Clinical and Experimental Health Sciences

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REVIEW

Clinical and Experimental Health Sciences

Cardiovascular Disease Risk Factors and Knowledge Level in Nursing Students

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ABSTRACT

Objective: In recent years, cardiovascular disease (CVD) and CVD-related deaths have been increasing in the young population in Turkey. Nursing students should be informed about CVD and should reduce their own risk of this disease. This study was conducted to determine the impact of the knowledge of CVD risk factors among nursing students on the results of risk assessments.

Methods: The population of study, which had a cross-sectional design, was composed of 587 students in nursing school at one university. The sample comprised 351 students who volunteered to take part in this study. The data were collected using the Cardiovascular Disease Risk Factors Knowledge Level (CARRF-KL) Scale, The Students' CVD Risk Factors Information Form, a physician scale, a tape measure, a digital blood pressure monitor, a glucometer and cholesterol and triglyceride measuring devices.

Results: The majority of the students (77.2%) were female, and the mean age was 20.77 years. The CARRF-KL Scale scores of the participants were low. In a significant majority of the sample, the anthropometric measurement results, blood pressure, respiration rate, pulse rate, blood glucose level, cholesterol level, and triglyceride level were within normal limits. As the knowledge level of CVD risk factors increased among the students, the blood glucose and cholesterol levels decreased.

Conclusion: Although they were in the minority, some of the nursing students in the sample had an increased risk of CVD. Health screening programs should be performed more often at institutions that educate health professionals. Strategies for reducing the CVD risk in the risk students should be conducted, and these students should be monitored closely.

Keywords: Assessment, cardiovascular disease, knowledge level, nursing students, risk factors

1. INTRODUCTION

Cardiovascular disease (CVD) is a major cause of mortality and morbidity especially in low to middle income countries. Eighty percent of CVD-related deaths occur in developing countries. The main risk factors for CVD are age, smoking, diabetes, hypertension, high levels of low-density lipoproteins (LDLs), high levels of triglycerides and homocysteine, low levels of high-density lipoproteins (HDLs) and obesity (1-4). Social behavioral models suggest that knowing the negative consequences of individual's behavior on his/her health is the basic factor in behavioral change. Since insufficient knowledge would cause insufficient motivation in lifestyle and behavioral changes, CVD prevention activities focus on community-based education programs (1).

Today, nearly half of the young population is at risk for CVD in the world (5). One of the goals of health for 21st century of the World Health Organization's (WHO's) European Region is "to provide young people with a healthier life and the ability to better fulfil their roles in the society in a healthy way by the year 2020". While this goal indicates the importance and necessity of health services that protect and improve young people's health, it requires early diagnosis, appropriate care, treatment and regular screening for CVD and other diseases associated with CVD-related deaths (6). The main purpose of CVD risk screenings is to detect the early signs of the disease and to decrease mortality and morbidity by identifying the CVD risk factors in healthy individuals (7).

Even though Turkey has a young population compared to other European countries, the CVD morbidity rate is higher (8,9). Recently, the prevalence of several CVD risk factors has increased among subsets of young adults. Factors known to increase the risk of CVD include age, obesity, central distribution of body fat, smoking, physical inactivity, hypertension, dyslipidemias and abnormalities in blood clotting for young people. Several screening studies on the prevalence of obesity in Turkey have shown that it is a major health problem, with increasing prevalence in younger age groups (10,11). While physical activity decreased (male: 2.3%, female: 6.3%) each year since 1990, hypertension incidence increased by 21.3% between 2003 to 2007 (12). Considering their ages, university students were expected to be at risk for CVD, and a screening program was designed. The effective risk management of CVD requires multidisciplinary cooperation in prevention and treatment as well as greater university student, his/her family, and community involvement and a skilled, proactive, and diverse workforce (9).

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CVD risk screening in nursing students is also important for several reasons: (1) the incidence rate of CVD in young people has increased, and nursing students are in this age group; (2) this group of university students is studying a health-related field, and they should be role models of health-related behaviours for other university students and the society in general; and (3) after graduation, these students will be responsible for the care and treatment of healthy and sick individuals in the community, and therefore, they themselves should be healthy; the first step toward developing positive health-related behaviours in individuals is to diagnose their current health risks (13).

Additionally, it is important that nursing students are informed about CVD so that they can protect themselves against this disease, have regular screenings and receive the necessary care and treatment if diagnosed with CVD. This knowledge and experience will allow nursing students to actively provide care and consulting services in health care after their graduation. Furthermore, students' knowledge level are very important for designing educational programs that provide information on CVD risk factors, thus determining the effectiveness of these programs and monitoring the CVD risk factors of participants (5,14). Despite the need for nurses to provide education to help reduce CVD-related morbidity and mortality in Turkey, no studies have been published of nursing students' knowledge and personal health behaviors related to CVD and related risk factors. In addition, it is important to determine the knowledge level of CVD risk factors and the ability of these students to obtain and evaluate their risk assessment results.

Objectives ;

This cross-sectional study was conducted to determine the knowledge of CVD risk factors/ and relationship students' have risk factors among nursing students. The research questions were as follows:

1. What are the knowledge levels of CVD risk factors and the risk assessment data of the students?

2. Does the level of knowledge regarding these risks affect the risk assessment data?

2. METHODS

2.1. Participants and setting

The study population comprised 587 nursing school students from one university during the 2010-2011 academic year and the sample comprised 351 students (59.8% of the study population) who volunteered to participate in this study. According to population and sample number, the results of present study reflected a 3.32 error rate and 0.95 confidence interval.

2.2.Instruments

Data were gathered using The Cardiovascular Disease Risk Factors Knowledge Level (CARRF-KL) scale and a risk assessment questionnaire.

Cardiovascular Disease Risk Factors Knowledge Level (CARRF-KL) Scale: This scale was prepared by Arikan et al (1), validity and reliability study was performed and the Cronbach's alpha coefficient was 0.85. The Cronbach's alpha coefficient from the data obtained in this study was 0.68. This result indicates a good reliability of the data. Of the 28 items in the CARFF-KL scale, the first four pertain to CVD characteristics, the next 15 pertain to risk factors and the last nine assess the effects of changes in risk behaviours. In this study, the items were presented in the form of a sentence that was either correct or incorrect, and the participants were asked to answer "Yes", "No" or "Do Not Know". Scoring was performed such that one point was given for each "correct answer", and zero points were given for each "Do Not Know" or "wrong answer". Six items in the scale (items 11, 12, 16, 17, 24 and 26) were coded inversely. The minimum score that could be obtained was zero, whereas the maximum score was 28. A higher score indicated a higher knowledge level (1).

The Students' CVD Risk Factors Information Form: This questionnaire was developed from the literature (5,15-19) by prior researchers, and it was used to record age, gender, class, using smoke and alchol, the height, weight, waist and hip circumferences, arterial blood pressure, pulse rate and respiration rate as well as blood glucose, cholesterol and triglyceride values.

2.3. Equipment and Calculations

Physician scale: This scale provides measurements based on gender. It measures height and weight, and it displays BMI value on a digital screen. Weights between 0 and 200 kg and heights between 90 and 200 cm can be determined accurately with a sensitivity of ±50 g and ±1 mm, respectively. The BMI of each student was recorded and classified using the scheme depicted in Table 1 (15,16).

Table 1. *BMI* Score Assessment Table*

	Males	Females
Underweight	<20	<19
Ideal Weight	20-25	19-24
Overweight	26-30	25-30
Obese	>30	>30

** Body Mass Index*

Tape measure: A tape measure was used to measure the waist and hip circumferences. The purpose of the Waist-To-Hip Ratio Test was to assess the waist-to-hip circumference ratio associated with coronary heart disease risk. In the test, the waist and hip circumferences were measured, and the waist circumference value was divided by the hip circumference value. Table 2 shows the acceptable and unacceptable

waist-to-hip circumference ratio ranges (16) and the waist-to-hip ratio classification scheme used in this study.

Table 2. *Waist/Hip Circumference Ratio Classification*

	Acceptable		Unacceptable	
	Perfect	Good	Mediocre	High
Male	< 0.85	0.85 – 0.90	0.90 – 0.95	> 0.95
Female	< 0.75	0.75 – 0.80	0.80 – 0.85	> 0.85

Digital arterial blood pressure measurement device: An upper arm digital blood pressure device was used to measure blood pressure accurately and comfortably. For an accurate measurement, the cuff that best fit the upper arm was selected (small/medium cuff=23-33 cm arm circumference, large/extra-large cuff=33-43 cm arm circumference). Measurements were taken in accordance with the standard procedures for measuring blood pressure. The measurement results were displayed on the screen as systolic and diastolic pressures in "mmHg" units. The pulse rate was also displayed on the screen of the device. Students' arterial blood pressure data were recorded as systolic and diastolic pressures, and values higher than 130 and 80 mmHg for systolic and diastolic arterial blood pressures, respectively, were considered high (15,17,19).

Glucometer: The glucometer used in this study measured the blood glucose level in blood taken from the fingertip. If the blood glucose level result was lower than 20 mg/dL or higher than 500 mg/dL, then "LOW" or "HIGH", respectively, appeared on the screen of the device. In this study, no low or high values were detected. Blood glucose levels were measured regardless of whether the students had eaten; however, depending on the time at which the students had eaten their last meal, their blood glucose level results were evaluated with respect to their fasting and postprandial durations. The students who had fasted for too long (12 hours or more) had their blood glucose measurements repeated postprandially. In classifying the blood glucose level values, 140 mg/dL was accepted as the upper limit (15,18), and the evaluation of the data was performed accordingly.

Cholesterol and triglyceride measurement device: Using device-specific cholesterol and triglyceride strips, the total cholesterol and triglyceride levels were measured in the capillary blood. This device performed the measurement using reflectance photometry. The measurement ranges of the device for cholesterol and triglycerides were 150-300 mg/dL and 70-600 mg/dL, respectively. When a value lower than the detection range of the device was detected, "LOW" appeared on the screen, and when a value higher than the range was detected, "HIGH" appeared. No high cholesterol or triglyceride values were found in the study. However, cholesterol values less than 150 mg/dL, which were labelled as "LOW", were recorded as 149 mg/dL in the database to perform calculations. Similarly, low triglyceride values were recorded in the database as 69 mg/dL. The duration of measurement was 180 seconds for cholesterol and between 45 and 174 seconds for triglycerides. The cholesterol values were classified as less than 200 mg/dL, between 200 and 239 mg/dL and greater than 240 mg/dL (20). The triglyceride values were classified as less than 200 mg/dL, between 200 and 400 mg/dL and greater than 400 mg/dL (18,19).

2.4. Data collection

The each student who agreed to participate in the study was taken into a room that was specifically allocated and designed for this study. First, the CARRF-KL scale was administered, and the students were asked to complete this form. Next, the height, weight, arterial blood pressure, pulse rate and respiration rate and blood glucose, cholesterol and triglyceride values were measured and recorded in the specified sections on the forms.

2.5. Ethical aspects of the study

The investigation was conducted in accordance with the principles outlined in the Declaration of Helsinki. Furthermore, the Istanbul University Cerrahpasa Faculty of Medicine Clinical Research Ethics Committee determined that the study was ethically compliant. An application was submitted to the director of the school where the data were collected with information on the purpose and content of the study, and written informed consent was obtained. The students that comprised the study sample were informed of the purpose of the study, its benefits and their roles in the study. Participants were informed of the voluntary nature of their participation in the study, and written and verbal consents were obtained. Before data collection began, the students were informed that they could decline to participate in the study at any stage.

2.6. Statistical analysis

The data obtained from the questionnaires were recorded in a database that was created and analysed using Statistical Package for the Social Sciences for Windows (SPSS 17.0). In the data analysis, ordinal variables were evaluated as arithmetic means with standard deviations (SDs) and minimum and maximum values, while nominal variables were evaluated as frequencies and percentages. The Spearman's rho correlation technique, Mann-Whitney U test and Kruskal-Wallis test were used to determine the relationships between ordinal variables, the difference between the means of two groups and the difference between the means of more than two groups, respectively.

3. RESULTS

In this section, the CARRF-KL scale scores, arterial blood pressure, blood glucose values, cholesterol values, triglyceride values, BMIs, and waist-to-hip ratios are outlined under two subheadings according to the questions addressed in the study.

The students' knowledge level of CVD risk factors, risk assessment data

It was determined that 77.2% of the students were women and 57.8% were between the ages of 20 and 22 years old. Also the mean age of the students was 20.77±1.98 years.

The students' mean CARRF-KL scale score was 9.44±3.23, and the knowledge level was low.

When the risk assessment data of the male students were analysed, 71.3% of the males were at an ideal weight according to BMI, 52.5% had a perfect waist-to-hip ratio, and 83.8% had an acceptable waist-to-hip ratio, thereby posing no CVD risk. For the male students, the mean BMI was 23.62±3.16, the mean waist-to-hip ratio was 0.84±0.55 (Table 3).

Table 3. *The Distribution of the CARRF-KL Scores of the Students According to the CVD Risk Factors Data (N=351)*

Risk Assessment Data	n (%)	CARRF-KL§ Mean±SD
Body Mass Index (Male)		
Underweight (<20) Ideal weight (20-25) Overweight (26-30) Obese (>30)	8 (10.0) 57 (71.3) 13 (16.3) 2 (2.5)	8.38±1.92 9.86±4.32 9.62±4.37 9.00±4.24
		x^2=0.65* p=0.89
Body Mass Index (Male) (Mean±SD) (Min.-Max.)	23.62±3.16 (16-39)	r=-0.12** p=0.30
Body Mass Index (Female)		
Underweight (<19) Ideal weight (19-24) Overweight (25-30) Obese (>30)	34 (12.5) 177 (65.3) 54 (19.9) 6 (2.2)	9.88±3.25 9.28±2.90 9.59±2.84 7.67±1.63
		x^2=3.17* p=0.37
Body Mass Index (Female) (Mean±SD) (Min.-Max.)	22.55±3.25 (16-34)	r=-0.02** p=0.75
Waist-to-hip ratio (Male)		
Perfect (<0.85) Good (0.85-0.90) Mediocre (0.90-0.95) High (>0.95)	42 (52.5) 25 (31.3) 12 (15.0) 1 (1.3)	9.67±4.35 9.56±4.22 10.08±3.23 6.00±0.00
		x^2=2.88* p=0.41
Waist-to-hip ratio according to acceptability level (Male)		
Acceptable Unacceptable	67 (83.8) 13 (16.3)	9.63±4.27 9.77±3.30
		Z=-0.57*** p=0.57
Waist-to-hip ratio (Male) (Mean±SD) (Min.-Max.)	0.84±0.55 (0.71-1)	r=-0.003** p=0.98
Waist-to-hip ratio (Female)		
Perfect (<0.75) Good (0.75-0.80) Mediocre (0.80-0.85) High (>0.85)	92 (33.9) 82 (30.3) 57 (21.0) 40 (14.8)	8.99±2.66 9.37±2.83 9.74±3.21 9.80±3.21
		x^2=2.17* p=0.54
Waist-to-hip ratio according to acceptability level (Female)		
Acceptable Unacceptable	174 (64.2) 97 (35.8)	9.17±2.74 9.76±3.19
		Z=-1.22*** p=0.22
Waist-to-hip ratio (Female) (Mean±SD) (Min.-Max.)	0.78±0.16 (0.63-0.96)	r=0.08** p=0.19
Arterial blood pressure (systolic)		
130 mmHg and ↑ 130 mmHg ↓	36 (10.3) 315 (89.7)	10.44±4.83 9.33±2.98
		Z=-0.74*** p=0.46
Arterial blood pressure (systolic) (Mean±SD) (Min.-Max.)	107.66±15.41 (66-173)	r=0.04** p=0.94
Arterial blood pressure (diastolic)		
80 mmHg and ↑ 80 mmHg ↓	81 (23.1) 270 (76.9)	9.49±3.28 9.43±3.22
		Z=-0.24*** p=0.81
Arterial blood pressure (diastolic) (Mean±SD) (Min.-Max.)	70.09±13.42 (42-105)	r=-0.02** p=0.65
Pulse		
Pulse (Mean±SD) (Min.-Max.)	84.18±13.06 (51-150)	r=0.02** p=0.71
Respiration		
Respiration(Mean±SD) (Min.-Max.)	20.03±2.08 (14-26)	r=0.006** p=0.90
Blood glucose		
140 mg/dL and ↑ 140 mg/dL ↓	16 (4.6) 335 (95.4)	9.00±3.12 9.46±3.23
		Z=-0.84*** p=0.40
Blood glucose (Mean±SD) (Min.-Max.)	100.72±19.87 (38-166)	**r=-0.118** p=0.027**
Cholesterol level		
200 mg/dL and ↑ 200 mg/dL ↓	4 (1.1) 347 (98.9)	8.75±2.99 9.45±3.23
		Z=-0.51* p=0.61
Cholesterol level (Mean±SD) (Min.-Max.)	152.28±9.57 (149-221)	**r=-0.142** p=0.008**
Triglyceride		
400 mg/dL and ↑ 200-400 mg/dL 200 mg/dL and ↓	1 (0.3) 11 (3.1) 339 (96.6)	6.00±0.00 9.36±4.43 9.45±3.19
		x^2=2.63* p=0.27
Triglyceride (Mean±SD) (Min.-Max.)	82.75±46.90 (69-458)	r=-0.06** p=0.23

§ CARRF-KL scale score was 9.44±3.23, Kruskal-Wallis, ** Spearman's rho correlation, *** Mann-Whitney*

When the risk assessment data of the female students were analysed, 65.3% were at an ideal weight according to BMI, 33.9% had a perfect waist-to-hip ratio, 64.2% had an acceptable waist-to-hip ratio, thereby posing no CVD risk. For the female students, the mean BMI was 22.55±3.25, the mean waist-to-hip ratio was 0.78±0.16 (Table 3).

When the other risk assessment data were analysed, 89.7% of students had a systolic arterial blood pressure less than 130 mmHg; 76.9% had a diastolic arterial blood pressure less than 80 mmHg;, 95.4% had a blood glucose level less than 140 mg/dL; 98.9% had a cholesterol level less than 200 mg/dL; and 96.6% had a triglyceride level less than 200 mg/dL. The mean systolic arterial blood pressure was 107.66±15.41 mmHg; the mean diastolic arterial blood pressure was 70.09 ±13.42 mmHg; the mean pulse rate was 84.18±13.06 beats/

min; the mean respiration rate was 20.03±2.08, breaths/min; the mean blood glucose level was 100.72± 19.87 mg/dL; the mean cholesterol level was 152.28±9.57, mg/dL and the mean triglyceride level was 82.75± 46.90 mg/dL (Table 3).

The effects of the students' knowledge of CVD risk factors on the risk assessment data

The CARRF-KL scale scores were not significantly associated with BMI, waist-to-hip ratio, arterial blood pressure, pulse rate, respiration rate, triglyceride values, or the separately classified blood glucose and cholesterol values ($p>0.05$). However, a statistically significant inverse relationship was found between CARRF-KL scale scores and the blood glucose and cholesterol values ($p<0.05$), such that an increase in the knowledge of CVD risk factors was associated with a decrease in blood glucose and cholesterol values (Table 3).

4. DISCUSSION

In recent years, CVD-related mortality has decreased due to efforts focused on health promotion, disease prevention and treatment development. However, CVD is still among the leading causes of death worldwide in both men and women. After CVD occurs, it follows a chronic course, and the associated care and treatments affect the patient and the patient's family biologically, psychologically, socially, culturally and economically. Therefore, as with any other chronic disease, it is important to emphasise the importance of protective health services for CVD and the control of CVD-related risk factors (21-23). In addition, it is important to consider the knowledge of CVD risk factors and to evaluate the risk assessment results of nursing students who will take an active role in preventive health services after their schooling is complete. In addition, despite widespread community screening for CVD risk factors, no studies have focused on the CVD risk factors in individuals who receive medical training and work in health services. Therefore, this study provides important information to the field.

In this study, the student's knowledge level of CVD risk factors was low. According to the data from the WHO, CVD was the fifth leading causes of death and disabilities in 1990 and is predicted to be the leading cause of death in 2020. The number of deaths is expected to rise to 25 million from 14 million (23). Therefore, society's awareness of CVD risk factors must increase. However, only health professionals can provide accurate, appropriate and comprehensive knowledge to the public (1,21). The low knowledge level of CVD risk factors among nursing students can be explained by their insufficient awareness of CVD. These findings suggest that nursing students may not be learning material from the courses covering CVD and may not be able to integrate the desired behaviour changes taught by these courses into their daily lives. Regardless of its cause, this finding indicates a problem that should be addressed and solved.

Because the reference ranges differ by gender, the BMI, and waist-to-hip ratio variables were analysed by gender. In terms of CVD, being male is an unchangeable risk factor. The prevalence of CVD among men is 4%, while it is 3.8% among women in Turkey (15). Women of reproductive age have 2.5-4.5 times less CVD risk than men; however, with menopause, the risk among women rapidly reaches the levels seen in men, especially after 50 years of age (15,22). The risk of developing CVD increases with BMI in men and women with age (24). The percentage of overweight and obese participants included in the sample was 18.8% for men and 22.1% for women. The percentage of men who had an unacceptable waist-to-hip ratio was 16.3%; this rate was 35.8% for women. These results suggest that the difference in CVD prevalence between men and women might disappear in the near future. It is known about the role of psychological factors in the development of heart disease in women (25). In the premenopausal time, women have extra protection from CVD, as evident by a 10 – to 20-year delay in theonset of CVD among women when compared to men (26,27). Recent data showed that mortality and morbidity of the women with regard to CVD are rising significantly since 1980 (28).

Increases in the students' knowledge of CVD risk factors were associated with decreases in blood glucose and cholesterol values. There are studies showing that as CVD knowledge level increases, disease results improve (14,29,30). The data obtained reflect this expected result. Researchers have studied the role in the development of the atherosclerosis and the increase of CVD risk for decades. Successful intervention programs in a number of countries have supported the casual link between the casual link between dyslipidemia and CVD by demonstrating that reductions in cholesterol lead to descreased CVD morbidity and mortality (31).

5. CONCLUSION

The knowledge of CVD risk factors among nursing students was low; female students were at a higher cardiovascular risk than male students and an increase in the knowledge of CVD risk factors had a positive effect on the anthropometric measurement results. To control the CVD risk factors among nursing students, the widespread implementation of this type of screening program and the monitoring of students assessed to be at risk is recommended. These recommendations and the results of this research were submitted to the school management, which highlighted those areas with identified problems.

Limitations of this study;

The study itself involved recruitment from only one site; this could quite markedly limited generalizability of the findings. On the other hand all nursing students at the studied school were expected to participate in the screening program; therefore, no sample calculations were made. However, because this screening contained invasive procedures, only the students who provided consent participated in the study, following the principle of voluntary participation. Therefore, students were not randomly included in the study. Also the CARRF-KL scale is a scale developed to measure the knowledge level of the individuals who did not have a health education

background. It is thought that the questions may have been easy for the nursing students, and therefore they may have expected distracters in the questions or have answered them incorrectly because the knowledge level of the students was lower than expected. Improvements are expected if similar studies are conducted with scales designed specifically for the students. Also, it would be better if the data obtained from the risk screening were examined while considering the individual and health-related behaviours of the nursing students.

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Clinical and Experimental Health Sciences

In Vitro Activity of Fosfomycin on Biofilm in Community-Acquired *Staphylococcus aureus* Isolates

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ABSTRACT

Objective: *Staphylococcus aureus (S. aureus)* is a significant pathogen microorganism that can lead to serious infections. In this study, we researched the activity of biofilm formation and fosfomycin on biofilm in community-acquired *S. aureus* isolates that were drawn from human noses.
Methods: Microtitration plate method was used to determine biofilm formation. The effect of fosfomycin on sessile cells was studied on biofilm matrix composed around plastic beads. The icaA, icaD, icaB, icaC, bap, eno, fnbA, fnbB, clfA, clfB, fib, ebpS, cna and mecA genes were screened by Polymerase Chain Reactions (PCR).
Results: *S. aureus* was isolated from 87 samples (13.2%) out of a total 658 nasal samples. We found that 10 of these isolates (11.4%) were methicillin-resistant *S. aureus* (MRSA). A total of 86 isolates had the ability to form biofilm. The biofilm inhibitor concentration (BIC) and minimum biofilm eradication concentration (MBEC) of fosfomycin were determined as 8 µg/ml and 32 µg/ml, respectively. In the molecular detection results of biofilm-related genes of these isolates, ica-dependent genes were determined to be quite high. However, no bap gene was observed to be positive in any of the isolates. Among the other genes, the most frequent genes to be declared positive were eno (97.6%) and fnbA (94.1%).
Conclusion: This study indicates that prevalence of biofilm genes in *S. aureus* isolates in nasal flora is high and fosfomycin is an effective anti-biofilm agent alone. However, to increase fosfomycin's efficiency, there is a need for more combination studies to make it more effective.
Keywords: *Staphylococcus aureus*, biofilm, fosfomycin, biofilm-related genes, nasal colonisation

1. INTRODUCTION

Staphylococcus aureus emerges as a pathogenic microorganism in many community-based and hospital-acquired infections (1). It leads to serious morbidity and mortality by causing various infections such as bacteraemia, infective endocarditis, septic arthritis, osteomyelitis and prosthetic joint and artificial graft infections (2, 3). In most staphylococcal infections, the agent is endogenous. Its colonisation in healthy humans' nasal mucosa is a risk factor for later infections (4). Nasal carriage of about 10%-40% has been reported for *S. aureus* in the human population (5).

The most pressing concern regarding *S. aureus* isolates today is their growing resistance to antibiotics (6, 7). Methicillin-resistant *S. aureus* (MRSA) isolates are common pathogens all over the world. However, community-acquired MRSA infections have increased the severity of the problem (1). With limited treatment options, decreased sensitivity and reports of resistance to vancomycin have become a problem (8). One of the reasons for antibiotic resistance in *S. aureus* isolates is their formation of biofilm (9). Biofilm is an important virulence factor because of survive in hospitals for a long time and antibiotic resistance (10).

Biofilm is a community formed by microorganisms residing in a living or inanimate surface that are embedded in an organic exopolysaccharide matrix of their own production and adhered to one another on a solid surface or interface (11, 12). Bacteria in biofilm are known to be 100-10,000 times more resistant to antibiotics, than their planktonic forms (13). Bacteria that do not die in the presence of antibiotics in the biofilm cause persistent infections that are difficult to treat (3). High morbidity and mortality rates associated with these infections are critical burdens that lead to high cost (9). Therefore, preventing these infections effectively and treating the infections are vital.

Fosfomycin trometamol, first obtained in Streptomyces cultures in Spain in 1969 and originally named fosfomycin, has been used for many years in the treatment of various infections, mainly urinary tract infections (14). Fosfomycin prevents the formation of UDP-NAMA by inhibiting the enzyme MurA and demonstrates its antibacterial activity by preventing the synthesis of the peptidoglycan layer (15). Recently, in addition to low resistance, its pharmacokinetic and pharmacodynamic advantages, in vivo activity, clinical efficiency, high level of tolerability and reliability and existence as a treatment option for infections other than

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urinary system infections caused by resistant bacteria are some of these remarkable features (16).

In this study, we examined the biofilm formation by community-acquired *S. aureus* nasal culture isolates and the effect of fosfomycin against adhered bacteria in the biofilm.

2. METHODS

2.1. Bacterial isolates

Samples of nose swabs were taken from 658 patients who were admitted to the Otorhinolaryngology polyclinic and had no history of hospitalisation prior to being involved in the 6-month study at the Kirsehir Ahi Evran University Education and Research Hospital. *S. aureus* isolation and identification of nose swab samples were done by using conventional methods (using mannitol salt agar (BD, USA), coagulase tube test) and the Vitek-2 system (bioMérieux, France). Methicillin resistance was tested with a cefoxitin (30µg) disk diffusion method in line with the recommendations of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) and was confirmed with the positive presence of the mecA gene in the isolates (17). The isolates were stored in 20% glycerol at - 80°C until run time.

Written informed consent was obtained from all participants. The study was performed in accordance with the Declaration of Helsinki's Good Clinical Practice guidelines and approved by the Turgut Ozal University Faculty of Medicine Ethical Committee (Ethical approval number-date: 99950669/32-9.01.2015).

2.2. Determination of biofilm production by microtiter plate assay

Biofilm formation was determined semi-quantitatively as defined in previous studies (18, 19). All isolates were incubated overnight at 37°C using Trypticase soy broth (TSB) supplemented with 2% glucose. TSB cultures of *S. aureus* isolates were diluted 1:100 with fresh TSB and 150 µl aliquots of each dilution were placed in 96-well plate. Three wells were used for each isolate. Plates were incubated for 48 h at 37°C. After incubation, the plates were washed 3 times with phosphate-buffered saline and 2% of crystal violet was used for staining. After washing the plates again with PBS, 150 µl of ethanol-acetone mixture (80:20) was put into each well and optical densities (OD) were determined by scanning at 540 nm. Using *S. aureus* ATCC 25923 (which forms strong biofilm) as a positive control and *E. coli* ATCC 25922 (which does not form biofilm) as a negative control, biofilm formation was determined in accordance with OD values (20). The isolates that gave absorbance values equal to or below the absorbance value of *E. coli* 25922 strains that did not form biofilms were evaluated as negative. The isolates that gave absorbance values equal to or above the absorbance value of *S. aureus* 25923 strains known to produce strong biofilms were identified as strong biofilm-producing isolates. The isolates with absorbance values between both controls were evaluated as moderate biofilm-producing isolates. Experiments were repeated 3 times.

2.3. Antibiotic study on biofilms

In order to investigate the efficacy of fosfomycin on biofilm, three MRSA isolates and three MSSA isolates were selected for the basis that produced a strong biofilm.

2.3.1. Antimicrobial agent and Minimum inhibitor concentration (MIC) determination

Fosfomycin was supplied as a dry powder for laboratory use by Sigma-Aldrich (St. Louis, MO, USA). A 0.20 µm filter-sterilised stock solution was prepared with fosfomycin at 5120 µg/mL. MIC values for fosfomycin of isolates were tested (0.5-64 µg/mL range) with an agar dilution method in accordance with the recommendation of EUCAST. An agar medium supplemented with glucose-6-phosphate (25 mg/L) was used for the antibiotic susceptibility testing of fosfomycin. The inoculum of each isolate contained 10^4 cfu/mL. The inoculated plates were incubated at 37°C for 24 h. The MIC was defined as the lowest antibiotic concentration that did not yield visible growth after overnight incubation. *S. aureus* ATCC 29213 was included in each assay as the control strain. Experiments were repeated 3 times.

2.3.2. Inhibition of biofilm formation

The effect of fosfomycin on the biofilm formed at this stage was examined. The isolates, which were incubated at 37°C for 24 h on TSB medium containing 2% glucose, were diluted to 1/100 and distributed to each well in the amount of 100 µl. After incubation for 48 h at 37°C, the microtiter plate was washed 3 times with PBS by aspirating the supernatant. Fosfomycin was added to wells in amounts of 100 µl by dilution in TSB as twofold increasing concentrations (0.5-128 µg/mL). Microtiter plates were incubated at 37°C for 20 h. Plates were washed after incubation and stained in crystal violet; then OD values were determined in the ELISA reader (BMG LABTECH, Germany) at 540 nm. The well in which the fosfomycin was not added was taken as the positive control well, and the well without the isolate was taken as the negative control well. All the isolates were run 3 times. Biofilm ODs at different concentrations of fosfomycin were compared and interpreted statistically.

2.3.3. Minimum biofilm eradication concentration (MBEC)

After all isolates were incubated overnight at 37°C in Glucose-TSB medium for biofilm formation, they were distributed in 200 µl, 96-well microplates with 1/20 dilution. Sterile plastic beads were placed in each well on the plates and incubated for 48 h at 37°C. Serial dilutions (0.5-128 µg/mL) of fosfomycin were made in freshly-prepared Mueller Hinton Broth (MHB) in another microplate. After biofilm-formed plastic beads were placed into each well of microplates with antibiotic dilution, they were incubated for 1 night at 37°C. The next

day, the beads in the wells were transferred to eppendorf tubes containing 200 µl MHB medium and vortexed for 5 min in the fast cycle to separate the biofilm layer. After this step, 100 µl of supernatant from the tubes was taken and added to wells containing 100 µl of MHB in a new microplate. The lowest concentration at which the growth was not found after overnight incubation at 37°C was determined as the biofilm eradication concentration (20). Experiments were repeated 3 times.

2.4. Detection of the icaA, icaD, icaB, icaC, bap, eno, fnbA, fnbB, clfA, clfB, fib, ebpS, cna and mecA genes by Polymerase Chain Reactions (PCR)

The genomic DNAs of *S. aureus* isolates were purified using the Genomic DNA Purification Kit (Thermo Fisher Scientific, USA). All primers used in this study are given in Table 1.

Table 1. *Primers were used in this study*

Genes	Sequences (5'-3')	T_m (°C)	Amplicon size (bp)	Reference
***mec*A**	F: GTAGAAATGACTGAACGTCCGATAA R: CCAATTCCACATTGTTTCGGTCTAA	50	310	Geha et al., 1994
***ica*A**	F: GAGGTAAAGCCAACGCACTC R: CCTGTAACCGCAAGTTT	58	151	Atshan et al., 2013
***ica*B**	F: ATACCGGCGACTGGGTTTAT R: TTGCAAATCGTGGGTATGTGT	57	140	Atshan et al., 2013
***ica*C**	F: CTTGGGTATTTGCACGCATT R: GCAATATCATGCCGACACCT	56	209	Atshan et al., 2013
***ica*D**	F: ACCCAACGCTAAAATCATCG R: GCGAAAATGCCCATAGTTTC	56	211	Atshan et al., 2013
bap	F: CCCTATATCGAAGGTGTAGAATTG R: GCTGTTGAAGTTAATACTGTACCTGC	57	971	Cucarella et al., 2004
eno	F: TGCCGTAGGTGACGAAGGTGGTT R: GCACCGTGTTCGCCTTCGAACT	58	195	Atshan et al., 2013
***fnb*A**	F: AAATTGGGAGCAGCATCAGT R: GCAGCTGAATTCCCATTTTC	56	121	Atshan et al., 2013
***fnb*B**	F: ACGCTCAAGGCGACGGCAAAG R: ACCTTCTGCATGACCTTCTGCACCT	58	197	Atshan et al., 2013
***clf*A**	F: ACCCAGGTTCAGATTCTGGCAGCG R: TCGCTGAGTCGGAATCGCTTGCT	58	165	Atshan et al., 2013
***clf*B**	F: AACTCCAGGGCCGCCGGTTG R: CCTGAGTCGCTGTCTGAGCCTGAG	58	159	Atshan et al., 2013
fib	F: CGTCAACAGCAGATGCGAGCG R: TGCATCAGTTTTCGCTGCTGGTTT	58	239	Atshan et al., 2013
***ebp*S**	F: GGTGCAGCTGGTGCAATGGGTGT R: GCTGCGCCTCCAGCCAAACCT	58	191	Atshan et al., 2013
cna	F: AATAGAGGCGCCACGACCGT R: GTGCCTTCCCAAACCTTTTGAGCA	58	156	Atshan et al., 2013

Methicillin resistance was confirmed by the presence of the mecA gene. Thirteen genes related to biofilm formation and microbial surface components recognising adhesive matrix molecules (MSCRAMMs) were analysed by PCR. The genes of icaA, icaB, icaC and icaD (intercellular adhesion genes A through D); bap (encoding biofilm-associated protein), eno (encoding laminin-binding protein); fnbA and fnbB (fibronectin-binding proteins A and B); clfA and clfB (clumping factors A and B); fib (fibrinogen-binding protein); ebpS (elastin-binding protein) and cna (collagen-binding protein) were determined in all *S. aureus* isolates.

The reaction mixtures of PCR were 25 µL in total volume, containing 1X Taq DNA Polymerase Buffer, 200 µM each dNTPs, 1.5 mM MgCl2, 0.2 pmol/µl forward and reverse primers, 200 ng genomic DNA and 2 U Taq DNA Polymerase (Thermo Fisher Scientific, USA).

PCRs were carried out with an initial denaturation step of 3 min at 95°C, followed by 35 cycles of denaturation (1 min at 94°C), annealing (1 min at the primer binding temperature calculated for each primer set) and extension (1 min at 72°C). The reactions were finalised by polymerisation for 5 min at 72°C. The PCR products were loaded in 1% agarose gel electrophoresis including ethidium bromide and were visualised under UV light.

2.5. Statistical analysis

In the statistical analysis, since the number of cases in the group did not satisfy the normal distribution conditions, the Wilcoxon sign test followed by a Freudian Variance Analysis was used for intragroup evaluation of the results obtained at different times. Chi-square test was used for the relationship between biofilm levels and gene presence. The Statistical Package for the Social Sciences (SPSS) version 20.00 (SPSS Inc., Chicago, IL, USA) statistical package program was used to analyse the data set. A value of $p<0.05$ was considered significant in the evaluation of the data.

3. RESULTS

S. aureus reproduced in 87 (13.2%) of the 658 nasal swab samples. Methicillin resistance was detected in 10 (11.4%) of the reproduced 87 isolates, and the presence of the mecA gene was confirmed. When biofilm formation was examined, it was determined that 86 of the 87 isolates produced biofilm. Biofilm formation was evaluated by taking control strains into account. When the results were evaluated in accordance with OD values of the positive controls and negative controls, strong biofilm formation was observed in (57.5%) 46 isolates and moderate biofilm formation was observed in (42.5%) 40 isolates.

Six of the positive *S. aureus* isolates identified as producing strong biofilms were selected (based on the amount of biofilm formed by *S. aureus* ATCC 25923, they had an equal and higher absorbance rate). Selected isolates were sensitive to fosfomycin, and their MIC values were between 1 and 2 µg/ml (MIC90, 2µg/ml). The effect of fosfomycin on the biofilms of the same isolates was also studied. The lowest BIC value of isolates for fosfomycin was 8 µg/ml. When OD averages according to fosfomycin concentrations were examined, a difference was detected, between 8 µg/ml of fosfomycin and 4 µg/ml of fosfomycin. This is shown in Figure 1.

