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Evaluation of the Effect of Hedonic Hunger on Nutrition Change Processes and Its Relationship with BMI: A Study on University Students

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ABSTRACT

Objective: The aim of this study is to evaluate the effect of hedonic hunger on nutritional change processes and its relationship with BMI in university students.

Methods: A questionnaire consisting of sociodemographic characteristics, questions about eating habits, Power of Food Scale (PFS) and Nutrition Change Processes Scale (NPCS) were applied to 1003 undergraduate students.

Results: Majority of the students were female and normal weight in terms of BMI. The median PFS and score of the obese students is higher than the normal ones. The median NPCS scores of obese students are higher than other BMI classifications (p< .01). The median scores of food available, food present and food taste sub-factors of PFS are statistically higher in obese students than in normal-weight students (p< .01). The sub-factors of NPCS that consciousness raising, dramatic relief, self-reevaluation, social liberation, contingency management, self-liberation, stimulus control median scores are statistically higher in obese students than in normal-weight students. As hedonic hunger increases, the nutritional change process increases by 13.7%. The increase in hedonic hunger affects the nutritional change processes positively by 46.1% (p< .001).

Conclusion: Hedonic hunger and nutrition change processes of obese students are higher than those of normal weight, and as hedonic hunger increases, the process of nutritional change increases, and the increase in hedonic hunger positively affects nutritional change processes.

Keywords: Hedonic hunger, nutrition assessment, body mass index, obesity

1. INTRODUCTION

Hunger is a biologically beneficial emotion, a metabolic impulse that reminds the individual of the need to seek and eat food. Considering the increasing prevalence of obesity in the world, it is seen that food consumption is based on pleasure as well as energy needs (1). This indicates two types of hunger, homeostatic and hedonic. Homeostatic hunger results from an energy deficit and develops independently of the flavor of the food and after at least 8 hours of nutrient deprivation. Hedonic hunger, on the other hand, is defined as an appetizing urge to consume delicious foods for pleasure, as opposed to physiological energy needs (2). In addition to individual differences such as age, gender, menstrual cycle, nutritional habits, and sensitivity to food cues, factors such as excessive food cravings, impulsivity, self-perception, and experiences affect hedonic hunger. The sensory properties of food, such as taste, color, aroma, texture, and even sound, and experience with these senses have a strong influence on the control of food intake. In addition, it can act as a tool to support excessive food consumption (3).

Frequent and excessive consumption of delicious foods such as fatty and sugary foods, as a result of hedonic hunger; causes an increase in the risk of many diseases such as eating disorders and obesity, hypertension, diabetes mellitus, cardiovascular diseases, non-alcoholic fatty liver disease, obstructive sleep apnea, and some types of cancer (4).

Hedonic hunger is difficult to distinguish from non-hedonic hunger and should be evaluated as soon as it occurs (usually within 2-3 hours of food intake). This time frame helps to understand hedonic hunger rather than homeostatic (2). In a study examining the relationship between hedonic hunger and eating attitude, it has been shown that a high-fat and sugary diet not only disrupts the homeostatic control of feeding behavior and body weight but also causes dysregulation of the brain hedonic system (5).

During the university years, the time spent away from home increases, different emotional states are triggered, and socio-economic conditions vary in which new eating behaviors are acquired that will also affect adulthood (6). Studies examining

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the nutritional attitudes of university students found that increased consumption of processed foods high in sugar, energy, and fat and a decrease in fruit-vegetable and whole-grain consumption in this population (7,8). This change in nutritional patterns may result in the triggering of hedonic appetite and hedonic hunger mechanisms. For this reason, it is necessary to acquire nutritional awareness and proper nutrition patterns for the protection and development of physiological and psychological health. In this context, it is important to examine the relationship between nutritional change processes and hedonic hunger among university students. This study aims to evaluate hedonic hunger in university students and to examine its effect on nutritional change processes.

2. METHODS

The population of this cross-sectional and descriptive study consists of 5064 undergraduate university students enrolled in Üsküdar University in the 2021-2022 academic year. The sample of the study was obtained by choosing a simple random sample. The sample size was calculated using the formula with a certain universe, and it was calculated that 400 university students would be sufficient for the sample size calculation made with a sampling error of 0.05 and a 95% confidence interval for the study to be carried out. The study started after the approval numbered 61351342/ January 2022-41 from the Non-Interventional Research Ethics Committee of Üsküdar University and was completed with 1003 students who participated voluntarily.

2.1. Body Mass Index

Body mass index (BMI) is frequently used in the evaluation of obesity in adults and obtained by dividing body weight (kg) by the square of height (m^2). According to the World Health Organization, a BMI value below 18.5 kg/m^2 is defined as underweight; being between 18.5-24.99 kg/m^2 is normal weight; between 25.0-29.9 kg/m^2 is defined as overweight and over 30 kg/m^2 as obesity (9).

2.2. The Power of Food Scale

The Power of Food Scale (PFS) is a scale that evaluates the psychological effects of living in an environment where delicious foods are abundant. It has been stated as an effective tool for measuring hedonic hunger (10). Ülker et al. have made the validity and reliability of the scale into Turkish in adults (11). It is a five-point Likert-type scale ranging from 1 (strongly disagree) to 5 (strongly agree) and has theree sub-factors (food available, food present, and food tasted) that measure responses to food proximity. Evaluation is made out of 5 points, and an average score of more than 2.5 indicates the presence of hedonic hunger and being affected by food (10).

2.3. Nutrition Processes of Change Scale

It has been developed by Prochaska et al. to determine how experiences affect people's eating habits. Validity and reliability studies in Turkish were conducted by Menekli and Fadıloğlu. The scale consists of 48 items and 12 sub-dimensions, and each item of the scale is evaluated with a grading score ranging from 1 (never) to 5 (very often). The subscales of this scale are consciousness raising, dramatic relief, environmental revaluation, self-reevaluation, social liberation, counterconditioning, helping relationships, contingency management, self-liberation, stimulus control, peer-to-peer system control, drug use. The highest score that can be obtained from the scale is 240, and the lowest score is 48 (12,13).

2.4. Statistical Analysis

Descriptive statistics for categorical variables are presented as frequency and percentage. The conformity of the numerical variables to the normal distribution was checked with the Shapiro-Wilk Test. The "Mann-Whitney U" Test was used for the comparison of two independent groups that did not have normal distribution, and the "Kruskal-Wallis H Test" was used for the comparison of more than two groups. Examining the relationships between the scales was determined by "Spearman's Rank Differences Correlation Coefficient". "Regression Analysis" was used to test the effect between variables. Regression analysis is the explanation of the relationship between two related variables, a dependent variable, and an independent variable, with mathematical equivalence (14). In all calculations and interpretations, the statistical significance level was considered as "$p< .05$" and hypotheses were established as bidirectional. Statistical analysis of the data was performed on the SPSS v26 (IBM Inc., Chicago, IL, USA) statistical package program.

3. RESULTS

Descriptive statistics of a total of 1003 university students participating in the study are presented in Table 1. Average age of students is 21.31±2.70 years. In terms of BMI classification, 75.5% of the students were normal weight, 19.1% were overweight and 5.4% were obese. 84.9% of female students and 53.5% of male students are of normal weight in terms of BMI. 67.5% of the students are studying in health related departments and 32.5% of them are studying in other fields. 35.4% of the students consume 1.5-≤2 L/d of water. 82.2% of the students skip meals and the most frequently skipped meal is lunch with 36.0%. 89.2% of the students do not use nutritional supplements.

Table 1. *Descriptive statistics of demographic and nutritional findings of university students*

	Female		Male		Total
Age	21.18±2.85		21.60±2.29		21.31±2.70
BMI	**n**	**%**	**n**	**%**	**n %**
Normal	596	84.9	161	53.5	757 75.5
Overweight	70	10.0	122	40.5	192 19.1
Obese	36	5,1	18	6.0	54 5.4
Department					
Health Science	474	67.5	82	27.2	556 55.4
Others	228	32.5	219	72.8	447 44.6
Daily Water Consumption (L/d)					
<1 L/d	121	17.2	11	3.7	132 13.2
1-≤1.5 L/d	198	28.2	54	17.9	252 25.1
1,5-≤2 L/d	230	32.8	125	41.5	355 35.4
2-≤2.5 L/d	103	14.7	70	23.3	173 17.2
2.5-≤3 L/d	50	7.2	41	13.6	80 8.0
>3 L/d	4	0.6	7	2.3	11 1.1
Skipping Meal					
Yes	587	83.6	237	78.7	824 82.2
No	115	16.4	64	21.3	179 17.8
Meal Skipped					
Breakfast	130	22.2	65	27.7	195 23.8
Lunch	241	41.2	54	23.0	295 36.0
Dinner	21	3.6	2	0.9	23 2.8
Mid-morning	138	23.6	95	40.4	233 28.4
Afternoon	39	6.7	16	6.8	55 6.7
Night	16	2.7	3	1.3	19 2.3

In PFS scores, the median score of female students [42 (15-75)] is higher than male students [38 (15-75)] (U=95276.5; p< .05), in terms of BMI classification, obese students (55[b] (20) – 75) mean score was higher than those with normal BMI classification (H=20.736; p< .001), the median higher than those who drank more than 3 L/day (46 (16-59) were 1.5-2 lt and 2.5-3 L and 2-2.5, respectively, It was found that the median score of the students who skip meals [42 (15-75)] is statistically higher than the students who do not skip meals [37 (15-75)] (U= 66362.5; p< .05), female students [91 (48-238)] mean score in NPCS scores is higher than male students [84 (48-225)] (U=90873; p< .001), BMI classification In terms of other BMI classification, the median score of obese students was 136.5[b] (63-204), higher (H=11.191; p< .01), the median score of those who drank more than 3lt./day (109 (52-138)) was higher than those who drank less water (H=31593; p< .001), the students who did not skip meals [98 (48 – 238)] score medians were higher (U=61031.5; p< .001) than students who did not skip meals [88 (48-225)], and the median score of those who skipped the night meal (101 (48-222) was statistically higher than those who skipped the morning meal was found to be high (H=25.273; p< .001) (Table 2).

In the comparison of PFS scores of university students according to their gender, the median score of female students [17 (6-30)] in the "Available Food" sub-factor score of the scale compared to male students [15 (6-30)] (U=92613.5; p< 0.01), the median score of female students [15 (5-25)] in the "Taste of Food" sub-factor score compared to male students [14 (5-25)] (U=97401.5; p< .05) and " The median score of female students [42 (15-75)] was found to be statistically higher than that of male students [38 (15-75)] in the "IDI Total" score (U=95276.5; p< .05). No significant difference was found in all other sub-factors (p> .05). Comparing the NPCS scores of university students by gender, the median score of female students [7.5 (4-20)] in the "Increase in Consciousness Level" sub-factor score of the scale compared to male students [6 (4-17)] (U=87235.5; p< .001), the median score of female students [9 (4-20)] in the "Dramatic Help Emotional Stimulation" sub-factor score compared to male students [8 (4-20)] (U=87345; p< .001), The median score of female students [8 (4-19)] in the sub-factor score of "Re-evaluation" compared to male students [7 (4-20)] (U=87723; p< .001), in the sub-factor score of "Self-Reassessment" the female students The median score of students [8 (4-20)] compared to male students [7 (4-20)] (U=96493.5; p< .05), "Contrasted Situation" sub-factor score of female students [8 (4-20)] mean score, compared to male students [7 (4-20)] (U=88688.5; p< .001), in the "Helpful Relationships" sub-factor score of female students [8 (4-20)] the median score compared to male students [7 (4-20)] (U=78138.5; p< .001), In the "Self-Liberation" sub-factor score, the median score of female students [8 (4-20)] compared to male students [7 (4-20)] (U=83403.5; The median score of female students [91 (48-238)] was found to be statistically higher than male students [84 (48-225)] in p< .001) and "NPCS Total" scores (U=90873; p< .001). No significant difference (p> .05) was found in all other sub-factors (Table 3).

