiency (11).

These improvements have resulted in a great change in the clinical workflow for dentists and dental technicians, as well as offering more treatment options to patients. Lithium silicate glass-ceramics reinforced with zirconia and composite constituted of a polymer-infiltrated ceramic are examples of these novel microstructures (12).

Many techniques have been carried out to improve the translucency and aesthetic properties of full-contour zirconia compared with conventional Y-TZP. These techniques included modifications on the fabrication processes, sintering temperature, addition of coloring liquids, increase in density and decrease in alumina content. A toughening mechanism of the transformation of tetragonal grains into the monoclinic phase leads to the high fracture toughness of zirconia, this transformation creates compressed stresses around defects, preventing their catastrophic diffusion. As a result, clinicians are now able to overcome one of the major problems associated to multilayered restorations as the issues regarding surface flaws and fracture of the low-strength veneering layer can be avoided by using monolithic zirconia restorations (12,13).

However, using monolithic zirconia restoration may arise other clinical complications which need to be taken in consideration, such as wear of the antagonist teeth and matching the aesthetic properties of the natural dentition. Although short-term data is available on high-strength zirconia materials, more research is still needed in cases of bruxism and periodontally compromised teeth (12,14). The null hypothesis suggests that thermomechanical fatigue would have significant influence on fracture resistance of all materials, however, monolithic zirconia groups would exhibit similar fracture loads but higher than veneered zirconia after thermomechanical fatigue.

The purpose of this in-vitro study is to evaluate fracture resistance of two monolithic and one veneered zirconia crowns on human molar teeth fabricated after thermomechanical fatigue.

2. METHODS

This study was approved by the ethic committee of Marmara University, Faculty of Dentistry in Istanbul, Turkey (Protocol number 260/2018).

A total of 72 extracted human molars, free of carries and restorations were selected for the study. Dental plaque, calculus and external debris were removed with an ultrasonic scaler and immersed in a germfree 0.1% thymol solution at room temperature for 1 day then all teeth were mounted individually in acrylic resin. The specimens were randomly divided into three experimental groups (n=24) according to restoration design.

All teeth were prepared according to a standardized protocol as follows: 1.2 mm chamfer finish line positioned 1 mm occlusal to the CEJ and 6° convergent axial walls. All sharp or internal line angles were rounded, and undercuts were avoided. Occlusal reduction of 1.5 mm was determined to all specimens (Fig. 1). All teeth were prepared by a single dentist, and standardized crown preparation was accomplished by fixing the dental handpiece in a parallelometer. A single-stage impression technique using putty and light-bodied vinyl polysiloxane (Zhermack Elite HD+, Badia Polesine, Italy) material was made then were poured with dental stone type IV (Fujirock EP, GC Europe, Leuven, Belgium).

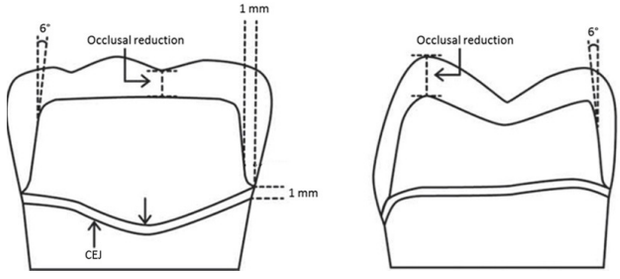

Figure 1. *Illustration of the abutment tooth of monolithic crowns showing width of chamfer finish line, convergent of axial walls and occlusal reduction.*

72 restorations were made using Cerec inLab CAD/CAM (Dentsply Sirona, Germany, Bensheim) system from three different materials; GC Initial (GC Europe, Leuven, Belgium), Dentsply Sirona TZI and Dentsply Sirona ZI (Dentsply Sirona, Germany, Bensheim).

A digital impression was taken for all dies using inEos X5. Then, the restorations design was made by single experienced dental technician. Monolithic restorations were designed with full anatomy and veneered restorations with anatomical design. A standard of 1.5

mm occlusal thickness was determined for all groups. For ZI group, a thickness of 0.8 mm was determined for the zirconia core and 0.7 mm for hand-layered veneering porcelain (IPS e.max Ceram A2, Ivoclar Vivadent, Schaan, Liechtenstein) to result in total occlusal thickness of 1.5 mm in a commercial dental laboratory (Optimal Dental Laboratory, Istanbul, Turkey). Thickness standardization was carried out by measuring and adjusting the thickness at 10 different points on the occlusal surface using the CAD software to insure the standardized occlusal thicknesses of 1.5 mm (Fig. 2).