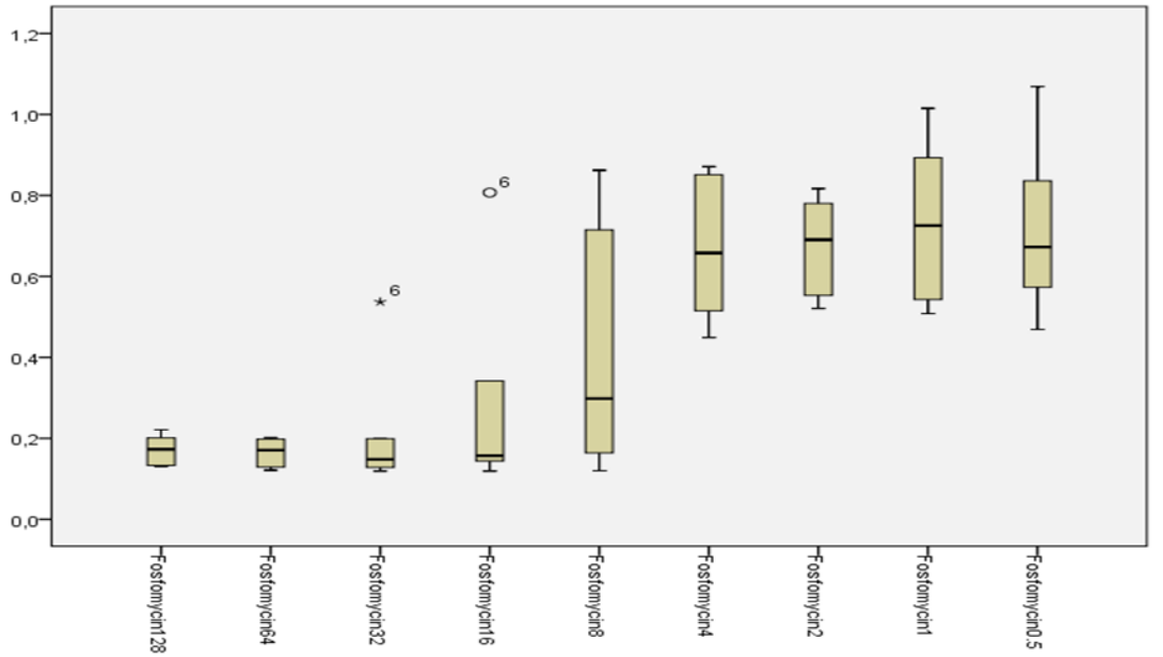

Figure 1. *Box graphical representation of average biofilm OD values of six clinical isolates of Staphylococcus aureus at different fosfomycin concentrations*

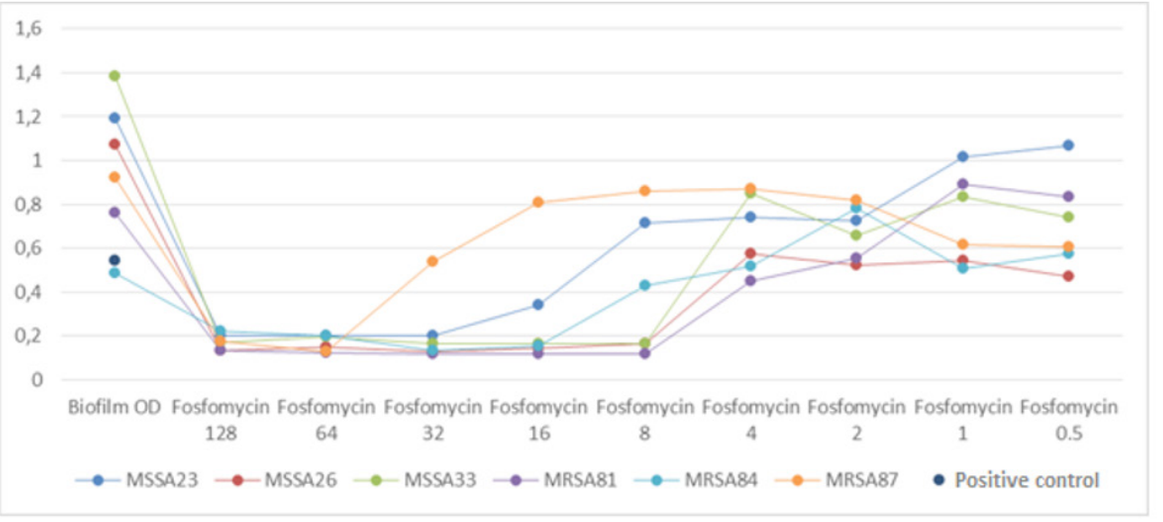

Figure 2. *Change of biofilm ODs according to fosfomycin concentrations of each isolate*

When the effect of fosfomycin concentrations on biofilm ODs are compared, it can be said that the difference between them is due to a statistically significant increase (Table 2).

Table 2. *Effect of Fosfomycin concentrations on biofilm ODs.*

	Fosfomycin16-Fosfomycin32	Fosfomycin8 - Fosfomycin16	Fosfomycin4 - Fosfomycin8	Fosfomycin2 - Fosfomycin4	Fosfomycin1 - Fosfomycin2
Z	-1,753[a]	-2,201[a]	-2,201[a]	-,105[b]	-,734[a]
p	0,08	0,028	0,028	0,916	0,463

Biofilm ODs changes according to fosfomycin concentrations are seen in Figures 1 and 2. ODs of the biofilm formation in accordance with methicillin resistance are shown in Table 3. No significant difference was detected between them in statistical analysis (p>0.05).

Table 3. *ODs of biofilm formation at different fosfomycin concentrations according to methicillin resistance*

	MRSA	MSSA
Biofilm OD	0,722±0,12	1,215±0,15
Fosfomycin128	0,170±0,02	0,160±0,20
Fosfomycin64	0,149±0,24	0,180±0,29
Fosfomycin32	0,262±0,13	0,164±0,20
Fosfomycin16	0,359±0,22	0,216±0,63
Fosfomycin8	0,471±0,21	0,348±0,18
Fosfomycin4	0,611±0,13	0,722±0,80
Fosfomycin2	0,711±0,82	0,634±0,59
Fosfomycin1	0,672±0,11	0,797±0,13
Fosfomycin0,5	0,671±0,82	0,759±0,17

The lowest MBEC value for fosfomycin of these isolates was found to be 32 µg/ml, which is 16 times the MIC90 value (Table 4).

Table 4. *Minimum inhibitory concentration (MIC) and minimum biofilm eradication concentration (MBEC) of the fosfomycin against six clinical isolates of Staphylococcus aureus.*

Isolates	Fosfomycin	
	MIC (µg/ml)	MBEC (µg/ml)
MSSA23	1	32
MSSA26	1	128
MSSA33	1	64
MRSA81	1	64
MRSA84	2	64
MRSA87	2	32

Prevalence of adhesion and regulation of biofilm-related genes in the 86 biofilm-positive *S. aureus* isolates were as follows: eno (97.6%), fnbA (94.1%), icaB (93%), icaD (91.8%), icaA (90.6%), icaC (84.9%), fib (75.5%), ebpS (58.1%), clfB (36%), clfA (15.1%), cna (4.7%). None of the isolates were bap or fnbB positive. However, no statistically significant difference was found between moderate and strong biofilm formation and presence of genes (p>0.05) (Table 5).

Table 5. *Relationship between biofilm level of isolates and gene presence*

	Moderate biofilm-forming isolates total=40 n (%)		Strong biofilm-forming isolates total=46 n (%)		*p*	X^2
Genes	**Negative**	**Positive**	**Negative**	**Positive**		
BAP	40 (100)	0	46 (100)	0	-	-
ICA-A	5 (12.5)	35 (87.5)	4 (8.6)	42 (91.4)	0,565	0,330
ICA-D	5 (12.5)	35 (87.5)	4 (8.6)	42 (91.4)	0,565	0,330
ICA-B	3 (7.5)	37 (92.5)	4 (8.6)	42 (91.4)	0,84	0,410
ICA-C	6 (15)	34 (85)	8 (17.4)	38 (82.6)	0,764	0,900
Fnb-A	4 (10)	36 (90)	2 (4.3)	44 (95.7)	0,305	1,053
Fnb-B	40 (100)	0	46 (100)	0	-	-
CLF-A	33 (82.5)	7 (17.5)	40 (86.9)	6 (13.1)	0,565	0,331
CLF-B	29 (72.5)	11 (27.5)	29 (63)	17 (37)	0,351	0,871
Fib	10 (25)	30 (75)	10 (21.7)	36 (78.3)	0,721	0,127
Ebps	16 (40)	24 (60)	22 (47.8)	24 (52.2)	0,466	0,531
Eno	2 (5)	38 (95)	3 (6.5)	43 (93.5)	0,764	0,090
Cna	39 (97.5)	1 (2.5)	43 (93.4)	3 (6.6)	0,377	0,780

4. DISCUSSION

S. aureus is the most adaptable and common human pathogen. Nasal carriage increases *S. aureus* infection risk by creating endogenous and exogenous sources (5). Biofilms that result in antibiotic resistance are heterogeneous microorganism populations. Biofilms lead to treatment failure since they are sources of infection. Determining the right antibiotic for biofilm formation and choosing a highly effective antibiotic for biofilm layers are significant steps in preventing the infections. In this study, we examined the effectiveness of fosfomycin on *S. aureus* isolates colonised in the nose and we also analysed biofilm-related genes. The effectiveness of fosfomycin on the biofilm formed by 3 MRSA and 3 MSSA isolates created a significant difference between concentrations. There was considerable biofilm inhibition at concentrations of 64, 32, 16 and 8 µg/ml according to the concentration of 4 µg/ml of fosfomycin, and the ODs were decreased. However, it was determined that for the total eradication of biofilm via fosfomycin, the effective dose should be as high as 16 times the value of MIC90 (32 µg/ml).

There are few studies in literature about the effectiveness of fosfomycin in biofilm medium formed by *S. aureus* isolates. In the studies, it was found that a combination of fosfomycin with other antibiotics showed strong in vitro activity against *S. aureus* isolates in biofilm; however, researchers are still trying to determine in vivo efficacy. Tang et al. (2) conducted a new study using a model of the methicillin-resistant *S. aureus* biofilm, and they specified that the antibacterial effectiveness of vancomycin combined with fosfomycin is better than vancomycin alone. Shi et al. (22) detected that fosfomycin and vancomycin are effective in-vivo synergistic bactericides to bacteria in biofilm of MRSA infections. Chai et al. (23), parallel with our study, observed that fosfomycin alone showed an activity of 8-32 µg/ml, depending on the concentration; however, linezolid and fosfomycin together is a stronger combination against MRSA biofilm both in vitro and in vivo. However, in a study conducted with biofilms on polystyrene and metal surfaces, the biofilm inhibitor concentration (BIC) value of fosfomycin was found to be quite high (>256) (24). It has also been reported that fosfomycin is effective in biofilm studies with *P. aeruginosa* and *E. coli* (25, 26).

Another important point that we want to emphasise is that these isolates are not hospital-originated. Nasal colonisation with MRSA is increasing in healthy societies. Nasal carriage is a major risk factor for community-aquired *S. aureus* infections (5, 27). Of the 658 outpatients without a previous hospital history, 13.2% were nasal *S. aureus* carriers and 11.4% of these isolates were MRSA. This rate was lower in our study compared with other studies conducted in our country. In these studies, *S. aureus* carriage was found to exist between 19.1% and 38%; however, MRSA carriage was observed to be much lower than our study (0%-5%) (28). In another study conducted in 2015, nasal carriage was noted to be 17.3% and 0.5% for *S. aureus* and MRSA respectively in healthy university students (29). MRSA carriage around the world has been increasingly reported (30-33). Especially in communities, the frequency of infections caused by MRSA has increased in the last decade (5). It is believed that the prevalence of MRSA carriage increases in a healthy population, and for this reason, the follow-up of the nasal carriage of MSSA and MRSA in healthy individuals is important (29).

Adhesive matrix proteins play a role in the first step of biofilm formation, which is attaching to the surface. Polysaccharide intercellular adhesin (PIA) production is controlled by the icaADBC gene cluster, and it is known that *S. aureus* isolates

harbouring this gene cluster are potential biofilm producers. Our results are compatible with all other studies that show ica-operon is present in almost all *S. aureus* isolates and that it is expression (34, 35). In addition to this, *S. aureus* may excrete a variety of adhesive matrix molecules that interact with the extracellular ligands of the host. Elastin-binding protein (ebpS), laminin-binding protein (eno), collagen-binding protein (cna), fibronectin-binding proteins A and B (fnbA, fnbB), fibrinogen-binding protein (fib) and clumping factors A and B (clfA, clfB) can be named as examples for these molecules (30). Eno (97.6%), fnbA (94.1%), fib (75.5%), ebpS (58.1%), clfB (36%), clfA (15.1%) and cna (4.7%) genes have been detected in our study respectively. fnbB has not been noted in any of the isolates. Athans et al. (35) found that these genes are highly positive in different clonal *S. aureus* isolates producing biofilms. Barbieri et al. (34) emphasised that *S. aureus* isolates that cause breast peri-implant infections in oncologic patients, expression these genes at high rates and the cna gene has an important role in these infections. When compared with other studies, gene prevalence in our study is quite high (36, 37). Also, no statistically significant difference was found between moderate and strong biofilm formation and presence of genes.

Biofilm-associated protein (bap) has been reported as one of the necessary structures for biofilm formation. Studies have shown that bap acts both in adhesion to the abiotic surfaces and in the intercellular adhesion steps (38). Studies on bap in *S. aureus* strains are restricted since the frequency of the bap gene presence is low. The bap gene was predominantly found in chronic bovine mastitis (39, 40). Vautor et al. (41) suggest that the prevalence of the bap gene is low because it is not yet as common among human and animal origin *S. aureus* isolates. No bap gene was detected in any of the isolates in our study as well. We believe that it is important to genotypically characterise the biofilm genes in order to better understand the complex biofilm process that leads to infections. One restriction of our study is that clonal typing was not performed in the *S. aureus* we isolated.

5. CONCLUSION

In conclusion, antibiotics are often ineffective eradicating *S. aureus* and resistance to topical antibiotics such as mupirocin has been reported (42, 43). We detected that methicillin resistance is significant in community-aquired *S. aureus* isolates obtained from the nose and fosfomycin is an effective antibiotic on biofilm; however, it can even become more effective when it is combined with other antibiotics. We also detected that the prevalence of biofilm-related genes is high. Especially in the infections caused by the species forming the biofilm layer, their treatment process is harder and takes longer, since the biofilm layer provides some advantages for bacteria in the protection phase from antimicrobial agents. For this reason, it is important to understand the biofilm mechanism formed by *S. aureus* bacteria. By understanding the processes of the genes and antibiotics involved in the biofilm mechanism, it will be possible to shed light on the production of a new generation of medicines for the treatment of *S. aureus* infections.

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Clinical and Experimental Health Sciences

Development of a Medical Error Scale for Nursing Students: a Methodological Study

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ABSTRACT

Objective: The purpose of this study was to develop the Medical Error Scale (MES) for the student nurses

Methods: This study was a methodological research. The study was conducted with 662 student nurses to develop a Medical Error Scale for student nurses. The scale development study was carried out in the nursing departments of the health sciences faculty of two universities; one in İstanbul and one in Trabzon. The data were collected with a questionnaire consisting of 6 questions about the demographic characteristics of the students and a draft scale including 94 items. In the analysis of the data, frequency, percentage, mean tests, confirmatory and exploratory factor analyses' tests were used.

Results: Content Validity Index of the scale was 0.82, Cronbach's Alpha was 0.94, Spearman-Brown was 0.93,Guttman coefficient were 0.92, the upper and lower 27% test was – 44.42 and p=0.000, and item-total item correlation values ranged from 0.36 to 0.68. The scale had seven subscales according to exploratory factor analysis. The results of confirmatory factor analysis were chi-square/degree of freedom 3.01, RMSEA= 0.055, CFI= 0.97, NNFI= 0.97 and NFI= 0.96 and the scale showed a good agreement with the subscales.

Conclusion: The scale was a valid and reliable tool to collect data on whether student nurses acted carefully or made medical errors during their patient-related practices.

Keywords: Nurse, Student, Scale, Medical Error, Malpractices

1. INTRODUCTION

In recent years, quality service delivery in national and international health care and patient safety has become an increasingly important and updated topic. Therefore, the prevention or reduction of medical errors and medical malpractices as much as possible, which is an important criterion for patient safety, should be considered as a priority (1, 2). In this respect, all healthcare workers, as well as anyone who provides direct or indirect services to the patient, are responsible for the prevention or reduction of medical errors (3).

When the studies on this topic were examined, it was seen that the vast majority of the studies were carried out by health professionals. However, medical malpractices can be carried out not only by health professionals but also by nursing students (4, 5). In some countries, as in the case of our country to close the nurse shortage in clinics, especially senior students are hired to work part-time and usually night shifts. This situation may cause students to put the safety of the patient in jeopardy by performing malpractices. Despite the legal and ethical dimensions of such an appointment or responsibility, this is an undeniable fact. The fact that students are performing an application on a patient without the supervision of the academicians, nurses or counselor nurses and that the patient is severely damaged as a result of the application can cause the student, the instructor, the nursing school and the institution to face the legal problems (6). In addition, medical malpractices can lead to an increase in financial burden due to the prolonged length of hospitalization time of patients, adverse events/ cases such as disability, death in patients, and compensation cases. For these reasons, as Alcan et al., (2012) pointed out that unforeseen events and errors that cause patient safety violations should be identified and analyzed with the aim of preventing errors in advance. Risks must be reduced to prevent errors by predicting situations that put patient safety in jeopardy. Moreover, risky situations and errors must be reported, the causes must be revealed, the proposals for the solution of the problem must be determined and lessons must be drawn from the events (2,7). In this direction, as Akgün and Al-Assaf (2007) also indicated that it was necessary to collect data and evidence with systematic approaches to demonstrate why medical errors arise to develop strategies to solve the problems in the health care system (8). The studies by Rodrigue et al., (2012) and Mira et al., (2015) revealed that the number of studies on what the medical and nursing students knew and what their attitudes were towards adverse events was very limited (9,10). In addition, as Vaismoradi et al., (2011) indicated that the curricula and instructional strategies of nursing students or their perspectives on their training should be assessed to strengthen them so as to ensure the safety of

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service (11). For this reason, it is important to develop valid and reliable scales that will make it easier for nursing students to see whether they are careful about medical errors or what mistakes they can make. Besides, it will be possible to determine in which areas students can make more errors with these scales or tools, take precautions towards risky areas, make measurements at periodical intervals and contribute to the solution of problems. In this context, the investigations of the measurement tools related to medical malpractices for students have reported that Tabbassum et al., (2015) developed a tool to measure medical errors for nursing students, but only its content validity was tested (12). Mira et al., (2015) also developed a measurement tool whose validity and reliability analyses were tested and it evaluated the knowledge and attitudes of medical and nursing students about patient safety during clinical trainings in five countries (10). In the study of Mansour (2015), a Likert-type scale was developed not related to individual medical errors but with the aim of evaluating the perceptions of nursing students about their awareness, skills and attitudes towards the patient safety education by performing exploratory factor analysis (13). As can be seen, a reliable and validated tool to deal with the medical errors or malpractices that can be done by nursing students was not found in the literature. As a result, it was reported that there was a limited number of studies on medical errors related to nursing students with large populations who are risk bearing and serving patients and that there is a gap in the literature concerning to the subject (14,15).

In this context, this study aimed to develop a valid and reliable medical error scale (MES) in order to investigate whether nursing students were performing medical malpractices related to their diagnosis, treatment and care practices during clinical applications, to identify common errors, types and causes of errors, whether students were careful about medical errors or medical malpractices, and to facilitate the identification of the areas where they were likely to have malpractices.

2. METHODS

2.1. Type of Study

This study was a methodological one as the Medical Error Scale (MES) was developed for the student nurses.

2.2. The Universe and Sampling of the Study

The universe of the study consisted of 1561 students studying in the nursing departments of two universities and sampling composed of 662 students selected by a simple random sampling method. In the factor analysis, 50 is considered very poor, 100 poor, 200 fair, 300 good, 500 very good and 1000 excellent for a sufficient sample size (16). For the test-retest study conducted within the scope of validity and reliability, 52 nursing students in a different university were included in the sampling. For the test-retest analysis, the sample should be at least 50 people (17).

2.3. Location and Characteristics of the Research

The scale development study was carried out in the nursing departments of the health sciences faculty of two universities; one in İstanbul and one in Trabzon.

2.4. Ethical Considerations

Written permission was obtained from the rectors and deans of the two universities to conduct the research on 15 July 2013 and 20 August 2013. The research ethics committee approval was received from the Ethics Committee of Non-Interventional Clinical Investigations of Haliç University on 09 September, 2013. Protocol number 06. In addition, nurse students' voluntary participation was ensured.

2.5. Data Collection Tools

In the scale development study, an information form and the Draft Medical Error Scale were used. The information form includes 6 questions about the nurses' age, gender, marital status, the type of high school they graduated from, and the names and classes of the universities they were studying in. The draft medical error scale was designed to determine whether student nurses performed patient applications safely during clinical practices, whether they made malpractices or medical errors or they were cautious about medical errors. It was developed by the researchers in the light of literature (18-30) under the following subheadings; care practices, medication administration, falling, infection and communication. In addition, this draft scale was prepared as a 5-point Likert-type scale consisting of 94 items which were evaluated as always (5), usually (4), sometimes (3), rarely (2), never (1).

2.6. Data Collection Process

During the first phase of the study, the data of face validity of the draft scale established with 94 items by Oztürk ve Kahriman with the help of the literature were obtained by interviewing 3 nurses through face-to-face interviews, and both the face and the content validity data were received by hand or by e-mails. For content validity, a guideline was submitted to the experts explaining the Lawshe technique, which introduced the scale and would be used for content validity. After the content validity process, the data regarding the exploratory (EFA), and confirmatory factor analysis (CFA) under the scope of the construct validity and the reliability analyses of the 85-item draft scale were received by hand from the second, third, and fourth – year nursing students at two universities, one in Trabzon and one in Istanbul by the researchers. The students were explained the aim of the study and how they should fill the scale. Afterwards, the data obtained from the students were entered into the statistical programs and the EFA, CFA and the reliability analyses were carried out. After the EFA and CFA analysis, the test-retest data, which is a reliability test, was conducted with a separate sampling including 52 student nurses studying at a university in Erzurum after the aim and the application process were explained. The students were asked to use nicknames and the 36-item scale was applied and received by hand with an interval of two weeks.

2.7. Statistical Analysis

Before all the tests carried out within the scope of the study, the central and prevalence criteria of the normal distribution of the data were checked and the Kolmogrov-Smirnov test was performed.

Percentage, mean tests and EFA and CFA were conducted for the validity analysis of MES. EFA was performed with Kaiser-Meyer-Olkin (KMO) and Bartlett test, anti-image correlation, Principal Components Analysis and Varimax Rotation. Chi-square, X2, RMSEA, CFI, NNFI, NFI tests were used for CFA. Besides, t-test and regression analysis were also used. For reliability analysis, Cronbach Alpha, item-total correlation tests and test-retest were performed.

Limitations of the study;

The limitation of the research was that it was carried out through the opinions of second, third, and fourth – year nursing students at two universities in Turkey. Another limitation is the use of a sample set of the exploratory factor analysis for confirmatory factor analysis.

3. RESULTS

According to the demographic characteristics of the student nurses, the average age of the students was 20.66±1.78 years. 60.9% (403) of them were studying at Karadeniz Technical University, Faculty of Health Sciences, Nursing Department and 39.1% (259) of them were studying at Marmara University Faculty of Health Sciences, Nursing Department. 81.7% (541) of them were female, 18.3% (121) were male, 97.4% were single and 2.6% (17) were married. 54.5% (361) were second-year students, 22.4% (148) were third-year students and 23.1% (153) were fourth-year students. 53.8% (356) were Anatolian/Super/Science High School, 35% (232) were Standard High School, 11.2% (74) were Vocational Health High School and other high school graduates (Table 1).

Table 1. *The demographic characteristics of the student nurses*

Demographic characteristics	Mean	SD
Age	20.66	1.78
University	**n**	**%**
KTU Faculty of Health Sciences	403	60.9
MU Faculty of Health Sciences	259	39.1
Gender		
Female	541	81.7
Male	121	18.3
Marital Status		
Single	645	97.4
Married	17	2.6
Class		
second-year students	361	54.5
third-year students	148	22.4
fourth-year students	153	23.1
Graduated sSchool		
Anatolian/Super/Science High School	356	53.8
Standard High School	232	35.0
Vocational Health and Other High School	74	11.2
Total	662	100.0

3.1. Face Validity

In terms of face validity of the draft scale, it was submitted to three student nurses and their opinions were requested to determine whether it was understandable, easy to read, expressed properly, accurately, clearly, whether the students were bored during filling, whether its statements were long, and how much time was spent. Afterwards, experts' opinions were asked for both face and content validity, and each item was evaluated by them. 6 items in the scale were improved to be more comprehensible.

3.2. Content Validity

Content validity means to what extent the test or scale items, specified as the sample, represent the conceptual main mass for a specific purpose. In other words, the more selected sample items represent the conceptual main mass, the more content validity they have (Şencan, 2005). Lawshe technique was used for content validity of a study and it consisted of setting up field expert groups, preparing candidate scale forms, receiving expert opinions and content indexes for the items, and formulating the form (31). In this direction, a total of 15 specialists who had worked as nurses and managers for many years in the fields of Nursing Fundamentals, Internal Diseases Nursing, Surgical Diseases Nursing, Obstetrics and Gynecology Nursing, Pediatrics Nursing, Nursing Management were requested to give their opinions. The experts evaluated each item as 'Necessary/ Appropriate', 'Need to be Improved' or 'Unnecessary/ Inappropriate' to assess the suitability of the materials for the purpose and conceptual structure. From the 94-item scale prepared as a draft, 9 items were excluded in line with the opinions of the experts and finally the scale was formed with 85 items. With the exclusion of 9 items due to their minimum value of the Content Validity Ratio (CVR) which was under 0.49 at $\alpha = 0.05$ significance level, the ultimate Content Validity Index (CVI) was determined as 0.82.

3.3. Construct Validity

Exploratory (EFA) and confirmatory (CFA) factor analyses were conducted to test the construct validity of the MES. Principal Components Analysis and Varimax Rotation Method with Kaiser Normalization were used for EFA. While the KMO (Kaiser – Meyer-Olkin) value of the 85-item draft scale was found as 0.935, the Bartlett test was χ^2= 12467.131 and p= 0.000 and anti-image correlation values were between 0.837 and 0.965.

In Rotated Component Matrix analysis for EFA, 7 rotations were performed. Twenty-six items (4,5,7,8,17,24,26,27,29,30,32,33,34,40,41,50,51,52,53,71,72,73,75,77,81,83) in the first rotation, 9 items (85,35,74,76,13,21,31,949) in the second, 4 items (48,37,6,12) in the third, 2 items (11, 25) in the fourth, 1 item (10) in the fifth, 3 items (38,14,28) in the sixth, and 4 items (22,23,36,39) in the seventh rotation were excluded since their loadings (49 items) were under 0.45. The scale was made up of 7 sub-dimensions and 36 items and it was free from overlapping items. The factor load values of the scale were between 0.559 and 0.827.

Table 2. *The mean values of factor items of MES and factor load values distributions*

Factor Name	Variance %	Items	Min.	Max	Mean	SD	Factor load value
F1 – Falling	13.45	59-66	1.38	5.0	4.20	0.69	0.602-.715
F2 – Blood and Blood Product Transfusion	11.92	42-47	2.0	5.0	4.62	0.54	0.686-.827
F3 – Patient Transfer	8.67	54-58	1.0	5.0	4.11	0.79	0.559-.754
F4-Medication Administration	8.25	15,16,18-20	1.0	5.0	3.89	0.74	0.640-.709
F5 – Communication	8.15	78-80,82,84	1.6	5.0	4.47	0.60	0.623-.700
F6 – Infection	6.92	67-70	2.5	5.0	4.56	0.52	0.598-.768
F7 – Care Practices	5.17	1-3	2.7	5.0	4.30	0.46	0.618-.807
Total	62.55						

Table 3. *Distribution of the items and their factor loadings according to the subscales of MES*

Item Number	Subscales of MES	Factor loads
Falling		
62	I tell the patient's companion that they should inform the nurse when they are leaving the patient alone.	0.715
65	I ensure that patient rooms or corridor floors are dried if they are wet.	0.696
63	I put the call button somewhere the patient can easily use.	0.685
66	I often visit the patients who have a risk of falling.	0.678
60	I tell the patient that s/he should inform a nurse when s/he wants to stand up.	0.673
64	I remove unused materials out of the patient's room.	0.655
61	For agitated patients, I place cushions on the side of the bed.	0.649
59	I lock beds and wheelchairs if they are not used.	0.602
Blood and Blood Product Transfusion		
45	I apply blood and blood products according to the technique.	0.827
44	Before applying blood and blood products, I check their expiration dates.	0.795
43	I control the label information of the product before applying blood and blood products.	0.791
46	I make sure of the blood type of the patient before using blood and blood products.	0.785
47	I observe the patient for possible complications after the application of blood and blood products.	0.738
42	I prepare blood and blood products according to the procedures.	0.686
Patient Transfer		
56	I confirm that the patient is not being transferred alone.	0.754
55	I always monitor my patients while they are being transferred.	0.741
57	I make sure that the patient is not discharged alone.	0.697
54	I ensure the transfer of the patient according to the protocol (with a stretcher / a wheelchair).	0.668
58	I check the availability of the care equipment and devices before transfer.	0.559
Medication Administration		
20	I administer medication after I learn its effects, interaction and side effects.	0.709
15	I give the patient's oral medication or be near him/her until s/he takes it.	0.705
16	I do not leave the medication with the patient so that s/he can take or apply it.	0.679
18	After the medication I monitor the patient for positive/adverse effects.	0.675
19	I do not administer medication without checking its expiration date.	0.640
Communication		
78	I clarify unclear and potentially problematic orders.	0.700
79	I write down any information related to the patient's treatment in the nurses' observation chart.	0.673
84	I record the name and surname, date and time of the physician instructing verbally/on the telephone.	0.669
82	I inform the physicians/ healthcare workers about the results of emergency critical tests.	0.651
80	I provide information on the patient's care and outcomes at shift changes.	0.623
Infection		
68	I put on and take off the gloves in due form.	0.768
69	I wash my hands and change the gloves from one patient to another.	0.750
67	I wash my hands properly before and after the practice.	0.671
70	I use materials and products exclusively for each patient.	0.598
Care Practices		
2	I check the local effects of the care practices that I apply.	0.807
3	I check the systemic effects of the care practices that I apply.	0.725
1	I verify the patient's identity for practices that I will perform.	0.618

In the scree-plot graph of the scale, the slope plateaued after the seventh point. This cut off point also supported the fact that the scale had 7 subscales (16) These 7 subscales accounted for 62.55% of the total variance of the scale together; the first subscale accounted for 13.45%, the second subscale 11.92%, the third subscale 8.67%, the fourth subscale 8.25%, the fifth subscale %8.15, the sixth subscale (6.92%), and the seventh subscale (5.17%) (Table 2). Factors were named after this step. The names of the selected factors/ scales were chosen from meaningful, expressive and well-known words, and the comments and names of factors were written in accordance with the theoretical basis (32).

The factor structure of the 36-item MES was tried to be confirmed with the CFA. For CFA, firstly, the items with non-significant t-values were examined, and it was determined that all the R coefficients and t-values of 36 items were significant and the model (factorial structure) was confirmed. The path diagram was presented in Figure 1.

Fit indices for MES's CFA are chi-square (χ^2)= 1766.95, chi-square/ degree of freedom (χ^2/df)= 3.01, Root Mean Square Error of Approximation (RMSEA)= 0.055, Comparative Fit

Index (CFI)= 0.97, Non-Normed Fit Index (NNFI)= 0.97, Normed Fit Index (NFI)= 0.96. When the coefficients indicating the relationship between the observed variables of the model showing the factorial structure of the scale and the factors were examined, it was concluded that all the coefficients were high. When the fit statistics calculated with the CFA was taken into consideration, the previously determined 7 factor-structure was generally determined to comply with the collected data.

To test the validity of the scale, the item-total correlation test which is an internal consistency test and an item discrimination procedure was performed, and for upper and

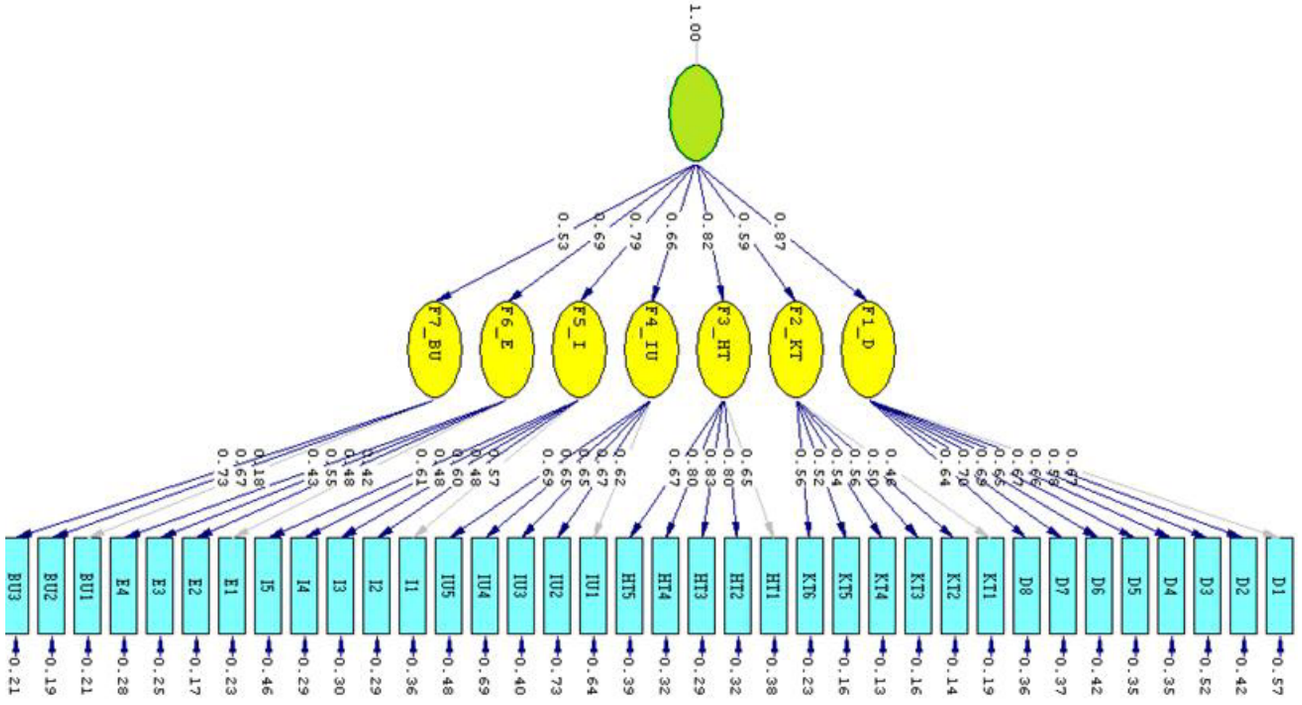

Figure 1. *Path diagram for MES. X^2=1766.95, X^2/df=3.01, RMSEA=0.055, CFI=0.97, NNFI=0.97, NFI=0.96. MES=Medical Error Scale, F=Falling, BT=Blood and Blood Products Transfusion, PT=Patient Transfer, MA=Medication Administration, C=Communication, I=Infection, CP=Care Practices.*

lower 27% quartiles the independent t-test was analyzed. Since the item-total correlation test is also a reliability test, it was presented within the scope of reliability tests. The t-test results for the upper and lower 27% quartiles of the scale were t= – 44.427 for total; t= – 26.386 for F1, t= – 16.729 for F2, t= – 28.388 for F3, t= – 20.005 for F4, t= – 19.540 for F5, t= – 19.310 for F6, t= – 17.010 for F7, and p values were statistically significant for each (p= 0.000).

3.4. Reliability Analysis

Cronbach Alpha, Spearman Brown and Guttman tests were tested to check the internal consistency of the draft scale within the scope of reliability. In addition, item-total correlation test which analyses both reliability and validity was performed. Test-retest analysis was used to determine the time-invariance of the scale.

The total Cronbach Alpha, Spearman-Brown, and Gutmann values of the scale were 0.94, 0.93, and 0.92, respectively. The Cronbach Alpha values for the subscales ranged from 0.71 to 0.91, while the Spearman Brown values ranged from 0.70 to 0.89 and the Gutmann value was between 0.70 and 0.90.

After these analyses, item – total item correlation values, which is also a substance analysis or substance discrimination process used for testing the internal consistency, reliability and validity of the scale, were found between 0.365 and 0.684 and significant (p= 0.000). In addition, in the sub-factors dimension, item-total correlation values were determined as 0.568-0.647 for F1, 0.504-0.555 for F2, 0.636-0.684 for F3, 0.485-0.556 for F4, 0.537-0.620 for F5, 0.511-0.561 for F6, 0.365-0.562 for F7, p= 0.000) and they were significant.

According to the test and re-test correlation values in the total of MES, there was a high positive correlation between the first and last application scores (r= 0.72) and this correlation was statistically significant (p= 0.000). In addition, there was no statistically significant difference between the two measurement scores (p= 0.482) in the comparison of these two application scores of the MES.