In comparing PFS scores of university students according to BMI groups, the median score of obese students [22.5 (8-30)] in the "Available Food" sub-factor score of the scale, compared to normal weight students [16 (6-30)] (H=43.203; p< .001), and the "Food Present" sub-factor score of obese students [14 (5-20)] the median score of obese students [18 (7-25)] in the "Food Taste" sub-factor score (H=50<796; p< .001) and according to normal weight students [9 (4-20)], statistically higher than normal weight students [14 (5-25)] (H=47.530; p< .001).

In comparing NPCS scores by BMI groups of university students, the "increase in consciousness" sub-factor score of the scale compared to obese students [12.5 (4-19)] score median, normal weight students [7 (4-20)] and fat students [7 (4-19)] (H=52.603; p< .001), the median score of obese students in the "Dramatic Relief " sub-factor score [13 (4-18)] compared to normal weight students [8 (4-20)] (H=67.630; p< .001), The median score of obese students [11 (4-19)] in the "Environmental Reevaluation" sub-factor score, according to fat students [7 (4-20)] (H=32.785; p< .001), and the "Self – Reevaluation" sub-factor score of obese students [13 (7-20)] according to normal weight students [7 (4-20)] (H=121.782; p< .001), the median score of obese students [14.5 (4-20)] in the " Social Liberation " sub-factor score, according to normal weight students [10 (4-20)] (H=119.430; p< .001), " Counterconditioning " sub-factor score of obese students [11.5 (4-19)] according to the median score, normal weight

students [7 (4-20)] and fat students [7 (4-20)] (H=57.627; p< .001), the median score of obese students in the " Helping Relationships " sub-factor score [12 (4-20)] compared to normal weight students [8 (4-20)] and fat students [8 (4-20)] (H=45.904; p< .001), The median score of obese students in the " Contingency Management " sub-factor score [11 (4-19)] compared to normal weight students [5 (4-20)] (H=58.686; p< .001), and the "Self-Liberation" sub-factor score of obese students [13 (7-20)] according to normal weight students [7 (4-20)] (H=95.474; p< .001), "Stimulus Control" is the median score of obese students [10 (4-17)] in the sub-factor score, according to normal weight students [5 (4-20)] and fat students [5 (4-20)] (H=48.726; p< .001), the " Peer-to-Peer System Control " sub-factor score of obese students [10 (4-19)] score median, the median score of obese students [6 (4-20)] and fat students [6 (4-20)] (H=48.533; p< .001) and obese students [7.5 (4-19)] in the "Drug Use" sub-factor score, it was found to be statistically higher than normal weight students [4 (4-20)]. (H=39.925; p< .001) (Table 4).

It was found that there was a very weak correlation between the PFS scores of the students and the NPCS scores (p= .137; p< .001) that was statistically significantly positive, and that there was a 13.7% increase in NPCS scores as the PFS scores of the students increased (Table 5).

Table 2. *Comparison of demographic and nutritional findings of university students, Power of Food Scale and Nutrition Processes of Change Scale scores*

	Power of Food Scale (PFS)			Nutrition Processes of Change Scale (NPCS)		
Gender	**Median (min-max)**	**U – *H***	**p**	**Median (min-max)**	**U – *H***	**p**
Female	42 (15-75)	**95276.5**	**0.014***	91 (48-238)	**90873**	**<0.001***
Male	38 (15-75)			84 (48-225)		
BMI						
Normal	40^{a} (15-75)			87^{a} (48-238)		
Overweight	47^{ab} (15-75)	**20.736**	**< 0.001***	94^{ab} (48-225)	**11.191**	**0.001***
Obese	55^{b} (20-75)			$136,5^{b}$ (63-204)		
Department						
Health Science	40 (15-75)	115561	0.056	89,5 (48-222)	124109	0.973
Others	43 (15-75)			89 (48-238)		
Daily Water Consumption (lt/d)						
<1 L/d	45^{bc} (15-75)			94^{b} (48-222)		
1-≤1.5 L/d	45^{bc} (15-75)			$80,5^{a}$ (48-170)		
1.5-≤2 L/d	42^{b} (15-75)	**40.291**	**< 0.001***	87^{ab} (48-238)	**31.593**	**<0.001***
2-≤2.5 L/d	34^{a} (15-75)			98^{bc} (48-225)		
2,5-≤3 L/d	37^{ab} (15-71)			98^{bc} (48-202)		
>3 L/d	46^{c} (16-59)			109^{c} (52-138)		
Skipping Meal						
Yes	42 (15-75)	**66362.5**	**0.035***	88 (48-225)	**61031.5**	**<0.001***
No	37 (15-75)			98 (48-238)		
Meal Skipped						
Breakfast	42 (15-74)			89^{ab} (50-207)		
Lunch	41 (15-72)			92^{ab} (48-225)		
Dinner	41 (22-71)	10.812	0.055	96^{ab} (58-202)	**25.273**	**<0.001***
Mid-morning	44 (15-75)			78^{a} (48-191)		
Afternoon	36 (15-74)			92^{ab} (50-191)		
Night	37 (15-72)			101^{b} (48-222)		

*U: Mann-Whitney U Test; H: Kruskal-Wallis H Test *p< 0.05;*

**a<ab<b; The difference between medians that do not have a common letter is significant (p< 0.05)*

Table 3. *Comparison of Power of Food Scale and Nutrition Processes of Change Scale Sub-Factor Scores of University Students by Gender*

PFS	Gender	Median (min-max)	U	p
Food available	Female Male	17 (6-30) 15 (6-30)	**92613.5**	**0.002***
Food present	Female Male	10 (4-20) 9 (4-20)	101712.5	0.347
Food tasted	Female Male	15 (5-25) 14 (5-25)	**97401.5**	**0.049***
NPCS				
Consciousness Raising	Female Male	7,5 (4-20) 6 (4-17)	**87235.5**	**< 0.001***
Dramatic Relief	Female Male	9 (4-20) 8 (4-20)	**87345**	**< 0.001***
Environmental Reevaluation	Female Male	8 (4-19) 7 (4-20)	**87723**	**< 0.001***
Self-reevaluation	Female Male	8 (4-20) 7 (4-20)	**96493.5**	**0.028***
Social Liberation	Female Male	10 (4-20) 11 (4-20)	99538	0.145
Counterconditioning	Female Male	8 (4-20) 7 (4-20)	**88688.5**	**< 0.001***
Helping Relationships	Female Male	8 (4-20) 7 (4-20)	**78138.5**	**< 0.001***
Contingency Management	Female Male	6 (4-20) 6 (4-20)	100996	0.254
Self-liberation	Female Male	8 (4-20) 7 (4-20)	**83403.5**	**< 0.001***
Stimulus Control	Female Male	5 (4-20) 5 (4-20)	104123	0.705
Peer-to-Peer System Control	Female Male	6 (4-20) 6 (4-20)	98058	0.066
Drug Use	Female Male	4 (4-20) 4 (4-17)	99147	0.092

*U: Mann-Whitney U Test PFS: Power of Food Scale, NPCS: Nutrition Processes of Change Scale *p< 0.05*
**a<ab<b; The difference between medians that do not have a common letter is significant (p< 0.05)*

Table 4. *Comparison of Power of Food Scale and Nutrition Processes of Change Scale Sub-Factor Scores of University Students by BMI*

PFS	BMI	Median (min-max)	H	p
Food available	Normal	16[a] (6-30)	**43.203**	**< 0.001***
	Overweight	18[ab] (6-30)		
	Obese	22,5[b] (8-30)		
Food present	Normal	9[a] (4-20)	**50.796**	**< 0.001***
	Overweight	12[ab] (4-20)		
	Obese	14[b] (5-20)		
Food tasted	Normal	14[a] (5-25)	**47.530**	**< 0.001***
	Overweight	17[ab] (5-25)		
	Obese	18[b] (7-25)		
NPCS				
Consciousness Raising	Normal	7[a] (4-20)	**52.603**	**< 0.001***
	Overweight	7[a] (4-19)		
	Obese	12,5[b] (4-19)		
Dramatic Relief	Normal	8[a] (4-20)	**67.630**	**< 0.001***
	Overweight	9[ab] (4-20)		
	Obese	13[b] (4-18)		
Environmental Revaluation	Normal	8[ab] (4-19)	**32.785**	**< 0.001***
	Overweight	7[a] (4-20)		
	Obese	11[b] (4-19)		
Self-reevaluation	Normal	7[a] (4-20)	**121.782**	**< 0.001***
	Overweight	8[ab] (4-20)		
	Obese	13[b] (7-20)		
Social Liberation	Normal	10[a] (4-20)	**119.430**	**< 0.001***
	Overweight	12[ab] (4-20)		
	Obese	14,5[b] (4-20)		
Counterconditioning	Normal	7[a] (4-20)	**57.627**	**< 0.001***
	Overweight	7[a] (4-20)		
	Obese	11,5[b] (4-19)		
Helping Relationships	Normal	8[a] (4-20)	**45.904**	**< 0.001***
	Overweight	8[a] (4-20)		
	Obese	12[b] (4-20)		
Contingency Management	Normal	5[a] (4-20)	**58.686**	**< 0.001***
	Overweight	7[ab] (4-20)		
	Obese	11[b] (4-19)		
Self-liberation	Normal	7[a] (4-20)	**95.474**	**< 0.001***
	Overweight	9[ab] (4-20)		
	Obese	13[b] (7-20)		
Stimulus Control	Normal	5[a] (4-20)	**48.726**	**< 0.001***
	Overweight	5[a] (4-20)		
	Obese	10[b] (4-17)		
Peer-to-Peer System Control	Normal	6[a] (4-20)	**48.533**	**< 0.001***
	Overweight	6[a] (4-20)		
	Obese	10[b] (4-19)		
Drug Use	Normal	4[a] (4-20)	**39.925**	**< 0.001***
	Overweight	4,5[ab] (4-17)		
	Obese	7,5[b] (4-19)		

*PFS: Power of Food Scale, NPCS: Nutrition Processes of Change Scale H: Kruskal-Wallis H Test; *p< 0.05*

**a<ab<b; The difference between medians that do not have a common letter is significant (p< 0.05)*

Table 5. *Correlation coefficients between PFS and NPCS*

		Total PFS	Total NPCS
Total PFS	**s**	1.000	0.137
	p	.	<0.001*
Total NPCS	**s**	0.137	1.000
	p	< 0.001*	.