Figure 2. *Restoration design and adjustment of occlusal thickness using inLab CAD software.*

After design, restorations were sent to a milling unit inLab MC X5 (Dentsply Sirona, Germany, Bensheim) and new set of CAD/CAM milling burs was used for each group. Then sintering was carried out for all groups with classic program using inFire HTC speed (Dentsply Sirona, Bensheim, Germany) according to the manufacturer instructions then all specimens were glazed.

Fit of crowns was evaluated by the same dental technician to ensure complete adaptation. All specimens were dried with oil free compressed air and cemented with dual-cure self-adhesive resin cement (G-Cem LinkAce, GC, Tokyo, Japan). Cementation was carried out individually to all crowns according to the manufacturer instructions as following: each restoration was coated with sufficient amount of cement then immediately seated on the prepared tooth and firm finger pressure was applied in the direction of insertion. Excess cement was removed using a surgical blade (AESCULAP no. 12, Aesculap AG & Co, Tuttlingen, Germany) after tack curing 1-2 seconds, then each surface was light cured using curing unit for 20 seconds and left for self-cure for four minutes.

Twelve crowns of each experimental group were subjected to thermomechanical fatigue (TMF) using chewing simulator (Willytec SD Mechatronic GmbH CS-4.4 Professional Line, Feldkirchen-Westerham, Germany) and the other 12 crowns were considered as control specimens without any fatigue. To simulate 1 year of clinical service, a total of 240 000 loading cycles was performed. The load was vertically applied to the central occlusal fossa of the crowns with a steel antagonist ball of 6 mm in diameter and at 1.7 Hz frequency. In addition, the simulator includes a thermocycling system, using magnetic valves in conjunction with a heating and cooling system controlled by PLCs. The test chambers were flooded using deionized water with a temperature of 5°C for 30 sec and –after evacuation– with a temperature of 55°C for 30 sec to result a total of 3000 thermal cycle (Fig. 3).

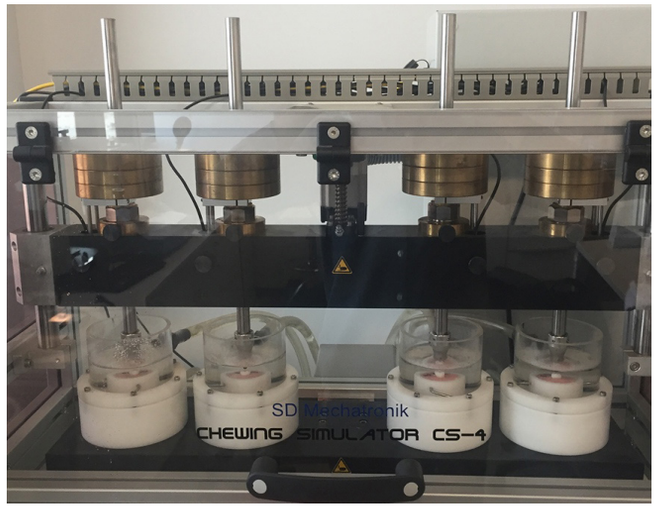

Figure 3.*Specimen fixed inside thermomechanical fatigue station.*

The fracture resistance test was performed with a universal testing machine (Shimadzu, model no:133.064.800195, Kyoto, Japan). A steel ball of 6 mm diameter at a crosshead speed of 1 mm/min was used for loading. All samples were loaded until fracture and the maximum breaking loads were recorded in Newtons (N).

The recorded data of fracture loads were statistically analyzed with a dedicated software (SPSS 23,00, SPSS Inc., Chicago, IL, USA). Loads at fracture were analyzed with the one-way ANOVA with descriptive and Post Hoc multiple comparisons according to Tukey. For all the statistical tests, the level of significance was set at P=0,05.

3. RESULTS

All specimens of TMF subgroups withstood thermomechanical fatigue in chewing simulator. Analysis of the results showed a statistically significant influence of thermomechanical fatigue on fracture resistance in both monolithic zirconia groups ($P<0.05$) while it didn't have statistically significant influence on veneered zirconia ($P>0.05$). Among all specimens, the highest fracture load was found in GC group (5001,81 N) and the lowest was found in ZI group (2117.37 N). Among only control groups, GC showed the highest mean fracture resistance value (4626,65 N) while TZI material showed the highest mean fracture resistance value (3459,27 N) among TMF groups (Table 1).