3.5. Evaluation of the Scale

The subscales of the scale, which was finalized with 36 items were as follows; Factor 1 – Falling (F1) 8 items (between 59 and 66); Factor 2 – Blood and Blood Transfusion (F2) 6 items (42-47); Factor 3 – Patient Transfer (F3) 5 items (54-58); Factor

4 – Medication Administration (F4) 5 items (15,16, 18-20); Factor 5-Communication (F5) 5 items (78-80,82,84); Factor 6 – Infections (F6) 4 items (67-70); Factor 7 – Care Practices (F7) 3 items (1-3) (Table 2 and Table 3). The total score range of the scale was 36-180. The scores close to 180 from 108 on the scale indicated that the student nurses were behaving in a controlled or cautious manner with respect to medical errors, while the scores close to 36 from 108 demonstrated that the student nurses might not be in control of medical errors. In order to be able to make a comparison, when divided by the number of items, these scores were found between 1 and 5 in terms of the sum scale and subscale levels. Accordingly, the scale scores were evaluated.

4. DISCUSSION

In institutions, timely detection of medical errors/ malpractices is important in order to identify problem areas by uncovering their causes and making measurements and to determine proposals for their solutions. In addition, measurements are necessary to make corrective initiatives by comparing the old and new results, to prevent the reoccurrence and to determine the progress. It is also proposed to examine the attitudes and behavior of patients and the other people involved in the health service provision besides these technical measures, in terms of their awareness level about patient safety so as to ensure patient safety, reduce medical errors, and eliminate or predict adverse conditions (33, 34). Additionally, the collection and measurement of the data through a measurement tool that will be able to make valid, accurate and consistent measurements are a necessity for the results to be valuable. When the measurement tools regarding the detection of the individual malpractices/ medical errors of student nurses or the carefulness of students about medical malpractice were investigated, it was seen that in some studies a survey (15,28, 29) or qualitative studies (35) were performed, and in a study the content validity of a measurement tool was done (12) but the number of validity and reliability studies was not sufficient. To fill this gap in the literature, there was a need to develop a valid and reliable tool which could guide to determine whether student nurses were careful about medical errors or whether they were making safe patient applications, in which areas they were making more errors, and to take precautions against risky areas, so a MES was tried to be developed for student nurses. In this respect, while face, content, and construct validity studies were performed for the validity of MES, internal consistency tests and item-total correlation analysis that is also a validity test and test/ re-test method called as a stability analysis were done for the reliability of the study.

The reliability is defined as the degree to which a scale measures a desired property in a consistent and stable manner, while the validity is to what extent a scale measures a desired property or whether it is appropriate for the property to be measured (36, 37). However, if a test is not valid, then there is no point in discussing its reliability. For this reason, validity is more important than reliability (37).

In this study, firstly, the face and content validity of MES were tried to be ensured. Although face validity has not been performed for a long time since it is indeterminate and subjective, some authors state that face validity is different from the content/scope, so it should be carried out (32). The face validity is used to determine that a tool is in appearance that includes the concept investigated. (38, 39). It takes the opinions of the researcher himself, then his close circle of friends, and the other people involved in the pilot study as to whether a scale measures what has been searched. In this process, it is necessary to ensure the conformity of the statements to the purpose and the education, culture and knowledge levels of the target group should not be forced. In addition, the readability, clarity and length analysis of the terms in the scale are performed (32). In this study, the researchers took three student nurses within the scope of face validity. Moreover, the face and content validity of the scale were performed together and the experts were requested to evaluate the face validity of each item in the scale. As a result, 6 incomprehensible, hard-to-read, and long items were rearranged and shortened to be more comprehensible.

The content validity of the scale was tested by Lawshe technique. In this technique, it is recommended that at least 5 and at most 40 experts should be consulted (31). In this study, 15 experts were consulted and 9 items were excluded from the draft scale consisting of 7 sub-scales. The Content Validity Index (CVI) of the scale, formed by 85 items, was 0.82. This value indicated that the content of the scale was acceptable or that the scale items represented a conceptual main structure for medical malpractices that students might make, because a value of 0.80 or higher for the CVI is considered as an acceptable criterion (12,40).

After the face and content validity, the construct validity of the scale was tested. The exploratory and confirmatory factor analyses were carried out for the construct validity, which identifies the theory or features, or theoretical structures that a scale measures (36,37,39). Factor analysis is a statistical approach that analyses the relationship between a large numbers of variables and explains these variables in terms of common fundamental dimensions (41). In order to be able to perform a factor analysis, there must be a significant correlation between the variables (41). The Bartlett test examines whether there is a sufficient correlation between the variables. The p value of this test is expected to be lower than the significance level of 0.05. This result shows that there is a sufficient level of correlation between the variables for factor analysis (41). KMO value showing whether the selected sample is sufficient for factor analysis was above expected limit with 0.93 in this 85-item scale and the Bartlett test was significant at an advanced level. If the value of KMO is bigger than 0.50, it is an indication that factor analysis can be carried out. As in our study findings, it is considered perfect if the KMO is higher than 0.90 (36, 41, 42). The question group's overall agreement with the KMO is measured by

factor analysis while each item/ question is measured by anti-image correlation and this value should not be less than 0.50. If this value is less than 0.50, it is recommended to exclude this item from the analysis (32, 36, 42). As for Hair et al., (2010) this value must be above 0.7. The Anti – Image Correlation values of the draft scale in this study were over 0.83. These results showed that factor analysis could be performed. Afterwards, an analysis of the rotated basic components was carried out for exploratory factor analysis. After 7 repeated analyses, 49 items with a load value under 0.45 were removed from the scale. The factor load value of 0.45 or higher is considered as a good criterion for selection (42, 43). In this respect, the scale was formed with 36 items and consisted of 7 sub-scales under the title of 'falling, blood and blood products transfusion, patient transfer, medication administration, communication, infection and care practices'. In the Scree plot, the plateau was formed after the seventh point to confirm these 7 subscales (16). The total variance of the scale was 62.5% and this value, which was explained by seven sub-dimensions. Accordingly, 37.5% of the scale could not be explained. However, the total variance of the scale is slightly higher than the desired level of variance (60%). Even for some researchers, the minimum variance explanation ratio is 50% (36).

After EFA, confirmatory factor analysis (CFA) was performed to confirm the structure. CFA is a method of analysis that shows whether a previously defined or constrained structure is verified as a model (16) or it means the verification of the theoretical structure or model (16, 32). CFA is actually used to test the researcher's theorem (44), that is, the researcher must theoretically know what the scale questions measure. But doing this with EFA and verifying it with CFA is a common practice. In other words, the items and subscales determined by performing EFA are regarded as a model and they are tested with CFA to make sure they are correct (44) In addition to this, compared with EFA, CFA is a stronger analysis method because it gives theoretically more reliable information about the validity of the model and factorial structure. In CFA, it is tried to prove that the observed and determined variables, based on the theoretical information, are related to the hidden factors and these hidden factors are related to each other. All assumptions about relationships in CFA are based on the previous research findings and theoretical knowledge. In other words, CFA is applied with the aim of testing and verifying the theoretical information. In this study, in contrast to EFA, the variables/ scale items were previously assigned/ placed in certain factors (32).

It was seen that t test values in CFA of MES were meaningful and no item was meaningless. For this reason, no item was excluded from MES (44). After this process, the goodness-of-fit tests were examined in CFA to evaluate the validity of the model. There are a wide variety of fit tests in the literature, but there is no exact consensus on which of these fit tests will be regarded as the standard (44). Some of fit tests are chi-square goodness, chi-square/degree of freedom, RMSEA, CFI, NFI, NNFI. Among them chi-square (chi-square goodness=χ^2) is the most commonly used one (44, 45). In the chi-square test, it is expected that the agreement between the data and the model is perfect, or the value obtained is close to zero and the value of p is not significant. Therefore, the large chi-square values obtained indicate how bad the agreement is (16). Since the chi-square value is influenced by the sample size, the value obtained by dividing the chi-square value by the degree of freedom is taken into account (16, 44). For our analysis, the chi-square/degree of freedom (χ^2/df) value was 3.01. This value showed that the scale items were in good agreement with the subscales because the chi-square/freedom value which is 3 and below in the large samples corresponds to a good agreement (16, 41, 46) and if it is 5 and below corresponds to acceptable agreement (16, 44, 47). In addition, if the sample size is 200 or smaller, it causes the chi-squared value to shrink and improves the agreement of the model (16). However, although the sample size was more than 200 in this study, the chi-square index showed good agreement. RMSEA value of MES was 0.055 which showed good agreement. The Root Mean Square Error of Approximation (RMSEA), one of the decentralized indexes, is a test used to detect population covariance in decentralized chi square distribution. RMSEA has a value between 0 and 1; a value which is less than 0.5 and 0.8 indicates a perfect and good agreement respectively (16, 44, 47). Other important fit tests are CFI, NFI and NNFI. Comparative Fit Index, one of the comparative fit indexes Comperative Fit Index (CFI) gives the difference between the null model and the established model, assuming there is no relationship between the variables. It compares the covariance matrix produced by the model which predicts no relation between hidden variables and the covariance matrix produced by the proposed structural equation model. CFI value is expected to be between 0 and 1, and CFI is considered perfect and good or acceptable if it is higher than 0.95 and 0.90 respectively (16, 44, 47). In this study, the fact that the CFI value explained in the study was 0.97 indicated that the agreement was perfect fit.

As for Normed Fit Index (NFI), it is similar to CFI in terms of its comparative models, but it makes comparisons without the obligation to comply with the assumptions/ quantiles required by the chi square distribution, the model is estimated by comparing the chi-square value of the independent model with the chi-square value of the model. Non-Normed Fit Index (NNFI) is calculated by adding the degree of freedom to the NFI value in small samples. It is similar to NFI but gives a value considering model complexity (16). Similar to CFI, NFI and NNFI have also a value of 0-1. A value close to 1 indicates perfect agreement while it means an inconsistency when it is close to 0 (16, 41). However, it is expected that both values will be 0.90 and over, which is a good agreement (16). In this study, the NFI value is 0.96, the NNFI value is 0.97, or in other words, these values have a good fit. As a result, when the coefficients, showing the relationship between the observed variables and the factors of the model showing the factorial structure of the scale, were examined, it was concluded that all the coefficients were sufficient. When the fit indexs calculated with CFA were taken into account, it

was decided that the previously determined structure of the scale provided overall agreement with the aggregated data.

After the factor analysis, internal consistency and item discrimination process were carried out. According to the difference between the item average scores of the upper and lower %27 quartile groups for the internal consistency and item discrimination power, the distinguishing level of the items in the scale was significant or the upper and lower groups of the items were well distinguished. In other words, the validity of the scale items was high, or it can be said that the student nurses distinguished their behaviors related to medical malpractices at a good level and they measured the same behavior. Significant differences between the groups are also considered as a sign of the internal consistency of the test (43).

After this process, the internal consistency was examined to test the reliability and homogeneity of MES, which was formed with 36 items. Internal consistency investigates whether items measure a particular conceptual structure consistently by using a single measurement tool and a single session (32). In another definition, it is the correlation of item scores forming a test with the score obtained from the whole test (37). The Cronbach Alpha, Spearman Brown, and Gutmann values, which show the internal consistency of the MES, were 0.70 and more or close to 1. In addition, the Cronbach Alpha value at the subscale level was between 0.71 and 0.89. The higher these values are, the more the items on the scale are consistent with each other and consist of the same items questioning the same characteristics (32, 48). A value of 0.70 or higher in the internal consistency coefficients is generally regarded as sufficient for the reliability of test scores (42, 43).

Item-total correlation explains the relationship between the scores from the test items and the total score of the test. If the relationship between the scores obtained from an item and the whole scale shows a positive correlation and a "sufficiently high" correlation, then that item is discriminatory or it is assumed that this item simulates similar behavior and is included in the scale (43, 48). The item-total correlation values of the 36-item scale were over 0.36. When the item-total correlation is interpreted, some limit values are taken as criteria. Correlation value should be 0.30 and above, and these items distinguish the individuals at a good level (32, 43).The high correlation values indicated that all scales were in the same structure.

Another test which is used to examine the reliability of the scales is the test-retest. The same form or scale for this test is administered to the same individuals twice under the same conditions but at different times (37). Test-retesting shows whether the measured characteristic of a test has changed according to the elapsed time, or how steady the test has measured over time, or whether similar responses have been reached (17, 48) According to the test-retest results in this study, it was seen that MES was stable or consistent in pre – and post-administrations at different times and that the scale is reliable in terms of continuity coefficient.

5. CONCLUSION

In this study which was conducted to find out whether nursing students were careful about medical malpractices or whether they made any medical errors, the face validity was provided by analyzing the clarity of each item and the length of the statements in the MES. For the content validity, it was determined that the content validity index was close to one or higher, or that the items in the draft scale were aimed at evaluating the attitudes and behaviors of the student nurses regarding medical errors/medical malpractices. For the explanatory factor analysis, through KMO, anti-image correlation test and Barlett test, it was determined that the sample selected for the entire question group was appropriate, each question/ item was appropriate for the factor analysis with, and there was a relation between the variables respectively. As a result of the analysis of the basic components, the scale was formed with 36 items after the rotation and 7 subscales were found as Falling, Blood and Blood Transfusion, Patient Transfer, Medication Administration, Communication, Infections, Care Practices. The variance ratio explained by these 7 sub-dimensions is practically acceptable. In addition, the structure/ model of this scale which was formed or determined by the explanatory factor analysis and consisted of these seven dimensions was confirmed by the confirmatory factor analysis or it was determined that overall agreement was generally good fit according to the results of the confirmatory factor analysis fit indices. Besides, the difference between the upper and lower 27% quartile groups was meaningful and the results of internal consistency analysis were high. All these results showed that the scale was valid. After the reliability analysis conducted following the validity analyzes, the fact that the Cronbach Alpha, Spearman Brown and Gutmann values of the scale were 0.70, the item total correlation values were above 0.30 and there was no difference between the test-retest results which showed time invariance revealed that 36-item scale was consistent and reliable.

In this respect, this scale can be used to accurately and consistently measure whether the student nurses are careful with regard to malpractices or whether they perform medical errors/ medical malpractices, in which areas they have problems, and where they need to be improved. In the areas where students are likely to make mistakes, the necessary precautions can be taken to prevent them. In addition, this study may be a guide or resource for further scale studies and may provide an opportunity to compare it with the previous scale studies. Besides, retesting the factor analysis of this scale in other samples and retesting its structure by administrating it concurrently along with similar scales could also enhance its validity.

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Clinical and Experimental Health Sciences

Effect of Dental Follicle Mesenchymal Stem Cells on Th1 and Th2 Derived Naive T Cells in Atopic Dermatitis Patients

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ABSTRACT

Objective: The purpose of our study is to investigate the immunomodulatory effects of Dental Follicle Mesenchymal Stem Cells (DF-MSCs) on lymphocytes isolated from peripheral blood of Atopic Dermatitis (AD) patients, a Th2 disease and psoriasis, a Th1 / Th17 disease and compare them with healthy individuals in vitro.

Methods: Patients with the AD (n = 9) and psoriasis (n = 6) who are followed up in Marmara University Pediatric Allergy and Immunology and Dermatology outpatient clinics and healthy subjects (n = 6) were included. Peripheral Blood Mononuclear Cells (PBMCs) were isolated from 20 ml of venous blood of all participants. Cells were cultured for 72 hours in the absence and presence of DF-MSCs with anti-CD3/anti-CD28 stimulation or without stimulation. At the end of this period, CD4+ and CD8+ T lymphocyte proliferation and cytokine levels from the culture supernatants were analyzed by flow cytometry.

Results: In the presence of DF-MSCs, proliferation ratio was suppressed in both CD4+ and CD8+ cells in AD and psoriasis patients ($p<0,05$). IFN-γ levels significantly increased in AD patients in the presence of DF-MSCs ($p<0,05$) whereas decreased significantly in psoriasis patients in the presence of DF-MSCs ($p<0,05$). IL-4 levels significantly decreased in AD patients in the presence of DF-MSCs ($p<0,05$) but remained unchanged in psoriasis patients ($p>0,05$). IL-10 increased significantly in both groups in the presence of DF-MSCs ($p<0,05$).

Conclusion: Our results support immunoregulatory effects of DF-MSCs on both AD and psoriasis which are Th2 and Th1 / Th17 dominant diseases respectively. Our evidence-based results demonstrated that DF-MSCs could have a beneficial therapeutic implication for inflammatory skin diseases.

Keywords: Mesenchymal Stem Cells, Immunoregulation, Soluble factors, Atopic Dermatitis, Psoriasis

1. INTRODUCTION

Atopic Dermititis (AD) is a chronic inflammatory skin disease in which immune responses are mediated by Th2 (T helper 2) cells (1-3). It is one of the most common skin disorders with an estimated prevalence of up to 20% of children and 3% of adults (4,5). It is characterized by xerosis, eczematous lesions, and severe pruritus (6,7). There is a complex interplay between genetic, environmental and immunological factors in the pathogenesis of AD (8). Psoriasis is, in contrast, a skin disease mediated mainly by Th1 and Th17 cells that produce IFN-γ and IL-17 (9-11).

Several first-line treatments are available for anti-inflammatory response to reduce the clinical symptoms of the AD and Psoriasis. Topical corticosteroids and systemic immunosuppressants and cytokine antagonis biologic therapies are used for treatment. However, none of these agents can provide a cure and have multiple side effects (12-15). Accordingly there is an unmet need for more effective and safe therapeutic options in AD management. Mesenchymal Stem Cells (MSCs) can be a promising candidate because they can regulate multiple factors simultaneously in response to the inflammatory conditions (16,17).

MSCs are the non-haematopoietic, multipotent stem cells with the capacity to differentiate into mesodermal lineage such as osteocytes, adipocytes and chondrocytes. MSCs express cell surface markers like cluster of differentiation CD29, CD44, CD73, CD90, CD105 and lack the expression of CD14, CD34, CD45 and HLA (Human Leucocyte Antigen)-DR (18). MSCs regulate the functions of various immune cells, including T cells, B cells, natural killer cells, monocyte/macrophages, dendritic cells, and neutrophils. T lymphocytes are the central mediators of many autoimmune and inflammatory diseases as well as of transplant rejection and graft-versus-host disease (19). Dental tissues provide a readily accessible source of MSCs (20). Among the dental origin MSCs, Dental follicle MSCs (DF-MSCs) show potent immunomodulatory properties which make them attractive approach for suppression of inflammatory conditions (21). Immunomodulation by MSCs is mediated by both direct cell-cell contact and release of soluble factors such as prostaglandin E2 (PGE2), indoleamine 2,3 dioxygenase (IDO), Transforming Growth Factor β (TGF-β), released in response to stimulation by inflammatory cytokines (22-24).

Several recent studies have investigated the effects of Umbilical Cord-MSCs ve Bone Marrow-MSCs on antibody

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secreted B cell and mast cell degranulation in AD patients. Current data supports different and robust modulatory effects of MSCs, particularly on T cell response (25). Furthermore, the preventive or therapeutic potency and the mechanisms of action can be altered by changing the sources of MSCs.

The purpose of our study is to investigate the regulatory potential of DF-MSCs on $CD4^+$T helper (Th) and $CD8^+$ T cytotoxic (Tc) cell proliferation, inflammatory and anti-inflammatory cytokine levels in AD patients and compare them wih psoriatics and healthy subjects.

2. METHODS

2.1. Study Subjects

Nine patients with an AD (mean age, 15,6 ±1,62 years) who fulfilled the criteria of Hanifin and Rajka (R) are included in the study. All patients showed positive cutaneous reaction to house dust mite. Patients showed total IgE of >400 IU/mL (mean, 2650±506,99 IU/mL). None of the patients had any systemic immunosuppressive treatment for at least 2 months before testing and don't have any other chronic disease. Six Psoriasis patients (mean age, 22,33±4,68 years) are included. Their mean serum IgE level was 19,5 ±6,09 IU/mL. None of the patients had any systemic immunosuppressive treatment for at least 2 months before testing. Six healthy controls (mean age, 24,5±3,03 years) with no history of atopy are included in the study as controls. Their mean serum IgE level was 17 ±1,2 IU/mL. (Table 1).

This study is approved by Ethics Committee of the Marmara University Medical Faculty in Istanbul, Turkey (Protocol No: 09.2016.196/70737436-050.06.04). Written informed consent was obtained from all patients.

Table 1. *Demographic data and clinical characteristics of the Patients. AD; Atopic Dermatitis patients (new diagnosed and non-treated), Ps; Psoriasis patients (new diagnosed and non-treated), C; Control group including HC. F: Female; M: Male; DF: Dermatophagoides farinae;DP: Dermatophagoides pteronyssinus, N: Negative.*

Patients	Age	Gender	Total IgE U/ml	SPT(mm)	Topical Therapy
AD1	18	M	2423	DF: 5*6, DP : 6*6	N
AD2	14	M	2748	DF: 6*5, DP:6*6	N
AD3	15	F	2105	DF: 3*3, DP:4*3	N
AD4	14	M	2867	DF: 5*5, DP: 4*4	Y
AD5	14	M	2374	DF: 6*6, DP: 5*6	N
AD6	13	F	3715	DF: 4*5, DP: 5*5	Y
AD7	22	M	2604	DF: 6*6, DP: 5*6	N
AD8	17	M	2031	DF: 4*5, DP: 6*6	N
AD9	18	M	2987	DF: 5*5, DP: 5*4	N
Ps1	28	F	28	N	N
Ps2	22	F	15	N	N
Ps3	17	M	11	N	N
Ps4	20	M	24	N	N
Ps5	19	M	20	N	N
Ps6	28	F	19	N	N
HC1	22	F	25	N	N
HC2	23	F	15	N	N
HC3	25	M	23	N	N
HC4	20	M	10	N	N
HC5	27	M	28	N	N
HC6	30	F	17	N	N

2.2. Isolation and culture of DF-MSCs

Dental follicle tissues were collected from 4 healthy volunteers aged between 19-25 years who have no abscess or inflammatory diseases. Third molar teeth were surgically removed before its eruption, and the dental follicle was extracted under sterile conditions. Dental follicle was cut into approximately 0,5 mm of diameter pieces and digested with 3 mg/mL collagenase type I (Sigma) in PBS (Invitrogen, USA) containing penicillin-streptomycin (Gibco, USA) for 45 min at 37°C. The obtained cell suspensions were washed for two times and resuspended in DMEM containing %10 Fetal Bovine Serum (FBS) and 1% P/S (referred as complete DMEM) and cultured in 25 cm^2 culture flasks. Non-adherent cells were removed by changing the cultivation medium.

2.3. Characterization and Determination of Multipotency of DF-MSCs

DF-MSCs were characterized and differentiated into osteogenic, chondrogenic and adipogenic lineages in the third passage, in order to determine the multipotency of these cells. To evaluate the expression of surface markers, DF-MSCs were trypsinized with 0,25% trypsin EDTA and washed in phosphate-buffered saline (PBS; Gibco, Gaithersburg, MD). After 15 minutes of incubation period with FITC or PE-conjugated mouse anti-human antibodies specific to CD34, CD45, CD14, CD29, CD44, CD73, CD90, CD105, or HLA-DR, cells were analyzed *via* flow cytometer (FACSCalibur; BD Biosciences, San Jose, CA) with CellQuest software. The mouse IgG served as isotype control.

DF-MSCs were stimulated with StemPro® Osteogenesis Differentiation (Gibco, Grand Island, NY), StemPro® Adipogenesis Differentiation Kit (Gibco) and StemPro® Chondrogenesis Differentiation Kit (Gibco) according to manufacturer's protocol to differentiate into osteocytes, adipocytes, and chondrocytes. Briefly, cells (1×10^5 /well) were seeded in 6-well plates and 3 days after seeding the cells were replaced with differentiation mediums. The cells were grown for 3 weeks, with medium replacement twice a week. Osteogenesis was detected by staining with Alizarin Red to determine extracellular calcium deposits. Adipogenesis was determined by Oil Red O to stain oil droplets produced by adipocytes. Chondrogenesis was assessed by Alcian Blue staining to determine extracellular proteoglycans. All Images of stained cells were captured by using a light microscope.

2.4. Isolation of PBMCs from Whole Blood Samples

Twenty milliliters of peripheral blood was collected in heparinized tubes prior to isolation procedure. Peripheral blood mononuclear cells (PBMCs) were isolated by density gradient centrifugation for 20 min at 2000 rpm. PBMCs were resuspended in complete culture medium (RPMI-1640, supplemented with 1% P/S, 10% FBS, all from Invitrogen) after cell counting by hemocytometer and adjusted to a final concentration of 5 x 10^5 cells/well in 48 well plates (26).

2.5. Co-culture of DF-MSCs with PBMC

In co-cultures, DF-MSCs (5x10 ^{4}/ well in a 48-well plate) were plated 48 h before the addition of 5x10^5 of PBMCs (in the ratio of 1:10) with complete RPMI-1640 medium (RPMI 1640 containing %10 FBS and 1% P/S) and were co-cultured for 3 days. T lymphocytes were stimulated using 0,5µg/ml anti-CD3 (eBioscience, San Diego, CA) and 0,5µg/ml anti-CD28 (eBioscience, San Diego, CA) antibodies (21).

2.6. Lymphocyte Proliferation Assay

In order to determine the effect of DF-MSCs on proliferative response of $CD4^+$ or $CD8^+$ T lymphocytes, PBMC was labeled with Carboxyfluorescein succinimidyl ester (CFSE) prior to culturing. Briefly, each 1-2x10^6 PBMC was diluted in 1 mL of PBS with 18 mM of CFSE and incubated for 6 minutes at 4°C in dark conditions. After incubation period cells were washed twice with culture medium (completeRPMI), supernatant was discharded and remaining cell pellet was resuspended in culture medium before culturing. Cells were analyzed for CFSE (FITC) signaling *via* flow cytometry after 3 days of culture period (27).

2.7. Analysis of Cytokine Expression Profiles

Supernatant from cultures was collected and stored at –80ºC until assayed. Samples were measured and analyzed for IFN-γ, IL-4 and IL-10 cytokine levels by Cytokine Bead Array (CBA) kit (BD Biosciences, USA) according to the manufacturer's protocol. Briefly, all the CBA kit contents and samples should be at room temperature at least 15 minutes. fifty microliters of culture supernatants, fifty microliters of capture beads and fifty microliters of detection reagent were added and incubated for 3 hours. After incubation, samples were washed for two times with cold PBS. Samples were acquired in a FACS Calibur flow cytometer (BD Biosciences) and analyzed using the FCAP Array v1.0.1 software (Soft Flow Inc.). Results were expressed as picograms per milliliter.

2.8. Down Regulation of IDO, PGE-2 and TGF-β

To explore the crucial factors responsible for the suppressive effect of DF-MSCs on T cytokine secretion, we inhibited the synthesis or the action of crucial factors using selective inhibitors. The crucial molecules or signals such as transforming growth factor (TGF)-β1, indoleamine-2, 3-dioxygenase-1 (IDO-1), and cyclooxygenase-2 (COX-2) were down-regulated with anti-TGF-β1 (0.5µg/ml) neutralizing antibody, 1-methyl tryptophan (MDT, 0,1 Mm,) 1-Methyl-D-tryptophan (1-MDT) and SC-58125 (50 µM,) respectively. After culture period, supernatants were analyzed for the cytokines levels *via* flow cytometry.

2.9. Statistical analysis

The statistical analysis was achieved by using GraphPad Prism 5 (GraphPad Software, La Jolla, CA, USA). One way analysis of variance (ANOVA) with Tukey's multiple comparisons was used for multi-group comparisons, and a two-tailed unpaired Student's t-test was used for comparisons between two groups, and a p-value of <0.05 was considered statistically significant.

3. RESULTS

3.1. Isolation, Characterization and Differentiation of DF-MSCs

The MSCs were isolated from dental follicle tissues. Their proliferation gradually formed small colonies in 3 days. The MSCs reached 70% confluency in the primary culture 7 days after plating for the first passage. Most of the MSCs exhibited fibroblast-like morphology at the P3 passage (Figure 1A).

The MSCs were analyzed *via* flow cytometry. These cells exhibited positive staining for CD29, CD90, CD 146, CD73 and

CD106 but were negative for CD34, CD45, CD14, CD28 and CD25 (Figure 1B).

The MSCs differentiated into osteocytes, adipocytes, and chondrocytes. First, the osteogenic differentiation capability was investigated *in vitro* during a twenty-eight-day culture period in osteogenic induction medium. The MSCs were stained with Alizarin red and the cells formed calcified bone nodule structures. Next, *in vitro* adipogenic differentiation capability was assessed by culturing the cells in adipogenic induction medium and staining with Oil Red O. Intracellular lipid droplets was observed in these cells. Chondrogenic differentiation capability was investigated *in vitro* following fourteen-day culture period in chondrogenic induction medium and cell differentiation into chondrocytes was confirmed with Alcian blue staining which exhibited intracellular proteoglycans in those cells (Figure 1C).

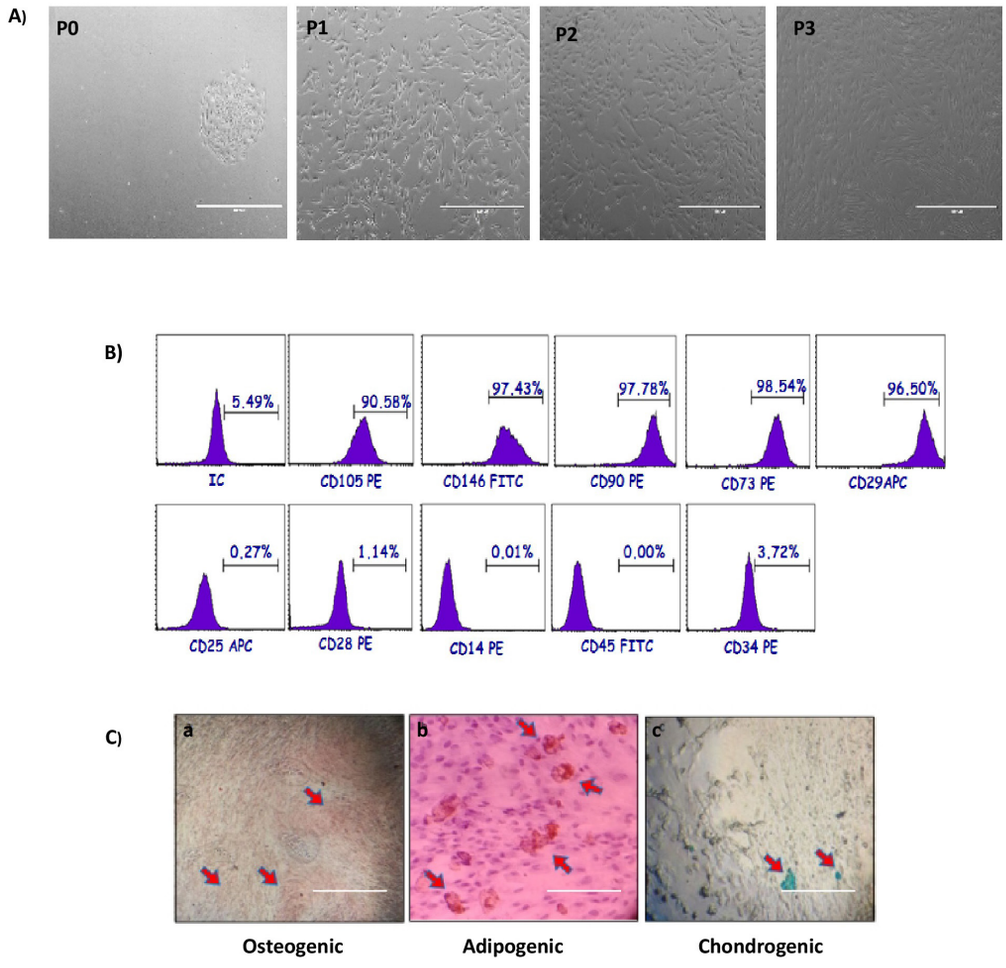

Figure 1. *Morphological appearance, characterization, and differentiation of DFSCs. A). Morphology of DFSCs in P0, P1, P2, and P3 (magnification = 10×). B). Representative flow cytometry analysis of positive surface markers CD105, CD146, CD90, CD73,CD29 and negative surface marker CD25,CD28, CD14, CD45,CD34 for DFSCs at the third passages. C). (a)Alizarin red staining of osteogenic induced DFSCs (b) Oil Red staining of adipogenic induced DFSCs (c) Alcian blue staining of chondrogenic induced DFSCs, scale bar = 1000 µm.*

3.2. DF-MSCs Decreased Proliferative Response of Lymphocytes in AD Patients

We investigated the immunomodulatory effect of DF-MSCs on $CD4^+$Th and $CD8^+$ T cell phenotypes in AD and Psoriasis Patients by CFSE cell labeling. According to our results, $CD4^+$Th proliferation capacity was significantly higher in AD and Psoriasis compared to healthy controls ($p< 0.01$, $p< 0.05$, respectively). $CD4^+$Th cells proliferation was decreased in the presence of DF-MSCs in both AD and Psoriasis group, but it was not shown a significant difference in healthy controls ($p< 0.01$, $p< 0.05$, $p>0.05$, respectively). $CD8^+$Tc proliferation capacity tended to increase in AD and Psoriasis compared to healthy controls but it is not statistically significant ($p>0.05$, $p>0.05$, respectively).

$CD8^+$Th cells proliferation was decreased in the presence of DF-MSCs in both AD and Psoriasis group, but it was not shown a significant difference in healthy controls ($p<0.001$, $p< 0.05$, $p>0.05$, respectively) (Figure2).

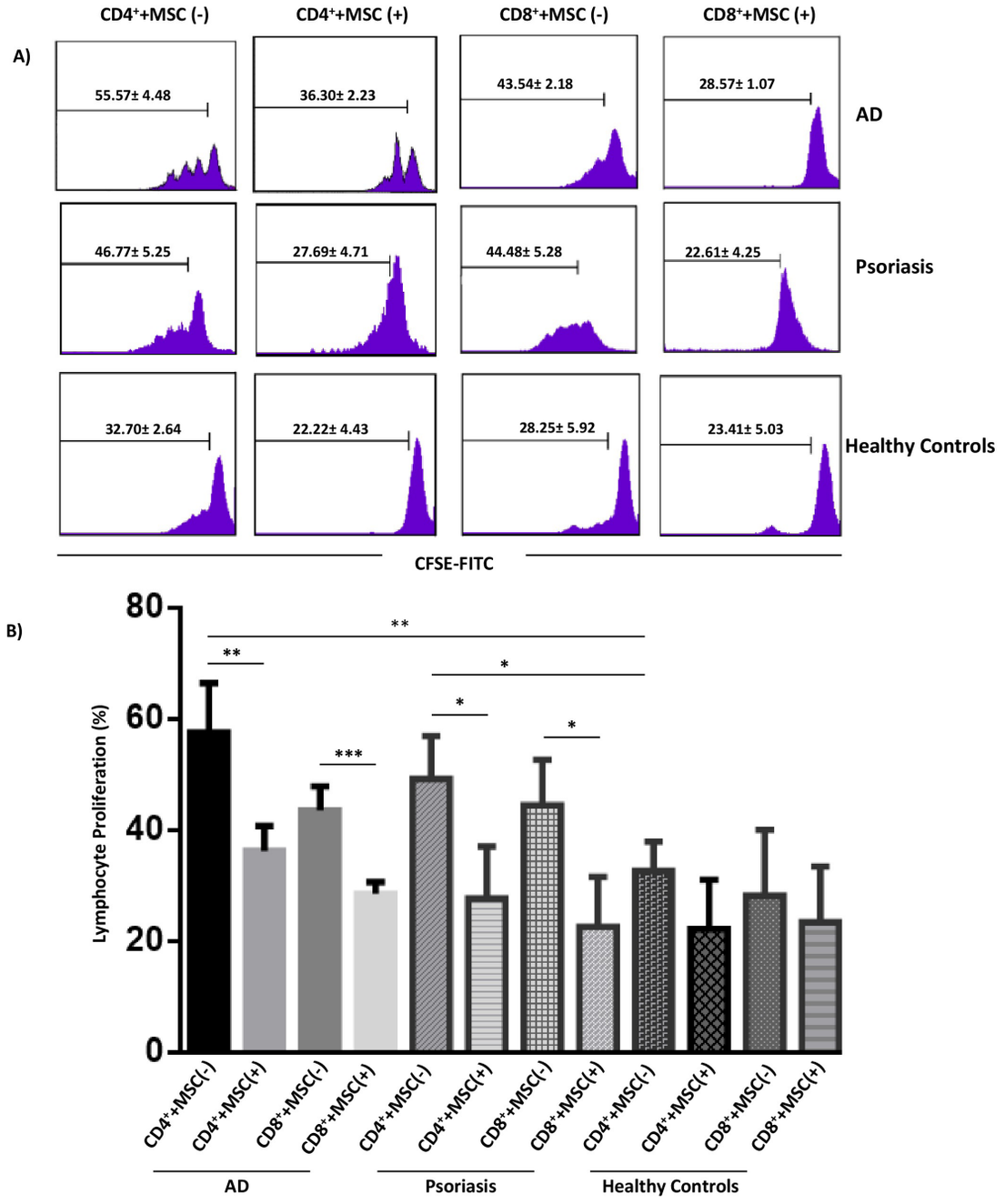

Figure 2. *Inhibitory effect of DF-MSCs on the proliferation of lymphocytes as detected by CFSE. A) Inhibitory effect of DF-MSCs on the proliferation of lymphocytes displayed by flow cytometry. B) Inhibitory effect of DF-MSCs on the proliferation of lymphocytes displayed statistically. *P < 0.05. Results are shown as mean ± SD.*

3.3. DF-MSCs Regulate the Inflammatory and Anti-Inflammatory Cytokine Production in CDmix-Stimulated PBMCs in AD Patients.

We investigated the immunomodulatory effect of DF-MSCs on Th cell phenotypes by evaluating IFN-γ levels for Th1 and IL-4 levels for Th2 cells, and IL-10 levels as an anti-inflammatory cytokine mainly produced by T regulatory cells. Culture supernatants were collected on 3 day of culture period and analyzed with CBA kit *via* flow cytometry. IL-4 levels were significantly high and IFN-γ levels were low in AD patients' PBMC cultures compared to healthy subjects and PS patients (p<0,05). DF-MSCs significantly decreased IL-4 levels and increased IFN-γ levels in co-cultures of AD patients (p<0,05), while decreased IFN-γ levels in co-cultures of PS patients (p<0,05). There was no significant difference between DF-MSC (-) and DF-MSC (+) groups in healthy controls (p>0,05). IL-10 levels were significantly increased with DF-MSCs in AD patients and PS patients (p<0,005 and p<0,01, respectively), and tend to increase in healthy controls but it was not significant. There is not significant difference in IFN-γ, IL-4 an IL-10 cytokine levels between PGE-2, TGF-β and IDO blockade culture supernatant in AD, Psoriasis and healthy controls (Figure 3).