*PFS: Power of Food Scale, NPCS: Nutrition Processes of Change Scale s: Spearman's Rank Correlation *p< 0.05*

It was found that the PFS scores of the students had a significant effect on their NPCS scores. PFS scores (β=0.461; t=5.916; p< .001); It accounts for 3.4% of who's scores (R2=,3.4; F=35.002; p< .001) (Table 6).

Table 6. *The effect of PFS scores on NPCS scores*

	Model	β	Std. Error	t	p	F	p
NPCS	(Constant)	75.807	3.407	22.252	< 0.001*	**35.002**	**< 0.001***
	PFS	0.461	0.078	5.916	< 0.001*		
	R=0.184; R^2=%3.4; Corrected R^2=%3.4						

*PFS: Power of Food Scale, NPCS: Nutrition Processes of Change Scale, Std. Error: Standart Error, t: test statistic, F: test value *p<0.05*

4. DISCUSSION

University years are a critical life stage in which adult dietary habits are acquired and pave the way for the risk of non-communicable diseases (15). In this period, besides the physiological factors affecting food choices and nutritional habits, the evaluation of hedonic hunger has a significant impact on understanding the nutritional change processes (16).

With the NPCS sub-dimensions, individuals' seeking new information about nutrition and increasing awareness, activating feelings about unhealthy life results, and how they are affected by the social environment about these behaviors are measured. Our results was determined that the median NPCS scores of obese students were significantly higher than those of normal weight. There is no study in the literature examining the relationship between NPCS and BMI. In a cross-sectional study, it was determined that there is an inverse relationship between obesity and nutritional attitude and nutritional knowledge. In addition, individuals with poor self-regulation of eating were more likely to be obese (17). Laz et al. found that obese individuals are more likely to engage in nutritional knowledge and related healthy weight loss behavior (18). This situation can be explained by the fact that obese individuals have higher nutritional change processes in parallel with the wrong nutrition practices they apply in the processes of losing and gaining weight.

In this study, increase in consciousness level, self-reappraisal, and opposite-opposite state scores, which are among the sub-factors of NPCS, were found to be higher in obese patients. The higher NPCS sub-dimension scores in obese patients may be due to the increased search for new information about nutrition, re-evaluation of unhealthy eating behaviors and their desire to increase the tendency to healthy behaviors instead. The available data in the literature show that obese people are more exposed to environments that will create obesity risk factors compared to normal-weight individuals (19,20). Our results, which supports the literature, NPCS scores, which also measure the effect of the social environment on the nutritional behavior process, are higher in obese patients.

We found that although the majority of the students were of normal weight, the BMI values of male students were higher than females. In addition, the median PFS score of obese students was found to be significantly higher than those of normal weight. Similarly, in a cross-sectional study conducted in adults, a positive and significant correlation was found between BMI and PFS scores (21). In a recent study conducted in adults, a significant positive correlation was found between BMI and PFS total scores (22). In contrast to these, Burger et al. In the study, no relationship was found between BMI and PFS (23). We found that the medians of all sub-dimensions of PFS were significantly higher in obese individuals than in normal weights. In their study, Ribeiro et al. showed that each unit increase in PFS score doubles the probability of being obese. In addition, nutrient power scale total score and sub-factors of food availability and nutrient availability sub-factor scores were found to be higher in obese than in normal-weight individuals (24). Andreeva et al. Similar to our study, a significant relationship was found between BMI and all sub-dimension scores such as food availability, food availability and food taste, and it was observed that the scores were higher in obese patients (25). In a study conducted in adults, it was found that the food availability and food availability sub-factor scores were significantly higher in obese compared to normal-weight individuals, while there was no significant difference in food taste subscale scores (26). Although there are inconsistencies as a result of studies examining the relationship between PFS and BMI, PFS reflects cognitive preoccupation and motivation to consume delicious foods, namely hedonic hunger. In this context, the significant increase in BMI and PFS scores in this study can be explained by the fact that the consumption of these foods due to hedonic hunger is probably a part of the weight gain process.

The PFS total score, food availability and food taste subscale scores were found to be significantly higher in female students than in males. The other study have reported that women experience higher levels of hedonic hunger and reward eating than men. In the study of Aliasghari et al., hedonic hunger levels were higher in women even when BMI and physical activity factors were kept constant (21). In a systematic review, it was reported that women may be more reactive to visual food stimuli, especially when they are hungry (27). One study found that, compared with men, women showed significantly greater activation to high-calorie foods in cortical regions related to behavioral control and self-referential cognition (28). As a result, this shows that female students have higher levels of hedonic hunger and food exposure. The reason why hedonic hunger is higher in women can be explained by the neural activity response to food stimuli in areas related to brain reward.

In this study, it was found that women were more likely to seek new information about nutrition, to take action to change unhealthy habits, to learn healthy behavior, to believe in changing their habits, and to receive support. Worldwide, overweight and obesity are more common in women (9). This may result in women focusing more on thoughts about their bodies, their desire to learn about healthy nutrition, and their preference for diet practices (29). The increase in nutritional knowledge can promote healthy weight loss behaviors (18). The fact that women are more adaptable to nutritional change processes can be explained by the fact that they can facilitate weight control processes.

It was found that although most of the students skipped meals, they skipped lunch most frequently. Similarly, another study conducted with university students show that the most frequently skipped meal is lunch (30). On the contrary, in a recent study conducted with university students, it was observed that the breakfast meal was skipped most frequently and this was due to reasons such as not feeling hungry, stress, and not having time to eat (31).

According to this study, lunch, mid-morning and breakfast meals were found to be the most frequently skipped meals, respectively. However, when the PFS results are evaluated, it can be said that hedonic hunger indicators are more prominent among students who skip meals. In a recent study conducted by examining the data of the American National Health and Nutrition Evaluation Study, it was found that skipping meals led to more energy intake in the next meals; It has been stated that the quality of the food consumed will decrease and health may be adversely affected over time (32). In another study conducted with high school students in parallel, it was found that adolescents with a higher hedonic hunger index reported more unhealthy food and beverage intake during their 4 years in high school, and that as a result of hedonic hunger, the liking for unhealthy foods and beverages increased over time and it was possible to control the consumption of these foods. It has been stated that it may be possible to reduce the ability of the patient (33). According to this; as well as physiological hunger caused by skipping meals; Sensitization to sensory environmental stimuli such as appearance, taste, smell and sound may be associated with triggering hedonic hunger and may result in malnutrition behaviors.

In this study, while PFS scores were found to significantly affect NPCS scores; A one-unit increase in students' PFS scores was associated with a 13.7% increase in NPCS scores. The frequent preference of delicious foods due to hedonic hunger and the fact that these foods are usually high in energy, salty, sugary and fatty may cause the development or progression of diseases such as obesity, hypertension and diabetes (34). In a study, it was found that the number of weight loss diets is higher in individuals with hedonic hunger (35). In our study, although statistically weak correlation was observed, it was observed that as hedonic hunger levels increased, compliance in the food exchange process increased. In this context, it is a possible outcome that individuals who have increased anxiety about obesity-related complications after hedonic fasting take steps to develop healthy eating behaviors.

5. CONCLUSION

In addition to nutritious environments, hedonic hunger triggers (such as delicious foods, feelings and thoughts related to appetite) affect nutritional change processes, and in this way, it is important both for individual to cope with hedonic hunger and to improve social dietary patterns in determining where the nutritional change processes of individuals at high risk of non-communicable diseases, especially obesity, are located. In this context, extensive studies are needed targeting larger populations in different groups of society or involving individuals at risk of different non-communicable diseases.

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Clinical and Experimental Health Sciences

The Relationship Between Somatization and Depression Types: Comparison of Unipolar Depression and Bipolar Depression

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ABSTRACT

Objective: Somatic symptoms are more likely to be present in depression and anxiety, which causes to waste medical resources due to excessive hospital addmissions. It has been observed that the unclarity of qualitative and quantitative characteristics of somatization depending on the type of depression influences clinical practice less than expected. In the present study, it was aimed to determine the hallmarks of somatic symptoms in depression groups and to investigate the factors that might have an effect on somatic symptoms.

Method: One hundred consecutive patients (50 with Bipolar Depression (BD), 50 with Unipolar Depression (UD)) who met the criteria participated in the study. Patients were assessed for depressive symptoms with Montgomery Asberg Depression Scale and for somatic symptoms with Bradford Somatic Symptom Inventory. Clinical features were obtained by the clinician via Sociodemographic Data Form.

Results: It was found that no significant difference in somatization characteristics between the depression groups. (p> .05). Somatic symptom severity was higher in the UD group in the presence of psychiatric comorbidity (p= .013), but not in BD. Another prominent finding was that the severity of depression was noted the only predictor of severe somatization.

Conclusion: The results show that increased somatic symptoms are associated with the severity of depression, suggesting treatment of depression with somatization rather than differential diagnosis should be primary concern.

Keywords: Unipolar depression, bipolar depression, somatic symptom

1. INTRODUCTION

Depressive episodes are common requirements as a main criteria to diagnose for both major depressive disorder and bipolar disorder (1). Although hypomanic episodes are mainly clear to be distunguished, subthreshold elevation symptoms that may be difficult to be remembered by patients, which results in misdiagnosis (2) or delaying diagnosis for bipolar disorder. There is some evidence that differantial diagnosis of bipolar disorder (BD) from unipolar depression (UD) is difficult in the early stages of the disease. It has been reported that 40% of patients diagnosed with BD have been previously diagnosed with unipolar disorder (3,4). Delayed initiation of treatment at illness onset in BD is more likely to result in, social, cognitive and functional limitation than UD (5).

Major depression has been suggested by a number of studies to be accompanied by medically unexplained somatic symptoms over the years (6,7). Comorbidity studies of depression and somatization indicated that; the more somatic symptoms, the more the likelihood that a patient suffer from depression (8,9). Indeed, patients can make their first medical application to their primary care phsycian for only somatic complaints (7). Moreover, a close relationship between depression accompanied by somatic symptoms and poor clinical outcome due to residuel somatic symptoms has been established in some studies (10,11). As a result of these findings, authors have tended to focus on medically unexplained symptoms as a primary concern for the treatment of UD.

In a meta-analysis, it was suggested that the vast majority of bipolar spectrum disorder (BSD) patients present with medically unexplained physical symptoms, which are reported to be significantly more common than in the general population and other psychiatric conditions. (12). Data from another research reported that somatic symptoms occur more frequently in recurrent major depression and BD than in depression not other specified. (13). Although somatic symptoms have been reported more common in UD than BD (13,14), a number of earlier studies suggest that patients

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with BD have more somatic symptoms compared to unipolar depressive patients (15,16).

Clinicians are more likely to evaluate somatic symptoms in the case management of UD, which can be neglected in the challenging prognosis of BD. Moreover, we have noticed that a group of patients with bipolar disorder referred to their somatic complaints as an early sign of their depressive episode in our clinical practice. Although the differences between UD and BD in terms of somatic symptoms have been shown in some studies, researches examining medically unexplained somatic symptom with a comprehensive scale are still scarce in Turkish population. The present study thus aims to elaborate overall screening of somatic symptoms in the UD and BD groups, to investigate the diagnostic role of the symptoms, and the predictors of somatization. We hypothesized that 1 – patients with BD have at least as many somatic symptoms as those with UD, 2 – somatic symptoms are associated with depression severity for both depression types, 3 – depression type is a predictor of somatic symptom severity beyond depression severity.