Table 1. *Fracture loads of each group (Mean, SD, Minimum, Maximum).*

Group		Mean (N)	SD (N)	Minimum (N)	Maximum (N)
GC (n:24)	C (n:12)	4626,65	267,93	4218,04	5001,81
	TMF (n:12)	3297,67	330,69	2864,77	3870,49
TZI (n:24)	C (n:12)	4602,55	449,81	3549,84	5001,59
	TMF (n:12)	3459,27	522,23	2858,71	4141,09
ZI (n:24)	C (n:12)	2958,43	460,53	2397,82	3552,77
	TMF (n:12)	2868,58	408,84	2117,37	3489,03

The results of ANOVA showed statistically significant difference between experimental groups (Table 2). Tukey results showed that both monolithic zirconia materials exhibited statistically similar fracture loads in control (p=1,000) and TMF groups (p=0,931) but higher than veneered zirconia in both groups. Although, after TMF only TZI specimens showed statistically significant difference (p=0,048) from veneered zirconia control group (Table 3).

Table 2*. One-way ANOVA test.*

	Sum of Squares	df	Mean Square	F	*P*
Between all subgroups	18 824 129 ,599	2	9 412 064 ,799	21,736	,000
Within subgroups	29 878 212 ,783	69	433 017 ,577		
Total	48 702 342 ,381	71			

Table 3. *Post Hoc Tukey tests.*

Group		Sig.	95% Confidence Interval	
			Lower Bound	Upper Bound
GC (C)	GC (TMF)	**,000**	831,2010	1826,7624
	TZI (C)	1,000	-473,6815	521,8799
	TZI (TMF)	**,000**	669,5985	1665,1599
	ZI (C)	**,000**	1170,4443	2166,0057
	ZI (TMF)	**,000**	1260,2926	2255,8540
GC (TMF)	GC (C)	**,000**	-1826,7624	-831,2010
	TZI (C)	**,000**	-1802,6632	-807,1018
	TZI (TMF)	,931	-659,3832	336,1782
	ZI (C)	,353	-158,5374	837,0240
	ZI (TMF)	**,023**	-68,6890	926,8724
TZI (C)	GC (C)	1,000	-521,8799	473,6815
	GC (TMF)	**,000**	807,1018	1802,6632
	TZI (TMF)	**,000**	645,4993	1641,0607
	ZI (C)	**,000**	1146,3451	2141,9065
	ZI (TMF)	**,000**	1236,1935	2231,7549
TZI (TMF)	GC (C)	**,000**	-1665,1599	-669,5985
	GC (TMF)	,931	-336,1782	659,3832
	TZI (C)	**,000**	-1641,0607	-645,4993
	ZI (C)	**,048**	3,0651	998,6265
	ZI (TMF)	**,011**	92,9135	1088,4749
ZI (C)	GC (C)	**,000**	-2166,0057	-1170,4443
	GC (TMF)	,353	-837,0240	158,5374
	TZI (C)	**,000**	-2141,9065	-1146,3451
	TZI (TMF)	**,048**	-998,6265	-3,0651
	ZI (TMF)	,995	-407,9324	587,6290
ZI (TMF)	GC (C)	**,000**	-2255,8540	-1260,2926
	GC (TMF)	**,023**	-926,8724	68,6890
	TZI (C)	**,000**	-2231,7549	-1236,1935
	TZI (TMF)	**,011**	-1088,4749	-92,9135
	ZI (C)	,995	-587,6290	407,9324

In the present study, two fracture patterns were observed among monolithic zirconia groups; total and crack (Fig 4). While all TZI specimens' pattern was only total fracture, GC group specimens showed two patterns; 7 total fracture and 5 crack for both control and TMF groups (Table 4).