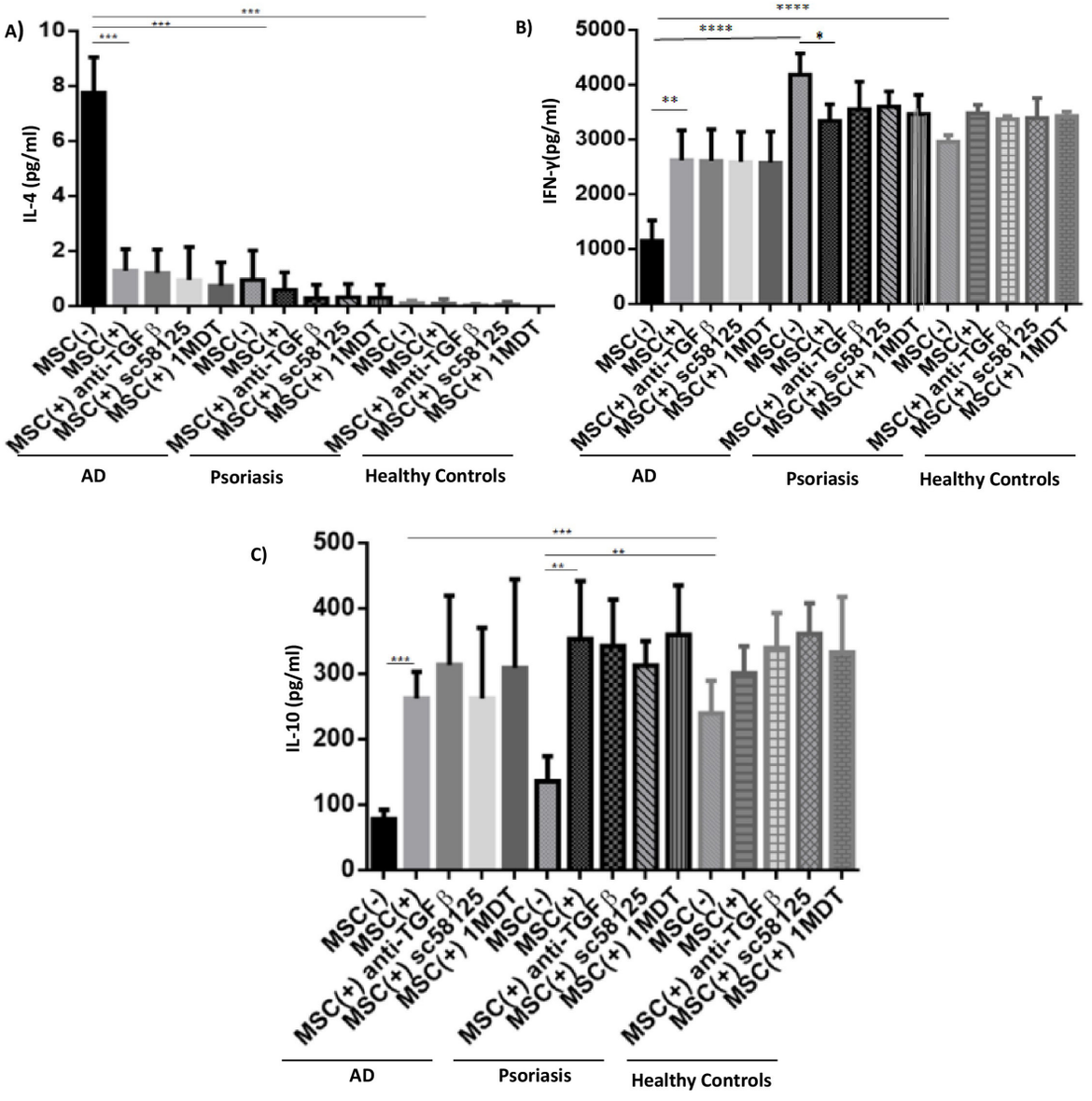

Figure 3. *DF-MSCs-modulated cytokine levels in the supernatants of CDmix-stimulated PBMCs. PBMCs (5 × 105 cells/well) collected from patients with the AD, Psoriasis, and HC were cultured in a 48-well plate following CDmix (0.5 µg/ml, 0.5 µg/ml) stimulation in the presence or absence of DF-MSCs (5 × 104/well) for 3 days. The levels of IFN-γ, IL-4, and IL-10 in the supernatants of CDmix-stimulated PBMCs were determined using flow cytometry. DF-MSCs significantly decreased IL-4(A) (p≤0.001) but significantly increase IFN-γ(B) and IL-10(E) levels (p≤0.01, p≤0.001, respectively) in AD patients. DF-MSCs significantly decreased IFN-γ(B) (p≤0.05) but significantly increase IL-10 levels (E) (p≤0.01) in Psoriasis patients. IL-4 was not shown a significant difference in presence of DF-MSCs in Psoriasis patients. *P < 0.05. Results are shown as mean ± SD.*

4. DISCUSSION

AD is a common chronic skin disease. The currently available therapeutics are limited, and AD management becomes challenging in most cases. To develop better therapeutics for the treatment of AD, many studies have focused on the pathogenesis of AD. Historically, AD was considered a Th2 cell-mediated disease. Experimental and clinical evidence suggests that Th2 cells and their related cytokines like IL-4 and chemokines are critical for the pathogenesis of AD (1-3). Therefore, many researchers have targeted Th2 cells or its signature cytokine IL-4 to treat AD. Indeed, human anti-IL-4 antibody (Dupilumap) effectively treats AD (28). Recently, MSCs have been shown to have immunosuppressive effects Furthermore, it has been proposed that MSCs could inhibit Th2 cell differentiation by reducing IL-4 cytokine level (29). These discoveries of immunosuppressive function and the ability to inhibit lymphocytes have driven scientists to test the possibility that MSCs can be used for the treatment of allergic inflammation diseases because Th2 cells are significant targets of allergic inflammation diseases. In this study, we demonstrated that DF-MSCs could inhibit T cells proliferation capacity and reduce IL-4 cytokine levels in AD patients.

Immunomodulatory effects of MSCs make them new candidates as a cellular therapy for the prevention and treatment of various inflammatory diseases (30). Mesenchymal stem cells can be isolated from many different tissues which exhibit remarkable tissue regenerative and immunosuppressive properties (31). Among these cells, dental tissue MSCs represent a source that is easily accessible and have the potential to differentiate into other tissue cell lines (32). Yıldırım and Colleagues show that DF-MSCs reduced inflammatory response compared to other dental sources (21). We investigate DF-MSCs immunomodulatory effect in inflammatory skin diseases for the first time.

MSCs suppress the response of T cells to stimulation factors. In a previous study, Bone marrow-derived stem cells (BM-MSCs) suppress T cell proliferation in OVA albumin induced atopic dermatitis mouse model (17). Although Th2-type $CD4^+$ Th cells appear to be significant in AD pathogenesis, $CD8^+$ Tc cells represent the dominant effector cell responses in the development of the allergic skin inflammation (33). To examine the $CD4^+$Th and $CD8^+$Tc cell in AD pathogenesis, we investigated the proliferative response of both T cell types in the presence of DF-MSCs. According to our results, DF-MSCs decrease $CD4^+$Th and $CD8^+$ Tc cell response in AD patients. This suppression was seen in the $CD4^+$Th and $CD8^+$ Tc cells of Th1/Th17 –type psoriasis patients. For the first time, we investigate the role of DF-MSCs on the response of $CD4^+$Th and $CD8^+$ Tc in two different inflammatory skin disease.

Na and colleagues showed that BM-MSCs suppress both IL-4 and IFN – γ through decreasing T-bet and GATA-3 expression (17). According to Fu and colleagues, BM-MSCs increased IFN – γ cytokine levels in allergic rhinitis with Th2-skewed eosinophilic inflammation but reduce IL-4 levels (25). After of Lymphocytes with DF-MSCs, the supernatants were collected, and cytokines analysis were performed. In our study, DF-MSCs suppressed the expression of IL-4 whereas the expression of IFN – γ and IL-10 was increased in AD patients. DF-MSCs increased IFN – γ levels in Th1/Th17 skewed Psoriasis patients, but IL-4 levels were not changed. According to our results, DF-MSCs provides immunomodulation according to the characteristics of lymphocyte in the culture.

5. CONCLUSION

These inhibitory actions might contribute to the therapeutic effects of DF-MSCs on both AD and Psoriasis patients. To our knowledge, these findings provide the new perspective that MSCs manipulation is a potential novel strategy for the treatment of inflammatory skin disease.

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Clinical and Experimental Health Sciences

Investigation of Potential Anticarcinogenic Effects of Corilagin in Lung Cancer Cells

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ABSTRACT

Objective: Lung cancer (LC) is the most extensive reason of cancer associated deaths in men and women in the world. LC categorizes into two main groups due to their molecular clinicopathological features and therapeutic responses. Non-small cell lung cancer (NSCLC) is the main subgroup that consists of nearly 85% of all lung cancer types. Corilagin, a biologically active ellagitannin, could be extracted from Phyllanthus species which are known as Chinese medicinal plant. It has been recently shown that Corilagin could exert anti-inflammatuar and antioxidative effects in different experimental cancer models. However, the molecular effects of Corilagin in NSCLC remain unclear.

Methods: In this study, the antiproliferative and apoptotic effects of Corilagin were identified by WST-1 cell proliferation test, caspase-3 and mitochondrial membrane potential (MMP).

Results: We found that Corilagin significiantly suppressed the proliferation of NSCLC cells. Furthermore, we also showed that Corilagin could contribute apoptosis by inducing activity of caspase-3 molecule and loss of MMP.

Conclusion: Taken together, our study first showed that Corilagin could be a new treatment method for NSCLC after verifying its effects with *in vivo* and clinical studies.

Keywords: Corilagin; antiapoptotic; non-small cell lung cancer

1. INTRODUCTION

Lung cancer (LC) is the most extensive reason of cancer associated deaths in men and women in the world (1). LC classifies into two subgroups based on their molecular clinical behaviors and therapeutic responses (2). Non-small cell lung cancer (NSCLC) is the main subgroup that consists of nearly 85% of all lung cancer types (3). NSCLC is less responsive to chemotherapeutics in contrast to small cell lung cancer (SCLC) (4). Despite of technological developments on surgical and diagnosis methods, the survival rate of NSCLC patients at metastatic stage is still poor (5-8). Therefore, recent studies turn towards the biomarker and drug researches to find effective and non-toxic treatment methods (9-11).

Corilagin involves in biologically active tannin family which could be extracted from *Phyllanthus* species. (12-15) Previous studies determined that Corilagin could show anti-hypertensive and anti-atherogenic features in experimental models of cardiovascular diseases (16-18). Furthermore, Corilagin could play an important role as a radical scavenger in superoxide anion system. Regarding to its health-beneficial impacts, researchers started to work about whether Corilagin had anticarcinogenic effects for cancer cells with *in vivo* and *in vitro* studies (19-23). However, the effects of Corilagin and its molecular mechanisms on LC remain unclear.

To our knowledge, there is no established study about the antitumor effects in NSCLC cells. Herein, our goal was to understand cellular and molecular effects of Corilagin in NSCLC cells as exploring novel and efficient diagnostic methods.

2. METHODS

2.1. Cell Culture

The A549 cell line was kindly picked up from Yusuf Baran (Department of Molecular Biology and Genetics, Izmir Institute of Technology). The cells were maintained in RPMI-1640 enriched with fetal bovine serum and penicillin/streptomycin (37°C,5% CO_2).

2.2. Corilagin Treatment

A549 cancer cells took exposure of dimethyl sulfoxide (DMSO) vehicle (1%) alone or increasing doses of of Corilagin (5, 10, 25, 50 and 100 µM).

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2.3. Cell Culture Reagents

The medium, FBS, and penicillin/streptomycin were bought from GIBCO Life Technologies (Thermo Fisher; USA). Corilagin was purchased from Sigma Aldrich (Sigma Aldrich; Darmstadt, Germany). The WST-1 was obtained from Roche Life Sciences (Roche Life Sciences; Germany). The Caspase-3 assay was purchased from manufacturer (BioVision; USA). The JC-1 Mitochondrial Membrane Potential Detection assay was obtained from Cayman Chemical (Cayman Chemical; Ann Arbor, MI, USA).

2.4. WST-I Assay

The cellular impacts of Corilagin in NSCLC cells was determined by using WST-1 assay. The cells were seeded in 96-multiwell plates as a density of 1×10^4 cells/well. Following incubation period (24h, 48h, 72h), cells were treated with increasing doses of Corilagin (5-100 µM) or vehicle (DMSO, 1%) alone as a control. Ten microliters of WST-1 solution were mixtured with cells and then incubated for 4 h at 37°C. The results were read at 450 nm wavelength by using spectrophotometry. Cellular viability was calculated by comparison of proliferation vs. untreated cells (control, 100%).

2.5. Caspase-3 Activity

The colorimetric Caspase-3 assay was to used to determine the apoptotic changes after Corilagin exposure. First, the cells were seeded in 96-well plates ($5x10^5$ cells/well). After incubation period (24h), cells were induced with Corilagin (50 or 100 µM) or control vehicle (DMSO, 1%) for several time points (24-72h). After each time point, the Corilagin stimulated cells underwent lysis process by adding 50 µL (chilled) and incubated for ten minutes. The supernatants were mixed with 50 µL Reaction Buffer and 5 µL DEVD-Pna substrate then waited two hours for incubation. The results were read at 405 nm wavelength by using spectrophotometry.

2.6. The JC-I assay

The JC-I assay was used to examine the loss of MMP after Corilagin exposure (50 or 100 µM). Briefly, the Corilagin stimulated cells (5×10^5 cells/2 mL) were collected with centrifugation (1000 rpm, 10 min). After homogenously mixing the remained pellets with 200 µL medium& 20 µL JC-1 dye, the cells were incubated for 30 minutes at 37°C and centrifuged. The final pellets were resuspended in 320 µL buffer and then 100 µl of each sample was seeded in a 96-well plate as triplicate. The results were read at Green/red (510 nm/585 nm) wavelengths by using ELISA reader.

2.7. Statistical Analysis

Statistical Package for the Social Sciences software package was used to determine statistical analyzes (revision 11.5 SPSS Inc., Chicago, IL, U.S.A.). The Mann-Whitney U test was used to find the mean value of results. The findings were thought statistically meanful if the p value was smaller than 0.01 or 0.05.

3. RESULTS

3.1. Corilagin Suppresses A549 Cell Proliferation

We determined that Corilagin stimulation (50 and 100 µM) had statistically significant impacts in A549 cells (*p<0.05,**p<0.01). IC50 values of Corilagin were calculated and found as 0.7 mM (Figure 1).

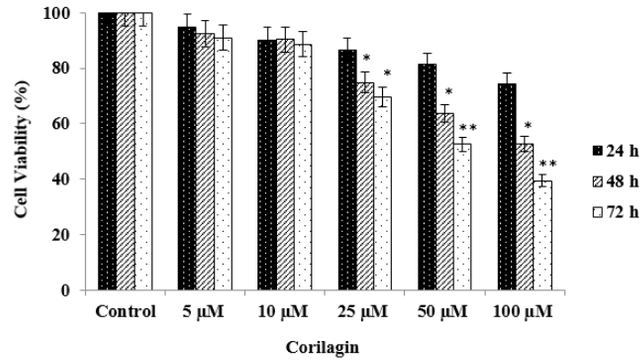

Figure 1. *Effects of Corilagin on cell proliferation. WST-1 proliferation was performed using triplicate samples in three independent experiments. Statistical significance was determined using two-way analysis of variance, and p < 0.05 was considered significant (* p < 0.05; ** p < 0.01).*

3.2. Corilagin Improves Caspase-3 Enzyme Activity

We found that there was a 1.5-fold increase in caspase-3 activity after Corilagin treatment (48 h, 50 µM). Furthermore, we also showed that that there were 2.6-fold (48 h) and 2.1-fold (72h) changes in caspase-3 activity for several time points after 100 µM Corilagin treatment (Figure 2).

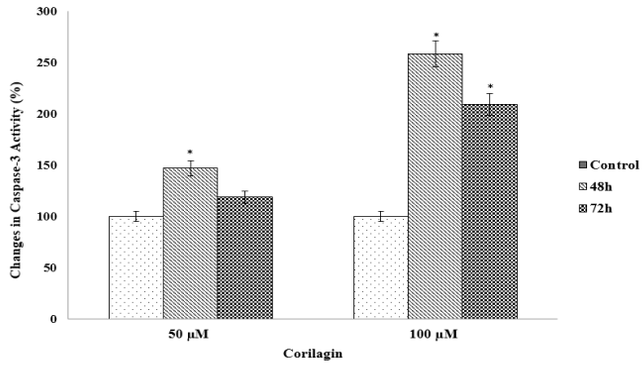

Figure 2. *Effects of Corilagin on caspase-3 activity in A549 cells. Changes in caspase-3 enzyme activity in response to increasing concentrations of corilagin in A549 cells. The results are the means of two independent experiments. p < 0.05 was considered significant*

3.3. Corilagin Induces the Loss of Mitochondrial Membrane Potential

The results showed that there was a 1.90-fold changes in the loss of MMP after 50 µM corilagin treatment. In addition, we also found that there were 2.1-fold (48 h) and 1.90-fold (72 h) increases in loss of MMP after 100 µM Corilagin treatment (Figure 3).

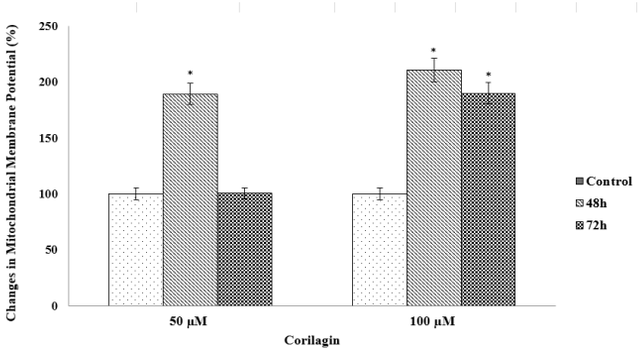

Figure 3. *Effects of Corilagin on membrane potential in A549 cells. Loss of mitochondrial membrane potential (MMP) in response to increasing concentrations of corilagin in A549 cells. The results are represented as the means of two independent experiments. $p < 0.05$ was considered significant.*

4. DISCUSSION

In this study, our goal was to examine antiproliferative and apoptotic effects of Corilagin in NSCLC for the first time. Herein, we identified that Corilagin could inhibit cell proliferation of A549 lung cancer cells while promoting apoptotic event like stimulation of caspase-3 activity and loss of MMP. Our finding showed correlation with previous studies. Hau and colleagues verified that Corilagin had antitumoral effects by suppressing tumoral growth in both in vivo and in vitro studies (20). They also showed that Corilagin had no side or toxic effects on liver with using alanine transaminase (ALT) and aspartate transaminase (AST) tests. Furthermore, Gambari and collegues found that combine effects of Cisplatin-Corilagin or Doxorubicin-Corilagin could sensitize Hep3B cells by increasing antitumoral effects of chemotherapeutics (21).

It had been also reported that Corilagin could cause cellular arrest at G2/M checkpoint to decrease pAKT signaling while promoting p53 gene expression in hepatocellular cancer cells (HCC) (22). Taken together, it could be concluded that Corilagin could use as a new therapeutic method for hepatocellular cancer treatment.

In our previous study, we showed that Corilagin had antiproliferative, apoptotic and genomic impacts in ovarian cancer cells. Due to results of bioinformatic analysis, we found that the phosphatidylinositol signaling system could be modulated through dose and time-dependent Corilagin stimulation. The data of Jai and collegues was also supportive for our findings (23). According to their study, Corilagin could stimulate G2/M cell arrest in ovarian cancer cells in line with studies in HCC. Moreover, they demonstrated that Corilagin could directly inhibit TGF-B secretion to suppress activation of SMAD and ERK/AKT signaling pathways.

There were only two studies which had investigated the reparative impacts of Corilagin exposure in experimental models. The first study was about determination the behavior of Corilagin against tobacco consumption (24). Muresan and collegues discovered that Corilagin could reverse cellular disruption between intercellular junctions through promoting expression of connexin proteins in lung epithelial cells (24). In the second study, Wang and collegues examined the impacts of Corilagin in experimental pulmonary fibrosis model. They showed that Corilagin could repair damages in lung epithelial cells by stimulating cytokine secretion and TGF-B activation (25).

5. CONCLUSION

In conclusion, we suggested that Corilagin has potential anticarcinogenic properties to use as a new therapeutic on NSCLC treatment. Further studies will help us to understand the molecular mechanism underlying corilagin stimulation in cancer models.

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Clinical and Experimental Health Sciences

The Knowledge and Attitude about Diarrhea of Mothers of Students Attending an Elementary School in a Suburban Area in Istanbul

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ABSTRACT

Objective: Diarrhea is one of the main cause of morbidity and mortality among children age under 5 years in Turkey though diarrheal disease control program has been performing and death ratio due to diarrheal disease under 5 years has been decreasing year by year in Turkey. This study is undertaken to evaluate knowledge, attitude and practice of mothers about childhood diarrheal diseases in a suburban area.

Methods: This descriptive study was carried out in a primary school in a suburban area of İstanbul. One hundred and forty four mothers of children included accepted in the study with response rate of 85%. A questionnaire form was applied for data collection. Epi-Info programme was used for statistical analysis. In addition to the descriptive statistical methods, for the comparison of qualitative data a chi-square test was used .

Results: In our study, the percentage of mothers who could define diarrhea correctly was 40%. Abdominal pain and watery defecation were the main signs which the mothers understand their children have diarrhea. Most of the mothers indicated that microbes as the cause of diarrhea. One of every two mothers stated that they would take their children to the doctor as they notice their child has diarrhea. Among the homemade treatments we asked, Potatoes cooked in boiling water and banana were the most frequent answers. Only one of mothers mentioned about using drugs at home for diarrhea and percentage of hearing about ORS was 21.5%. Eleven percent of mothers mentioned they believed to decrease liquid intake would be an effective practice in the treatment of diarrhea. Education level was very influential about knowledge and attitude about diarrheal disease.

Conclusion: This study reveals the importance of continuous health education of mothers as well as the need for raising their status especially schooling in communities. Circulating of correct information is also important as well as educating population.

Keywords: Childhood diarrhea, Mother, Knowledge, Istanbul, Turkey

1. INTRODUCTION

Diarrhea is defined as the passage of three or more loose or liquid stools per day (or more frequent passage than is normal for the individual) (1). The incidence of diarrheal diseases varies with the seasons and child's age. As the age is increased, vulnerability is decreased. Incidence is highest in the first two years of life and declines as a child grows (2).

Diarrheal disease is one of the leading causes of mortality and morbidity particularly in developing world. Diarrhea is one of the main cause of morbidity and mortality among children age under 5 years in Turkey though diarrheal disease control program has been performing and death ratio due to diarrheal disease under 5 years has been decreasing year by year in Turkey (3-5). In many developing countries, most diarrhoeal episodes are treated at home, and mothers are the key caregivers to under-five children. They are the ones who decide about the type of food given to the child and the overall management of the disease. Therefore, their knowledge about this common disease is critically important. Awareness of and perception towards diarrhoea, and individual as well as household actions to prevent and/or manage the disease, have paramount importance to reduce diarrhoea-related morbidities and mortalities (6, 7).

This descriptive study is undertaken to evaluate knowledge, attitude and practice of mothers about childhood diarrheal diseases in a suburban area. This study will provide information about diarrhea and also provide insight about maternal role and role of the health workers on control of diarrhea. The knowledge about diarrheal disease may contribute to health care provider to empathize with mothers' point of view and dealing with the situation more effectively. Also there is limited number of studies about this topic in Turkey (8, 9) and our study will contribute to this literature gap.

2. METHODS

This descriptive study was carried out in a primary school in a suburban area of İstanbul. Due to this school is in the education district of medical faculty and relevant for our study, we selected this school. Convenient sampling method was used. Total number of students who had been attended in this school was 420. We invited 170 mothers of students to participate in our study and 144 of these mothers accepted to participate in the study. Response rate was 85%. Mothers of children were invited to the school for interview. The questionnaires formed by examining related literature were

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applied by the sixth year students of medical faculty who were trained by the researchers to standardize the data collection. Ethics committee permit and institute permit were obtained to perform our research.

2.1. Statistical Analysis

Epi-Info programme was used for statistical analysis. In addition to the descriptive statistical methods (mean, standard deviation, and frequency) for the comparison of qualitative data a chi-square test was used. Outcomes were analyzed within 95% confidence intervals with a significance level of $p<0.05$.

3. RESULTS

The mean of mothers' age was 33.2±5.9 with range of 22-55. The mothers' educational level was poor, 11.8 % illiterate, 3.5 % literate, 37.5% of them being elementary school graduates, 15.3 % secondary school graduates and 19.4 % is high school graduates.

Mothers responded the question of "What does diarrhea mean?" in the following way. The percentage of mothers who could give the definition as " watery defecation more than 3 times a day" was 40% (Table 1). Abdominal pain (60.4%) and watery defecation (59.0%) were the main signs which the mothers understand their children have diarrhea.

***Table 1**. Distribution of the Responses of the Mothers to the Question of "What Does Diarrhea Mean?"*

Diarrhea definition	n	(%)
Watery defecation more than 3 times a day	54	40.0
More than 5 defecation	49	36.3
I don't know	29	21.5
Bloody defecation	3	2.2
Total	135	100.0

Most of the mothers indicated that microbes as the cause of diarrhea. "Cold" was expressed as a cause by 42.4 % of mothers (Table 2).

***Table 2.** Distribution of the Responses of the Mothers the Question of "What Causes Diarrhea?"*

Responses*	n=144	(%)
Microbes	112	77.8
Drinking dirty water	63	43.8
Cold	61	42.4
Dirty and spoiled foods	46	31.9
Antibiotic use	36	25.0
I don't know	12	8.3
Other **	10	6.9
Other diseases	9	6.3

** more than one answer could be chosen, ** worsening of immune system, emerging teeth, eating from market, dirty toilet, fatty foods, hot weather*

One of every two mothers stated that they would take their children to the doctor as they notice their child has diarrhea; 42.8 % of the mothers expressed that they would give some kind of foods to their children at home. Among the homemade treatments we asked, Potatoes cooked in boiling water (86.1%) and banana (79.2%) were the most frequent answers. Leblebi, rice cooked in boiling water, Salty ayran, Peach, Cola – aspirin, Coffee-lemon, warm water with honey, boiled egg, apple, coffee-yoghurt, lime, maya-water were the other choices for diarrhea used by mothers (Table 3). Only one of mothers mentioned about using drugs at home for diarrhea and percentage of hearing about ORS was 21.5%.

***Table 3.** Distribution of the Responses of the Mothers to the Question of "How Do You Feed a Child with Diarrhea"*

Nutrition in Diarrhea	n=144	(%)
Potatoes cooked in boiling water	124	86.1
Banana	114	79.2
Roasted chickpeas	51	35.4
Rice cooked in boiling water	47	32.6
Salty ayran	29	20.1
Peach	22	15.3
Cola – aspirin	22	15.3
Coffee-lemon	21	14.6
Other *	18	12.5
Carrot juice	9	6.3

** warm water with honey, boiled egg, apple, coffee-yoghurt, lime, maya-water*

More than half of the mothers (65.7 %) indicated the necessity of more liquid food, but there were still mothers who believed to decrease liquid intake would be an effective practice in the treatment of diarrhea (11.1 %) (Table 4).

***Table 4.** Distribution of the Responses of the Mothers to the Question of "How Do You Feed Your Children While He Has Diarrhea?"*

Responses	n=144	(%)
I give more liquid food	94	65.7
I give more solid food	59	41.0
I give less liquid food	16	11.2
I feed as usual	18	12.5
Other*	7	4.8

** I feed less fatty foods, I give fruits, I feed according to my doctor's recommendations*

Education level was very influential about knowledge and attitude about diarrheal disease. While almost half of the mothers graduated from elementary school and above (44.7 %) could define the diarrhea as 'Watery defecation more than 3 times a day', these knowledge level was 15.0 % among the mothers not literate or literate (Table 5). Also the percentage of the mothers who graduated from at least elementary school (72.7 %) differed from the mothers who had not graduated from any school (28.6 %) regarding to giving more liquid food as their children has diarrhea (Table 6).

Table 5. *Relation Between the Mothers' Educational Level and Knowledge about Diarrhea Definition*

Mothers' Educational Level	Knowledge about diarrhea definition									
	I don't know		Watery defecation more than 3 times a day		Watery defecation more than 5 times		Bloody defecation		Total	
	n	%	n	%	n	%	n	%	n	%
Literate or illiterate	11	55.0	3	15.0	5	25.0	1	5.0	20	100.0
Graduated from at least elementary school	17	14.9	51	44.7	44	38.6	2	1.8	114	100.0
Total	28	20.9	54	40.3	49	26.6	3	2.2	134	100.0

p<0.001, chi-square=18.480

Table 6. *Relation Between The Mothers' Educational Level and Feeding Their Children with More Liquid Food When They Have Diarrhea*

Mothers' Educational Level	Feeding their children with more liquid food when they have diarrhea				Total	
	Yes		No			
	n	%	n	%	n	%
Literate or illiterate	6	28.6	15	71.4	21	100.0
Graduated from at least elementary school	88	72.7	33	27.3	121	100.0
Total	94	66.2	48	33.8	142	100.0

p<0.001, chi-square= 15.592, OR= 0.15 (95% CI= 0.054-0.419)

4. DISCUSSION

Participant mothers' educational level was poor. Percentage of mothers defining diarrhea correctly was 40% and most of them was higher educational level. Most of the mothers indicated that microbes as the cause of diarrhea. Mothers prefered solid foods such as boiled potatoes (86.1%) and banana (79.2%) for their children with diarrhea. More than half of the mothers (65.7 %) indicated the necessity of more liquid feeding food and this attitude was also highly related with higher educational level. Most of mothers had not heard about ORS.

Despite the negative consequences of illness not only sanitary but also economically and the considerable therapeutic benefits of ORS, application in developing countries has been remaining so low and nonly 43.0% of children under five in the 'least developed countries' are receiving ORS for the treatment of diarrhea (10). Since 1980's World Health Organization and UNICEF have been issuing the program named Early Treatment of Diarrhea but unfortunately diarrhea is still a leading health problem for children. Insufficient and inaccurate knowledge about diarrhea is still affecting the appropriate attitude in preventing and treatment of diarrhea. In our study, we too found that there are still wrong practices and believes about treatment of diarrhea in communities.

In spite of continuous effort for public education especially in primary health care services, in this study it is found that knowledge about diarrhea such as hearing about ORS (22,6%), giving more liquid food (65.7%), defining diarrhea correctly (40.0%) is so deficient. Results of a research reported from Turkey indicated that 40.2% of mothers heard about ORS (9) but asking about ORS usage represented lower rate (6.6%) in another study from our country (8) and percentage of giving more water was almost the same to our study result (65.2%). Also many mother (84.4%) mentioned that they continued breast feeding during diarrhea of children (8). The knowledge that breastfeeding should be continued during diarrhea was only 47.0% in an interventional study performed in Delhi. But, it is promising to see this percentage increased to 90.0 % after intervention for further interventional studies that can be planned in our country (11).

It is also observed that mothers of the children with diarrhea give more fluids in a considerable percentage (65.7%), but still there are mothers who make no changes in the fluid intake of their children with diarrhea (12.6%), and who decrease the fluid intake (11.2%).

It is revealed that educational level plays an important role in deciding to take the child having diarrhea to a doctor. Although in our study mothers having higher education (high school, university graduates) were not represented, graduates of primary school were found to have a considerable important impact on mothers' attitude towards usage of health care facilities. This effect may be attributed to defining role of education of mothers, women's status in communities and mothers' freedom to get into contact with the outdoor environments.

In a study carried out in Belgium majority of mothers were found to prefer home available fluids (78.7 %) and 81.0 % of the mother who attended our study also prefer giving different types of treatments to their children at home. There are many types of traditional feeding practices in Anatolia to treat the child at home, such as bitter, well steeped tea, well steeped tea with pepper, raw onion, lemon salt, banana, salty ayran, kola, boiled potatoes, leblebi, wheat in oil, to keep the child warm etc (12). Especially rice boiled in water is one of the classical menu for diarrheal diseases in the Turkish culture. Ulaş et al. reported that besides more liquid and breast feeding, boiled potatoes (87.6%) and rice (62.6%) were most preferred solid foods for feeding during diarrhea. In our study, potatoes cooked in boiling water (86.1 %) and banana (79.2 %) are the most preferred feeding style supporting our cultural practices. Similar to our culture, a study in Kosovo revealed that rice water (19.6 %), bananas (15.9 %),tea (7.5 %),potatoes (6.5 %) are commonly used to treat diarrhea (13).

"Cold" is one of the important causes of many diseases in the Turkish culture. It is shown that acute respiratory disease, urinary diseases, rheumatological diseases and headache

are thought to be caused by cold in the Turkish culture (12). This study also supported this believe expressing 'Cold' as a cause by 42.7 % of mothers. If the real cause of a disease is not known, preventive measures as well as the appropriate treatment can't be performed seriously. When the TDHS data is compared between the years 1998 and 2008, slight increase in diarrheal disease frequency is observed (30.0 % to 23.0 %) indicating decrease of the poor sanitation practices and the use of contaminated water supplies. In our study, the mothers expressed that dirty-spoiled foods (32.2 %) and dirty water (44.1 %) can cause diarrhea and they seemed to be aware of the diarrhea causing effect of unsafe foods and drinks sold in open markets and school canteens. This awareness also exist in Belgaum reported by results that drinking contaminated water (80.3 %) and eating contaminated food (68.42 %) expressed as cause of diarrhea (14).

5. CONCLUSION

This study reveals the importance of continuous health education of mothers as well as the need for raising their status especially schooling in communities. Circulating of correct information is also important as well as educating population. Eventually planning and performing our study as a descriptive study was a limitation. So that, the results represent the research sample and cannot be generalized for population.

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Clinical and Experimental Health Sciences

Aesthetic Rehabilitation of Enamel Hypomineralization with Microabrasion and Direct Composites (18 Month-Follow-up Report)

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ABSTRACT

Objective: This case report represents a direct, prepless treatment of discolored anterior teeth due to Molar-Incisor Hypomineralisation (MIH) defect following microabrasion and vital bleaching.

Methods: Following clinical and radiological examinations, the discolored, teeth were microabraded with a microabrasive agent containing 6.6% HCl (hydrochloric acid) and silicone carbide particles. Then the teeth were bleached by using 40% hydrogen peroxide. Finally direct composite restorations were performed with A2 shade. Polishing procedure was done by using polishing discs and spiral wheels.

Result: The restorations were evaluated in terms of retention, marginal integrity, marginal discoloration, anatomical form, secondary caries, surface texture, shade match, and postoperative sensitivity according to 'The Modified United States Public Health Service' (USPHS) criterias at 3rd, 9th and 18th months. Nevertheless, it was detected slight abrasion at 18-month follow-up on the labial surfaces of teeth #11 and #21, all the scores were considered as acceptable.

Conclusion: The microabrasion, vital bleaching and direct composite restoration combination is considered as a promising treatment method for MIH effected teeth under the conditions of this study.

Keywords: Microabrasion, Direct Composite, Bleaching, MIH, Aesthetic dentistry

1. INTRODUCTION

Tooth eruption begins after completing certain stages of development. During this phase, the formation of dental tissues; enamel, dentin and cement may not be fully completed due to some external or internal factors, resulting in developmental anomalies. The anomalies especially located at anterior region also causes some problems about aesthetics.

In this case report, a 21-year-old female patient applied to Marmara University faculty of Faculty of Dentistry, Department of Restorative Dentistry Clinic with unwilling aesthetic outlook due to discolorations on her anterior teeth (Figure 1). Clinical examination revealed hypomineralized sections were observed on labial surfaces of both maxillary and mandibular anterior dentition. Cavities and yellow-brown discolorations were observed in incisal thirds of maxillary canines and in middle thirds of maxillary central incisors, limited to enamel tissue (Figure 2). At radiographic examination no periapical lesions were detected and all the teeth were considered as vital. Minimally invasive preaparations followed by single shade composite veneers were selected as the treatment plan.