2. METHODS

2.1. Participants

Two groups in which fifty patients with BD and fifty patients with UD ranging in age from 18 to 65 years, both of whose the duration last depressive episodes are at least four weeks, were included. Subjects who were referred to Marmara University Pendik Training and Research Hospital Psychiatry Outpatient Clinic were considered for recruitment in the study. Psychiatric diagnoses, which include primary diagnosis and psychiatric comorbidities, were made according to the Structured Clinical Interview for the Diagnostic and Statistical Manual of Mental Disorders (DSM)-IV Axis I Disorders (SCID-I) administrated by the same psychiatrist. Exclusion criteria were: (I) diagnosed with psychotic disorders; (II) psychotic featured depression; (III) cognitive decline suggestive of a clinical mental retardation and demantia; (IV) the patients who are illiterate; (V) a major physical health problem; (VI) recent use of substance and (VII) pregnancy. The first 50 consecutive patients from each group fulfilling the selection criteria took part in the study. The present study was conducted in accordance with the Declaration of Helsinki. The Marmara University Ethics Committee approved the study at 06.01.2017 with protocol code “09.2017.094” and all of the patients gave informed consent.

2.2. Measures

2.2.1. Sociodemographic data form

Sociodemoghraphic and clinical characteristics of the subjects were assessed with a structured data form prepared by the researchers which includes the sociodemografic variables (age, sex, marital status, income etc.), and clinical variables (onset of illnes, duration of last episod, smoking use etc.). The medical comorbidities were recorded to the data form according to the patient’s self-report.

2.2.2. Montgomery Åsberg Depression Scale (MADRS)

The severity of depression was assessed with the Montgomery Åsberg Depression Scale (MADRS). The scale is rated by the clinician and consists of ten items, of which nine are based on psychiatric history and one on clinician observation (17). The adaptation of Turkish version of the scale yielded valid and reliable outcome (18).

2.2.3. Bradford Somatic Inventory (BSI)

BSI was administrated to assess the severity and the quality of somatic symptoms. The BSI scale contains wide range of somatic symptoms in a 44-item questionnaire and self-rated (19). Every symptom is scored up to three points whether the frequency of the items less or more fifteen days over the past month. The validity and reliability of BSI was demonstrated in the Turkish population (20). The cut-off value “forty points” for severe somatization stated in original study was used to transform the value of BSI total score to a binominal variable in the present study.

2.3. Statistical Analysis

Shapiro-Wilk test, absolute skewness/kurtosis values and the histograms were used together to explore the normality of distribution. T test was conducted to analyze the differences between two independent groups. Chi-square test (or Fisher’s exact) was run to examine the relations between nominal variables. Comparisons among more than two independent subgroups were conducted by one-way ANOVA tests (or Kruskal-Wallis H test). Pearson’s and Spearman’s correlation tests were used for correlation analysis takin into account whether the normally distributed or not.

3. RESULTS

Mean age was 37.98±10.14 in the unipolar group and 39.58±10.09 in the bipolar group. The bipolar group consisted of 29 females (58%) and 21 males (42%), and the unipolar group consisted of 40 females (80%) and 10 males (20%). There was significantly difference in terms of gender distrubition between two groups. The majority of participants in both groups were graduated under elemantary school, unemployed and from low-income population. There were no statistically significant differences between the unipolar and BD groups in terms of age, marital status, educational status. All the sociodemographic characteristics and clinical features of the patient groups are presented in Table 1.

Even though depression and somatic symptoms scores were higher in the bipolar group, there were no significantly differences between two groups (respectively p= .101; p= .475). There were also no significantly differences between two groups in terms of BSI factors and the number of somatic symptoms which was calculated regardless of the severity of somatic symptoms. The results of comparison of two groups with regard to depression scores and the level of somatic symptoms are shown in Table 2.

Table 1. *Sociodemographic data and clinical features of participants*

	UD N (%)	BD N (%)	p
Age (mean±SD)	37.98±10.14	39.58±10.09	.431
Gender			.017
Female	40 (80)	29 (58)	
Male	10 (20)	21 (42)	
Marital Status			.766
Married	34 (68)	34 (68)	
Single	9 (18)	11 (22)	
Widow/Divorced	7 (14)	5 (10)	
Education			.833
Primary	19 (38)	15 (30)	
Elemantary	13 (26)	13 (26)	
High School	9 (18)	11 (22)	
University and beyond	9 (118)	11 (22)	
Number of depressive episode (mean±SD)	2.86±2.58	8.2±6.13	.001[a]
Onset age of disease (mean±SD)	25.32±8.0	30.26±10.17	.008[a]
Duration of last episode (week) (mean±SD)	62.66±101.98	17.62±29.52	<.001[a]
Use of additional medicine			.629[c]
(-)	40 (80)	38 (76)	
(+)	10 (20)	12 (24)	
Medical comorbidity			.683[c]
(-)	29 (58)	31 (62)	
(+)	21 (42)	19 (38)	
Psychiatric comorbidty			.107[c]
(-)	35 (70)	28 (56)	
(+)	15 (30)	22 (44)	
Alcohol consumption			.695[b]
(-)	47 (94)	46 (92)	
(+)	3 (6)	4 (8)	
Smoking			.548[c]
(-)	28 (56)	25 (50)	
(+)	22 (44)	25 (50)	

[a]Student t Test [b]Fisher Exact Test [c] Chi-Square Test UD: Unipolar Depression BD: Bipolar Depression SD: Standard Deviation

Dividing all participants into two groups as patients with medical comorbidity and without medical comorbidity, it was not found significantly differences between the groups in unipolar and BD groups (respectively p= .113; p= .928). Another way of making the former result more reliable was that the somatic symptoms between unipolar and bipolar groups was compared among participants who did not have a medical illness. In this condition, there was no difference in terms of BSI scores as well (p> .05; not demonstrated). As for psychiatric comorbidity, the severity of somatic symptoms was found significantly higher in the participants with psychiatric comorbidity in only unipolar group but not in bipolar group (respectively p= .013; p= .807). There were no significant differences in any groups with regard to depression severity in terms of medical and psychiatric comorbidity (p> .05). The data of all the comparisons was shown in Table 3.

3.1. Correlation Analysis

In bivariete levels, Correlation analysis revealed that there were positive, mild to modarate, statistically significant correlations between BSI total score and MADRS total score in UD (r=.427, p=.002), in BD (r=.476, p<.001) and in all participants (r=.453, p<.001).

Table 2. *Comparison of depression and somatic symptoms scores*

	UD	BD	p[a]
	mean±SD	*mean±SD*	
MADRS	25.1±6.93	27.14±5.28	.101
BSI-44	39.36±18.23	42.06±19.38	.475
BSI-44 (number of symptoms)	26.28±9.46	26.70±11.49	.842
BSI-44 Factors			
Head	3.96±2.35	3.78±2.6	.717
Chest	3.48±2.42	3.36±2.65	.813
Abdomen	6.04±4.1	7.26±4.4	.155
Fatigue	3.72±1.63	4.3±1.5	.067
Heat	2.36±1.24	2.18±1.24	.470
Globus	2.72±2.19	2.82±2.41	.829
Frequency	6.94±4.1	6.86±3.9	.921
Panic	8.38±4.21	9.08±4.51	.424

[a]Student-t Test UD: Unipolar Depression BD: Bipolar Depression SD: Standard Deviation BSI-44: Bradford Somatic Inventory MADRS: Montgomery-Asberg Depressiın Rating Scale

3.2. Regression Analysis

The predictors of severe somatization were examined using multiple logistic regression model. The dependent variable was reconstructed by dividing the BSI score into two categories with a cut-off score of 40, which resulted in binominal variable: severe (n=45) and non-severe (n=55). The independent variables were age, gender, marital status, duration of education, additional medical illness, additional psychiatric illness, duration of last depressive episode, membership of depression group, depression severity (total score of MADRS). The only significant variable was MADRS total score [OR (95% CI): 1.136, (1.050-1.229), p= .001] remained in last step (Nagelkerke R^2 =0.208, p< .001).

Table 3. *Comparison of depression and somatic symptoms according to medical and psychiatric comorbidties*

BSI-44 total score		All Part.	UD	BD	BPD-I	BPD-II
Medical Comorbidity						
(-)	***Mean±SD***	39.55±18.36	36.37±18.22	42.51(18.50)	40.16(19.16)	44.00(19.64)
	median	-	35	43	38	43
(+)	***Mean±SD***	42.45±19.36	43.47±17.83	41.31±21.55	44.72±19.22	36.62±20.94
	median	-	43	43	43	35
	p	**.452**[a]	**.113**[b]	**.928**[b]	**.608**[b]	**.515**[b]
Psychiatric comorbidity						
(-)	***Mean±SD***	38.33±16.93	35.02±15.20	42.46±18.32	40.16±19.16	44.00±19.64
	median	-	33	43	38	43
(+)	***Mean±SD***	44.75±21.16	49.46±21.10	41.54±21.08	44.27±19.22	36.62±20.94
	median	-	43	39	43	35
	p	.121[a]	.013[b]	.807[b]	.563[b]	.373[b]
MADRS total score		**All Part.**	**UD**	**BD**	**BPD-I**	**BPD-II**
Medical Comorbidity						
(-)	***Mean±SD***	26.58±6.17	24.68±7.45	27.58±5.35	26.66±6.28	28.15±4.77
	median	-	24	28	27.5	29
(+)	***Mean±SD***	26.03±5.73	25.66±6.25	26.42±5.22	26.27±5.25	26.62±5.52
	median	-	24	26	26	27.5
	p	.901[a]	.472[b]	.588[b]	.880[b]	.938[b]
Psychiatric comorbidity						
(-)	***Mean±SD***	25.57±6.11	24.34±6.98	27.10±4.46	26.66±6.28	28.15±4.77
	median	-	23	28	27	27
(+)	***Mean±SD***	27.05±6.36	26.86±6.96	27.18±6.28	26.27±5.25	26.62±5.52
	median	-	26	27	27	27
	p	.257[a]	.098[b]	.837[b]	.927[b]	.943[b]

[a]*Student-t Test bMann Whitney U Test UD: Unipolar Depression BD: Bipolar Depression BPD-I: Bipolar Disorder-1 BPD-II: Bipolar Disorder-2 SD: Standard Deviation BSI-44: Bradford Somatic Inventory MADRS: Montgomery-Asberg Depressiın Rating Scale*

4. DISCUSSION

The objective of this study was to compare the medically unexplained somatic symptoms between UD patients and BD patients. We also aimed to explore the variables that might predict somatic complaints in both BD and UD groups.

The number of depressive episodes were significantly higher in the BD group than the UD group in the present study, which is consistent with the findings reported by Forty and the colleagues (21). In another study comparing patients with BPD-II and UD, past major depressive episodes were found to be in favor of the unipolar group (22). In addition, the duration of the last episode was higher in UD group; which is also consistent with the results of a number of studies comparing the longest episodes of depressive episodes (21,23). However, some exclusion criteria in our study such as the presence of psychotic symptoms which might have an effect on episodic duration and chronicity, suggest the possibility of making a difference according to the results we would expect to encounter in the natural course of the diseases.