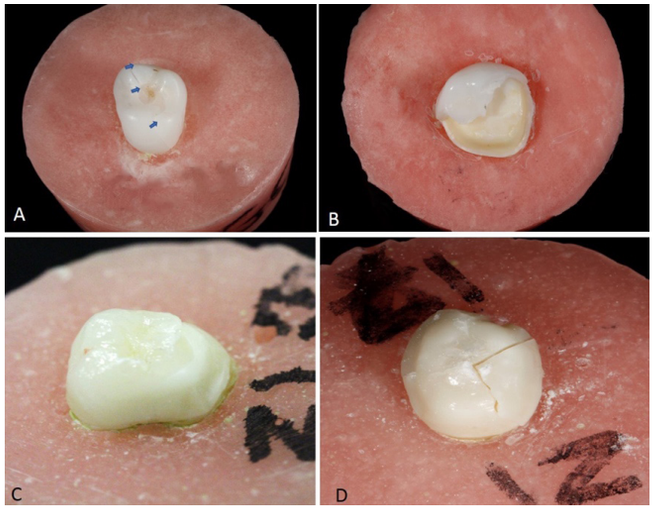

Figure 4. *Failure patterns of monolithic crowns (A: crack, B: total fracture) and veneered crowns*

(C: fracture of lingual cusp porcelain D: fracture of porcelain and core)

Table 4. *Failure patterns of experimental groups.*

Material		Failure patterns during fracture test				
		P	P + C	P+C+T	Crack	Total
GC	C	-	-	-	5	7
	TMF	-	-	-	5	7
TZI	C	-	-	-	0	12
	TMF	-	-	-	0	12
ZI	C	7	3	2	-	-
	TMF	9	2	1	-	-

**P: porcelain fracture, C: core fracture, T: tooth fracture*

Three fracture patterns were observed in veneered group; porcelain fracture (P), porcelain and core (P+C), porcelain and core with tooth fracture (P+C+T) (Fig. 4). In ZI control group 7 P, 3 P+C and 2 P+C+T were observed, on the other hand 9 P, 2 P+C and 1 P+C+T were observed in ZI TMF group. No failure was observed during TMF test in the chewing simulator, but wear occurred at contact points of some specimens (Table 4).

4. DISCUSSION

The results of the present study led to partially reject the first hypothesis, as thermomechanical fatigue significantly decreased fracture resistance of only monolithic zirconia

while it didn't have significant effect on veneered zirconia. On the other hand, the second hypothesis was accepted as monolithic zirconia groups exhibited similar fracture loads but higher than veneered zirconia.

The increase of esthetics' interest has led to the production of metal-free restorations. Dental ceramics exhibits several adequate features like biocompatibility which makes them excellent choice to simulate the features of natural teeth. Bilayer systems demonstrated several drawbacks including the low strength of the veneering material, multistep manufacturing process, and the weak bond between coping and veneer layer as the most common reported complication is chipping or cracking of the porcelain veneer. Therefore, efforts to overcome this complication have included improving the veneering ceramic firing protocol, modifying the core design, using the over-pressing technique, and using CAD-CAM veneering (CAD-on) and monolithic restorations. Thus, it seemed appropriate to provide actual evidence on fracture rates of all-ceramic zirconia crowns comparing monolithic and veneered zirconia restorations.

The abutment material plays a crucial role in evaluating the strength of dental restorative materials as it affects the mechanical properties and fracture resistance. Heintze et al.(15), and Preis et al.(6), used PMMA (Poly methyl methacrylate) abutments to test the fracture probability of all-ceramic crowns with a chewing simulator. These abutments can be a dependable artificial alternative that helps for a better standardization in fabricating identical restorations for more reliable comparison as mentioned by Dinesh et al.(16) study in 2015. On the other hand, Nakamura et al. (17) and Güngör et al. (5) used plastic Frasaco tooth as abutments. Lopez-Suarez et al.(18) used metal abutments in their study on metal ceramic, monolithic and veneered zirconia restorations. In the present study, natural teeth were used as abutments to ensure relevant strength data comparable with the clinical conditions.

Nakamura et al.(17) studied the effect of different cements on fracture resistance of monolithic zirconia crowns, they used zinc phosphate cement, glass ionomer cement, self-adhesive resin-based cement and resin-based cement. Their results didn't show influence of cement type on fracture strength. Preis et al.(6), used dual-curing resin (Variolink) to cement monolithic and veneered zirconia, while Sorrentino et al., 2016 used dual-cure self-adhesive universal resin cement (G-Cem LinkAce) to cement monolithic zirconia. This resin cement contains unique phosphate monomers that chemically bond to zirconia, for a strong and stable bond. The literature data emphasize the clear advantageous effect of phosphate monomers on bond strength zirconia/luting cements associated with mechanical pretreatments (airborne particle) in order to achieve enduring bond values (18,19). In the present study, dual-cure self-adhesive resin (G-Cem LinkAce, GC, Tokyo, Japan) was chosen and cementation was carried out according to manufacturer instructions.