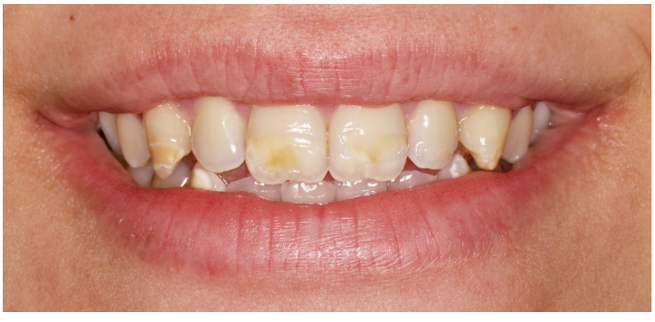

Figure 1. *Initial (Extraoral)*

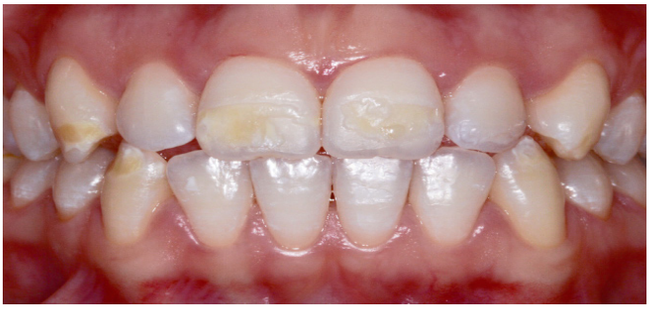

Figure 2. *Initial (Intraoral)*

DOI: 10.33808/clinexphealthsci.599718

2. METHODS

At first appointment the teeth were isolated with rubber dam and microabrasion were applied to the teeth numbered 13, 11, 21, 43 with a microabrasive agent (Opalustre, Ultradent, USA) containing 6.6% HCl (hydrochloric acid) and silicon carbide microparticles (Figure 3). The procedure was done at low speed for 60 seconds with a set of specially brushed tires and the cycle was repeated for 5 times. After applying a fluoride-containing polishing paste was applied on the teeth with low speed polishing tires, the rubber dam was removed and the teeth were cleaned (Figure 4). Vital office bleaching was done with an office bleaching agent (Opalescence Xtra Boost, Ultradent, USA) at the second appointment (Figure 5). Restorative treatment appointment was arranged after 2 weeks of bleaching procedure. Firstly the shade selection was considered by using button technique as A2 shade (Ceram-X One, Dentsply, USA). Then the cavitated enamel surfaces were etched with 37.5% phosphoric acid (Gel Etchant, Kavo-Kerr, USA) for 30 seconds and the surfaces were rinsed and dried. A universal adhesive agent (Single Bond Universal, 3M ESPE, USA) was applied, slightly refined and polymerized for 20 seconds. A2 shade composite resin was applied to whole the prepared surfaces by free-hand layering technique (Figure 6). Surface finishing procedure was done by using a red-banded diamond burr in low speed under water cooling. In marginal contouring and interdental polishing, interdental polishing strips I different grain sizes (Epitex, GC, Japan) were used. Surface polishing procedure was done by using polishing discs (Optidisc, Kerr, USA) and and spiral polishing discs (Twist Dia, Kuraray, Japan) in different grains according to the manufacturer's guidelines. The patient was informed about oral hygiene and called for the recalls at 3, 9 and 18 months.

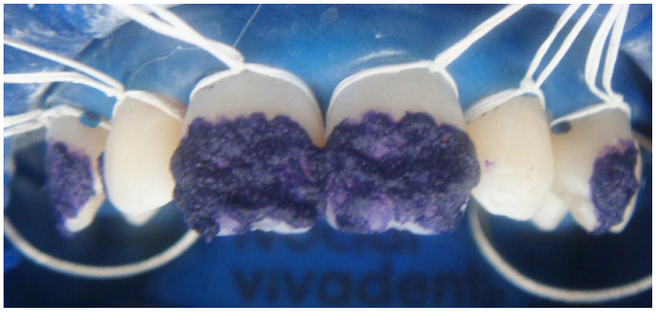

Figure 3. *Microabrasion*

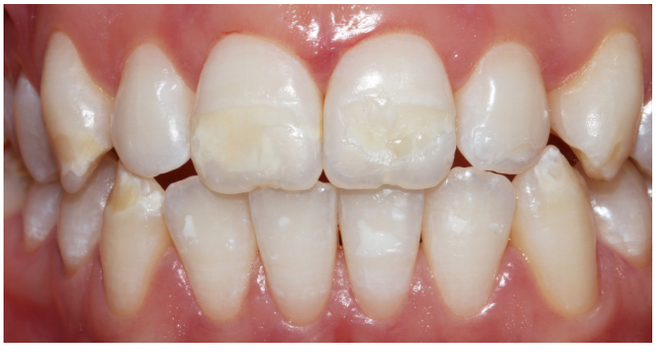

Figure 4. *Immediate after microabrasion*

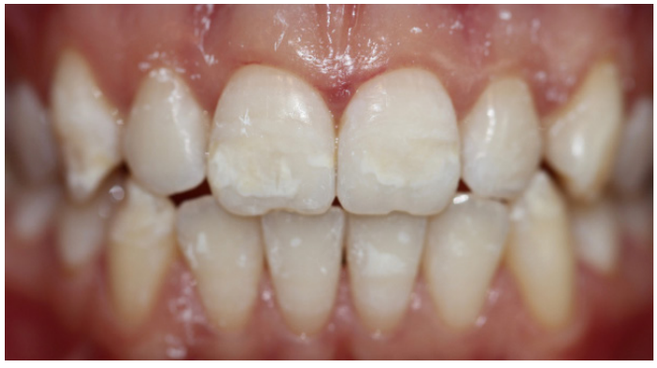

Figure 5. *Immediate after vital bleaching*

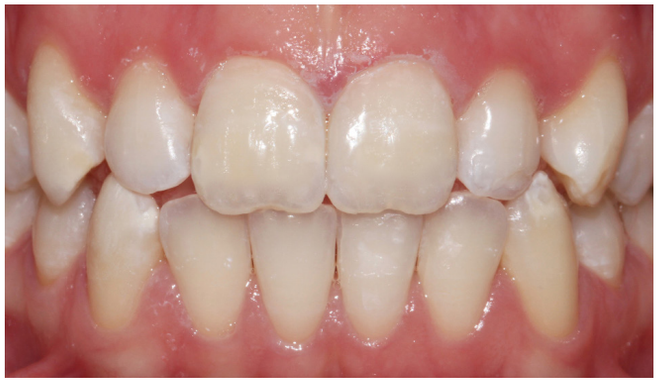

Figure 6. *Immediate after the restorations*

2.1. Statistical Analysis

The success rate of the restorations were evaluated according to the modified USPHS criterias in 3rd, 9th and 18th months recalls (Table 1).

3. RESULTS

The following outcomes were assessed; retention, marginal integrity, marginal discoloration, anatomical form, secondary caries, surface texture, shade match and postoperative sensitivity. As a result, at 3 (Figure 7) and 9-month-follow-ups (Figure 8) , all the scores were acceptable as Alpha (A). All the restorations were considered as stable and compatible with the surrounding tissues in color and contour.

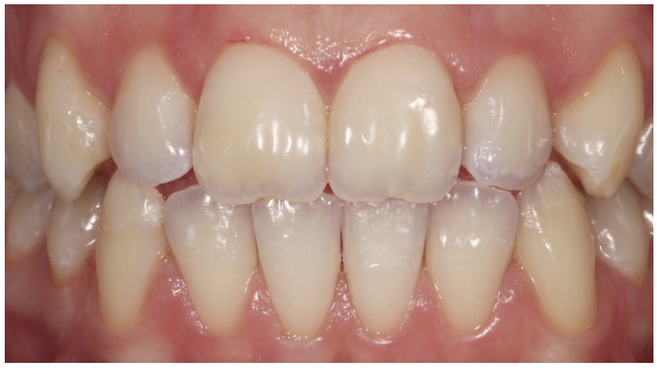

Figure 7. *Three months follow-up*

Table 1. *The Modified USPHS Criterias Scores of 3rd, 9th and 18th month follow-ups*

Category	Scores	Criteria	Re-call (Month)		
			3	9	18
RESTORATION	**Alpha (A)** **Bravo (B)** **Charlie (C)**	Restoration is present Restoration is partially lost Restoration absent	A	A	A
MARGINAL INTEGRITY	**Alpha (A)** **Bravo (B)** **Charlie(C)** **Delta (D)**	Resin-enamel interface is excellent; restoration closely adapted to the tooth No crevice is visible at margins Crevice at margin, enamel exposed Restoration is mobile, fractured or missing	A	A	A
MARGINAL DISCOLORATION	**Alpha (A)** **Bravo (B)** **Charlie (C)** **Delta (D)**	No discoloration on the margin between the restoration and the tooth structure Slight staining can be polished away Obvious staining cannot be polished away Gross staining	A	A	A
ANATOMICAL FORM	**Alpha (A)** **Bravo (B)** **Charlie (C)** **Charlie (C)**	Restoration continuous with existing anatomical form and margins Restoration is slightly overcontured or oncontoured Restoration is undercontoured, dentin or base exposed Restoration is missing	A	A	A
SECONDARY CARIES	**Alpha (A)** **Charlie (C)**	No evidence of caries contiguous with the margin of the restoration Caries evident contiguous with the margin of the restoration	A	A	A
SURFACE TEXTURE	**Alpha(A)** **Bravo (B)** **Charlie (C)** **Delta (D)**	Smooth surface Slightly rough or pitted Rough, cannot be refinished Surface deeply pitted, irregular grooves	A	A	B
SHADE MATCH	**Alpha (A)** **Bravo (B)** **Charlie (C)** **Delta (D)**	Restorations matches the shade and translucency of adjacent tooth structure Discoloration between restoration and tooth structure within the normal range of tooth Discoloration between restoration and tooth structure outside the normal range of tooth Unacceptable color, shade and translucency	A	A	A
POSTOPERATIVE SENTITIVITY	**Alpha (A)** **Bravo (B)** **Charlie (C)**	No postoperative sensitivity Postoperative sensitivity Postoperative sensitivity with treatment need	A	A	A

Acceptable Scores: Alpha (A), Bravo (B); Unacceptable Scores: Charlie (C), Delta (D)

However at 18-month-follow-up, slight abrasions were detected on the labial surface morphology of teeth #11 and #21 and surface textures were scored as Bravo (B) (Figures 9 and 10). The restoration – tooth compatibility was also checked by using high contrast dental photography technique (Figure 11). Abraded surfaces on the restorations of both maxillary central incisors were approved. Moreover gloss retention of the same teeth was determined as low and in need of re-polishing comparatively.

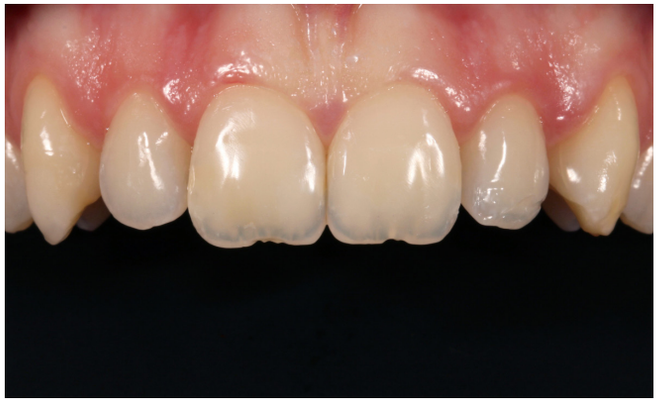

Figure 8. *Nine months follow-up*

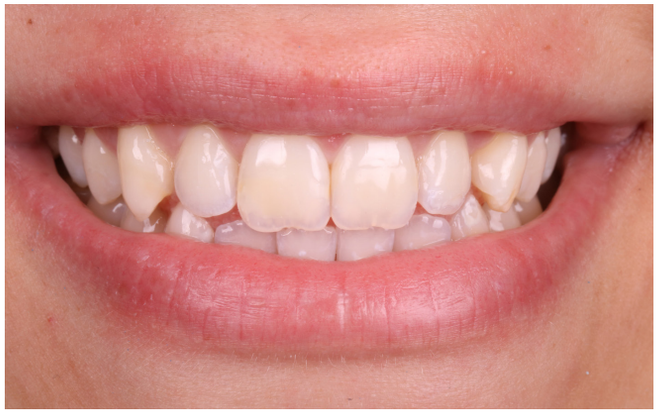

Figure 9. *Eighteen months follow-up*

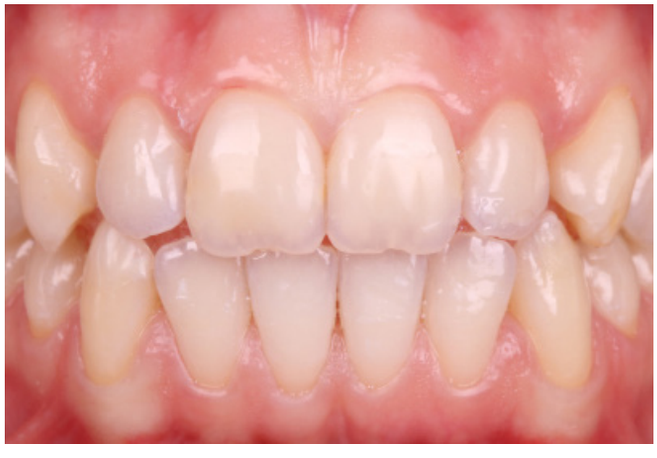

Figure 10. *Eighteen months follow-up*

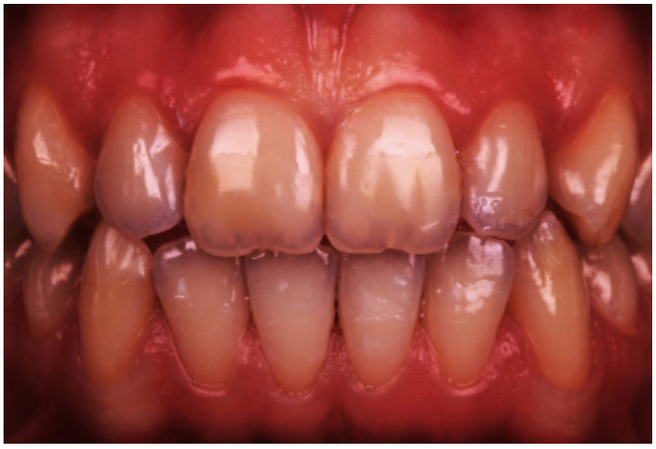

Figure 11. *Eighteen months – high contrast control*

4. DISCUSSION

The aetiology of developmental enamel defects may be congenital, acquired or unknown. Congenital defects such as amelogenesis imperfecta have genetic basis however In the case of acquired defects the aetiology is usually unknown (1). Trauma or excessive use of fluoride can be examples of the known aetiologies. Molar-Incisor Hypomineralisation (MIH) in the case presented is an example of the acquired defects with unknown aetiology (2).

Possible pre, peri and post-natal complications during formation of dental hard tissues, may cause temporary or permanent damages to ameloblasts which may result in enamel defects (3). In cases of MIH, there is hypomineralisation defects on first permanent molars, frequently combined with affected incisors (4). The term "cheese molar" has been used for this specific enamel defects based on this clinical appearance.(5) The color of these enamel defects may seem as creamy-white to yellowish-brown and there is always a clear border between affected and sound enamel tissues(6). Suckling et al. reported a total of 9.9% of the diagnosed MIH children gave a history of high fever/fever of unknown origin (7).

Beyond these information there is still no standard treatment which can be recommended for all MIH-affected teeth (8). As the minimally invasive dentistry concept has been accepted also in field of caries management, dentists should avoid operative treatment wherever possible (8). Lygidakis et al. recommended composite resin as the restorative material for fully erupted MIH-affected teeth in children, in long run (9). However, in such cases, as the affected MIH enamel is less sensitive to etching (10-12), which is precisely needed for retention (13,14), the affected enamel should be removed during preparation. William et al. supported that by suggesting removing all affected or discoloured enamel to achieve the best possible adhesion (15). On the contrary, Mathu-Maju and Wright indicated in a 12 months follow up study that the complete removal of affected enamel in 6 to 9 years old children is not justified, even though the value of such short-term clinical studies are limited (16).

In the case presented the discolored, hypomineralysed enamel defects on anterior teeth were removed minimally invasively by using microabrasion technique. Some researches consideres enamel microabrasion, a conservative and non-destructive method, as one of the best treatment options for both intrinsic fluorosis stains and extrinsic superficial enamel stains (17,18). This technique usually causes no postoperative sensitivity (19). In the case two sessions of vital bleaching with 40% hidrogen peroxide was done on the teeth after microabrasion as some researchers suggested to do vital bleaching after enamel microabrasion since it promotes microreduction of the enamel surface (20). Microabrasioned teeth may develop a darker yellowish shade after the treatment as remaining slightly thinner and translucent enamel surface leads underlying dentin shade to appear more. So, as done in this case, it has been suggested to wait for surface remineralization accompanying with optical improvement of enamel for several weeks after microabrasion therapy before bleaching (21,22).

After microabrasion, the treatment of the teeth was completed using direct composite resin. Despite need for significant clinical time, this method is more conservative compared to indirect options (23). The resin used in this case has supra-nano-size inorganic fillers, that give high mechanical resistance, low polymerization shrinkage value, good polishing and optical properties to the material (24-26). Polishing is a clinical key step to maintain color stability as well as the surface roughness of the restoration (27-29). Interdental polishing and surface polishing of the restorations were done with recently developed diamond embeded interdental strips and spiral rubber discs in the case presented.

5. CONCLUSION

In the case presented, direct composite resin restorations following microabrasion and vital bleaching were done without any preparations. Althought 18 months is still a short time to evaluate, the results indicate that the microabrasion, vital bleaching and direct composite restoration combination can be a promising treatment method for MIH efected teeth under the conditions of this study.

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Clinical and Experimental Health Sciences

Efficiency of Rapid Antigen Test in Diagnosis of Acute Streptococcal Tonsillopharyngitis

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ABSTRACT

Objective: Group A beta hemolytic streptococcus (GABHS) is the most common bacterial cause of acute tonsillopharyngitis. Cases with bacterial infection suspicion should undergo rapid antigen test (RAT) and/or throat culture test in addition to clinical criteria, since it may lead to serious complications.

Method: A total of 220 adult and pediatric patients admitted to the emergency department between April-May 2016 with complaints of fever and sore throat, and diagnosed as acute tonsillopharyngitis were prospectively enrolled to the study. All participants had Centor score ≥2 and they underwent RAT. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of RAT results of both groups were compared. Throat culture was considered as gold standard method.

Results: Mean age of the study population was 22,5±16,9 (1-72) years. 57% (n=61) of the pediatric group, and 42.5% (n=48) of adult group were male. Sensitivity of RAT in adults and pediatric group was 62% vs. 57%, and specificity of the test was 96% vs. 97%, PPV was 55% vs. 80%, and NPV was 97% vs. 93%, respectively.

Conclusion: We found low sensitivity and PPV values of RAT. Also, low levels of sensitivity decrease the possibility of RAT to be a diagnostic tool for the diagnosis of GABHS.

Keywords: Pharyngitis, streptococci, emergency care, rapid antigen test, culture

1. INTRODUCTION

Acute tonsillopharyngitis is a benign and self-limiting disease characterized with sore throat and difficulty in swallowing. It is the cause of 1-2% of total outpatient clinic administrations in United States which makes 12 million patients (1). These applications cause needless antibiotic prescriptions (2). Most of tonsillopharyngitis cases are viral in origin however; bacterial etiology must be excluded because of its serious complications (3). The most common cause of bacterial tonsillopharyngitis is Group A beta hemolytic Streptococcus (GABHS). Certain diagnosis and rapid onset of antibiotherapy is crucial since it may lead to serious complications such as acute rheumatic fever and acute glomerulonephritis (4).

Clinicians should consider rapid antigen test (RAT) and/or throat culture test in addition to clinical diagnostic criteria in patients with bacterial infection suspicion while establishing streptococcal tonsillopharyngitis diagnosis (5). Thus, a number of scoring system criteria were developed to help identifying bacterial agent. Modified Centor Criteria is most commonly used one. Throat culture positivity rate is 56% in patients with 4 criteria, whereas that probability is 2.5% in patients with 0 criteria for the diagnosis of GABHS (6). But sensitivity (12%-92%) and specificity (30%-93%) of this criterion are reported variously (7).

Although culture is the gold standard diagnostic test for streptococcal pharyngitis this takes 2 days for the isolation of the microorganism. Sensitivity of culture in GABHS identification is reported as 90-95% (8). Nevertheless, laboratory requirements, cost, and duration of throat culture limits its use as a diagnostic tool in low-income or developing countries. On the other hand, RAT is a diagnostic test with high sensitivity and specificity which gives result within minutes (9). Rapid antigen test enables early initiation of treatment and subsequently symptomatic relief within 48 hours. Furthermore, this precludes spreading of the microorganism and needless prescription of antibiotics (10).

Rapid antigen test has been used in Europe and United States for a long time; however, it is not commonly used in our country, especially in the emergency departments yet. Thus, in this study we aimed to investigate diagnostic value of RAT for GABHS on patients admitted to emergency department with acute tonsillopharyngitis according to Centor classification, its relation with throat culture results, and its effect on antibiotic prescription rate.

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2. METHODS

A total of 220 adult and pediatric patients admitted to the emergency department of a state hospital with fever and sore throat complaints between April-May 2016 and diagnosed with acute tonsillopharyngitis were prospectively enrolled to the study. Demographic data, complaints, physical examination findings and vital signs of the participants were recorded. All patients applying Centor criteria which includes 38≥ fever by history, tonsillar exudates, tender anterior cervical adenopathy, age and absence of cough, acute rhinorrhea were divided into two: above (adults) and below (pediatric) 18 years of age. All participants had ≥2 score according to Centor classification. Exclusion criteria were as follows: antibiotic usage in last 2 weeks, patients with a history of acute rheumatic fever or acute glomerulonephritis. Patients with Centor score 0-1 were discharged after symptomatic treatment and recommendations. Rapid antigen test was applied to the ones with Centor score ≥2. Two study groups (adults vs. pediatric group) were compared in terms of sensitivity, specificity, PPV and NPV of their RAT results. Throat culture test was accepted as gold standard method. Furthermore, the clinicians were asked about their antibiotic prescription decision after physical examination before they noticed the RAT results. Both groups were compared in terms of RAT results and the clinician's decision on antibiotic prescription. The present study was compatible with ethical rules of Helsinki Declaration. All participants or their parents signed informed consent form. Local ethics committee of Necmettin Erbakan University, Meram Faculty of Medicine approved the study protocol (date: 2017, number: 872).

2.1. Microbiologic Method

Two throat swabs were carefully (avoiding to touch tongue, uvula, or elsewhere) collected from oropharynx of each patient by an experienced doctor who evaluated and examined the patient. The first collected swab was exposed to RAT which is an immunochromatographic test used to calitatively detect StrepA antigen (Turklab Strep A Test/ İzmir/Turkey). Second swabs were taken into Stuart agar which contains sheep blood (BioMerieux/İstanbul/Turkey) seeded and then incubated for 24 hours. Catalase test was performed on the reproducing and beta hemolysis making colonies while culture plates were evaluated. Catalase-negative ones were exposed to PYR (L-pyronidonin beta naphthylamide). PYR (+) colonies were taken into passage and loaded into automated antibiogram and identification device (Vıtek®2;Healthcare/bioMerieux/USA). The bacterial strains identified as *Streptococcus pyogenes* were reported as 'GABHS (Strep. pyogenes) reproduction'. Other identified strains were reported as 'normal oral flora reproduction' (11, 12).

2.2. PYR Method

PYR-impregnated papers were immersed to distilled water and inseminated with beta hemolysis catalase-negative bacteria which degrade L-pyronidonine beta naphthylamide by the naphthylamidase enzyme. Then indicator is added 2 minutes later. Pink color indicated the presence of PYR. Enterococci, S.pyogenes and Staphylococcus lugdunensis are also PYR-positive. Thus, if the detected colonies are catalase-negative and PYR-positive, they are identified as S. pyogenes by the automated bacteria identification (13).

2.3. Statistical Analysis

All data was analyzed with SPSS 21.0 software (IBM Turk Limited Company /Istanbul/Turkey). All variables were summarized by descriptive variables. Kolmogorov-Smirnov test was used to verify that continuous variables were normally distributed. Continuous variables were listed as mean ± standard deviation, and categorical variables were listed as percentages (%). Chi-square test was used to compare categorical variables of independent groups. McNemar test was used for comparison of categorical variables of 2 dependent groups. Sensitivity, specificity, PPV, and NPV of RAT results of both groups were calculated. Statistical significance was defined as $p \leq 0.05$.

3. RESULTS

Mean age (minimum-maximum) of the study population (n=220) was 22.5 ± 16.9 (1-72) years. Mean age of the pediatric group (n=107) was 7.9±4.3 years, and that of adult group (n=113) was 36.3±12.2 years. Fifty-seven percent (n=61) of the pediatric group, and 42.5% (n=48) of the adults were male. The antibiotic prescription rate of the clinicians who had examined the patient and unannounced about the RAT (-) and throat culture (-) results of the patient was 54.2% (n=58) in pediatric group, and 43.3% (n=49) among adult group (107 of 220 (48.6%) patients in total). Pediatric and adult groups were comparable in terms of RAT results (p=0.450) and throat culture results (p=0.104). There was a statistically significant difference between study groups in antibiotic prescription decision rates on the basis of clinical findings (p=0.005) (Table 1 and 2). Rapid antigen test sensitivity was 62% vs. 57%, specificity was 96% vs. 97%, PPV was 55% vs. 80%, and NPV was 97% vs. 93% in adult vs. pediatric group, respectively. Table 3 and 4 demonstrates false-positive and false-negative RAT results of RAT in adult and pediatric groups, respectively.

Table 1. *Decision to prescribe antibiotics in childhood*

		Culture		Total
		Positive	Negative	
Decision of doctors	Antibiotic Writing	14	61	75
	Antibiotic isn't Writing	0	32	32
Total		14	93	107

Sensitivity:100%; Specificity:34%; PPV:18%; NPV:100% ; Consistency: 42%

Table 2. *Decision to prescribe antibiotics in adults*

		Culture		Total
		Positive	Negative	
Decision of doctors	Antibiotic Writing	8	51	59
	Antibiotic isn't Writing	0	54	54
Total		8	105	113

Sensitivity:88%; Specificity:51%; PPV:13%; NPV:100% ; Consistency: 54%

Table 3. *RAT results in childhood*

		Culture		Total
		Positive	Negative	
RAT	Positive (+)	True (+) 8	False (+) 2	10
	Negative (-)	False (-) 6	True (-) 91	97
Total		14	93	107

Sensitivity:57%; Specificity:97%; PPV:80%; NPV:93% ; Consistency: 90% RAT:Rapid Antigen Test

Table 4. *RAT results in adults*

		Culture		Total
		Positive	Negative	
RAT	Positive (+)	True (+) 5	False (+) 4	9
	Negative (-)	False (-) 3	True (-) 101	104
TOTAL		8	105	113

Sensitivity:62%; Specificity:96%; PPV:55%; NPV:97% ; Consistency: 93% RAT:Rapid Antigen Test

4. DISCUSSION

We found RAT sensitivity as 62-57%, specificity as 96-97%, PPV as 55-80%, and NPV as 97-93% when we applied RAT and throat culture in adult and pediatric patients having Centor score ≥2 in our study. When we compared the HAT and culture outcomes of adult and pediatric patients, the results were clinically similar. Besides, our results regarding test sensitivity and PPV values were much lower than those specified by the manufacturer (sensitivity: 97.3%, specificity: 99%, PPV: 98.6% and NPV: 97.5%).

A rapid, easy and readily available method using throat swab to detect streptococci, RAT, is being used as a diagnostic tool in recent years. This test has advantageous of giving result within minutes during diagnostic study and has a sensitivity of 70-80% and a specificity of 95% when used with the gold standard throat culture test. However, RAT sensitivity may differ between 75% to 95% according to the kit and the study design (14). This variation was thought to arise from study groups, culture methods, laboratory performance and difference in disease spectrum. One of the most important factors affecting test sensitivity is gathering throat swab correctly. The American Academy of Pediatrics (AAP) guideline reports that RAT sensitivity differs between 70%-95% according to the swab gathering technique and staff experience (15). Besides, colony amount on the swab may affect RAT sensitivity. A number of recent study emphasize that RAT sensitivity varies according to disease severity (16, 17).

When we consider variation in RAT sensitivity, IDSA, AAP, the American Heart Association (AHA) and other recent guidelines recommend verifying negative RAT results with throat culture which has higher sensitivity (18). Studies evaluating RAT and throat culture report RAT sensitivity as 87-96.7%, RAT specificity as 95-100%, PPV as 84.5-95%, and NPV as 95.1-100% (9, 19). Gurol et al. found RAT sensitivity as 64.6% which is much lower than the value (95%) declared by manufacturer, but higher than the value (58%) reported in literature (58%) (20). Çamurdan et al. reported RAT sensitivity as 97.2% in their study and they state that this difference may be originating from the trademark of the kit they used (21). A study by Cardoso et al. demonstrated that PPV of RAT is 44.9% and 32.9% of the cases were prescribed with unnecessary antibiotic (11) The most probable explanation of this low sensitivity in our study depends on various factors that affect RAT sensitivity such as swab collecting technique, variety in staff experience, throat flora of patient at that moment, and inadequate time allowed to each patient in emergency departments, as mentioned in the literature.

Araujo et al. detected a false-positive rate of 32.6% (n=15) and speculated that this had arisen from improper technique of rapid test they used or from cross-reaction of other groups with streptococci. Also, they pointed out that usage of eau for oral hygiene prevents microorganisms to reproduce in culture media properly (22). We found false-positive result in only 3.5% (n=4) of adult group and in 1.8% (n=2) of pediatric group. On the other hand, our study demonstrated that if we didn't administer RAT; clinicians would decide to prescribe unnecessary antibiotics to 43.3% (n=49) of adults and 54.2% (n=58) of pediatric patients according to clinical findings. Consequently, usage RAT precludes prescription of unnecessary antibiotics especially in pediatric patients.

Mayes et al. point out the fact that if false-negative rate of RAT decrease below 2.4%, it can replace throat culture (23). According to Infectious Diseases Society of America (IDSA), because streptococcal pharyngitis and acute rheumatic fever are very rare for children < 3 years old, there is no need for further diagnostic studies in this age group except for selected children in GABHS pharyngitis (24). But Çamurdan et al. reported false-negative rate of RAT as 6.1% which is lower than that of many previous studies, and stated that negative test results should be verified with throat culture in countries where GABHS infection complications are frequently encountered such as Turkey (21). The study by Araujo Filho et al. reported NPV of RAT as 94.2% and 6% (n=2) false-negative result. They speculated that this might originate from restricted amount of antigens located in oropharynx (22). Darrow et al. proposed the explanation of RAT negativity in culture (+) patients as insufficient amount of swab material collection during RAT procedure, besides rarely presence of scarce amount of colonies in culture

media (25). We found false-negative RAT result rate only 2.6% (n=3) among adults and 5.7% (n=6) in pediatric group. Thus, only 9 in 220 patients (4%) did not receive antibiotic treatment although they needed. Throat culture results were followed-up and patients with positive culture results were recalled. Hence, it is important to keep in mind that RAT results should be re-evaluated concomitantly with culture results especially in countries where RAT sensitivity is low and affected by many factors, and GABHS infection and its complications are frequently encountered like our country.

There is no international consensus currently on RAT usage in streptococcal tonsillopharyngitis diagnosis. Nonetheless, these kits are being commonly used in Europe and United States (26). There are a number of advantages of RAT though variations in its sensitivity rates. Application of this test in laboratory, clinics and emergency departments is easy which give result in less than 15 minutes. In this way, RAT provides rapid and reliable diagnosis of GABHS and decrease nonsuppurative complications of the infection and precludes unnecessary antibiotic prescription rates (20). Maltezou et al. reported 61% decrease in unnecessary antibiotic prescription rate with RAT use (12). Improper antibiotic usage leads to drug side effects, antibiotic resistance development, and consequent increase in health service cost. Rapid antigen test can provide economic gain in health expenditures if its sensitivity is high enough (27).

Limitations of this study;

1- The cost of throat culture test performed at emergency departments is not included in health insurance in our country. Also, RAT cost is relatively high which limits patient number included into study.

2- The factors that affect oral flora (such as oral hygiene, smoking, use of eau for oral hygiene, etc.) were not evaluated during throat swab collection which was done and evaluated at emergency department by unstandardized various health staff. This might influence test results.

3- The participants had not been followed-up in terms of drug side effects, antibiotic resistance, or hospital re-admission. Thus, we could not make an extensive calculation of cost effectiveness.

5. CONCLUSION

We found lower sensitivity and PPV of RAT in patients admitted with acute tonsillopharyngitis than those declared by the manufacturer and by previous studies in the literature. Whereas, our RAT specificity and NPV of RAT were higher than those reported before. Thus, low levels of sensitivity decrease the possibility of RAT to be a diagnostic tool for the diagnosis of GABHS. But all factors affecting RAT sensitivity should be kept in mind. Additionally, low false-positivity rate of RAT decreases unnecessary antibiotic prescription tendency of doctors at emergency department who decide according to clinical criteria only. Further extensive studies with higher patient population are needed to clarify the benefits of RAT use in GABHS diagnosis.

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Clinical and Experimental Health Sciences

Three Enemies of Circadian Rhythm: Anxiety, Sleeplessness and Pain in Patients Following Open-Heart Surgery

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ABSTRACT

Objective: This study aimed to determine the relationship between preoperative anxiety and postoperative pain and perioperative sleep quality in open-heart surgery patients.

Methods: It was a cross-sectional study. The research sample included 126 patients who underwent open-heart surgery for the first time and remained in intensive care for a maximum period of 48 hours. All patients' procedures were performed with cardiopulmonary bypass and sternotomy. Data were collected using a Patient Identification Form, developed by the researcher and used to determine patients' characteristics; the Anxiety Specific to Surgery Questionnaire (ASSQ), used to determine patients' anxiety level; the Pittsburgh Sleep Quality Index (PSQI), used to measure perioperative sleep quality, and the Numeric Pain Scale (NPS), used to determine postoperative pain levels.

Results: Open-heart surgery patients experienced moderate levels of anxiety (27.28±8.48), moderate postoperative pain (4.30±2.29) and poor sleep quality (10.27±4.23) perioperative period. In this paper, a significant, weak, and positive correlation between ASSQ score and postoperative NPS score ($r=0.318$, $p<0.05$) was found; no correlation between the ASSQ score and perioperative PSQI score was found. It was determined that 90.48% (n = 114) of patients who underwent open heart surgery had poor sleep quality and there was no relation between preoperative anxiety and postoperative sleep quality.

Conclusion: The authors concluded that preoperative anxiety impacts postoperative pain but has no effect on sleep quality for open-heart surgery patients in Turkish people.

Keywords: Heart surgery, nursing, pain, sleep, anxiety

1. INTRODUCTION

Currently, open-heart surgery (OHS) has a wide range of interventional uses for several cardiac diseases. Despite its ability to increase length of life and quality of life, OHS remains a significant source of anxiety. Anxiety is a reaction to the body's stressors; mild anxiety is associated with increased alertness and complete rational thinking, while moderate anxiety is associated with mouth instability, palpitations, increased respiratory rate, increased heart rate, muscle tension, and intentional activity (1,2). The anxiety experienced by cardiac surgery patients may have several causes. Uncertainty over the date of the surgery is an important cause of anxiety in prospective OHS patients and previous studies show how postoperative patients' anxiety is reduced (3-5) as patients may experience anxiety due to a fear of death and pain (5). Some studies have reported a fear of death before surgery was greater than the fear of death during surgery (3,6).

Anxiety has a variety of outcomes including pain, increased use of analgesics, sleeplessness, surgical complications and higher mortality rates (7-9). Additionally, anxiety can lead to hypertension and thereby impair circadian rhythm and can lead to hypertension (10), with some studies reporting higher postoperative pain levels and increased use of analgesic drugs due to anxiety (8, 9). One study (n=180) examined the impact of preoperative anxiety and educational level on mortality over a ten-year period, concluding that preoperative anxiety was associated with mortality (11). In another study (2011), the relationship between pre – and postoperative anxiety and anxiety and quality of life was investigated among Coronary Artery Bypass Grafting (CABG) patients (n=187), a positive correlation was found between preoperative and postoperative anxiety ($p=0.000$) between the two groups (12).

Acute postoperative pain following OHS is one of the most severe discomfort-inducing postsurgical conditions; OHS patients may experience pain due to sternotomy, intercostal drain, and vein or artery graft removal. Pain leads to adrenalin secretion, arteriole construction, increased after-load, and decreased cardiac output resulting from tension. Acute postoperative pain is one of the most disturbing complaints resulting from OHS, and is associated with a risk of negative postsurgical conditions (13,14). Uncontrolled pain can lead

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to postponed wound recovery, depression and chronic pain (15-17), as well as to the development of complications such as atelectasis, pneumonia and deep vein thrombosis which may affect postoperative coughing deep breathing exercises and mobilization (13). One study (n=371) found that 85% of patients experienced moderate-to-severe pain from (numerical pain score ≥4/10) within the first four days, with 70% of the patients experiencing pain from sternotomy wounds (18). Other studies have shown that patients generally experienced the most pain from coughing and deep breathing (19,20). While pain score was found 5.3, after coughing it was found to be 6.6 as measured by Numeric Pain Scale (0-10).

One of the postoperative effects of preoperative anxiety experienced in patients who have undergone OHS concerns sleep quality. Sleep is a condition wherein an organism's bodily reactions and behavioral activity slow down; as its central nervous system and body passes assume a passive resting state. Conversely the brain remains active during sleep, undertaking neurophysiological recovery and depositing what it has learned while it was awake. Rest, like recovery, is a necessary condition for the body's physical and mental functions (21). OHS can impact a patient's sleep in two ways; the length of the surgical procedure can affect the patient's sleep quality, while the anxiety experienced before the surgery can disrupt the patient's circadian rhythm and causes problems in the patient's sleep–wake cycle (7). This may be risky for patients undergoing OHS as sleeplessness negatively affects cardiac function and delay patient recover times (22). Additional factors such as pain, applied procedures, and clinical environment characteristics can cause sleeplessness during the postoperative period. Several studies have reported sleep problems in OHS patients both before and after surgery pre – and postoperative period (23,25).

Patients who undergo OHS remain in intensive care units (ICU) for at least one night following the procedure. The intensive care setting patients' sleep wakefulness by disturbing their circadian rhythm. Lighting, patient care activities, monitor alarms, and other, patient related-factors contribute to this situation. The natural environment of the patients is deteriorating (26-28). One study showed how many patients within ICU experienced sleeping disorders and disrupted circadian rhythms (29). Elliott, Rai, and McKinley (2014) used polysomnography to determine sleep quality over a 24-hour period, reporting that patients (n=43) experienced sleeplessness due to noise, light and nursing interventions in their ICU (30). A further study reported that sleep duration on the 3rd night and the 4th night following surgery, patient's sleeping quality decreased postoperatively compared to the preoperative period (31). Kamalipour et al. (2014) conducted a study on patients undergoing OHS (n=188) reporting that 14.8% still experienced sleep problems three months after their surgery (32). Hedges and Redeker (2008), conducted a study on patient sleep-quality by comparing two patient groups (n=48) one treated off-pump (n=81) and one treated on-pump (n=48) who had undergone CABG surgery; the researchers found that sleep quality was better in the early postoperative period in the group of patients who were treated off-pump surgery in the early period (33).