The present study suggests that somatic symptoms were not associated with group of depression. Several studies have suggested that somatic symptoms were higher in UD rather than BD. Perlis et al. found that the somatic subscale scores of the Hamilton Anxiety Scale, which provides relatively wider range of somatization screening, were significantly lower in bipolar patients (14). Similarly, another study corresponding to our design found that recurrent depression and bipolar depression were associated with fewer somatic symptoms than other types of depression. (13). On the contrary, some earlier reports pointed out that unexplained somatic symptoms are more common in BD than UD (15,16). Similar findings to our results have been presented in a previous investigation. Hantouche and Akiskal showed that there was no difference between unipolar and bipolar-II groups with regard to somatic symptoms in the study in which Hamilton Depression Scale was used for assesing the level of somatic symptoms (24). The contradiction among all these findings appeared to be related to the distinctive impact of the rating scales and sample sizes. To our knowledge, there was no study in which the comparison of somatic symptoms was directly evaluated between the unipolar and bipolar groups using the rating scale that is capable of determining the symptoms in the context of temporality beside quality. A single study that considers the aforementioned marks seemed to be proximate to our design (13) . Also, it might be another reason why all these results were so conflicting that some variables could not be involved in analysis such as subtypes of depression, level of anxiety, alexithymia, hypocondrisis etc. The data of present study, likewise, contains severity of depression but

not the level of anxiety. In the literature, the studies are more likely to show that somatic symptoms associate with UD than BD but not enough to clarify the association between somatic symptoms and type of depression. In our sample, that there was no significant difference between unipolar and bipolar groups in terms of MADRS scores is in favor of interpreting the comparison of somatic symptom, by which effect of depression severity is not considered. Moreover, it is widely known that there is a significant correlation between somatic symptoms and severity of depression (25,26). Notwithstanding, because of that psychiatric comorbidity and anxiety level are not included in the present study, it remains unclear to what extent the effect of depression severity on the level of somatic symptom which is observed equally for both of groups contributes to clarification of comparison.

Another finding of the present study is that somatic symptoms were unrelated to the medical comorbidity in which all participants were divided into two groups with regard to whether medical comorbidity exists or not. Somatic complaints are expected to be more common in the presence of medical comorbidity (27). The reason why this difference was not seen in our study might be exclusion of the patients with decompensated medical illness, which prevents us from observing the effect of natural coexisting of somatic complaints and medical morbidity on the results. In addition, our unexpected results may arise from examining the relationship between somatic symptoms and medical illness without considering the type and severity. On the other hand, aforementioned finding allows us to evaluate properly the somatic symptoms on which the hypothesis focused.

As to psychiatric comorbidity, the severity of somatic symptom was found significantly higher among the participants with psychiatric comorbidity than those not (respectively p= ,013; p= ,807) in only unipolar group but not in bipolar group. It can be assumed that the effect of psychiatric comorbidities on somatic complaints may be varied by type of disorder. In this sense, it is also possible that the psychiatric comorbidities in the unipolar group might be clustered in such a way that it can affect the level of somatic symptoms more than those in bipolar group. Should think over the causes of the assumption, the fact that the number of female participants were higher than that of the bipolar group might be regarded as a confounding factor. Psychiatric comorbidity was reported to be higher in females in previous studies (28). Indeed, while disruptive behavior antisocial behavior and adjustment disorder are reported to be more frequent in males; anxiety and somatization were more frequent as psychiatric comorbidities in women with UD (29). Nevertheless, it is possible that the result found in the unipolar group was not corresponded with that in the BD group due to sample selection, sample distribution and sample size.

We aimed to determine particularly the predictors of somatization severity in the binominal regression model. Depression severity was the unique predictor of severe somatization In a previous study including patients with UD, being unmarried and severity of depression in patients were found to be predictors of somatic symptoms in a linear regression model in which depression severity and sociodemographic were included together (30). However, when anxiety scores were added to model, the severity of depression was shown to be no longer significant. Although our findings are supported by some aspects of these results, it was emphasized that high level of depression hindered well-established relationship with somatic symptoms (30). In a study conducted by Haug and the colleagues, likewise, the associations of somatization with anxiety and depression were not more prominent for any of them, whereas the association was stronger in the presence of comorbid anxiety and depression. (25). When viewed from this aspect, depression might not be noteworthily considered as a strong predictor of somatic symptoms per se in the absence of the anxiety level in our model.

Some studies have reported that female gender is a predictor for somatic symptoms in patients with major depression as well as in the general population (31). Our regression results were not congruent with the prior reports in terms of gender. However, the differences of female/male ratio between the groups makes this finding difficult to compare with the existing evidence. In a study with similar male/female ratios, the predictive effect of being female varies in the regression models in which firstly only sociodemographic data was added and psychologic factors were entered additionally in second step (30). There is a need for further studies with larger sample size using similar methodology in which subtle factors relavant to the patients or the disorders are unveiled.

4.1. Limitations

Some limitations should be considered to make the interpretation of these results more reliable. The small size of sample and the higher ratio of female/male in the UD group were the two most important ones. Another caveat was that the level of anxiety, comorbid psychiatric disorders and psychiatric drugs which are likely to have an effect on somatic symptoms are not included separately in the analysis. The cross-sectional design of our study requires to replicate our findings prospectively. Although patients with decompensated medical illnesses were not included in the present study, classification of medical comorbidity remained limited. Another major limitation is that BPD patients who have not yet been diagnosed were erronously deemed in the UD group, which can be reduced by defining subgroups in participants with UD in further studies.

5. CONCLUSION

Considering the contradictions in the relationship between somatic complaints and depression types, the present findings converged moderately with previous results. Despite its limitations, this study is the first to screen of somatic symptoms at a broad level across the depression

groups with a specific scale. Studies with larger sample sizes and evaluating the confounding factors may help to better understand the relation between affective disorders and somatic symptoms and the utility of somatic symptoms to differentiate between UD and BD. We hope to delineate the importance of somatic symptoms in depression types though we failed to confirm our hypothesis, knowing that the most challenging aspect of BPD is early diagnosis.

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Clinical and Experimental Health Sciences

Experience, Thoughts, and Attitudes of Orthopaedic Surgeons about Chronic Postsurgical Pain after Arthroplasty

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ABSTRACT

Objective: Arthroplasty constitutes a major procedure commonly used in orthopaedic and traumatology surgery, and chronic pain develops in 7%–34% of patients after arthroplasty. This study aims to evaluate the experience, thoughts and attitudes of orthopaedic surgeons about chronic postsurgical pain after arthroplasty.

Methods: The survey was sent to orthopaedic surgeons via the Google survey system. The questionnaire consisted of 17 questions in total; four were related to demographic information, two asked about the surgeons' experience, three asked for their thoughts, and eight queried their attitudes.

Results: The study evaluated 319 participants. All the orthopaedic surgeons reported that they routinely prescribe for postoperative pain control when discharging patients after arthroplasty. Of those surveyed, 69.7% have used multimodal analgesic therapy to treat chronic postsurgical pain after arthroplasty. Consultation rates were 5% and 20% for psychiatry and pain physician, respectively. A weak positive correlation was found between the rate of referring patients with chronic postsurgical pain after arthroplasty to pain physician and the beliefs of orthopaedic surgeons that pain physician can help patients with chronic postsurgical pain after arthroplasty who are unresponsive to pharmacological treatments ($p = 0.008$, $r = 0.148$).

Conclusion: Orthopaedic surgeons employ pre-emptive approaches to chronic postsurgical pain after arthroplasty and engage in pharmacological management of chronic postsurgical pain after arthroplasty, but they need to advance further with multidisciplinary treatments and directions.

Keywords: Chronic postsurgical pain, arthroplasty, orthopaedic surgeon, multidisciplinary pain management, multimodal pain treatment

1. INTRODUCTION

Utilisation rates of arthroplasty surgeries have increased exponentially, in population-based studies it has been reported as 8,200 per 100,000 (1-3). The survival of the joint replacement has been considered as an indicator of the success of arthroplasty, for many years (4). Recently, pain reduction has been to be considered as a parameter in evaluating the success of the operation, and, as a result of this new parameter, success rates have decreased (4, 5).

Chronic postsurgical pain is defined as 'the pain is localised to the surgical or referred area, persisting at least three months after surgery'. Chronic postsurgical pain is characterised as not being present before surgery or that has different characteristics or increased intensity from preoperative pain (6). Chronic postsurgical pain after arthroplasty (CPSP after arthroplasty) is seen in 7% to 23% of patients after hip arthroplasty, 10% to 34% after knee arthroplasty (7) and 16% to 29% after shoulder arthroplasty (8). CPSP after arthroplasty negatively affects a person's general activity level, working status, mood, relationships, sleeping and enjoyment of life (9).

Predictive factors in the development of chronic postsurgical pain and approaches that reduce the risk of development have been identified. Thanks to surgeons' awareness of chronic postsurgical pain, it is now possible to minimize the risk of developing this pain and combat it in case the pain develops (10). The general notion of chronic pain suggests that surgeons should focus on the patients' pain in their clinical practices, trainings, and researches, undertake the pharmacological management of pain regarding dose, side effects, and complications, receive support from pain clinics that offer pharmacological and interventional options in the management of patients, and emphasize multidisciplinary

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pain management (11). On the other hand, it has been reported that CPSP after arthroplasty has been neglected in the medical literature and needs more attention (12). The need to improve awareness of CPSP after arthroplasty is evident (13).

Although it is known that CPSP after arthroplasty affects people's lives negatively and that preventive and therapeutic approaches for this type of pain have been established in general terms, to the best of our knowledge, there is no study in the literature that reveals the clinical approaches of orthopedic surgeons regarding this subject. This study aimed to evaluate the experience, thoughts and attitudes of orthopaedic surgeons about CPSP after arthroplasty.

2. METHODS

2.1. Ethical Considerations

This cross-sectional study was conducted in accordance with the Declaration of Helsinki and in line with the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement. The ethical approval was obtained from Ethics Committee of University of Health Sciences Turkey, Van Research and Training Hospital (No: 2020/24 Date: December.11.2020).

2.2. Data Collection

The study was conducted between December 2020 and April 2021. The survey was sent to orthopaedic surgeons via the Google survey system, and was sent to the Turkey Orthopaedics and Traumatology Association (TOTBİD) by e-mail. The study universe was composed of all orthopaedic surgeons actively working in Turkey, and it was aimed to reach the entire universe.

Orthopaedic surgeons with at least two years of experience who agreed to participate in the questionnaire and who completed the questionnaire in full were accepted as the sample. Those who did not answer the questionnaire, did not agree to participate in the study or answered the survey incompletely were excluded from the study.

One orthopaedic surgeon and one pain physician designed a survey to evaluate CPSP after arthroplasty, focusing on orthopaedic surgeons' thoughts, attitudes, and experiences. The physician based the questions to address important topics related to CPSP after arthroplasty. The survey included four questions to assess demographic characteristics (years of experience in orthopaedic surgery, type of health facility where they're employed, availability of a pain clinic in the hospital and city where they work) of the respondents; three questions related to thoughts of neuropathic pain component in CPSP after arthroplasty, the necessity of pharmacological treatment of CPSP after arthroplasty, and pain clinics effectivity on management of CPSP after arthroplasty; eight questions about attitudes towards management of early postoperative pain and assessment and management of CPSP after arthroplasty with their prescription preferences; two questions related to the frequency of use of loco-regional anesthesia in arthroplasty surgery working collaboration with a pain clinic and (Appendix).