The application of artificial aging has been an essential aspect in any in-vitro study regarding fracture strength to gain realistic results of fracture loads. Rosentritt et al. (20) reported that artificial aging should be performed combining thermal cycling with mechanical loading to simulate the oral environment. However, huge range of cycles' number and vertical loading values were performed in artificial aging data in the literature, with in-vitro studies performing 5 000 to 400 000 cycles (20-22,24). Certainly, many studies applied 1 200 000 cycles with 50N of vertical load for 5 years of service (6,21). For the present study, 240 000 cycles were selected to simulate 1 year of clinical service. The parameters of thermomechanical fatigue have been chosen in accordance to numerous other in vitro studies (26-28).

The results of the present study indicate a stable performance of zirconia-based crowns after 1 year of clinical service. The absence of failures during TMF as well as the high fracture loads of all groups evaluated in this study may be explained by the high mechanical properties of zirconia, especially high strength, hardness and resistance to crack propagation compared to porcelain. The toughness of zirconia has been addressed in the literature intensively, and it is attributed to a local "toughing transformation" from tetragonal to monoclinic phase upon external application of stress (6). Fracture loads of monolithic zirconia has been significantly decreased by thermomechanical fatigue compared to control groups, this result is consistent with similar studies in which they found that monolithic zirconia is clearly affected by thermomechanical fatigue (4,29). This can be explained by the tendency of zirconia to low temperature degradation (LTD) which is mainly initiated in moist environment (4,30). On the other hand, although fracture loads of veneered zirconia slightly decreased after TMF, no statistical difference was observed in the present study when compared to control group. The result of this study differs from studies showing that thermomechanical fatigue reduces the fracture resistance in veneered zirconia (20-31). This difference could be due to the different methodologies employed, that include the type of the restoration analyzed (crown or FPD), the type of die employed, or the number of cycles and the force applied during the thermomechanical loading.

Accordingly, previous studies evaluating the fracture strength of all-ceramic monolithic crowns demonstrated excellent performance of the monolithic design over the veneered one (11,12,38). The enhanced performance of monolithic crowns may be caused by the elimination of the interface between core and veneer, which is believed to be the fragile link in bilayer systems (3). Furthermore, fabricating CAD/CAM restorations involves high quality material with a minimum flaw compared to the manual veneering process. The results of the present study showed that both monolithic zirconia materials exhibited statistically similar fracture loads but higher than veneered zirconia in both control and TMF groups.

In the present study, the predominant fracture pattern of monolithic crowns was total fracture while only 20% had crack. This result was expected, since these crowns have only one material layer which leads to a bulk structural fracture.

These patterns are in accordance to the studies by Sun et al.(36) and Nordahl et al.(37). On the other hand, veneering layer fracture was the predominant fracture pattern in veneered zirconia group similarly to other studies (5,36,38). The procedures of conventional layering technique and the low mechanical features of the veneering material may be the reason for chipping failure pattern.

All evaluated materials in the present study showed values that surpasses the predictable average maximum loads with safe margin, demonstrating adequate fracture resistance, as the lowest mean value (2868,58 N). Nevertheless, fractures in veneering layer, which is a popular complication in the clinical practice, have been reported by several clinical studies rather than complete fractures. In summary, monolithic zirconia crowns arise to be a considerable alternative, especially in cases with previous fractured restorations. More clinical evaluation is needed, to assert the outcome of this in-vitro study.

Limitations of the study

In the present in vitro study, standardized conditions were provided for every experimental procedure, however, limitations of this study may include: the use of natural teeth helped to simulate clinical conditions, that did not ensure optimal standardization of the abutments. A further limiting factor may be the use of steel antagonist ball instead of human tooth as antagonists in chewing simulator. Although the steel ball sphere assured a standardized antagonistic condition (6). Analyzing with prolonged TMF simulation duration might be necessary to obtain better evaluation of the in-vitro performance of the different groups of zirconia-based materials.

CONCLUSION

Within the limitation of the present study, the following can be concluded: monolithic zirconia exhibits higher fracture resistance than veneered zirconia. However, thermomechanical fatigue has shown more significant influence on fracture resistance of monolithic zirconia than veneered. Therefore, they could be used in load-bearing areas without the chipping problem as frequently observed in their veneered counterpart.

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