If the negative effects of anxiety remain unresolved, expectations regarding faster patient recovery and better comfort will not reach the desired level. Hence this research hopes to determine the relationship, if any, between preoperative anxiety and postoperative pain and sleep quality in OHS patients (34-36). The purpose of this study is to determine the relationship between preoperative anxiety and postoperative pain and sleep quality in OHS patients.

2. METHODS

This was a descriptive, cross-sectional study. Data were collected after receiving Ethical Committee Approval, as well as written and oral permission from all patient participants. The research sample contained 126 first-time OHS patients who remained in intensive care for a maximum period of 48 hours. Some patients developed complications within the ICUs and hence stayed there for more than 48 hours. All patient participants could understand and converse in Turkish, received no psychiatric diagnosis and had no neurological problems, none of the patients were given sleeping pills throughout the study period. All patients were hospitalized at least one day before surgery; all procedures were performed with cardiopulmonary bypass and sternotomy. The data were collected in July and December 2015.

There are three research questions to this study:

1. What are the anxiety scores of the patient before OHS?
2. Is there any relationship between mean preoperative anxiety scores and mean postoperative pain scores for OHS patients?
3. Is there any relationship between means preoperative anxiety scores and means sleep quality index scores for OHS patients?

2.1. Sample selection

Calculation of the sample size was based on the study by Karancı and Dirik (2003), which described preoperative anxiety level score in emergency surgery patients. Herein, an effect size of 0.2 was accepted to be clinically significant (37). A sample size of 126 of 80% power was calculated to detect an effect size of 0.2 at a $p<0.05$ level of significance using mean of anxiety level of scores. Sample power was detected of 96% as a middle effect size using Gpower software program respectively.

2.2. Ethics

Both hospitals included in this study and Dokuz Eylul University's Ethics Committee granted their permission and approval (number 528). All participants provided

informed consent before responding to the questionnaires. All information within this and its questionnaires remains confidential, and participants were informed of this before they gave consent.

2.3. Data collection

Data were collated using a Patient Identification Form, developed by the researcher and used to determine patients' characteristics; the Anxiety Specific to Surgery Questionnaire (ASSQ), used to determine patients' anxiety levels; the Pittsburgh Sleep Quality Index (PSQI), used to measure perioperative sleep quality, and the Numeric Pain Scale (NPS), used to determine postoperative pain levels. ASSQ data were collected 24 hours before surgery because patient anxiety levels are heightened during this period. PSQI and NPS data were evaluated during the postoperative period when the patients were brought into the service (24-72 hours after surgery). Postoperative data were evaluated at the same time, in the morning, after breakfast and while nursing care was being provided to the patient.

2.4. Measurements

The Patient Identification Form consists of questions developed to determine the socio-demographic characteristics-such as the age, gender, education level and profession of the patient-as well as the date of their surgery, their the total number of long of stay in the hospital prior to the surgery and current comorbid diseases of patients.

The ASSQ was developed by Karancı and Dirik (2003) to evaluate intraoperative and postoperative anxiety in surgical patients. This questionnaire consists of 10 questions with responses given per a five-point Likert scale (from "1: I disagree completely", to "5: I agree completely"). The ASSQ score is obtained by addition of the scores replies given to all the articles. The highest possible score for the questionnaire is 50. There is no a breakpoint. The original Cronbach's alpha coefficient for this questionnaire was 0.79 (37); the coefficient was calculated as 0.78 for the current study.

The NPS scale provides a numerical categorization of pain severity; the scale ranges from zero ("no pain") to 10 ("unbearable pain") (38).

The PSQI was developed by Buysse et al. (39); a validity and reliability test for this questionnaire was performed by Agargün, Kara and Anlar (1996) a Turkish study (40). This is a self-report questionnaire consisted of 19 items evaluating sleep quality or sleep disorder over a month. The questionnaire was comprised of 24 questions, 19 self-report questions and five further questions that were answered by the patient's spouse or roommate.

There are seven components to 18 scored questions within the questionnaire: subjective sleep quality, duration until falling asleep, duration of sleep, habitual sleep activity, sleep disorder, use of sleep medication and daytime function disorder. These questions are answered using points ranging from 0–3, with higher points reflecting poorer sleep quality. Each of the seven major headings were first evaluated within themselves, then added up. A total points score of five or above is regarded to reflect poor sleep quality (40).

2.5. Statistical analysis

Data analysis was completed using the SPSS 16.0 program. In the study, patients' ASSQ-, PSQI – and NPS-score averages were calculated according to gender using a t-test marital status social security scores were calculated using a in Mann–Whitney U test; ASSQ, PSQI and NPS score averages were calculated according to education, profession, surgical intervention and chronic diseases using a Kruskal-Wallis test. Pearson correlation analysis was used to determine the relationship between patients' ASSQ, PSQI and NPS scores.

3. RESULTS

66.7% (n=84) of the patients were male. The average age of all patients is 58.81±12.17 are male, of which 57.9% (n=73) underwent CABG. While 28.6% (n=36) of the participators had hypertension, their hospital stay was found to be 10.28±5.41 in days.

The mean anxiety score of patients was 27.28±8.48 (Table 1). Patients' mean PSQI scores were 10.27±4.23; 66.7% (n=84) of all patients whose mean age was 58.81±12.17 were male, of which 57.9% (n=73) underwent CABG; 28.6% (n=36) of the participants had hypertension, while the mean hospital-stay time for all patients was 10.28±5.41 days (Table 1).

A difference was detected in terms of gender in patients' ASSQ scores ($p=0.005$, $p<0.5$) (Table 1). The mean anxiety score of patients were significant regarding both their educational level and profession ($p=0.008$ and $p=0.027$, $p<0.05$); an advanced analysis determined this difference was caused by literate group and housewives. A difference between mean ASSQ scores were significant in having chronic diseases ($p=0.010$, $p<0.05$) (Table 1). A weak and positive, statistically significant relationship between patients' mean anxiety and mean pain scores was found ($r=0.318$ $p<0.05$), no statistically significant relation was found between mean anxiety score and mean sleep quality scores ($r=0.129$ $p>0.05$) (Table 2). Within the study 93.7% (n=118) of patient participants were found to have poor sleep quality (Table 3).

Table 1. *Investigation of Anxiety Specific to Surgery Questionnaire by Socio-Demographic – Clinical Characteristics of Patients (n = 126)*

		Anxiety Specific to Surgery		Pittsburgh Sleep Quality Index		Numeric Pain Scale	
Socio-Demographic Characteristics	Number (percent)	x̄ ± SD	p	x̄ ± SD	p	x̄ ± SD	p
Gender							
Female	42 (33.3)	30.35±8.56	**0.005***	11.64±4.03	**0.01***	4.97±2.27	**0.021***
Male	84 (66.7)	25.75±8.06		9.59±4.18		3.97±2.24	
Level of Education							
Literate	7 (5.5)	36.57±5.74	**0.008***	13.00±2.51	0.194	5.42±2.63	0.158
Elementary school	55 (43.7)	28.38±8.80		10.78±4.02		4.63±2.14	
Junior high school	19 (15.1)	27.21±8.53		9.84±4.12		4.21±1.71	
High school	19 (15.1)	24.78±8.80		9.68±4.19		4.00±2.30	
University	26 (20.6)	24.34±8.53		9.23±4.89		3.61±2.75	
Profession							
Housewife	29 (23.1)	31.72±7.83	**0.027***	12.44±3.69	**0.016***	5.31±2.07	0.068
Officer	18 (14.3)	25.05±9.32		9.50±3.32		4.44±2.99	
A retired	42 (33.3)	26.88±7.83		10.59±3.52		3.78±2.01	
Workers	9 (7.1)	24.55±11.14		8.55±4.44		4.55±3.28	
Freelancer	27 (21.4)	25.62±7.53		8.66±4.15		3.96±1.78	
Student	1 (0.8)	25.00		7.00		2.00	
Chronic diseases							
Hypertension	36 (28.6)	28.05±7.63	**0.010***	9.44±4.22	0.22	4.33±2.37	0.572
Diabetes Mellitus	13 (10.3)	21.61±5.73		9.00±4.16		3.61±2.36	
Diabetes Mellitus and Hypertension	23 (18.3)	31.56±8.67		11.6±3.93		4.65±2.40	
Other	11 (8.7)	27.90±10.08		11.63±4.78		5.09±2.42	
No	43 (34.1)	25.90±8.36		10.32±4.15		4.11±2.12	
Planned surgery							
CABG	73 (57.9)	26.68±8.22	0.438	10.06±4.30	0.487	4.06±2.25	0.289
VS	47 (37.3)	27.72±9.11		10.76±4.01		4.68±2.25	
CABG+VS	6 (4.8)	31.16±7.30		9.00±5.17		4.33±3.14	
Total		27.28± 8.48		10.27±4.23		4.30±2.29	

*CABG: Coronary Artery Bypass Surgery, VS: Valve Surgery * p < 0.05*

Table 2. *The Relationship between Anxiety Specific to Surgery Questionnaire, Postoperative Numerical Pain Scale and Pittsburgh Sleep Quality Index Mean Rates (n = 126)*

Variable	Anxiety Specific to Surgery Mean
The Mean of Numeric Pain Scale Scores	**r: 0.318** **p:** 0.000*
The Mean Pittsburgh Sleep Quality Index Score	**r:** 0.129 **p:** 0.151

**p < 0.05*

Table 3. *Perioperative Pittsburgh Sleep Quality Index Scores (PSQI) of Patients with Anxiety Specific to Surgery Questionnaire Means*

PSQI Score	Number (Percent)	ASSQ x̄±SD	p
5 scores and above	**118 (93.7)**	27.37±8.56	0.814*
0-4 scores	8 (6.3)	26.00±7.55	

**p > 0.05*

4. DISCUSSION

In our research, patient anxiety was determined to be at a moderate level (27.28±8.48). An examination studies mentioned herein reveal that a moderate level of anxiety is commonly experienced by patients scheduled for OHS. In one study preoperative anxiety levels were found to be %55 (41). Whether a surgery is planned or conducted as an emergency intervention also impacts the patients' preoperative anxiety levels. Karancı and Dirik (2003) found that the ASSQ scores of patients who underwent emergency surgery to be 27.54±8.95 (37). Fındık and Yıldızeli Topçu (2012) included sample patients from urology, emergency and general surgery departments in their research study which examined the effect of type of surgical procedure on preoperative patient anxiety levels; the study found that mean ASSQ scores of patients who underwent a planned surgery to be 23.76±7.12 (42). Although OHS is a planned surgery, the mean ASSQ scores of patients in this study were found to be 27.28±8.48, similar to those of Karancı and Dirik (2003). This outcome can

be interpreted as a sign that anxiety levels are higher in OHS patients compared to general surgery and urology patients; this may be attributable to the heart being a vital organ and so patients may feel the fear of death more acutely.

In this study, a difference based on gender and profession was found among patient's ASSQ scores, and female anxiety levels, especially those of housewives, were seen to be higher. Additionally, differentiation of anxiety scores based on educational level was found to stem from the literate group. Yılmaz et al. (2011) also reported a difference between preoperative anxiety scores in terms of gender and the educational level, for those patients scheduled to undergo cardiac surgery (43). The level of anxiety was higher in women than in men, and this may be associated with their roles and responsibilities; women find it easier to express their concerns than men due to the structure of Turkish society in general, wherein men feel as if they have to portray themselves as strong. Consequently, it is more difficult for men to express their feelings even express any feeling of anxiety. Conversely, several other studies indicate no difference in the levels of anxiety according to sex (44).

When the patient's ASSQ scores were examined, the highest score of questionnaire was observed to be given to the thought of overcoming postoperative pain and discomforts. The mean fear of postoperative pain and death scores were shown to be the greatest causes of causes of preoperative anxiety. These two results show a similarity with another study result, namely, the relationship between preoperative anxiety and postoperative pain. Similarly, Rosiek et al. (2016) detected anxieties in patients related to cardiac surgery, pain, postoperative complications and anesthesia prior to their OHS procedure (41). Other studies conducted in the field support the results the current study, emphasizing pain, sense of discomfort and fear of death as the most frequently encountered causes of preoperative anxiety (45).

Patient's mean score for death-related anxiety was moderate. During the research, patients had activity intolerance and dyspnea. Many coronary artery disease patients in particular had a recent acute myocardial infarction history. In this study patients may have become less sensitive to death due their history regarding myocardial infarction; they stated that they thought surgery to be a cure. Conversely, heart valve patients state that they never think of death and thought their OHS surgery to be a salvation since as disease prevents them from completing many daily activities. It was observed that frequent visits made by the surgeon to the patient, providing information related to surgery, relieved patients and increased their trust in the physician, thereby decreasing anxiety. Researchers conducted on this subject have drawn attention to the fact that patients who received preoperative information and education show lower degrees of anxiety and thus fewer related complications (35).

In this study, patients were reported moderate levels of pain (4.30±2.29) after surgery. Khan et al. (2012) detected patients' mean pain scores to be 4.26, 48-hours after surgery (27). Mathai and Sams (2015) detected that 68% of the patients who underwent cardiac surgery experienced mid-level pain (46). Mirbagher Ajorpaz et al. (2014) determined patients' mean postoperative pain scores to be 6.32±0.21 (47). Pain scores in this study were lower than that of existing literature; this is potentially attributable to difficulties patient's ability to describe their pain. Patients might perceive postsurgical pain as a normal condition and refrain from expressing their true coping level.

In our study a statistically weak, positive and significant relation was detected between preoperative ASSQ scores and postoperative NPS scores ($r=0.318$, $p<0.05$); as preoperative anxiety increases, so does the patients' postoperative pain. Sidar, Dedeli and Iskesen (2013) examined the relationship between anxiety and pain and determined that anxiety plays a particular and significant role in pain perception (36). Khan et al. (2012) examined pre – and postoperative pain levels and detected a positive relationship between the two (27). The authors of the current study thought preoperative anxiety levels would increase the perception of pain caused by physiological effects. Besides, nurses and relatives of the patient are likely to visit them more regularly if they are experiencing higher levels of pain. It makes the patient feel more confident.

After OHS, sleep problems are frequently experienced, particularly by patients who remain in the hospital, sleep quality is considered very low (10.27±4.23). The adverse effect of this on the circadian rhythm can result in increased anxiety levels to deterioration in patients' sleep patterns (7, 48). In our study 93.7% (n=118) of all individuals were found to have experienced poor sleep quality, though no relationship between preoperative anxiety and perioperative sleep quality was found. These outcomes suggest that sleep problems are caused by other factors. In corroboration with the findings of this study, Özkaya et al. (2013) stated that patients with high postoperative pain experienced significantly more severe sleep problems and found other factors causing sleep problems such as stuffiness in the room, medical devices attached to body, crowding in the room, and noise (49). In another study a compulsory supine sleeping position (74.3%), pain (47.1%) and disease-related anxiety (12.9%) were included as factors effecting postoperative sleep in cardiac surgery patients (50).

5. CONCLUSION

The study concluded preoperative anxiety has a little bit impact on postoperative pain, but no effect on sleep quality for OHS patients. Pain remains a major problem influencing patients' recovery. In this study, patients had very bad sleep quality and so their circadian rhythms were affected. Health professionals have a key role to play to management of three enemies of circadian rhythm. They must investigate prevention methods for preoperative anxiety, postoperative pain and perioperative sleep problems for OHS patients. Nurses must collaborate the team and the role of patient advocacy must be foregrounded.

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Clinical and Experimental Health Sciences

In Vitro Comparison of the Effectiveness of a Resin Infiltration System and a Dental Adhesive System in Dentinal Tubule Penetration

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ABSTRACT

Objective: The aim of this study was to assess and compare the dentin tubule penetration effectiveness of a dental adhesive and a resin infiltration system used with two different surface treatments.

Methods: Dentin specimens were obtained from 42 impacted lower right wisdom tooth, 2 of these specimens were used to detect the effects of surface treatments. Two different surface treatments (37% phosphoric acid and 17% EDTA) were applied to the samples to compare the dentin tubule penetration effectiveness of a dental adhesive –which had been using for treatment of dentin hypersensitivity - and a resin infiltration system. Scanning electron microscopy was used to investigate the tubule penetration effectiveness. For statistical analysis, Kruskal Wallis and Mann Whitney U and One Way Analysis of Variance (ANOVA) and Tukey HSD tests were used.

Results: ICON had shown significantly more resin penetration intensity and more resin penetration depth than Adper Single Bond 2 ($p<0.05$). Phosphoric acid treatment groups had shown significantly more penetration intensity than EDTA treated groups ($p=0.001$).

Conclusion: According to the results of this study, it can be concluded that tubuler penetration effectivenes of ICON resin infiltration system is better than Adper Single Bond 2 adhesive system.

Keywords: Dentin sensitivity, resins, adhesives, microscopy

1. INTRODUCTION

Dentin hypersensitivity is defined as a “short, sharp pain arising from exposed dentin in response to thermal, evaporative, tactile, osmotic or chemical stimuli” (1-5). For dentin sensitivity to develop, the dentinal tubules leading from the dentin surface to the pulp must be open (2).

A number of theories have been used to explain dentinal hypersensitivity. The most widely accepted mechanism is described by the “Hydrodynamic theory,” proposed by Branstrom and Astron in 1964 (5-7).

Two basic approaches are used to treat dentin hypersensitivity. The first is to occlude dentinal tubules, preventing the disturbance of hydrodynamic fluid and blocking neural transmission in the pulp (8, 9). This approach involves filling the dentinal tubules or forming a precipitate on their surfaces (10). Because the agents used to treat sensitivity generate a superficial precipitate on the tubules’ surface, no single desensitizing agent is considered ideal for managing dentin hypersensitivity (5, 10-13).

Infiltration resins are generally recently developed materials that are used to treat early enamel lesions (caries) and white spot caries-like lesions. These materials can effectively penetrate the enamel (14, 15). The purpose of this study was to investigate the effectiveness of resin infiltration in occluding tubules and treating dentin hypersensitivity by assessing the penetration of resin into dentinal tubules.

In this study, scanning electron microscopy (SEM) was used to compare the dentinal tubule penetration of a dentin hypersensitivity dental adhesive treatment with that of a resin infiltration system. These treatments were combined with two dentin surface pre-treatments, 37% phosphoric acid and 17% ethylenediaminetetraacetic acid (EDTA).

Two null hypotheses were tested. The first null hypothesis was that the surface pre-treatments would have no effect on resin penetration. The second null hypothesis was that triethyleneglycol dimethacrylate (TEGDMA) containing the resin infiltration system and bisphenylglycidyl dimethacrylate (BisGMA) containing the adhesive system would show similar levels of penetration.

2. METHODS

This study used 42 impacted caries-free human third molar teeth and a protocol approved by the ethics committee with the No. 36290600/03. The teeth were obtained from

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individuals aged 23–30 years. Teeth that had been cracked or damaged during extraction were excluded. Only teeth extracted within 1 month prior to the study were used and these were stored in distilled water. A microtome was used to cut the teeth at their roots 3 mm below the enamel–cement junction. The pulp was removed using an excavator. The buccal enamel layer, cement, and superficial dentin were removed using a drill, and the prepared surfaces were polished using 320, 400, 600, 800, and 1000 grit abrasives, all while cooling the samples with water. The cervical dentin surfaces were examined and the samples stored in distilled water before the treatments were performed. The samples were dried with a gentle stream of air and randomly divided into two groups, each of which was then divided into two subgroups (n = 10). To visualize the tubular openings, a dentin sample from each subgroup was treated with 17% EDTA for 1 min and 37% phosphoric acid for 15 s and examined using SEM. The materials used in the study are listed in Table 1.

Table 1. *The materials and product details used in the study.*

Material (Manufacturer)	Application Technique	Composition
ICON Dry (DMG, Hamburg, Germany)	Apply the etched enamel surface and set for 30 seconds. Dry with oil-free and water-free air.	%99 Ethanol
ICON Infiltrant (DMG, Hamburg, Germany)	Apply an ample amount of Icon-Infiltrant onto the etched surface by turning the shaft and set for 3 minutes. Remove excess material with a cotton roll and dental floss. Light-cure Icon-Infiltrant for 40 seconds. Repeat the application and set for 1 minute. Remove excess material and light-cure for a minimum of 40 seconds.	TEGDMA-based resin, initiators and stabilizers
Adper Single Bond 2 (3M ESPE, Germany)	3. f or 10 sec onds Apply etchant for 15 seconds. Rinse for 10 seconds. Blot excess water using a cotton pellet or mini-sponge. After blotting, apply 2-3 consecutive coats of adhesive for 15 seconds with gentle agitation using a fully saturated applicator. Gently air thin for 5 seconds to evaporate solvents. Light-cure for 10 seconds.	Bis-GMA, HEMA, dimethacrylates, ethanol, water, photoinitiator, methacrylate functional copolymer of polyacrylic and poly (itaconic) acids, silica particles
Panora 200 Phosphoric Acid (Imıcryl, Konya, Turkey)	Apply dentine surface and set for 15 seconds. Dry with oil-free and water-free air for 10 seconds.	37% Phosphoric Acid
EDTA Solution (Werax, Turkey)		17% Ethylene diamide tetra acetic acid, sodyum hydroxide, distile water

Group 1a samples were treated using 37% phosphoric acid plus Adper Single Bond 2 (3M, Neuss, Germany). The vestibular surfaces of the samples were treated with 37% phosphoric acid to remove the smear layer. Following rinsing and air-drying, Adper Single Bond 2 was applied and the samples were light-cured for 20 s, in accordance with the manufacturer's instructions.

Group 1b samples were treated using 17% EDTA plus Adper Single Bond 2. The vestibular surfaces of the samples were treated with 17% EDTA for 60 s to remove the smear layer. Following rinsing and air drying, Adper Single Bond 2 was applied and the samples were light-cured for 20 s, in accordance with the manufacturer's instructions.

Group 2a samples were treated using 37% phosphoric acid plus Icon (DMG, Hamburg, Germany). The sample surfaces were treated with 37% phosphoric acid for 15 s. The samples were then treated using Icon Dry and allowed to stand for 30 s, prior to air-drying for 5 s. The resin was applied using a circular motion to the sample surfaces for a duration of 3 min. A gentle stream of air was applied for 5 s and the samples were light-cured for 40 s. The resin was applied again for 1 min and the samples were light-cured for 40 s.

Group 2b samples were treated using 17% EDTA plus Icon. The vestibular surfaces of the samples were treated with 17% EDTA for 60 s to remove the smear layer. Icon resin was then applied using the procedure described for group 2a samples.

All of the prepared samples were incubated in distilled water for 24 h at 37°C.

The samples were sectioned longitudinally, and each cross-section surface was treated with 37% phosphoric acid for 5 s to remove the smear layer that had formed during sectioning. Samples were then treated with 5.25% NaOCl for 3 min to remove all organic content. All samples were rinsed with distilled water for 1 min, desiccated for 24 h, and sputter-coated with gold for visualization using SEM.

A total of 80 sample surfaces were initially evaluated under low magnification. For each sample, the cervical region closest to the pulp was photographed at 700× magnification, including the treated surface. The resin density was rated by two observers who had been blinded to the treatments. The scoring system (0-3) used was described by Moradi et al. (16, 17), and the scores are defined below:

0 = Resin was not observed in any of the tubules examined.

1 = Resin was observed in less than half of the tubules examined.

2 = Resin was observed in more than half of the tubules examined.

3 = Resin was observed in all of the tubules examined.

The first eight images (10% of a total of 80 images) were evaluated together as part of the calibration process. The remaining images were evaluated independently. Cohen's kappa coefficient was used to assess inter-rater agreement (18). The scores assigned to the images viewed during the calibration process were not included in the kappa analysis. A consensus was reached by discussion for those images that had no inter-rater agreement. The scores determined by

consensus were used in the statistical analyses. The greatest depth of resin penetration was measured in the region closest to the pulp in each image using the SEM device software. The scores and penetration depths were evaluated statistically.

2.1.Statistical analysis

The data were evaluated using SPSS (ver. 21.0; SPSS, Inc., Chicago, IL, USA). Differences in penetration densities among the groups were assessed using a Kruskal–Wallis test, and inter-group comparisons were made using a Mann–Whitney U-test. As Shapiro-Wilk test showed dependent variables were normally distributed (Table 2), one-way analysis of variance (ANOVA) and Tukey's honest significant difference (HSD) test were used to compare penetration depths. To analyze inter-group differences, 95% confidence intervals were calculated. A p-value < 0.05 was considered statistically significant.

Table 2. *Tests of normality for penetration depth values*

Tests of Normality[b]							
	Groups	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
Penetration depth	Phosphoric Acid +Adhesive	,173	20	,118	,913	20	,072
	Phosphoric Acid +ICON	,124	20	,200*	,950	20	,369
	EDTA+ICON	,128	20	,200*	,907	20	,055

*. This is a lower bound of the true significance, a. Lilliefors Significance Correction, b. Penetration depth is constant when Group = EDTA+Adhesive. It has been omitted.

3. RESULTS

The effects of the surface pre-treatments on the smear layer were evaluated in samples that were not included in the study groups. There were more open dentinal tubules in samples treated with phosphoric acid than in those treated with EDTA, as shown in Figures 1 and 2, respectively.

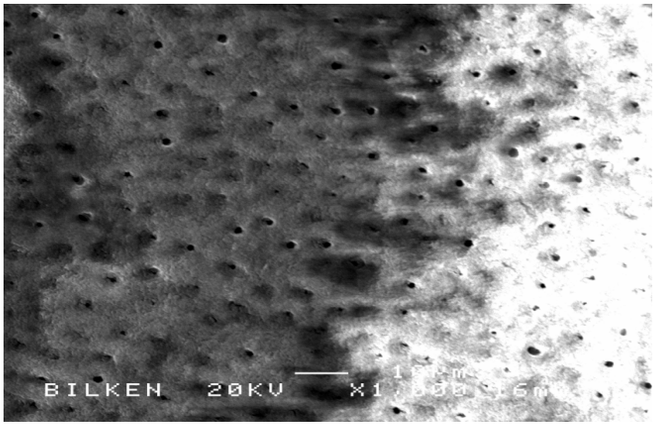

Figure 1. *Image of dentin surface treated with 37% phosphoric acid*

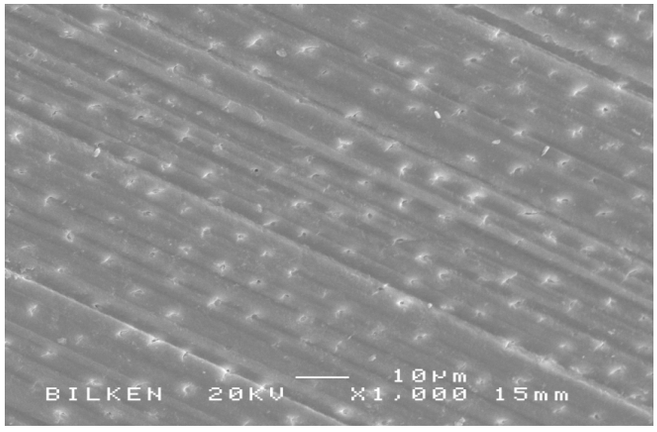

Figure 2. *Image of dentin surface treated with 17% ethylenediaminetetraacetic acid (EDTA)*

The kappa value for inter-rater agreement of 0.79 indicated strong agreement. Surface images at 700× magnification and penetration depth measurements from one sample in each group are shown in Figures 3-9. We did not find resin in any of the EDTA plus adhesive group sample images.

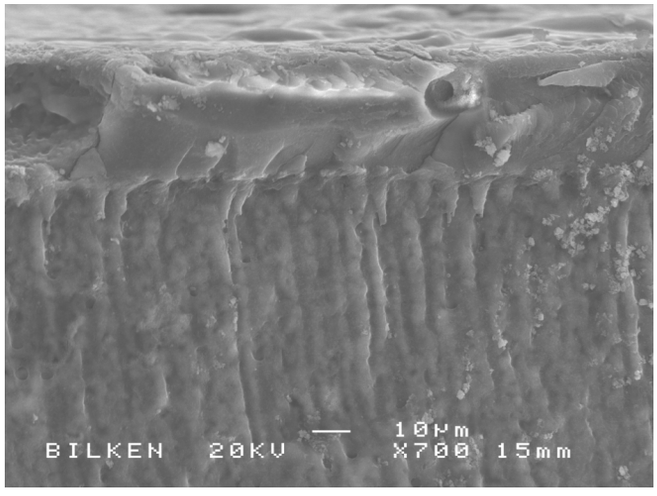

Figure 3. *Scanning electron microscopy (SEM) image of a group 1a (phosphoric acid plus adhesive) sample at 700× magnification*

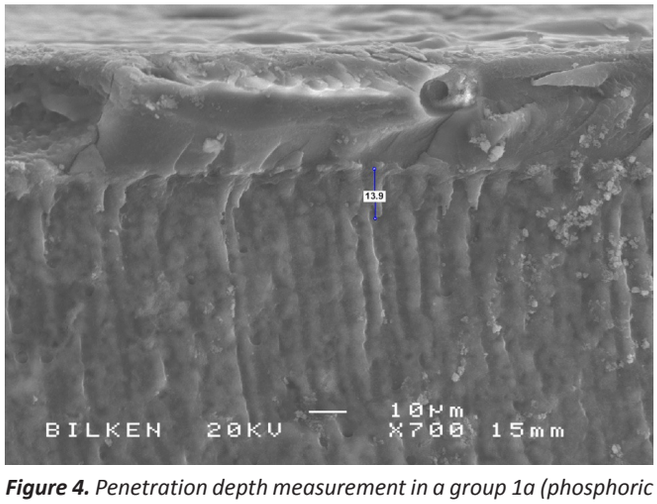

Figure 4. *Penetration depth measurement in a group 1a (phosphoric acid plus adhesive) sample*

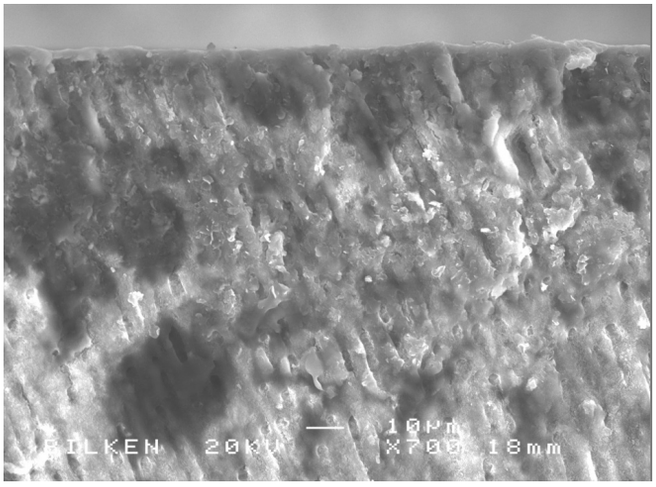

Figure 5. *SEM image of a group 1b (EDTA plus adhesive) sample at 700× magnification*

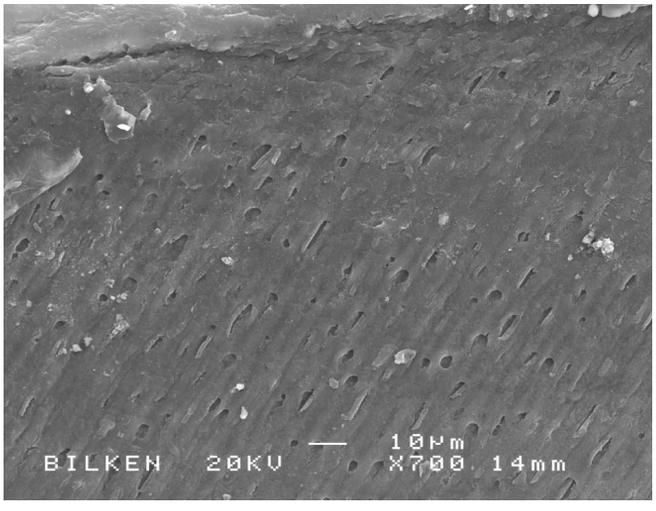

Figure 6. *SEM image of a group 2a (phosphoric acid plus Icon) sample at 700× magnification*

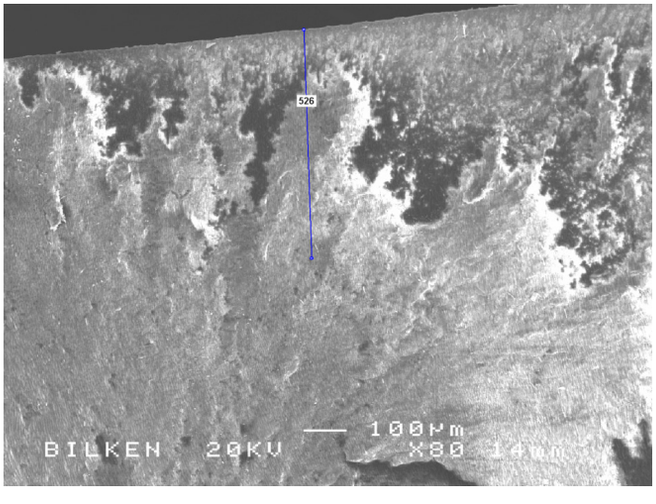

Figure 7. *Penetration depth measurement in a group 2a (phosphoric acid plus Icon) sample*

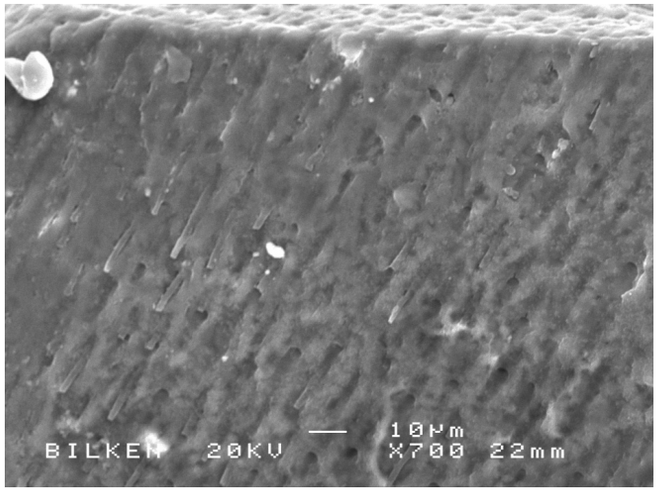

Figure 8. *SEM image of a group 2b (EDTA plus Icon) sample at 700× magnification*

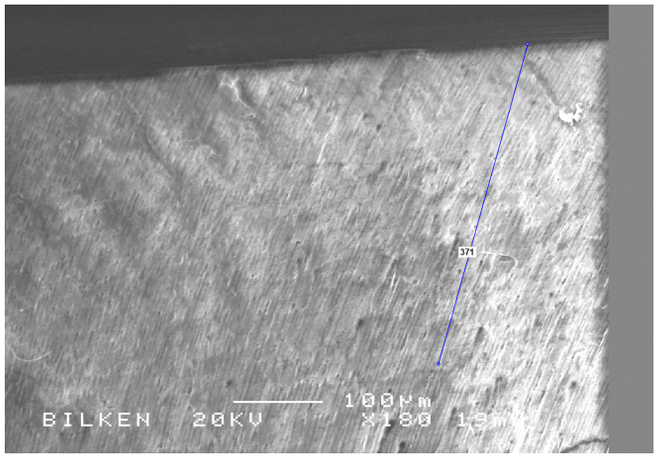

Figure 9. *Penetration depth measurement in a group 2b (EDTA plus Icon) sample*

Comparing the penetration densities using a Kruskal–Wallis test demonstrated significant differences among the study groups (p=0.000). There was a significant difference in penetration density between the phosphoric acid and EDTA surface pre-treatment groups (p=0.001). Additionally, the Icon group demonstrated significantly more penetration density than did the adhesive group (p=0.000). (Table 3, Table 4)

Table 3. *Mean ranks of study groups for penetration density*

Groups	N	Mean Rank
Group 1a (Phosphoric Acid+Adhesive)	20	35,60
Group 1b (EDTA+Adhesive)	20	12,50
Grup 2a (Phosphoric Acid+ICON)	20	61,75
Grup 2b (EDTA+ICON)	20	52,15

Table 4. *Test statistics of Kruskal Wallis and Mann Whitney U for penetration density*

Variable	Penetration Density	
Kruskal Wallis	1a, 1b, 2a, 2b*	Chi-square= 55,325 df=3 Asymp. Sig.= ,000
Mann Whitney U	Phosphoric acid vs EDTA	U=473,000 Z=-3.256 Asymp. Sig.= ,001
Mann Whitney U	Adhesive vs ICON	U=142,000 Z= – 6,551 Asymp. Sig.= ,000
Mann Whitney U	1a vs 1b*	U=40,000 Z=-4,954 Asymp. Sig.= ,000
	1a vs 2a*	U=45,000 Z=-4,377 Asymp. Sig.= ,000
	1a vs 2b*	U=97,000 Z= – 2,947 Asymp. Sig.= ,003
	1b vs 2a*	U= ,000 Z=-5,888 Asymp. Sig.= ,000
	1b vs 2b*	U= ,000 Z= – 5,831 Asymp. Sig.= ,000
	2a vs 2b*	U= 130,000 Z= – 2,063 Asymp. Sig.= ,039

**Statistically significant difference between groups*

Multiple comparisons using the Mann–Whitney U-test demonstrated significant differences between penetration densities of groups 1a and 1b, groups 1a and 2a, groups 1a and 2b, groups 1b and 2a, groups 1b and 2b, and groups 2a and 2b (p=0.000, p=0.000, p=0.003, p=0.000, p=0.000, and p=0.039, respectively). (Table 4)

The mean and standard deviation for penetration depts of each group is listed in Table 5. The results of the multiple inter-group penetration depth comparisons are shown in Table 6. Group 2a samples had the deepest level of resin penetration, followed by group 2b, group 1a, and group 1b, in decreasing order.