2.3. Statistical Analysis

The SPSS 20.0 (IBM Corporation, Armonk, New York, United States) program was used to analyse the variables obtained in the study. The data collected contained continuous, categorical and ordinal variables. Continuous data were examined for normality of distribution using the Kolmogorov-Smirnov test. Data with non-normal distributions were summarised as medians with 25-75% percentiles. Categorical data were summarised in numbers and percentages. The relationship between nominal variables and continuous variables was evaluated with Point-Biserial analysis. The relationship between sequential categorical variables and continuous variables was assessed using Spearman's Rank Order Correlation analysis. The relationship between categorical variables was analysed using chi-square or Fisher exact tests.

3. RESULTS

A total of 334 physicians agreed to participate in the study; those with less than two years of experience in orthopaedic surgery were excluded (n = 15). The study was evaluated out of 319 participants.

The physicians' average experience in orthopaedic surgery was 10.0 (2.0–43.0) years, and the health centres where they work are shown in Table 1. In total, 35.4% (n = 113) of the orthopaedic surgeons worked in a clinic collaboration with the pain clinic in their residency, 51.7% (n = 165) had a pain physician in the health centre where they currently work and 79.0% (n = 252) had a pain physician in the city where they work.

Table 1. *Health centres where orthopaedic surgeons work*

Health Centre Type	Percentages (Frequencies)
Public hospital	29.4% (81)
Private center	17.2% (55)
Training and research hospital	32.6% (104)
University hospital	24.8% (79)
Total	100.0% (319)

The orthopaedic surgeons reported that loco-regional anaesthesia was administered at rates of 80.0 % (50.0–95.0) in the arthroplasty surgeries. Orthopaedic surgeons stated that they prescribe for postoperative pain control when discharging patients after arthroplasty at rates of 100.0 % (100.0–100.0). Their choices for painkillers for early postoperative pain are shown in Table 2.

Table 2. *Pain killer agents preferred by orthopedic surgeons for early postoperative pain*

Pain killers	Percentages (Frequencies)
Paracetamol and/or non-steroidal antiinflamatory drugs (1)	84.6% (270)
(1), and antidepressant combinations	2.2% (7)
(1), and gabapentinoids combinations	11.6% (37)
(1), and combinations of gabapentinoids and antidepressants	1.6% (5)
Total	100.0 (319)

The surgeons' thoughts were asked about neuropathic pain as a component of CPSP after arthroplasty, and interventional treatments of pain clinics for intractable CPSP after arthroplasty. The survey also queried their attitudes about concentration on pain, after excluding possible septic causes, as well as wound problems in patients who presented with chronic pain at the third and 12th months after arthroplasty. The answers, which were given on a Likert scale, are shown in Table 3. Orthopaedic surgeons declared that they use a 0.0% (0.0–20.0) validated scale or questionnaire to assess chronic pain after arthroplasty. Prescription preferences of orthopaedic surgeons for CPSP after arthroplasty are shown in Table 4. Consultation rates of orthopaedic surgeons for CPSP after arthroplasty to pain physician and psychiatry clinics are shown in Table 5.

There was a difference in orthopaedic surgeons' agreement to consider on the chronic pain after excluding possible underlying septic causes and wound problems between the patient groups at the third and 12th months ($p = 0.022$, $Z = 2.289$).

There was a statistically relation between orthopaedic surgeons' had been in coordination with the pain clinic during their residency and their referral of patients with CPSP after arthroplasty to a pain physician today ($p < 0.001$ $r = 0.209$). There was a statistically significant weak positive correlation between the rate of referring a patient with CPSP after arthroplasty to a pain physician and the presence of a pain physician in the health centre and/or city where she/he is currently working ($p = 0.01$ $r = 0.185$).

There was a weak positive correlation between the rate of referral of patients with CPSP after arthroplasty to pain physician and the belief of orthopaedic surgeons that pain physician can help patients with CPSP after arthroplasty who are unresponsive to pharmacological treatments ($p = 0.008$, $r = 0.148$).

Table 3. *Orthopaedic surgeons' thoughts and attitudes about CPSP after arthroplasty*

Likert	Percentages (Frequencies)				
	3. month*	12. month†	Neuropathic‡ pain	Prescription for CPSP§	Pain Physician||
1 Strongly disagree	8.5% (27)	10.1% (32)	4.1% (13)	11.9% (38)	4.4% (14)
2 Do not agree	18.2% (58)	11.6% (37)	9.7% (31)	18.2% (58)	6.6% (21)
3 Unsure	21.9% (70)	24.1% (77)	26.0% (83)	32.6% (104)	11.3% (36)
4 Agree	24.5% (78)	21.6% (69)	29.5% (94)	19.4% (62)	22.6% (72)
5 Strongly agree	27.0% (86)	32.6% (104)	30.7% (98)	17.9% (57)	55.2% (84)
Total	100.0% (319)	100.0% (319)	100.0% (319)	100.0% (319)	100.0% (319)

**: What is your approach, after excluding possible septic causes and wound problems, for the patient who comes with chronic pain in the third month after total/partial arthroplasty surgery? Do you agree with the proposition 'I'll consider on the pain'?*

†: What is your approach, after excluding possible septic causes and wound problems, for the patient who comes with chronic pain in the 12th month after arthroplasty? Do you agree with the proposition 'I'll consider on the pain'?

‡Do you agree with the proposition that patients who describe chronic pain after arthroplasty have neuropathic pain?

§: 'I think that patients who describe chronic pain after arthroplasty should receive routine pharmacological treatment for pain.' Do you agree with this proposition?

||: 'Despite the appropriate adequate pharmacological treatment I have given, there may be interventional pain procedures that the pain physician can employ.' Do you agree with this proposition?

Table 4. *Orthopaedic surgeons' preferred painkillers for CPSP after arthroplasty*

Pain killers	Percentages (frequencies)
Paracetamol and/or Nonsteroid Anti-Inflammatory Drugs (1)	23.2% (74)
Antidepressants	2.2% (7)
Gabapentionoids	5.0% (16)
(1) with Antidepressants	10.7% (34)
(1) with Gabapentinoids	40.4% (129)
(1) with Antidepressants and Gabapentinoids	8.8% (28)
Antidepressants with Gabapentinoids	1.3% (4)
Combinations with Opioids	8.5% (27)
Total	100,0% (319)

Table 5. *Number of orthopaedic surgeons to consult with patients experiencing chronic pain after arthroplasty*

Clinics	Median	25-75% percentiles
Pain Clinic	20.0	5.0-55.0
Psychiatry	5.0	0.0-10.0

4. DISCUSSION

In the present study, nearly all of the orthopaedic surgeons declared to prescribe medications for early postoperative pain control. More than half of the orthopaedic surgeons proclaimed that chronic pain should be focused on after excluding surgical complications, neuropathic pain might be a component of CPSP after arthroplasty, and preferred multimodal therapies for CPSP after arthroplasty management while they declared using validated scales to pain assessment seldomly and referred patients with CPSP after arthroplasty to pain and psychiatry clinics rarely. Moreover, the rate of referral of patients to pain clinics by orthopaedic surgeons was associated with the fact that surgeons worked with pain specialists during their residency, the presence of a pain specialist in the center where they are working, and the thought that pain specialists could be helpful.

The present study determined that nearly all of the orthopaedic surgeons prescribed medications for early postoperative pain control. The surgeons mainly used paracetamol and/or non-steroidal anti-inflammatory drugs, although 15% preferred gabapentinoids and antidepressant drug combinations; none of the physicians stated that they chose opioids. In the literature, Thomazeau et al. (14) showed that the severity of acute postoperative pain after knee arthroplasty is associated with CPSP after arthroplasty at the sixth month. Clarke et al. (15) concluded that the conversion of acute postoperative pain to chronic postsurgical pain could be prevented by using gabapentinoids, based on their systematic research and meta-analysis. Kinney et al. (16) found a relationship between increased postoperative opioid consumption and the development of chronic postsurgical pain. In this study, orthopaedic surgeons have been shown to prescribe appropriate pharmacological choices predictive of CPSP after arthroplasty development to manage early postoperative pain.

In the present study, the orthopaedic surgeons declared using validated scales rarely. In pain management, pain intensity and pain impact on functionality and quality of life are significant issues, and inappropriate pain assessment is one of the physician-related causes for chronic postsurgical pain development (17). Tools available to assess CPSP after arthroplasty include: Western Ontario and McMaster Universities' Osteoarthritis Index pain scale, which is a disease-specific questionnaire assessing pain severity, functionality and stiffness; Oxford Knee Score, a joint and disease-specific questionnaire that has a predictive value for outcomes of knee replacement; Brief Pain Inventory Short-Form, a generic questionnaire with pain severity and pain-related impacts; Short-Form McGill Pain Scale, a generic questionnaire assessing pain severity and affects; and the PainDETECT and Douleur Neuropathique-4, tools that evaluate the presence of neuropathic pain (18). In the present study, it can be concluded that orthopaedic surgeons did not sufficiently benefit from the available validated scales that may be useful for assessing the severity and impact of CPSP after arthroplasty.

In this study, the orthopaedic surgeons reported that loco-regional anaesthesia was used in joint arthroplasty at a frequency of 80%. Regional anaesthesia reduces the mortality and morbidity rates of arthroplasty (19). Oldman et al. (20) reported that regional anaesthesia practices in orthopaedic cases are associated with supportive attitudes of orthopaedic surgeons, and a Cochrane review declared that regional anaesthesia prevents chronic postsurgical pain (21). Epidural or spinal anaesthesia, plexus blocks and peripheral nerve blocks/infusions are regional anaesthesia options used in arthroplasty surgeries (22-24). In the present study, the high rate of regional anaesthesia in arthroplasty surgeries is a promising finding.

In this study, more than half of the orthopaedic surgeons declared that chronic pain should be focused on, after excluding surgical complications, when questioned about the postoperative period of 3 to 12 months. Wylde et al. (25) formed a core set for CPSP after arthroplasty, which proposes detailed questioning to evaluate pain intensity, temporal aspects of pain, the definition of pain, pain impacts on daily life/physical function/emotional life, use of pain medication, improvement in pain relief and satisfaction. Such sets in routine clinical follow-up can help chronic pain be defined beyond the subjective perceptions of orthopaedic surgeons and can increase their awareness.

In this study, 60.2% of orthopaedic surgeons thought that neuropathic pain might be a component of CPSP after arthroplasty and that 67.3% of orthopaedic surgeons prefer gabapentinoids and antidepressant agents as monotherapy or combinations in the treatment of CPSP after arthroplasty. Neuropathic pain is observed in 7% to 34% of patients with CPSP after arthroplasty (7, 9), and gabapentinoids and antidepressants are recommended in the treatment of chronic neuropathic pain (26, 27). Based on the findings of this study and the literature, it can be said that some patients with neuropathic pain were under-evaluated. Still, orthopaedic surgeons are successful in prescribing choices that are useful for possible neuropathic pain presence.

In the present study, 69.7% of the orthopaedic surgeons preferred multimodal therapies for CPSP after arthroplasty. Treatment with acetaminophen, NSAIDs, alpha-2-delta modulators (gabapentin, pregabalin), NMDA-receptor antagonists (ketamine), alpha-2 adrenergic agonists (clonidine, dexmedetomidine) and opioids are recommended for chronic postsurgical pain management (28). The preference rate of orthopedic surgeons for multimodal analgesics in the management of arthroplasty-related CPSP after arthroplasty was one of the encouraging findings of this study.

In the present study, the orthopaedic surgeons requested a pain clinic consultation at a frequency of 20% in patients with CPSP after arthroplasty in cases of refractory to pharmacological treatments. There are many interventional therapy choices for CPSP after total knee replacement, although they lack evidence (29). Erdem et al. (30) used ultrasound-guided genicular nerve pulsed radiofrequency in patients with CPSP after total knee replacement and found that after three months of follow-up, patients showed improvement in pain intensity, stiffness and physical function. Qudsi-Sinclair et al. (31) reported that both genicular nerve block and radiofrequency neurolysis successfully improved pain and functionality in CPSP after knee arthroplasty. Albayrak et al. (32) reported clinical improvement with TENS and lumbar dorsal root ganglion radiofrequency in patients who developed CPSP after knee arthroplasty. Kim et al. (33) reported significant relief of pain and gain in physical function during 24 months of follow-up using the ultrasound-guided femoral nerve cold radiofrequency therapy in CPSP after hip arthroplasty. Zabolotsky et al. (34) showed that pain and analgesic consumption decreased and range of motion increased for two months after C4-5-6 dorsal root ganglion pulsed radiofrequency in a patient with severe chronic pain after shoulder arthroplasty. Orthopaedic surgeons may refer patients to pain physicians more frequently to give a chance to various interventional pain treatments in the management of CPSP after arthroplasty.

The present study showed that orthopaedic surgeons referred their patients with CPSP after arthroplasty to psychiatry at a rate of 5%. There is a need for psychiatric solutions in chronic postsurgical pain management, and Acceptance and Commitment Therapy, Cognitive Behavioural Therapy and Mindfulness are psychiatric treatment methods helpful for chronic postsurgical pain treatment (35). In the present study, orthopaedic surgeons did not focus enough on psychiatric consults in patients with CPSP after arthroplasty.

This study revealed that, orthopaedic surgeons were considering on CPSP after arthroplasty more strongly when at postoperative 12th months than in the postoperative third months. The frequency and severity of chronic postsurgical pain decreases with increasing follow-up time (36). However, orthopaedic surgeons' interest in CPSP after arthroplasty did not diminish with time.

In this study, a statistically significant relationship was found between history of collaboration with pain clinics in their residency programme, the presence of a pain physician in the health centre or city where orthopaedic surgeons are currently working and whether patients were referred to a pain physician. Orthopaedic surgeons' rate of referring patients with CPSP after arthroplasty to a pain clinic is related to accessibility and their past experiences.

One of the limitations of this study is that it is subjective, since physicians' experiences and attitudes were obtained from physicians' own self-evaluation and therefore, the rates given regarding preferences and experiences may not reflect the exact truth. More realistic findings can be obtained with studies that scan the official data.

5. CONCLUSION

Orthopaedic surgeons employ pre-emptive approaches to CPSP after arthroplasty and are engaging well in pharmacological management of CPSP after arthroplasty, but they need to advance further with multidisciplinary treatments and directions.

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Clinical and Experimental Health Sciences

The Assessment of the Neurological Development of Infants with Prenatal COVID-19 Exposure

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ABSTRACT

Objective: The effects of prenatal coronavirus 2019 disease (COVID-19) exposure on the infantile neurological development are unknown. It has been considered that the inflammatory, thrombotic, and vascular changes occurred in the placentas taken from pregnant women diagnosed with COVID-19 and the inflammatory nature of COVID-19 infection may lead to negative obstetric and neurological events. We aimed to assess the potential neurological effects of prenatal COVID-19 exposure on the infant.

Methods: The present study included 2–12-month-old infants born to women with positive real-time reverse transcription polymerase chain reaction test results for COVID-19 from the population of pregnant patients under routine follow-up. The neurological examinations and the Denver II Developmental Screening Test (DDST II) were performed for 41 infants aged 2–12 months to assess the neurological effects of prenatal COVID-19 exposure.

Results: The average gestational age of the infants was 38.7 ± 1.9 weeks, and the average birth weight was 3198 ± 543 g. Eight of the infants had a history of hospitalization in the neonatal intensive care unit. The neurological examination and the neuromotor development of 40 (96%) infants were normal for their age group. Only one infant had abnormal neurological examination and DDST II result.

Conclusion: The findings of the study suggest that prenatal COVID-19 exposure has no negative neurological impact on infants. Long-term prospective studies with larger sample sizes are needed for more comprehensive assessment of the neurological effects of prenatal COVID-19 exposure on the infants.

Keywords: COVID-19, infant, Denver II Developmental Screening Test

1. INTRODUCTION

The effects of COVID-19 infection on the developing fetus are still unknown, and vertical transmission has been shown to be uncommon in case reports. However, evidence for placental and fetal infection has been reported (1). Inflammatory, thrombotic, and vascular changes have been observed in placentas collected from pregnant women diagnosed with COVID-19, and the inflammatory nature of COVID-19 infection may cause adverse obstetric and neonatal events during pregnancy (2,3).

Long-term neurological effects may occur in a fetus that has been exposed to endometrial inflammation and placental changes due to COVID-19 infection. Therefore, this study was conducted to evaluate the possible neurological effects of prenatal exposure to COVID-19 on the infant.

2. METHODS

In this prospective study, infants aged 2–12 months born to women who had a positive COVID-19 real-time reverse transcription-polymerase chain reaction (RT-PCR) test taken from nasopharyngeal or oropharyngeal swab samples during pregnancy were included. All the pregnant women were under routine follow-up at the Gaziosmanpaşa Training and Education Hospital, Department of Obstetrics and Gynecology. Medical treatment was administered to pregnant women who were not suitable for outpatient treatment.

Infants were evaluated in the pediatric neurology department of Haseki Sultangazi Training and Education Hospital during the postnatal 2–12 months between June and August 2021. Data regarding comorbidities, smoking history, gestational week of the COVID-19 infection, severity of the disease, history of hospitalization, gestational week, birth weight, birth history, and history of hospitalization in the neonatal

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intensive care unit were collected and recorded. Neurological examination of all the infants was performed by one pediatric neurologist. The DDST II was administered to all infants by one child development specialist. Cranial imaging by ultrasound was performed for all infants.

2.1. Denver II Developmental Screening Test

Denver II Developmental Screening Test, which is used to follow the developmental stages of children and for early detection of abnormal situations, is the detailed version of the Denver Developmental Screening Test (DDST), which is standardized and used in Turkey. The Denver test was developed by Frankenburg and Dodds in 1990 (4). In Turkey, a standardization study was conducted by Anlar, Yalaz, Bayoğlu, and Denver II started to be used in 2011.

This test consists of 4 parts and 134 items. The following areas are evaluated: personal social emotional development, fine motor development, language development, and gross motor development. The results of the DDST II test are classified as either normal, suspicious, abnormal, or untestable.

2.2. Statistical Analysis

SPSS version 22.0 (IBM Corp., Armonk, New York, USA) was used in the analysis of the data. Data are expressed as percentage, mean ± standard deviation, or median (minimum–maximum). The Kolmogorov–Smirnov test was used to analyze the conformity of parametric data with normal distribution.

2.3. Ethics committee

Ethics committee approval dated 26.05.21 and numbered 05-2021 was obtained from the Health Sciences University, Haseki Training and Research Hospital, Clinical Research Ethics Committee. Written informed consent was obtained from the infants' parents.

3. RESULTS

The average age of mothers in this study was 30.6 ± 5.6 years. Diabetes mellitus was diagnosed in two pregnant women and celiac disease was diagnosed in one pregnant woman, one of the pregnant women was a thalassemia carrier, and two mothers were smokers. The mean gestational week at which mothers were diagnosed with COVID-19 was 22.4 ± 10.2 weeks. The distribution of mothers according to the trimester in which the diagnosis of COVID-19 was made was 27% in the first trimester, 39% in the second trimester, and 34% in the third trimester (Table 1). Seven mothers had a history of hospitalization due to COVID-19. Among the remaining 34 mothers, 2 were asymptomatic and 32 had mild-moderate symptomatic COVID-19 disease.

The average age of the 41 infants with prenatal exposure to COVID-19 was 5.3 ± 3.3 months; 22 infants were female and 19 were male. The average gestational age of the infants was 38.7 ± 1.9 weeks, and the average birth weight was 3198 ± 543 g. Eight of the infants had a history of hospitalization in the neonatal intensive care unit (Table 2). Neurological examinations were normal in 40 infants. Head circumference percentiles were in the normal range. No pathology was detected during transfontanelle ultrasonography. Neuromotor development was normal and, according to the DDST II, partial social emotional development, fine motor development, language development, and gross motor development were normal for the infants' ages. The mother of one of the infants had diabetes, and the infant was intubated for 1 day and hospitalized in the neonatal intensive care unit for 17 days due to low APGAR scores and respiratory distress. In this infant, hypotonicity and neuromotor developmental delay were present upon neurological examination; he could not roll over (prone to supine), did not respond to affection, and did not follow moving objects with his eyes. One infant was delivered at the 27th gestational week and weighed 1300 g at birth. Findings that were compatible with periventricular leukomalacia were observed on cranial magnetic resonance imaging in this infant. The neurological examination of this infant was normal at the fifth month of age but long term developmental evaluation is needed. Six infants had history of hospitalization in the intensive care unit due to hyperbilirubinemia (2/6), sepsis (2/6), dehydration (1/6), and transient tachypnea of the neonate (1/6). Neurological examinations and the DDST II scores of these infants were normal for their ages. One infant had grade 1 intracranial hemorrhage that was revealed during transfontanelle ultrasonography. No pathology was detected during transfontanelle ultrasonography of the other infants.

Table 1. *Demographic characteristics of pregnant women diagnosed with COVID-19.*

Age (mean ± SD) (years)	30.6 ± 5.6
Body mass index (mean ±SD) (kg/m^2)	29.3 ± 6.0
Smoking (n, %)	3 (7)
Gestational week of COVID-19 diagnosis	(4–39)
Trimester of COVID-19 diagnosis (n, %)	
First	11 (26)
Second	16 (39)
Third	14 (34)
Medication taken during infection (n, %)	17 (41)
Gestational week of birth (mean ± SD) (n, %)	38.7 ± 1.9

SD:Standart deviation.

Table 2*. Characteristics of infants with prenatal COVID-19 exposure.*

Sex (n, %)	
Female	22 (54)
Male	19 (46)
Age (mean ± SD) (months)	5.3 ± 3.3
Birth weight (mean ± SD) (gr)	3198 ± 543
NICU history (n, %)	8 (19)
Neurological examination normal (n, %)	40 (98)
DDST score normal (n, %)	40 (98)

SD:Standart deviation.