Table 5. *Mean and standard deviation values of penetration depths*

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
					Lower Bound	Upper Bound
Group 1a (Phosphoric Acid+Adhesive)	20	15,685	10,9048	2,4384	,0	42,3
Group 1b (EDTA+Adhesive)	20	,000	,0000	,0000	,0	,0
Grup 2a (Phosphoric Acid+ICON)	20	818,950	396,8596	88,7405	201,0	1461,0
Grup 2b (EDTA+ICON)	20	621,750	294,6923	65,8952	269,0	1384,0
Total	80	364,096	438,4873	49,0244	,0	1461,0

**The mean difference is significant at the 0.05 level.*

Table 6. *Tukey's honest significant difference results for the multiple inter-group comparisons among the penetration depth results*

	(J) Groups	Mean Difference (I-J)	Std. Error	Sig.
Phosphoric Acid+Adhesive	EDTA+Adhesive	15,68500	78,17608	,997
	Phosphoric Acid +ICON	-808,26500*	78,17608	,000
	EDTA+ICON	606,06500*	78,17608	,000
EDTA+Adhesive	Phosphoric Acid +Adhesive	-15,68500	78,17608	,997
	Phosphoric Acid +ICON	818,95000*	78,17608	,000
	EDTA+ICON	-621,75000*	78,17608	,000
Phosphoric acid+ICON	Phosphoric Acid +Adhesive	803,26500*	78,17608	,000
	EDTA+Adhesive	818,95000*	78,17608	,000
	EDTA+ICON	197,20000	78,17608	,064
EDTA+ICON	Phosphoric Acid +Adhesive	606,06500*	78,17608	,000
	EDTA+Adhesive	621,75000*	78,17608	,000
	Phosphoric Acid +ICON	-197,20000	78,17608	,064

**The mean difference is significant at the 0.05 level.*

Tukey's HSD test showed there was no significant difference between the penetration depth values in the phosphoric acid plus adhesive group and the EDTA plus adhesive group samples (p=0.997). Similarly, there was no significant difference between the penetration depth values in the phosphoric acid plus Icon group and the EDTA plus Icon group samples (p=0.064). There were statistically significant differences among all other groups (p<0.05).

When all the surface treatments were compared, the samples in groups pre-treated with phosphoric acid showed deeper penetration, but the differences between these samples and those in the groups pre-treated with EDTA were not statistically significant (p=0.280).

The Icon group samples showed a significantly deeper level of penetration than the adhesive group samples (p=0.000).

4. DISCUSSION

In this study, the penetration of a highly effective enamel infiltration resin and an adhesive system used in sensitivity treatment were compared using different surface pre-treatment procedures (14, 15). The Icon manufacturer's instructions recommend removing the hyper-mineralized layer on the surface of the tooth enamel with HCl. However, due to the differences in the enamel and dentin mineral content, the cellular structure of dentin, the risk of pulpal inflammation, and the lack of reports in the literature describing HCl application to the surface of dentin at different concentrations and for different durations, we did not use HCl. Instead, we used phosphoric acid and EDTA for dentin surface pre-treatments.

EDTA is usually applied clinically at a concentration of 15–17% and can remove the smear layer in less than 1 min (19).

We looked at the smear removal procedures used in similar studies and decided to apply 17% EDTA solution to the dentin surface for 1 min in our study (20).

Ersöz and Özyurt described how 37% phosphoric acid removed tubular plugs and peritubular dentin in addition to removing the smear layer on the surface of dentin; the openings became significantly wider and funnel-shaped after they were emptied (21). In our study, the samples pre-treated with phosphoric acid showed more extensive and deeper resin penetration. SEM images showed that the application of 17% EDTA for 1 min did not widen the tubules sufficiently to allow resin infiltration. These results demonstrate that at the concentrations and durations used in this study, phosphoric acid is more effective than EDTA for removing the superficial smear layer. Therefore, the first null hypothesis, which states that the different surface pre-treatments would not affect resin penetration can be rejected.

Sauro et al. investigated the application of similar experimental adhesives to dentin samples pre-treated with either 5% EDTA or phosphoric acid and found that the resins infiltrated a smaller area in EDTA-treated samples compared with those treated using phosphoric acid (22).

To optimize the penetration of hydrophobic monomers (e.g., BisGMA), the collagen matrix in the demineralized dentin may be treated with ethanol rather than water. This is the basis of the ethanol-wet bonding technique (23). As a result, the acidified collagen matrix may be less hydrophilic and phase separation of hydrophobic monomers may be prevented (24). Following the surface pre-treatment procedures, we applied a primer containing 99% ethanol to the surfaces of samples to be treated with Icon, in accordance with the manufacturer's instructions. It is likely that this ethanol-wet bonding step used in our study enhanced resin penetration in the samples treated with Icon.

In their sensitivity treatment study, Ünlü and Bala concluded that the inclusion of both water and ethanol as solvents in Single Bond enhanced the material's properties (25). They also said that the presence of hydroxyethyl methacrylate (HEMA) may have meant that the dentinal tubules were blocked more effectively by the Single Bond reagent. However, Ünlü and Bala also described how many of their patients' tooth-sensitivity problems recurred (25). This suggests that adhesives may not be ideal for long-term sensitivity treatment. It is likely that this long-term failure in sensitivity treatment is due to superficial blocking of the dentinal tubules that is subsequently reversed by brushing or dietary acid (5, 11-13). Therefore, although ethanol and HEMA may be effective in treating short-term dentin sensitivity, they are not sufficient for successful treatment in the long term. The depth of resin penetration may be insufficient.

The BisGMA monomer is one of the main monomers used in adhesive dentistry and is highly viscous (26). The low-viscosity reagent TEGDMA is added to dilute viscous resins, enhancing their infiltration capacity (27, 28). In this study, we compared the penetration of an adhesive containing BisGMA with Icon containing TEGDMA. Statistical analysis showed there were significant differences among all groups ($p<0.05$). Resin penetration was most effective in the phosphoric acid plus Icon group samples, probably due to the highly effective penetration properties of TEGDMA. This was followed by the EDTA plus Icon group samples, the phosphoric acid plus adhesive group, and the EDTA plus adhesive group samples, all in decreasing order of penetration. We found that the Icon resin infiltrated samples in both surface pre-treatment groups more effectively than did the Adper Single Bond 2 reagent. This is probably because Icon contains TEGDMA, which has a higher penetration coefficient than the combination of HEMA and ethanol present in Adper Single Bond 2. Penetration depth of resin may also be affected by viscosity of the materials used. Adper Single Bond 2, is a filled adhesive resin with low viscosity. The size of the fillers are approximately 5 nm (29) but it was proved that these small nanofillers could not penetrate into the interfibrillar space of 20 nm to form the hybrid layer (30, 31). In an in vitro study, Araújo et al. revealed that addition of hydrophobic monomers and solvents (mainly ethanol) into TEGMA blends resulted in decreased penetration depth (32). Altohugh all materials used in this study are manufacturing as low viscosity materials, our results could be attributed to their different monomer and solvent compositions.

The resin penetration depth measurements demonstrated that the phosphoric acid plus Icon group samples showed the deepest penetration, followed by the EDTA plus Icon, and phosphoric acid plus adhesive group samples, in decreasing order. No resin penetration was found in the EDTA plus adhesive group samples. Therefore, the second null hypothesis, which states that Icon and Adper Single Bond 2 would show similar levels of dentin penetration was also rejected.

Griffiths et al. investigated adhesives containing different monomers on dentin pre-treated with phosphoric acid and found that the resin penetrated deeper when the adhesive contained TEGDMA compared with other adhesives (33). We demonstrated that Icon containing TEGDMA penetrated dentin deeper and more effectively than did Adper Single Bond 2 containing BisGMA and HEMA, in samples that had been pre-treated with phosphoric acid.

TEGDMA has a low degree of monomer conversion and when it penetrates parts of the tooth close to the pulp there is a risk of adverse outcomes, including pulpal inflammation and necrosis (28). TEGDMA is typically applied to the enamel surface; toxicity studies will be required to evaluate its safety for use in the cervical region or near the pulp. In addition to assessing the depths of penetration investigated here, more comprehensive studies should also be performed to investigate how effectively these reagents block dentinal tubules. The limitation of this study was to evaluate the penetration with a 2D SEM image. It could be insufficiant to give precise results and further studies are needed to evalute the penetration in 3D manner.

5. CONCLUSION

Within the limitations of this *in vitro* study we concluded the following. For removing the superficial smear layer, treatment with 37% phosphoric acid for 15 s is more effective than treatment with 17% EDTA for 1 min. The Icon resin infiltration system penetrates dentinal tubules more effectively than does the adhesive system tested. Treatment of dentinal hypersensitivity is an area of active research and more *in vitro*, *in vivo*, and clinical follow-up studies will be required to determine the ideal treatment materials and methods.

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Clinical and Experimental Health Sciences

Association Between Onychomycosis and Foot Ulcers in Patients with Diabetes Mellitus

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ABSTRACT

Objective: Diabetes mellitus (DM) is associated with many serious health complications such as diabetic foot ulcer (DFU). Onychomycosis increases the risk for foot disorders and limb amputation in DM patients, and if untreated, can result in tissue degradation and DFU. Therefore, this study aimed to determine the association between the incidences of onychomycosis and DFU.

Methods: This study included 40 DM patients with DFU (study group) and 40 DM patients without FU (control group). Samples were obtained from the most affected part of the nail. The deep-nail plaque of the right toe was preferred in patients with normal toenail appearance. In addition, mycological examinations were conducted. Values of $p<0.01$ were considered as significant.

Results: No significant difference was observed between the two groups with respect to age, sex, and hemoglobin A1c levels ($p<0.01$). However, the incidence of onychomycosis and use of insulin were significantly higher in the study group than in the control group ($p<0.01$ and $p<0.001$, respectively).

Conclusion: Onychomycosis might be associated with development of FU in patients with DM. By treating onychomycosis early, foot amputation can be prevented.

Keywords: Diabetes Mellitus, Diabetic Foot, Risk Factors, Onychomycosis

1. INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disease that affects all age and socioeconomic groups.The number of people with diabetes was estimated to be 285 million in 2010 and is projected to increase to 439 million by 2030 (1). In patients with DM, local and systemic infections occur frequently and with higher severity as compared to the normal population (2). Two such infections onychomycosis and tinea pedis comprise the majority of these infections (3,4). In this study, we focused on onychomycosis, which is the most common nail disease with an incidence of 2–26% worldwide (5) and 15.8–26.0% in Turkey (6,7). Notably, this fungal infection is responsible for 30% of all superficial fungal infections(8).

Foot ulceration is one of the most hazardous complications of DM, as it is the most common cause of non-traumatic foot amputation (vascular disease, diabetes, or combination of both) worldwide. Patients with diabetes are 15–20 times more prone to amputation than those without DM (9). The prevalence of diabetic foot ulcers (DFUs) ranges from 4% to 10% in hospitalized patients, and the lifelong risk of developing FUs may reach 25% in patients with diabetes(10). Once an FU develops, the risk of foot amputation increases due to rapid wound progression, and approximately 5% of diabetics develop FUs each year and 1% have require amputation (11).

Onychomycosis increases the risk for foot disorders and limb amputation in patients with diabetes in addition to affecting the cosmetic appearance of the foot (12). Therefore, optimum treatment is mandatory for diabetic patients with onychomycosis (13).

Many studies have thus far assessed the prevalence of onychomycosis in patients with diabetes. Gupta et al. reported a prevalence of 26% for onychomycosis in diabetic patients (12), whereas Mayser et al. reported a prevalence of 59% for the same (14). Impaired sensation in diabetic patients may indicate the presence of traumatic and onychomycotic lesions, which may become a potential portal of entry for bacterial infections that can aggravate DF (15). Therefore, in this study, our aim was to determine the relationship between the incidence of onychomycosis and that of DFU in patients with diabetes.

2. METHODS

2.1. Patients

The study was conducted at the general surgery, orthopedics, internal medicine, and microbiology clinics of the hospital

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between February 2013 and July 2013. The study group composed 40 patients with DM and FU and the control group comprised 40 patients with DM but without FU.

Inclusion criteria for the study were as follows: no receipt of oral and/or topical antifungal therapy in the last two months and no current immunosuppression. All patients included were aged > 18 years and had similar treatment statuses. Patients with gestational diabetes and excessive peripheral vascular disorder were excluded from the study.

All data were obtained from patients by face-to-face interview. Approval was obtained from each patient by using a form that included information on age, sex, hemoglobin A1c (HbA1c) levels, and treatment status (intake of oral anti-diabetic drugs versus subcutaneous insulin).

2.2. Laboratory data

The HbA1c levels (%) were detected by high-performance liquid chromatography (HPLC) (Agilent 1200 Series, UV, Shimadzu Class-VP, Kyoto, Japan). The normal range was 4.4–6% of the reference range. The HbA1c measurements were performed in a specific order: 0.1 mM ethylene diamine tetra acetic acid and 25 mM Tris-HCl were mixed with 500 mL distilled water to prepare the hemolysate (pH:8.7). Thereafter, 1 mL hemolysate was added to 5µL complete blood and incubated for 20 min (37.2°C) to allow the erythrocytes to disintegrate. The mixture was then loaded into the HPLC instrument mentioned above.

2.3. Sample processing

Samples were obtained from the most-affected part of the nail. After cleaning the area with 70% alcohol, the affected part was scraped using a scalpel. The deep-nail plaque of the right toe was preferred for patients with normal toe appearance. However, the most-affected site was preferred for diabetic patients with onychomycosis. Sterile plastic containers were used for preservation of samples during transport.

Two drops of 20% KOH were added and a coverslip was applied. Thereafter, the sample was incubated for 3h until the specimen softened. The results of direct microscopic examination were recorded as positive or negative for fungus. Sabouraud dextrose agar with chloramphenicol (Merck, Germany) was used for culture plating of the sample in the clinical microbiology laboratory. The agar plates were incubated for 4 weeks at room temperature (26°C), after which the observations were recorded.

2.4. Ethical consideration

Approval from the local ethics committee was obtained for this study (10.12.2012-2012/90) and written informed consent was obtained in accordance with the Declaration of Helsinki from all patients. Privacy rights of the patients have been observed.

2.5. Statistical analysis

The Number Cruncher Statistical System (NCSS) 2007&Power Analysis and Sample Size (PASS) 2008 statistical software (Utah, USA) program was used for statistical analysis. Statistical measures such as mean, standard deviation (SD), frequency, ratio, minimum and maximum were used to evaluate the data. Student's *t*-test was used to compare the normally distributed groups, and Fisher's exact test or Yates' Continuity Correction test (Yates corrected QHI-square) was used for analysis of qualitative data. Values of $p<0.01$ were considered significant.

3. RESULTS

The sex distribution was similar in both groups, and the ages in both groups ranged from 40 to 81 years (mean ± SD, 60.19±9.40 years). There was no significant difference between the two groups with regard to age and sex ($p> 0.05$).

Importantly, the prevalence of onychomycosis was 40% in the study group, i.e., DM patients with DFU, and 12.5% for the control group, i.e., DM patients without DFU (Table 1). The HbA1c levels ranged between 5.5% and 14.3%, with a mean of 7.82% ± 1.64%. The mean HbA1c value in the study group was 7.66% ±1.42%, whereas that in the control group was 7.98% ± 1.84%. Furthermore, 40% of all patients (n=32) were receiving oral anti-diabetic drugs, and the remaining 60% (n=48) were receiving subcutaneous insulin. Direct microscopic examination of the samples yielded a negative result in 86.2% (n=69) of the patients and a positive result in the remaining 13.8% (n=11) of patients. In addition, culture results were negative in 74% of the patients and (n=59) and positive in 26% patients (n=21). The HbA1c levels and microscopic examination results did not show any significant difference between groups ($p>0.05$).

Table 1. *Characteristics of patients in the study group (diabetic patients with diabetic foot ulcers) and control group (diabetic patients without diabetic foot ulcers)*

		Total (n=80)	Study group (n=40)	Control group (n=40)	*p*
		Mean±SD	Mean±SD	Mean±SD	
Age (years)		60.19±9.40	60.10±9.88	60.28±9.03	0.934[a]
HbA1c		7.82±1.64	7.66±1.42	7.98±1.84	0.387[a]
		n (%)	n (%)	n (%)	
Sex	Female	40 (50.0)	16 (40.0)	24 (60.0)	0.118[b]
	Male	40 (50.0)	24 (60.0)	16 (40.0)	
Drug use	OAD	32 (40.0)	8 (20.0)	24 (60.0)	0.001[b,**]
	Insulin	48 (60.0)	32 (80.0)	16 (40.0)	
Microscopy results	Negative	69 (86.2)	33 (82.5)	36 (90.0)	0.516[b]
	Positive	11 (13.8)	7 (17.5)	4 (10.0)	
Culture	Negative	59 (74)	24 (60.0)	35 (87.5)	0.003[b,**]
	Positive	21 (26)	16 (40.0)	5 (12.5)	

*[a]Student's t-test, [b]Yates' Continuity Correction Test or Fisher's Exact Test **p<0.01, OAD, oral anti-diabetic drugs; HbA1c, hemoglobin A1c; SD, standard deviation*

The diabetes medication status was found to be significantly different between the groups ($p<0.001$): Insulin usage was

approximately 6 times higher in the study group than in the control group (odds ratio: 6.0;95% confidence interval [CI]: 2.207–16.313). In addition, the culture results were significantly different between groups (p=0.003; p<0.01; odds ratio for culture positivity: 4.667; 95% CI:1.685–12.927; Table 1).

4. DISCUSSION

DM is associated with various disabling health complications, especially DFU (16,17). DF is the infection, ulceration, and deep tissue destruction related with neurological peripheral vascular and/or metabolic complications of DM (18). Nearly one-third of all patients with DM develop onychomycosis(12), which is responsible for 50% of all nail problems (19) and 30% of all superficial fungal infections (20). As such, it is an important factor of morbidity.

In the literature, standard microscopic examination of tissue of DFU has shown varying rates of positive results, ranging between 10% and 75% (21-23). In our study, the rate of positive results for microscopic examination was 13.8%, but that by cultural studies was 26%. The major cause of false-negative results of microscopic examination is use of the uninfected part of the nail that does not contain any fungal hyphae (21,24). In addition to previous topical or systemic treatment, the quality and amount of nail sample obtained are important (21,25). Therefore, culture of the sample is recommended to confirm the diagnosis in suspected cases. In our study, all samples were subjected to both standard microscopic examination and culture in order to reduce the false-negative results.

Several controlled (26-30) and non-controlled studies (12,31) have evaluated the relationship between onychomycosis and DM. The frequency of onychomycosis worldwide varies widely, i.e., 6%–85%. Some studies have reported that DM promotes onychomycosis (31), while others have reported that the frequency of onychomycosis does not vary between patients with diabetes (14.4%) and those without diabetes (14%) (29). In the current study, we found that the frequency of onychomycosis was 26% among the 80 patients with DM. In particular, 40% of patients in the study group had onychomycosis. This high prevalence of onychomycosis could be because macro – and microangiopathy in patients with diabetes with hypoxia and impaired immunity may facilitate the development of onychomycosis (27,32).

Foot infections occur frequently in individuals with DM and dramatically increase the risk of hospitalization and amputation (33). Intravenous insulin infusions are the preferred method for achieving and maintaining glycemic control in critically ill patients and for the majority of non-critically ill patients. For some non-critical patients, scheduled subcutaneous administration of insulin with basal, nutritional, and correction components is preferred for achieving and maintaining glucose control. Additionally, non-insulin anti-hyperglycemic agents are avoided for most hospitalized patients requiring therapy for hyperglycemia (34). In this study, the majority of patients with DFU were hospitalized because they presented with infected FUs. Therefore, insulin usage in these patients was significantly higher (p<0.001) than that in the control group.

In this study, we found a significant relationship between onychomycosis and FU. It is known that onychomycosis promotes progression of DFU (35). Sharp, brittle nails can gouge the skin, creating a portal for entry of bacteria. Onychomycosis is often associated with tinea pedis, which can create fissures in the skin, paving the way for bacterial infections. The incidence of secondary infection in diabetic patients with onychomycosis and those without onychomycosis was found to be 15% and 6%, respectively (36). Both fungal and bacterial secondary infections, including paronychia and cellulitis, may be caused by injury to adjacent skin from the mycotic nail. Patients are usually unaware of these injuries or infections (12,20). Blisters may arise due to the pressure applied by thickened nails and hyponychium. Peripheral neuropathy may contribute to deterioration of simple erosions and blisters to cellulitis or osteomyelitis of the underlying bone (12,20). Because the risk of amputation increases with onychomycosis, it is imperative for clinicians to examine the feet of diabetic patients and when the presence of infection is suspected, the clinician should obtain a sample for diagnosis.

Despite our important findings, our study has a few limitations that need to be addressed. First, the size of the study population was small. Second, we excluded patients with excessive peripheral vascular disorder, but included some patients with mild peripheral vascular disease; therefore, the influence of slight obstructions (asymptomatic, incomplete blood vessel obstruction) in the development of DFU was not excluded.

5. CONCLUSION

Onychomycosis might cause DFU in DM patients when left untreated and once developed, may require limb amputation. It is usually asymptomatic in otherwise healthy persons but may be responsible for progression to FU in patients with DM. Therefore, patients with diabetes should be routinely checked for onychomycosis.

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Clinical and Experimental Health Sciences

The Effects of Loneliness on Menopausal Symptoms

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ABSTRACT

Objectives: This study was carried out to investigate the effect of menopausal symptoms on the loneliness of women in the menopausal period.
Methods: The study sample consisted of 546 women who applied to the menopause polyclinic of a state hospital in Istanbul and agreed to participate in the study. Data were collected using the Personal Information Form, UCLA Loneliness Scale and Menopausal Symptom Rating Scale (MRS).
Results The mean age of the women included in the study was 52.70 ± 6.39. The mean scores of menopausal symptoms of women included in the study; were found to be higher in women with low education level (p = 0.001), women with big family structure (p = 0,002) and women with low income (p = 0,001). It was determined that women with less education (p = 0,015), women not working (p = 0,001) and women with low income (p = 0,001) were found to have higher mean loneliness scores. Correlation analysis revealed that the mean scores of menopausal symptoms were increased (p = 0,000) as the loneliness level average of women increased. Women with the most severe mean scores of menopausal symptoms were found to have the highest mean level of loneliness (p = 0,000).
Conclusion: Women with high levels of loneliness were found to have experienced severe menopausal symptoms. It was determined that as women's level of loneliness increased, menopausal symptoms increased.
Keywords: Menopause, loneliness, women's health

1. INTRODUCTION

Women spend nearly 1/3 of their lives in menopause and the period after menopause due to prolonged life span (1-3). In this period, women live a lot of change in their family, work and social lives besides some roles and responsibilities as wife, mother, business woman, grandmother and friend. Women in the menopausal period are exposed to significant physiologic changes concurrent with social changes. Radical family and social environment changes (divorce, children leaving house, being a grandmother, lost relatives, etc.) with increased age and in the beginning of the menopausal period could prolong the adaptation period to menopause and pose a developmental crisis. A crisis that cannot be managed well could cause feeling vasomotor and emotional symptoms deeply, decrease the productivity of women and increase costs in healthcare (4-6). The main physiological changes in the menopausal period are due to the effect of decreasing follicle activity and loss of circulating estrogen. However, physiological changes cause menopausal symptoms of different severity depending on the cultural differences in each population. When menopause is perceived as loss of femininity, symptoms could be more frequent and severe; however, when it is perceived as a process that leads the requirement of contraceptives to disappear, the severity of symptoms may be lower (4,7). For this reason, the meaning of menopause for the women is quite important in terms of the severity of symptoms (4,7-11).

Although menopause is a natural and physiological process, it needs follow up and support due to its risks. Because of the importance of estrogen in mood and cognitive regulation, menopause is a powerful predictor of depression and mood symptoms in middle-aged women. In this period, the most common symptom among mood symptoms is the feeling of loneliness. Loneliness is a painful and undesirable feeling in itself, but it also leads to many different problems. Loneliness could lead to a lot of negative health problems such as – particularly – depression and sleep problems, disturbance in HPA (Hypothalamus-pituitary-adrenal) axis and cardiovascular risks (12-16). It is very important to reduce the feelings of loneliness and share feelings in menopause. When literature is reviewed, it is seen that studies that have investigated the effects of loneliness on menopausal symptoms (17,15) are limited. This study aimed to evaluate the effects of loneliness on menopausal symptoms in women in their menopausal period.

2. METHODS

This study is a cross sectional and descriptive study.

2.1. Participants

This study was conducted with women who applied to the menopause polyclinic between October 2016 and October

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2017, were voluntary to participate to the study, spoke Turkish and did not have any communication problems. All women included in the study were in the postmenopausal period. The mean age of menopause was 45.37±5.74 (Min:30-Max:57) years. Data were collected from a total of 570 women. After excluded the forms that were not completely filled out, the sample consisted of 546 women. The study was conducted at Zeynep Kamil Women and Child Diseases Research and Training Hospital, which is the largest Women and Child Diseases Hospital in Istanbul's Anatolian side. Istanbul is the most crowded city in Turkey which receives immigration the most. It is foreseen that the diverse set of data obtained due to the public service of the hospital where the study was performed, would reflect the characteristics of the country as a whole.

Before the study, permission was obtained from Zeynep Kamil Woman and Child Diseases Research and Training Hospital Clinical Research Ethical Committee (171-12.23.2016). Moreover, women who met the criteria of the study were informed about the objective, method and contributions of the study, and their verbal consent was also obtained. They were explained that they could leave the study when they wanted.

2.2. Data collection and tools

All participants were asked to fill in the questionnaires by face-to face interviews: Sociodemographic Information Form, Menopause Rating Scale (MRS) Turkish Version and UCLA Loneliness Scale Turkish Version.

The Sociodemographic Information From was prepared in relation to the literature by the researchers. The form included 13 questions on the participants' age, marital status, occupational status, number of births, menopause characteristic, etc. All menopausal women who applied to the polyclinic due to menopausal symptoms in the premenopausal, perimenopausal and postmenopausal periods were included in the study. The menopausal periods were determined by using the WHO's criteria.

The Menopause Rating Scale (MRS) has 11 items that are scored as a Likert-type scale. The scale can determine the symptoms of menopause as somatic, psychological and urogenital, and at the same time, provide information on the quality of life of the women. A higher total score in the scale indicates an increase in the severity of menopausal symptoms from one side, while it affects the quality of life negatively on the other. The validity-reliability study of the scale in Turkey was conducted by Gürkan (18). The Cronbach's Alpha value of the scale was 0.96 in this study.

The University of California Los Angeles Loneliness Scale (UCLA-LS) has 20 items as a Likert-type scale. 10 items are positive sentences showing satisfaction from social relationships, and 10 items include negative sentences showing dissatisfaction from social relationships. The validity-reliability study of the scale in Turkey was conducted by Demir (19). The Cronbach's Alpha value of the scale was 0.91 in this study.

2.3. Statistical Analysis

After all data were collected, they were analyzed by using the Statistical Package for Social Science (SPSS), version 21.0. Score means ±SD for MRS and UCLA-LS and frequency and percentages of the demographic characteristics were determined. The data were tested for suitability for normal distribution by histogram and One-Sample Kolmogorov-Smirnov Test. The statistical significance of the scores for MRS and UCLA-LS were compared with student's t-test and one-way ANOVA in terms of the sociodemographic characteristics. Menopause total score and UCLA-LS total score were evaluated by using Pearson Correlation Analysis. One-way ANOVA test was used to evaluate menopausal complaint levels and loneliness scores.

3. RESULTS

A total of 546 women between 38 and 65 years of age participated in this study. Their mean age was 52.70±6.39 (min:38-max:65), and 83.2% of them (n=454) were in the spontaneous menopause group. The demographic characteristics of the women are seen in Table 1.

Table 1. *Participants characteristics (n= 546)*

Characteristics;	%	n
Education level		
≤8	73,8	403
>8 years	26,2	143
Marital status		
Single	34,8	190
Married	65,2	356
Family type		
Small family	83,3	455
Big family	16,7	91
Presently working		
Yes	25,6	140
No	74,4	406
Income status		
Low	11,5	63
Balanced	75,3	411
Income more than expense	13,2	72
Menopause reason		
Spontaneous	83,2	454
Surgical	16,8	92

It was determined with the student's t-test that the mean menopause complaint scores were higher in the women whose education levels were 8 years and shorter (p=0.001), who lived in a big family (p=0.002), and did not work at a regular job (p=0.001). The MRS score was lower in the women who went through menopause by surgery (p=0.001). Moreover, it was found with the one-way ANOVA test that the mean MRS score was higher in the women with low income (p=0.001) (Table 2).

Table 2. *Comparing characteristics of participants with MRS scores (n= 546)*

Characteristics;	%	n	MSD	p
Education level			Mean±SD*	
≤8	73,8	403	21,64±13,76	**p=0,001** t=8,14**
>8 years	26,2	143	10,96±12,63	
Marital status				
Single	34,8	190	19,38±14,51	p=0,52 t=0,63**
Married	65,2	356	18,56±14,14	
Family type				
Small family	83,3	455	18,02±13,89	**p=0,002** t=3,04**
Big family	16,7	91	22,97±15,41	
Presently working				
Yes	25,6	140	12,78±12,50	**p=0,001** t=6,01**
No	74,4	406	20,94±14,24	
Income status				
Low	11,5	63	26,00±9,75	**p=0,001** F=9,997***
Balanced	75,3	411	18,24±14,90	
Income more than expense	13,2	72	16,04±11,88	
Menopause reason				
Spontaneous	83,2	454	20,50±13,65	**p=0,001** t=6,22**
Surgical	16,8	92	10,68±14,49	

**Mean; Average SD: Standard deviation, ** Student t test, ***One-way ANOVA*

It was determined as a result of the student's t-test that the mean loneliness scores were higher in the women whose education statuses were 8 years and shorter (p=0.015), and did not work at a regular job (p=0.001). Additionally, it was found by the one-way ANOVA test that the mean loneliness score was higher in the women with low income (p=0.001) (Table 3).

Table 3. *Comparing characteristics of participants with UCLA – LS scores (n= 546)*

Characteristics;		%	n	UCLA	p
Education level				Mean±SD*	
	≤8	73,8	403	48,15±14,84	**p=0,015** t=2,44**
	>8 years	26,2	143	44,62±14,56	
Marital Status					
	Single	34,8	190	48,85±15,45	p=0,15 t=1,44**
	Married	65,2	356	46,55±14,47	
Family type					
	Small family	83,3	455	46,80±14,64	p=0,13 t=1,48**
	Big family	16,7	91	49,32±15,66	
Presently working					
	Yes	25,6	140	43,10±14,15	**p=0,000** t=3,86**
	No	74,4	406	48,65±14,81	
Income status					
	Low	11,5	63	50,12±15,12	**p=0,001** F=6,716***
	Balanced	75,3	411	47,76±14,90	
	Income more than expense	13,2	72	41,63±12,86	
Menopause reason					
	Spontaneous	83,2	454	46,67±14,68	p=0,053 t=1,94**
	Surgical	16,8	92	49,95±15,35	

**Mean; Average SD: Standard deviation, ** Student t test, ***One-way ANOVA*

In the correlation analysis, it was found that menopause complaint levels increased with increased loneliness scores (p=0.000 r=0.27), and as a result of the one-way ANOVA test, it was determined that the group that had the most severe menopause symptoms also had the highest loneliness scores (p=0.000 F=16.745) (Table 4). In explanation of loneliness levels in the women, the significance of menopause complaint levels was confirmed by the regression analysis (F=43.499 p=0.000). The values related to the regression (Beta) coefficient (t=6.595 p=0.000) were statistically significant.

Table 4. *Comparing MRS with UCLA-LS Scores (n= 546)*

Scale		%	n	UCLA-LS	p
Menopausal complaint level (MRS)					p=0,000 F=16,745*
	No complaint	20,7	113	46,72±15,47	
	Mild	18,7	102	40,89±12,91	
	Middle	10,4	57	40,45±13,80	
	Severe	35,2	192	49,31±13,80	
	Heavy severe	15,0	82	55,54±14,01	

** One-way ANOVA*

The mean MRS score of the women was 18.84±14.26 (Min:0, Max:44). It was determined that the somatic, psychological and urogenital subscale scores in the women increased with increased loneliness scores (p=0.000) (Table 5).

Table 5. *MRS with UCLA-LS Score Means of participants (n= 546)*

	MRS				UCLA
	Total	Somatic	Psychological	Urogenital	
Mean± SD	18,84±14,26	6,95±5,20 r=,23/p=000*	8,86± 7,02 r=,24/p=000*	3,03± 2,91 r=,32/p=000*	47,22±14,83 r=,27/p=0.000*

**Comparing UCLA and MRS sub scales by pearson correlation*

4. DISCUSSION

When the literature is screened, it is seen that there are limited studies that investigated menopausal complaint levels and loneliness in women in their menopausal period. In this study, it was determined that loneliness increased menopausal complaint levels similar to the results of the study by Fernández et al. (15). In the study by Fernández et al. (15), the mean MRS score (10.3±4) was lower than our result. Besides this, the mean UCLA score was three times higher (47.22±14.83) than the other study (18.4±12.75), as an unexpected result. A high mean UCLA score is an unexpected situation for Turkish women. Being alone in Turkish society is not an accustomed status. From childhood to old age, life continues in a strong social support network, where family and social and environmental connections are close. However, women could be traumatized, and menopausal period could be more difficult for them because of some reasons like the effect of migration from the village to the city, increased education level, increased number of working women, living in a small family type instead of traditional big family, increased number of old people and becoming alone by children leaving the house. It may also be due to the fact that women could undertake a lot of roles in this period, and

some economic inadequacies and limited social environment could make them more self-enclosed (20). Indeed, culturally determined attitudes are reported to affect menopause perception and experience (10, 21).

According to Fernández et al. (15), loneliness is a common complaint, while women go through menopause, and the menopausal period is more difficult in women who have high levels of loneliness. Loneliness is also a trigger factor for depression (16,22). Loneliness could cause very negative health problems such as sleep problems and cardiovascular problems (14-16,23). Sharing loneliness could decrease the development of depression and make an important contribution to the healthcare system (22). It is debated whether depression is a cause or effect in the menopausal period (24). Although it is suggested that menopause could not lead to psychiatric disorders by itself (25), going through menopause increases the risk of depressive symptoms (26-32). Lee and Kim (33) reported that women who had high menopausal complaint levels were at risk of depression by three times more. Retirement, children leaving home and losing relatives are other important situations that should be considered in depression within the menopausal period (34). However, it was a limitation of the study that depressive symptoms in women were not investigated here. Thus, relationships between loneliness, depressive symptoms and menopausal symptoms could not be determined.

In similarity to the literature, menopausal symptoms increased with decreased education status (20,26,33,35), and they caused more negative mental health (11,29). Additionally, in these people, social support levels were lower (7). It is believed that, as the level of education of women increases, this may lead to economic prosperity and expansion of social environments and the opportunity to express their feelings.

When the literature was reviewed, it was suggested based on some studies the family type did not affect the severity of menopausal symptoms (20,36). In another study, it was found that incompatible familial relations could predict depressive symptoms (37). Besides this, another study determined that perceived social support from the family decreased menopausal symptoms (38). In our study, it was seen that the quality of support, regardless the number of people in the family, was important. The extended family type is more common in families that are dependent on their cultural values in Turkey. Moreover, the process of passing to the small family type to the big family type takes place by being affected from western culture. The small family type is especially common with increased education levels.

In this study, menopausal symptoms were higher in the women with low income as in the case in the relevant literature (4,10,11,20,33,39,40). Poor economic level could affect health-seeking behavior negatively (10,41). Hence, Istanbul is a very huge and expensive city and it has transportation problems. Even if women with poor education level apply to menopause clinics, they are hesitant to share information about their emotional state with healthcare personnel. Additionally, there is a culture of sexuality and emotion in the Turkish family structure, often not shared with healthcare personnel and waiting for problems to be solved spontaneously by themselves.

Psychosocial interventions in coping with physical, psychologic and social changes in women in their menopausal period could provide benefits for the quality of life of women and better health outcomes. Psychosocial interventions should be supplied in healthcare services for women as a part of comprehensive care (6,17,26,42-44). Thus, women should be examined not only about their menopausal symptoms but also about their mood within a holistic view. In this study, we evaluated the loneliness statuses of women in their menopausal period. However, we did not carry out any intervention about how a woman copes with this period or how these issues should be managed. As in other diseases, early recognition of mental problems is very important, and besides physical symptoms, mental health of women in the menopausal period should be follow up. A lot of women who are not able to receive sufficient healthcare services have to take care of themselves in their menopausal period, which is a fragile stage in terms of mental health. So, we thought that every woman in their menopausal period should receive holistic healthcare services, even though it is not possible within Turkey's conditions.

5. CONCLUSION

It was determined that women who had high loneliness levels experienced menopausal symptoms more severely, and menopausal symptoms increased with increased loneliness levels. Menopausal symptoms were higher in women who had low education levels, lived in big families, did not have a regular job, had low income. Loneliness was felt more intensely in women with lower levels of education, less regular employment and lower economic income.

In this study, loneliness was found to be an important predictor of the severity of menopause symptoms. The common risk group for both loneliness and menopause symptoms was low education, non-working and low income women. Women in the risk group should be follow up more closely and early interventions to protect mental health should be done.

That the study data were based on self-reporting is an important limitation in the research. It should not be forgotten that the function of the scales and of its scores are meant to serve as a guide to psychotherapists and physician. Another important limitation to the study was that it was conducted at one hospital and did not include women from outside these facilities. Because the study only encompassed information gleaned from women applying to the one hospital in Istanbul, this data cannot be generalized to all women in the menopause stage.

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Clinical and Experimental Health Sciences

In Vitro Gap Changes After Porcelain Firing Cycles of Three and Four Unit of CAD/CAM Milling, Laser Sintering and Cast Metal Ceramic Restorations

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ABSTRACT

Objectives: Marginal adaptation changes during the veneering process is an important factor in the clinical success of metal framework techniques such as conventional cast metal cores (LW), CAD-CAM Metal Milling (MM) and Direct Metal Laser Sintering (DMLS). The aim of this study is to evaluate the marginal fit changes between three- and four- unit's metal ceramic fixed partial dentures (FPD's) fabricated by Lost Wax (LW), CAM Metal Milling (MM) and (Direct Metal Laser Sintering) DMLS metal framework techniques after porcelain firing cycles (PFC).

Methods: A total of 60 stainless steel three and four unit FPD's models were fabricated. Specimens were randomly divided into three groups to fabricate metal ceramic FPD's frameworks with LW, MM and DMLS techniques. Before and after PFC, cross-sections from silicone replicas were obtained, sectioned, examined and measured with a light microscope. The statistical analysis was done with Mann-Whitney U and Kruskal Wallis and Wilcoxon Signed Ranks tests. Results were evaluated at 95 % of confidence interval and $p<0.05$ level.

Results: There was a statistically significant difference between the three and four unit of FPD's, before PFC for LW and MM (p:0.000) and DMLS (p:0.019)'s groups and only DMLS (p:0.006)'s group was statistically significant after PFC. The mean marginal gaps of LW technique was higher than the MM and DMLS's techniques before PFC. After PFC, DMLS's technique results were higher than LW and MM technique but, no statistically significant difference was found between the marginal gap values of the three and neither for four units of DMLS's FPD's.

Conclusion: PFC decreases the mean marginal gap of the LW and MM group, but, there was a slight increase for DMLS group. However, all the marginal gap values obtained were in clinical acceptance level for three and four-units FPD's for all tested specimens.

Keywords: Marginal gap, lost wax, CAD/ CAM, laser sintering, porcelain firing cycle

1. INTRODUCTION

In 21th century, metal fused to porcelain restorations were usually used in clinical practice and are still a gold standard in fixed partial dentures. The success of fixed partial dentures (FPDs) depends a lot of factors (1). Marginal fit plays an important role for a dental restoration. The degradation of cement in oral cavity can result in loss of marginal seal, retention of plaque, development of secondary dental caries and development of periodontal disease (2). McLean and von Fraunhofer (3) suggested that the maximum gap should be 120 µm. Goldin et al (4) reported that the clinically acceptable marginal gap should be between 40-120 µm.

Metal ceramic restorations can be fabricated from the noble and non-noble alloys. Non-noble alloys (Ni-Cr and Co-Cr alloys) were used instead of noble alloys due their lower cost (5, 6). Because of the allergic reactions to Ni-Cr alloys, more biocompatible Co-Cr alloys were developed (7). Lots of technique are available for producing Co-Cr copings. CAD/CAM systems were developed and it was a possible alternative to conventional lost-wax technique (LW). Milling the frameworks from a block of Co-Cr (MM) and sintering metal powders by using direct metal laser sintering (DMLS) were two fabrication methods of digitized technique (8). Short manufacturing time, elimination of casting shrinkage and easy production of complicated shapes were the advantages of CAD/CAM techniques (9, 10). CAD/CAM restorations can be affected by precision of scanner, precision of milling machine and data transformation process (11) Gonzalo et al. (12) reported that better marginal fit values with CAD/CAM processing of ceramic systems than with the conventional LW technique. Sundar et al (10), Xu et al. (13), Kim et al. (14, 15) reported that metal copings manufactured by DMLS showed good marginal fit. Kane et al. (16) pointed out that MM Co-Cr copings (NobelProcera; Nobel Biocare) were shown clinically acceptable marginal fit in the range of 52-113 µm.

The major problem with metal ceramic restorations is the marginal fit. The marginal fit changes during the ceramic firing is a big problem for the metal ceramic restorations (17). The contraction occurred due to the porcelain firing cycles, margin and alloy type are important factors contributing to distortion. Sundar et al. (10) reported that ceramic firing procedure has minimal effect on metal laser sintered crowns. The aim of this study was to evaluate and to compare the changes on the marginal gaps of three and four-unit posterior metal ceramic restorations fabricated by using three different techniques as conventional LW, MM and DMLS after PFC. The

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null hypotheses was that the marginal fit changes during the firing cycles will be less in MM and DMLS's than the LW's technique for the three and four-units FPD'S.

2. MATERIALS AND METHODS

Three different techniques as conventional LW, MM and DMLS were chosen for evaluation. Materials used in this study were shown in Table 1.

Table 1. *Materials tested*

Material	Product Name (Manufacturer)	Ingredient's	Batch number
Impression Material	Panasil Putty Soft (Kettenbach, California, USA)	Polyvinlysiloxane additional silicon	11121
Impression Material	Panasil Initial Contact Light (Kettenbach, California, USA)	Polyvinlysiloxane additional silicon	13411
Impression Material	Panasil Initial Contact X-Light (Kettenbach, California, USA)	Polyvinlysiloxane additional silicon	13401
Metal	Magnum Ceramic S (Mesa, Brescia, Italy)	Metal blocks (Ni, Cr and Mo)	0546
Metal	Yena CoCr 10 mm (Yenadent, Istanbul, Turkey)	Metal blocks (Co, Cr and Mo)	
Metal	EOS CobaltChrome SP2 (EOS, Munich, Germany)	Metal blocks (Co, Cr and Mo)	9011-0018
Wax	Waxwire (Bego, Bremen, Germany)	Wax sprue	40085
Wax	Bego Kronenwachs (Bego, Bremen, Germany)	Modelling wax	40115
Investment	Hera Moldavest (Heraeus, Germany)	Phosphate bonded investment	66009780

2.1. Specimen preparation

Master abutments were prepared by CNC machining (Chevalier FBL-1233, Chang Hua, Taiwan) using stainless steel with 360° 1mm rounded shoulder finish line, 10 mm diameter for molars and 5mm for premolars, 5 mm axial height, 10 % axial taper, and flat occlusal surface (18). Prepared master abutments were fixed on a metal plate using screws to obtain three – and four-unit FPD's framework. (Fig.1 and Fig.2).

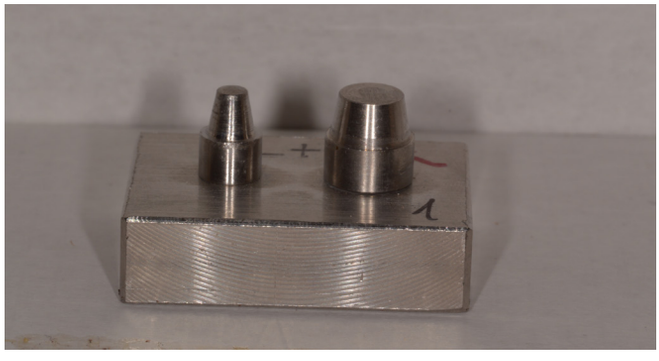

Figure 1. *Three-units master abutments*

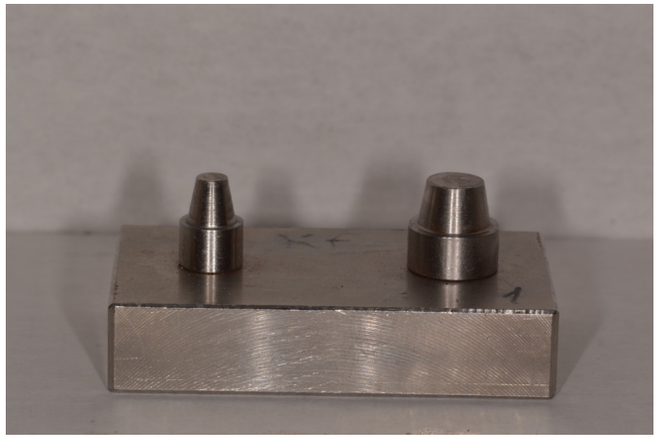

Figure 2. *Four-units master abutments*

In total of 30 three-unit and 30 four-unit FPDs were fabricated with LW, MM and DMLS's techniques (n: 10).

Conventional lost-wax method (LW) (Group 1) (n:10): Two layers of die spacer (30 µm) (Isowachs, Labor – Scheftner, Mainz, Germany) were applied on each metal abutments specimens to eliminate the dimensional stability problem caused by the impression material. To fabricate a 0.4 mm. uniform thickness of coping, pattern wax (Bego Kronenwachs, Bego, Bremen, Germany) was used. The thicknesses of patterns were checked by using a metal gauge. A sprue was attached to the completed wax coping (Waxwire, Bego, Bremen, Germany), invested (Hera Moldavest,,Heraeus, Gerrmany) and casted with Ni-Cr alloy pellets (Mesa Magnum Ceramic S, Brescia,Italy). All the casting were performed according to the manufacturers' instructions.

Metal Milling technique (MM) (Group 2) (n:10): 3D laser scanner (D800; 3Shape A/S, Copenhagen, Denmark).was used to scan each metal model and CAD software program (Dental DesignerTM; 3Shape A/S, Copenhagen, Denmark) was used to design the coping with 0.4 mm in thickkness. The internal space was set 30 µm from the 1 mm upper margin. The completed design was saved as an STL file and send to the milling machine (Yenamak D40, Yenadent, Istanbul, Turkey) to mille the Co-Cr alloy copings with metal blocks (Yenadent, Istanbul, Turkey).

Direct metal laser sintering (DMLS) (Group 3) (n 10): Metal laser sintering technology (EOS M270) (EOS, Munich, Germany) was used to fabricate 0.4 mm in thickness with internal relief of 30 µm specimens with a biocompatible Co-Cr alloy in powder form, designed specifically for metal porcelain restorations. (EOS CobaltChrome SP2, Munich, Germany). 3D scanner (Scanner 7Series) (Dental Wings 7Series 3D Scanner, Montreal, Canada) was used to scan the die specimens. The CAD design of the coping was obtained as an STL data which was used in EOS RP tools software (EOS RP Tools; Magics RP, Munich, Germany) for fabrication of copings.

Vita VMK Master (Vita Zahnfabrik, Bad Sackingen, Germany) and Vita VMK 95 Metall Keramik Dentine, (Vita Zahnfabrik,

Bad Sackingen, Germany) were used as veneering material for all groups.

2.2. Marginal gap evaluation

Marginal gap values were measured at two different times. The initial measurement was performed before and the second and final measurement was measured after the ceramic firing cycles. Any internal adjustments were done after ceramic firing cycles for each tested specimens.

The silicone replica technique was used (Panasil Kettenbach, California, USA) to observe and compare the marginal gap changes between the initial and final measurements. The frameworks of the FPDs were filled with extra light body silicone impression material (Panasil Initial Contact X – Light Kettenbach, California, USA); then were placed onto the metal abutments specimens. Finger pressure was used during setting of the silicone impression material. Then, thin silicone film replicas and copings were removed together from the abutments. To stabilize the silicone films representing the space between abutment teeth and frameworks, a light body silicone (Panasil Initial Contact Light Kettenbach, California, USA) was injected on the light body silicone replicas and immediately during working time, retainers put into boxes filled with heavy body silicone (Panasil Putty Soft Kettenbach, California, USA).

Silicone replicas were sectioned in both of the mesio-distal and bucco-lingual directions in one time with a razor blade. Initial and final silicone replicas were examined under a binocular stereomicroscope (Leica Optic microscope, Leica Cambridge Ltd., Cambridge, England) at a magnification of ×48 to obtain marginal gap values. From each segment, 9 different marginal gap values were measured. A total of 36 measurements were obtained per one abutment (Fig.3).

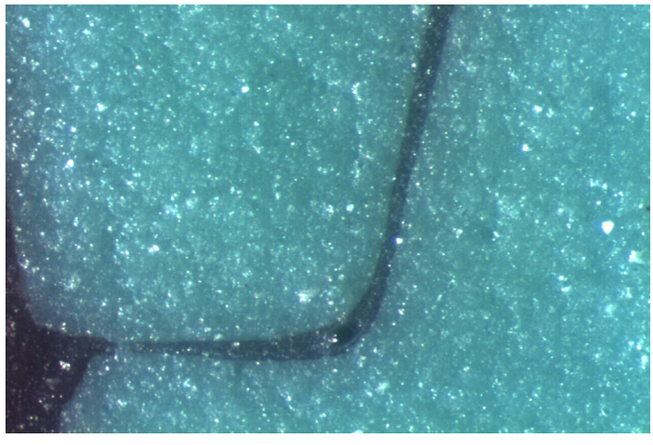

Figure 3. *Stereomicroscope image of marginal gaps measurements points*

2.3. Statistical analyses

SPSS for Windows, version 15.0 (SPSS Inc., Chicago, IL, USA) was used for the statistical analysis. To compare quantitative data without normal distribution, for two groups Mann-Whitney U, for more than two groups Kruskal Wallis test were used. Wilcoxon Signed Ranks tests were used for the statistical analysis of intra-group comparison of non-normal distribution parameters. The results were evaluated in 95% confidence interval (p<.001)

3. RESULTS

The minimal, maximal and mean marginal gap values of the three and four – unit FPD's for LW, MM and DMLS's groups and the Mann – Whitney U statistical analysis results were shown in Table 2. Tables 3 shows the comparison of three and four unıts FPD's. The Wilcoxon Signed Range test results were given in Table 4.

Statistically significant difference was found between the three and four-unit of FPD's, before porcelain firing process for LW (p:0.000) and MM (p:0.000) and DMLS (p:0.019)'s groups and only DMLS (p:0.006)'s group was statistically significant after PFC. Mean marginal gap values of three and four-units FPD's fabricated by LW, MM and DMLS before PFC for three and four-units were 98.68 µm, and 68.72 µm; 82.74 µm and 71.10 µm; 63.97µm and 70.42 µm and after porcelain firing cycle 64.58 µm and 65.70 µm; 63.69 µm and 61.95 µm; 64.27 µm and 71.64 µm respectively.

Table 2. *The mean marginal gap values of the three and four – unit fixed partial dentures for LW,MM and DMLS group (*p<0,05)*

			N	Min	Max	Mean	SD	p
LW *Group 1*	**Before porcelain firing cycles**	**Three-unit**	10	21.96	306.72	98.68	28.94	**0.000***
		Four-unit	10	13.49	136.14	68.72	10.27	
	After porcelain firing cycles	**Three-unit**	10	17.49	115.56	64.58	8.34	0.589
		Four-unit	10	13.13	105.12	65.70	5.86	
MM *Group 2*	**Before porcelain firing cycles**	**Three-unit**	10	28.75	161.88	82.74	11.28	**0.000***
		Four-unit	10	20.86	120.60	71.10	5.02	
	After porcelain firing cycles	**Three-unit**	10	17.15	104.32	63.69	6.03	0.168
		Four-unit	10	15.13	99.78	61.95	4.00	
DMLS *Group 3*	**Before porcelain firing cycles**	**Three-unit**	10	13.13	117.18	63.97	8.12	**0.019***
		Four – unit	10	14.55	119.96	70.42	9.73	
	After porcelain firing cycles	**Three-unit**	10	15.14	133.06	64.27	6.14	**0.006***
		Four-unit	10	15.23	130.30	71.64	12.05	

Table 3. *The Kruskal Wallis statistical test result of the three-unit FPD's before and after porcelain firing cycles (*p<0,05)*

			Mean	SD	p
3 units-FPD's	Before porcelain firing cycles	LW	98,68	28,94	0,000*
		MM	82,74	11,28	
		DMLS	63,97	8,12	
	After porcelain firing cycles	LW	64,58	8,34	0,994
		MM	63,69	6,03	
		DMLS	64,27	6,14	
4 units-FPD's	Before porcelain firing cycles	LW	68,72	10,27	0,780
		MM	71,10	5,02	
		DMLS	70,42	9,73	
	After porcelain firing cycles	LW	65,70	5,86	0,001*
		MM	61.95	4.00	
		DMLS	71.64	12.05	

Table 3 shows that there was statistically significant difference (p: 0.000) between the technique when the three-unit FPD's compared. The mean marginal gaps of LW technique was higher than the MM and DMLS's techniques before PFC. The comparison of the mean marginal gap of the four-units FPD's revealed statistically significant difference (p: 0.001) after PFC. The DMLS's technique results were higher than LW and MM technique. There were no statistically significant difference between other cycles for three and neither four unit's technique.

Statistically significant decrease was observed between the marginal gap values of three-unit FPD's manufactured with LW and MM and also, with four-units FPD's manufactured with MM. The initial marginal gap values were higher than the final values (*p*: 0.000). No statistically significant difference was found between the marginal gap values of the four-units FPD's and neither for three or four units of DMLS's FPD's (Table 4).

Table 4. *Wilcoxon statistical analysis's results of the all the tested group (*p<0,05)*

	p
LW – 3 units-FPD's	**0,000***
LW – 4 units-FPD's	0,191
MM – 3 units-FPD's	**0,000***
MM – 4 units-FPD's	**0,000***
DMLS – 3 units-FPD's	0,970
DMLS – 4 units-FPD's	0,502

4. DISCUSSION

The null hypotheses was rejected. The marginal fit changes will be less in LW and MM than DMLS's for the three and four-unit FPD'S during the firing cycles.

Development of CAD-CAM systems is an important innovation in dentistry. CAD-CAM systems provide time reduction by eliminating many disadvantages of conventional lost wax technique such as investing and burn-out process. Precision of scanner, 3D design and the precision of fabricating machine effect marginal fit of dental restorations (19). 3D design data is saved as an STL (stereolithography) file. STL format characterizes the surface structure of a solid 3D model (20). In the literature, few studies have been reported about the marginal fit of multiple-unit FPDs s fabricated by DMLS and computer-aided milling (10).

In the presented study, marginal fit of three-unit and four-unit FPDs fabricated by DMLS system using Co-Cr alloy powder and computer-aided milling system using Co-Cr metal block was compared with that of three-unit and four-unit FPDs by the LW method using Ni-Cr alloy. Because of that Ni-Cr alloy has been widely used for metal casting until present, as a control group Ni-Cr alloy was chosen for casting as Kim et al's (14,15) and Sundar et al's (10).

In vitro studies provide optimizing and standardizing circumstances for an experimental study (21). In the literature, there were many in vitro studies which investigate marginal fit (1, 5, 10). Regish et al. (22) fabricated a standardized metal master die simulating a prepared crown. Souza et al. (23) reported that with using rounded shoulder finish line significantly lower marginal discrepancy valueswas obtained than that of tilted and large chamfer finish lines. Tsitrou et al. (24) reported better marginal fit values with shoulder finish line than chamfer finish line. In the present study, standardized stainless steel master dies simulating prepared crowns with shoulder finish line for three-unit and four-unit FPDs were manufactured. All the FPD's restorations were prepared on the related metal models to eliminate problems could be occurred during the impressions procedures for LW's group and the scanning and designing procedures for MM and DMLS's groups and to compare the metal framework techniques. In LW group, two layers of die spacer (Isowachs, Labor – Scheftner, Mainz, Germany) were applied corresponding to 30 µm which was the relief area of the MM and DMLS group.

The number of data is important for the evaluation of marginal gap values. The number of measurements per crown and specimens size used in the literature has varied considerably (25-27). In some studies, measurements numbers are between 4 and 12 and this might be misleading (26, 28, 29). Groten et al. (30) reported that number of measurements per specimen should be minimum 20-25 and ideally 50. For the reliability of measurement, in the presented study before and after ceramic firing stage, 36 measurements were performed for each specimen, totally 72 measurements performed.

In the literature, different techniques were used to measure marginal fit of dental restorations; silicone replica, cross-sectioning, direct-view, profilometry and micro CT (31). Quante et al. (32) used a silicone replica technique to examine the gaps of Co-Cr copings under a microscope. Many researches (33-36) have used the silicone replica

technique for the marginal fit study's. It is a non-destructive technique and it has a good reliability and precision. Örtrop et al. (5) used a stereomicroscope and digital photos to evaluate the marginal and internal gaps of Co-Cr three-unit bridges for posterior teeth. Reich et al. (34) and Harish et al. (37) used light microscope with ×48 magnification to take images. In the presented study, the silicone replica and the stereomicroscope (Leica Optic microscope, Leica Cambridge Ltd., Cambridge, England) at ×48 magnification was used to evaluate the marginal gap measurements.

Dental restorations must have a good marginal fit to be clinically successful (38). In the presented study, all marginal gap measurements were within the clinically acceptable range as reported in many studies (3, 39). According to the three-unit FPDs results of the current study, before porcelain firing stage, mean marginal fit of DMLS (63,97 µm) was less than the mean marginal fit of LW (98,68 µm) and MM (82,74 µm). After porcelain firing stage, higher mean marginal fit values were observed in LW (64,58 µm), compared to MM (63,69 µm) and DMLS (64,27 µm). According to the four-unit FPDs results of the this study, before porcelain firing stage, mean marginal fit values were similar in all groups; LW (68,72 µm), MM (71,70 µm) and DMLS (70,42 µm). After porcelain firing stage, mean marginal fit value of DMLS (71,64 µm) was slightly higher than LW (65,70 µm) and MM(61,95 µm).

In the literature, there were a lot of in vitro investigations (10, 13, 15, 20, 37) which were evaluated and compared the marginal fit changes of metal-ceramic crowns fabricated with different techniques. Unfortunately, there were limited study with three-units (1, 5, 14) and few study [40] with four-unit. FPD's. Örtrop et al. (5) evaluated and compared the marginal and internal fit of three-units FPD's in Cr-Co fabricated by LW, milled wax with lost-wax (MW), MM and DLMS' techniques before the PFC. Best fit was found for DMLS group (84 µ), followed by MW (117 µ); LW (133 µ) and MM (166 µ). These results were parallel with the presented studies results. The MM groups (82.74 µ) results were higher than the DMLS groups (63.97 µ) which was the lowest mean marginal gap values obtained. Örtrop et al. (5) reported the highest values for MM groups (166 µ), nerveless in the presented study LW group (98.68 µ) results were higher than all the other groups. The reason for this discrepancy could be related to the milling machine which manufacturers were different. And also, Örtrop et al. (5) used 50 µ's cement thickness, but in the presented study 30 µ's die spacer was applied as a cement thickness. Olivera and Saito (41) reported that the thickness of the die spacer could be affecting the adaptation of the restorations. Kim et al. (14) compared and evaluated the marginal and internal gap of three-nits FPD's fabricated by using DMLS system with that of three-unit FDP's by a LW method and reported that the DMLS results (130.6 and 133.1µ) were higher than the LW results (81.7 and 81.8 µ).These results were not compatible with the presented study. Kim et al. (14) used epoxy resin model, but in this study stainless steel models were used. It may be the reason for this difference. Nesse et al. (1) examined and compared the internal and marginal fit of cobalt-chromium metal frameworks of three-unit FPD's fabricated by LW, MM and selective laser melting (SLM). Direct-sight technique was used to evaluate the marginal fit. Scored 1-5 was used and they reported that the MM group had the best overall fit, followed by the LW and SLM groups. These results were parallel with the initial results of the presented study, before the PFC the mean marginal gap values of the MM technique were lower than the others.

In the literature, there were no study which compare the marginal adaptation of MM and DMLS methods. Bayramoglu et al. (40) was compared the marginal and internal marginal fit of three different restorative materials and the effect of veneering/pressing on the material used for 3 – and 4 – unit implant supported FPDs. The mean marginal gap values obtained were75.4 µm µand 103.82 µm for the three and four unit FPD's fabricated by LW respectively. The four-unit's mean marginal values were higher than the three-unit's. In the presented study, the results were 64.58 µm and 65.70 µm respectively. There were a slight difference between the results, but all the values were in clinically acceptable level.

In the presented study, statistically significant decrease was observed for the mean marginal gap values in three-units LW and in three and four units MM group after porcelain firing cycles (*p*: 0.000). The results of the four units LW and three or four units DMLS group were changed slightly and no statistically significant difference was found. Sundar et al. (10) reported similar results after ceramic firing with Co-Cr alloy copings fabricated by DMLS, the change was not statistically significant.

In the literature, no consensus exists on the effect of ceramic firing cycles on the marginal fit of FDPs (18). Quante et al. (8) reported that the ceramic firing changed the gap of the crown slightly. These changes, increasing or decreasing the marginal gap after ceramic firing, support the idea that the porcelain firing cycles distorts the metal substructure (17). In the literature, previous study (42,43) were in agreement in two areas, first of all the distortion occurs during the thermal cycling process and second one the timing of the deformation mostly occurs during the oxidation of the alloy. Patil et al. (44) observed that small changes continue during the porcelain application process. The results of the presented study show that the ceramic firing cycles changed the marginal gap of the three and four-unit FPD's manufactured by LW, MM and DMLS's techniques .The gap values were decreased after PFP for LW and MM, slight increase were seen in DMLS's group. The minimum and maximum mean marginal gap values obtained were in 61.95 µm and 71.64 µm range. Fortunately all the marginal gap values obtained were in clinically acceptable level. Therefore, the three methods tested in this study, LW, MM and DMLS, could be used safely in the clinic with regard the marginal gap values. Further clinical study is needed to evaluate the survival of these new techniques as MM and DMLS.

5. CONCLUSION

Within the limitations of this study, it can be concluded that PFC decreases the mean marginal gap of the LW and MM group, but, there were a slight increase for DMLS group. However, all the marginal gap values of all tested techniques were in clinical acceptance level for three and four-units FPD's after PFC.

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Clinical and Experimental Health Sciences

Fatigue and Physiotherapy in Liver Transplant Recipients

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ABSTRACT

Liver transplantation surgery is a life-saving treatment option for life-threatening end-stage liver diseases and acute liver failure. While the health-related quality of life of liver transplant recipients is related to the success of the transplantation procedure; fatigue, malnutrition, loss of muscle mass, decrease in exercise capacity, negative respiratory and metabolic changes as the findings of organ failure are among the causes of functional loss in the posttransplant period. The prevalence of the fatigue, as the frequently experienced symptom in the end-stage liver diseases, varies depending on the specific forms of liver disease and it adversely affects pre and postoperative functional levels of the patients, liver transplantation results and also survival. The severity of the fatigue decreases after the transplantation surgery, but it continues to be seen as the most compelling clinical symptom experienced during the postoperative first year. Current literature data emphasize the necessity of physiotherapy programs and active early mobilization which applied in the early period in the intensive care unit following transplantation surgery and physiotherapy and rehabilitation approaches including strength and endurance training contributes to the functional level in this population. These findings suggest that there is a need for structured physiotherapy programs to increase muscle strength and exercise capacity and prevent fatigue.
Keywords: Exercise training, fatigue, liver transplantation, physiotherapy

INTRODUCTION

Transplantation surgery is considered to be the best liver replacement therapy and a life-saving treatment option for life-threatening end-stage liver failure (1). Although the liver function and survival are improved after transplantation; surgical stress response, physical and psychological problems due to the pathology affect the quality of life negatively (1,2). Many recent studies have focused on the relationship between health-related quality of life and the success rate of transplantation procedure states that transplantation surgery decreases scores representing physical activity parameters of quality of life. The decrease in the level of physical activity is reported to be associated with cachexia, diminishing muscle strength and exercise capacity, loss of range of motion, osteoporosis, malnutrition, pain, arthritis and physical fatigue (1,3,4).

Fatigue has been listed as the most commonly experienced symptom in patients with chronic liver disease (5); it leads to complications, adversely affecting results of liver transplantation as well as survival (6,7). Ney et al. reported that the level of physical activity which is reported by the subjects who have liver failure was low and the major barrier was fatigue (8). Although the severity of fatigue is decreased by transplantation surgery, it remains to be the most challenging clinical symptom experienced within the first postoperative year (9,10). Van den Berg-Emons et al. reported that fatigue is an ongoing symptom in 44% of recipients in the post-transplant period up to 15 years (11).

The pathogenesis of fatigue is not clear in chronic diseases and liver transplant recipients, however, it is multifactorial in general (5). Since many factors such as age, gender, the level of physical activity, sleep quality, cardiorespiratory fitness, anxiety, and depression are associated with fatigue (12,13); physiotherapy which applied in the early and late postoperative period is extremely important. There is a necessity to introduce patient-specific and structured exercise programs for liver transplant recipients (14,15).

CLINICAL AND RESEARCH IMPACTS

Liver Transplantation

Liver transplantation is the procedure in which normal functioning liver tissue from either a living or cadaveric donor is replaced electively in cases with chronic liver diseases or acute fulminant insufficiency (16). Currently, the only proven, definitive treatment modality for the end-stage liver disease is liver transplantation. However, the selection of liver-transplant candidates is extremely important for the success of transplantation (17). Liver failure is characterized

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by the progressive deterioration of hepatic functions in acute or chronic progress. The process itself is critical due to the complications of the disease rather than the liver disease alone; therefore, there is a high mortality risk. In the course of a pretransplant evaluation, recipients should be evaluated thoroughly; thus the identification of high-risk patients is of utmost importance for the success of transplantation surgery. The most commonly used risk scoring methods are the Model of End-Stage Liver Disease (MELD) and Pediatric End-Stage Liver Disease (PELD) scores which is the specific form for children. Model for End-Stage Liver Disease (MELD), The United Kingdom Model for End-Stage Liver Disease (UKELD) (17-19) and Child-Pugh scores are other scoring systems. These systems aim to determine the severity of liver disease and predict the prognosis. The MELD score is known to be more predictive in short-term pretransplant mortality risk prediction (Grade II-1) (18). When MELD and Child-Pugh scoring systems are compared, some studies reported that both are similar for prognostic aspects in many cases and they both have distinctive features for some specific conditions and there is a need for further research to decide which scoring system should be used in which conditions (20).

Perioperative risk assessment includes evaluating comorbidities for liver transplantation such as coronary artery disease, pulmonary hypertension. Patients with hepatic insufficiency have malnutrition, myopathy, and poor physical performance and these secondary outcomes cannot be easily detected with standard cardiorespiratory tests such as 2-dimensional echocardiography and pulmonary function tests (1,18). In resting transthoracic 2-dimensional echocardiography, the normal left ventricular systolic function is defined as an ejection fraction (EF)>45% and according to the European Society of Cardiology guidelines, an elevated systolic pulmonary artery pressure (PAP)>36 mmHg is defined as the cutoff value for possible pulmonary hypertension (19).

Fatigue

The clinical definition of fatigue includes biological, psychosocial and behavioral processes and its' complex interactions along with the prevalence varies depending on the specific form of the liver disease (21,22). Cholestasis causes degenerative central nervous system changes adversely affecting the brain regions which regulates autonomic dysfunction and sleep pattern. These changes lead to many symptoms of fatigue and associated cognitive disorders (2). Besides, autonomic dysfunction contributes to these metabolic changes by limiting the reaction capacity of the muscle to increase in proton/lactate current from the cell and its excretion from tissues (23). Complications in cirrhotic patients such as sarcopenia and hepatic encephalopathy also appear as other causes that lead to fatigue (24,25).

Studies report that chronic liver inflammation is associated with changes in the central nervous system (CNS) emerging as behavioral modifications (26). Liver inflammation also causes changes in brain function. Abnormal central neurotransmission leads to behavioral changes when there isn't any pathological CNS tissue damage present (27). Neurotransmitters associated with central fatigue are corticotropin-releasing hormone (CRH), serotonin and noradrenaline (22). The liver is innervated by vagal nerve afferents that respond to immune mediators such as tumor necrosis factor (TNF α), interleukin-1 and interleukin-6 (22). The activated vagal nerves affect different regions of the brain potentially leads to subsequent behavioral changes. However, this neural pathway is also thought to play only a minor role in the mechanism of fatigue in chronic liver disease because post-liver transplant patients (in which the liver is deinnervated) often reported a very little change in their perception of fatigue (22,28).

Glial cells and neurons in the brain can produce cytokines leading to behavioral modifications including fatigue. Fatigue is also reported to be associated with alterations of basal ganglion neural activity (22). Stinton and Swain reported that there are still areas where density is even more decreased after a few months of the transplantation. The brain dynamics in those cases where cirrhosis had not recurred indicates that neurological damage may be permanent or recovery is very slow (22).

Despite the pathophysiological mechanisms explaining fatigue exists, there is still not enough data on fatigue's mechanism. Also, it is reported that liver transplantation does not eliminate fatigue. Therefore, further investigation is needed in liver transplant patients to predict fatigue factors and to plan exercise and rehabilitation programs for fatigue management.

Physiotherapy and Rehabilitation

Even though physiotherapy and rehabilitation programs have benefits in reducing fatigue severity; studies in the literature are limited regarding structured exercise programs.

Studies in liver failure cases are indicated reduced exercise capacity which is measured by maximal oxygen uptake (VO_{2max}) (29,30) and the inverse correlation between exercise capacity and liver disease severity is remarkable. The decrease in exercise capacity is not only associated with the severity of disease but also considered to be as a predictor of mortality after transplantation. Physical competence, which is one of the most important parameters affecting exercise capacity, is affected by many factors, mainly fatigue, and muscle strength. Studies have emphasized that therapeutic exercises applied to chronic liver failure patients improves physical fitness by improving cardiopulmonary functions (31). These studies especially pointed out a four-week physical training program is needed to improve VO_{2max} by enhancing physical activity level, skeletal muscle volume, and mass (32). Therapeutic exercise approaches are therefore extremely important in managing fatigue in end-stage liver failure (33,34).

Because of a long waiting period is added to the presence of sarcopenia in liver failure cases which are in the transplantation waiting list, rehabilitation programs

intending to reduce inactivity, improve muscle performance, increase exercise tolerance and prevent postoperative complications are of great importance in this population (29,35,36). Individualized and standardized physical activity programs are acceptable, effective and reliable in patients who are waiting for transplantation. Although however the positive effects of such programs on functional performance and quality of life are known, it is needed for prospective, comprehensive randomized trials to point out promising effects on the post-transplantation process, duration of hospital stay and six-months survival (37).

Studies reported that maximal oxygen consumption of post-transplant patients is 40-60% lower than expected (38). Beyer et al. had followed liver transplant recipients by supervised exercise program during their first postoperative year. Although they reported the improvement in cardiovascular and neuromuscular capacity, the maximal oxygen consumption, and muscle strength were 10-20% lower when compared with similar sex and age-matched healthy subjects (39).

Another study observing the relationship between physical fitness deficits and fatigue and health-related quality of life in liver transplant recipients showed that the cardiorespiratory fitness level was significantly impaired in the recipients and the prevalence of obesity was higher than the general population. Consequently, based on the relationship between cardiorespiratory level and fatigue, rehabilitation programs aimed at increasing cardiorespiratory fitness can increase the quality of life by reducing the severity of fatigue after transplant surgery (14).

Current literature data reveals that; loss of muscle mass due to metabolic and nutritional deficits, peripheral neuropathies and pulmonary complications emerging as a result of postural component influences emphasize intensive physiotherapy programs early after transplantation surgery (37,40). The research which evaluated the hemodynamic effects of physiotherapy in intensive care process after liver transplantation suggest that, acute cardiopulmonary responses caused by intensive care physiotherapy in liver recipients are in normal physiological limits (41).

Studies have proved that physical exercise can increase the quality of life in liver transplantation cases. Exercise programs improve the functional capacity based on reduced difficulties encountered in tasks of daily living and by increased patient orientation; as a result, they actively participate in their treatment process. Preoperative patient education, however, may be beneficial in the perioperative and post-transplant recovery period. Limongi et al. followed transplantation candidates for three months which are given patient educations preoperatively and observed improvements in their diaphragm's electrical activity and quality of life. But they also stated that further researches on the benefits of respiratory exercises after liver transplantation surgery are needed (42).

The research findings of Van Ginneken et al. similarly suggest that the effects of endorphins, the encouragement of the patient, positive feedbacks, and social interactions contributed to the post-transplant process; an active lifestyle, improved psychological well-being, and improvement of physical functioning increase the health-related quality of life (43).

There is a need for patient-specific structured and well-planned physiotherapy interventions to prevent the loss of muscle and bone, to cope with cardiovascular complications and excessive physical fatigue before and after liver transplantation. Specific exercise programs applied to this basis increase muscle strength and endurance, improve aerobic capacity, maximize physical activity level and optimize the health-related quality of life. Secondary beneficial effects of regular exercise in liver recipients are on sleep disorders, depression, and anxiety (15).

Recent studies reported that combined strength training programs and active early mobilization contributes to the postoperative functional performance by increasing activity participation, but it is seen that the number of studies indicating the effect of strength and endurance exercise training in the early period is inadequate (44,45).

A study which investigated physical capacities of liver transplant recipients by the six-minute walk test emphasizes the need for the aerobic exercise to improve physical performance (46). The moderate and high intensity concurrent supervised exercise training program which is administered at the postoperative 6^{th} months has positive effects on VO_{2max}, maximal strength, body composition and health-related quality of life in liver transplant recipients (47).

Rehabilitation programs, however, may be effective in reducing fatigue after liver transplantation; taking into account the factors related to fatigue is crucial while setting appropriate programs. Physiotherapy programs aimed at improving exercise capacity can help to reduce post-transplant fatigue and consequently increase the health-related quality of life (14). Garcia et al. noticed that aerobic exercises which include 30 minutes of continuous Treadmill workout and 24 sessions in total, make an increase by 19.4% in walking distances of the post-liver transplant cases (48).

Van den Berg-Emons et al. (11) suggest that fatigue complaints experienced by liver transplant recipients are physical primitively, not psychologically. Van den Berg-Emons et al. also suggested in another study (44) that the severe fatigue sensation of liver transplant recipients was associated with a lower level of daily physical activity. Researchers wanted to pay attention to the negative cycle due to a hypoactive lifestyle and reported that the increase in fatigue perception may lead to more hypoactivity as well as hypoactivity may lead to a decrease in exercise capacity.

CONCLUSION

In conclusion; fatigue affects pre – and postoperative functional levels and survival of liver transplantation cases negatively. It is proved in the literature that physiotherapy and rehabilitation approaches including strength and endurance exercise training programs contribute to the functional level of the present patient population; further research is needed (37,44,45).

Aerobic and resistive exercise training programs aimed at improving post-transplant overall health and survival should be essential in post-liver transplantation patients, helping to ensure that physical activity becomes a routine in the treatment plan (47, 49).

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