4. DISCUSSION

Infection response during pregnancy is associated with short – and long-term adverse effects on the infant by causing maternal immune activation and inflammation (5,6). As a result of maternal immune activation, proinflammatory cytokines, such as interleukin-1β, interleukin-6, and tumor necrosis factor α, can cross the placental barrier and trigger fetal immune response (7,9). This may lead to neurological effects and long-term problems like autism spectrum disorder in the offspring (10). No studies have yet been reported on neurodevelopmental outcomes for autism spectrum disorder that the childrren are still in infantile period. To evaluate the possible neurological effects of this immune response on infants, a detailed neurological examination and DDST II were performed on infants aged 3–12 months. In our study, signs of neurological involvement were found in only one infant.

It was observed that pregnant women with severe COVID-19 infection had an increased risk of cesarean delivery, hypertensive pregnancy disorders, and preterm birth when compared with asymptomatic patients (11). In another study, when women with COVID-19 were compared with uninfected women, the frequency of preterm birth and intrapartum fetal distress was significantly increased in women with symptomatic COVID-19 infection (12).

Fetal stress, preterm birth, and need for hospitalization in the neonatal intensive care unit can cause neurological involvement and neuromotor developmental delay in the infant during the neonatal period and beyond. In our study, one patient who was born at the 27th gestational week and had periventricular leukomalacia. Another patient had a diabetic mother and had neurological involvement after birth with low Apgar scores, respiratory distress, and hypotonicity and neuromotor developmental delay on neurological examination. The relationship between these findings and COVID-19 infection is unclear. To predict this relationship, there is a need for detailed studies examining the pathophysiology of fetal involvement in COVID-19. In our study, neurological examinations and DDST II scores were normal in all infants, except one.

In utero exposure to viral pathogens is a risk factor for direct infection of fetal tissues. Transplacental infection of the fetus by COVID-19 is yet to be proven exactly. COVID-19 has a neurotropism and direct viral neuroinvasion of the central nervous system through physical contact is a controversial topic. The relationship between neuroinvasion and the risk for neurodevelopmental disorders is still unknown.

Limitations of this study include a small number of children and short-term neurodevelopmental follow-up. Additional investigation of the placenta post-delivery would be helpful in evaluating the impact of direct viral infection on placental and fetal tissues.

5. CONCLUSION

In conclusion, although we did not find any evidence of short-term neurological effects on the infants in our study, long-term prospective studies are needed to elucidate these neurological effects.

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Clinical and Experimental Health Sciences

The Frequencies of Amino Acids in Secondary Structural Elements of Globular Proteins

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ABSTRACT

Objective: The frequencies of amino acids in proteins for different structural levels have been determined by many studies. However, due to the different content of data sets, findings from these studies are inconsistent for some amino acids. This study aims to eliminate the contradictions in the findings of the studies by determining the frequencies of the amino acids in all structural level of globular proteins.

Methods: The frequencies of the amino acids in overall protein, in secondary structural elements (helix, sheet, coil) and in subtypes of secondary structural elements (α-, π-, and 3_{10}-helices, and first, parallel and anti-parallel strands) were calculated separately using a data set including 4.882 dissimilar globular peptides. The frequencies of the amino acids were calculated as the ratio of the total number of a specific residue in related structure to the total number of all residues in the related structure.

Results: The frequencies of residues determined in this study is partially in consistent with the other studies. The differences are probably due to the data set contents of the studies. The frequencies of the amino acids in subtypes of secondary structural elements were determined for the first time in this study.

Conclusion: Variations in the frequencies of PRO residue in 3_{10}-helix structure and of ILE, LEU, and VAL residues in strands of sheet structure are valuable findings for the improvement of secondary structure prediction methods, as they can be used as secondary structural elements markers.

Keywords: amino acid, globular protein, secondary structure

1. INTRODUCTION

The frequencies of amino acids in proteins could provide important information about the nature of the proteins and this information can be used in many application areas of proteomics, molecular biology and bioinformatics. Because of this, the frequencies of amino acids for both overall protein structures (1-18) and specific peptide structures (19-39) are constantly being investigated. Despite the partial consistency in the results of these studies, there are some issues related to the data set and methodology that need to be clarified and improved.

Firstly, data set used in the studies must be homogenous in regard of protein main class (i.e., globular, membrane, and fibrous proteins). It must include only one type of protein class. Otherwise, the information on amino acid frequencies will be diluted and will not reflect the true nature of the residue abundance, as each protein class has very different amino acid content depending on the different sequence and structural characteristics of the class. Likewise, findings from studies based on specific species (10, 13, 16, 17) are not conclusive in this regard. In order to attain a more qualified level of homogeneity, proteins of extremophile organisms should also be removed from the data set.

Secondly, studies also require layering of data set at the level of subtypes of secondary structural elements of proteins. That is, frequencies of the amino acids in proteins should be determined for subtypes of helix (such as, α-, π-, and 3_{10}-helices) and of sheet (such as, first, parallel and anti-parallel strands) structures. This approach will provide further information about the residue frequencies. As stated in previous paragraph, this layering method will also prevent the information about frequencies from being masked which caused by subtype heterogeneity.

Third issue concerns the similarity of the proteins in data set. Similar proteins mostly have similar structure and residue content. Thus, similar proteins change the weights of the residue numbers towards their residue numbers, resulting

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in bias in residue frequencies. Since proteins with less than 25% similarity considered dissimilar, the similarity of proteins in data set should not exceed this value. Because the whole genome of a species includes many similar proteins (e.g. in human genome, there are many similar G-Protein-Coupled-Receptors [GPCR] and Guanine Nucleotide Binding Proteins [G proteins]), this issue especially needs to be taken into account in studies using the whole genome of a particular species.

This study investigates the frequencies of amino acids in globular proteins at different structural levels and aims to contribute to resolve the inconsistencies in the findings obtained from different studies. For these purposes, a comprehensive and qualified data set which were obtained from Nacar (40) was used in this study.

2. METHODS

2.1. Protein Data Set

Protein chain data set was obtained from Nacar (40). It contains 4,882 protein chains, all of which are globular peptides. Also, it does not include any protein of extremophile organisms. Protein structure files were downloaded from Protein Data Bank (41). The lengths of peptide chains are longer than 80 residues. The sequence identity of any peptide pair is smaller than 25%, ensuring that the peptides are not similar.

2.2. Peptide Sequences

Amino acid sequences of peptides were obtained from the ATOM or HETATM entries of the Protein Data Bank (PDB) files of the peptides. Modified residues were replaced with original residues by referring to MODRES entry and expression tags were removed using SEQADV entry data. In this study, amino acids were represented using the three-letter-code system of IUPAC-IUB.

2.3. Secondary Structural Elements

The regions corresponding to secondary structural elements of peptides were determined according to the data deposited in HELIX and SHEET entries of the PDB files of the peptides. The regions other than helix and sheet were considered random coil or loop.

2.4. Frequencies of Amino Acids

The frequencies of amino acids were determined for overall peptides and, for each helix and sheet types and their subtypes (α-helix, 3_{10}-helix and first, parallel, anti-parallel strands) separately. The frequencies of the amino acids were calculated as the ratio of the total number of a specific residue in related structure to the total number of all residues in the related structure.

3. RESULTS

3.1. Distribution of Lengths of Peptides

Length distribution of the peptides were shown in Figure 1. Lengths of peptides have a great variety, but most of them lay in range of 80-to-500 residues, roughly. Peptides of 1,000 residues or longer were group into a single group as the last item of the figure.

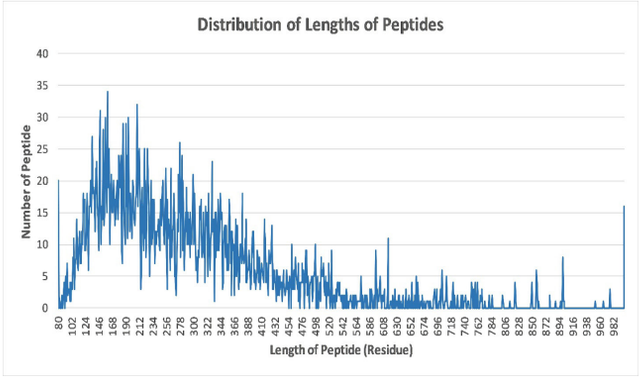

Figure 1. *Distribution of lengths of peptides. Most peptides are less than 500 residues in length. Peptides of 1.000 residues or longer were group into a single item.*

3.2. Distribution of Secondary Structural Elements

4.882 protein chains contain 1,419,498 amino acids. Of these amino acids, 613,334 (43.2 %) are located in the helix region, 344,676 (24.3 %) in the sheet region, and 461,488 (32.5 %) in the coil region. Also, percentages of secondary structural elements for each peptide of 4,882 chains were calculated and shown in Table 1 as mean value (Mean) and standard deviation (StdD). Despite the large standard deviation values, the latter values are in consistent with the former ones.

Table 1. *Distribution of secondary structural elements*

	Secondary structure	Mean (%)	StdD (%)
Helix	Overall	42.9	20.6
	α-Helix	37.8	20.6
	310-Helix	5.2	3.4
Sheet	Overall	22.6	13.9
	First Strand	4.0	3.3
	Parallel Strand	13.7	12.8
	Antiparallel Strand	4.8	5.4
	Coil	34.4	10.4

The dominant secondary structural element in globular proteins is helix structure and α-helix is the dominant subtype in helix. Second most abundant element is random coil. The less abundant one is the sheet structure. In sheet structure, parallel strand is the most abundant subtype.

Table 2. *Amino acid frequencies of proteins and secondary structural elements*

Amino acid	Protein (%)	Helix (%)			Sheet (%)				Coil (%)
	Overall	Overall	α	310	Overall	First	Parallel	Anti-Parallel	Overall
ALA	8.3	10.6	11.0	7.9	6.5	5.7	7.0	6.5	6.4
ARG	5.1	5.7	5.9	4.7	4.7	5.1	3.5	5.1	4.6
ASN	4.3	4.0	3.9	5.0	2.5	2.7	2.6	2.5	5.8
ASP	6.0	6.0	5.7	8.3	3.3	3.6	3.2	3.2	8.1
CYS	1.4	1.2	1.2	1.3	1.7	1.6	1.7	1.8	1.3
GLN	3.7	4.5	4.6	3.8	2.9	3.5	1.9	3.1	3.3
GLU	6.4	8.0	8.2	7.3	4.5	5.6	3.2	4.6	5.6
GLY	7.3	4.9	4.7	6.7	4.9	4.1	5.1	5.0	12.3
HIS	2.5	2.4	2.3	2.8	2.5	2.7	2.4	2.5	2.6
ILE	5.5	5.1	5.4	3.6	9.4	8.8	12.4	8.4	3.1
LEU	9.2	11.0	11.3	8.9	10.4	8.9	11.8	10.2	5.9
LYS	5.4	6.0	6.1	5.4	4.2	4.9	3.1	4.4	5.6
MET	2.1	2.5	2.6	1.9	2.2	2.0	2.4	2.2	1.6
PHE	4.2	4.0	3.9	4.3	6.0	5.9	5.9	6.1	3.0
PRO	4.8	2.8	2.2	6.9	2.0	2.6	1.4	2.0	9.6
SER	6.1	5.9	5.6	7.5	5.3	5.9	4.5	5.4	7.0
THR	5.6	4.7	4.8	4.4	6.7	7.1	6.0	6.8	5.9
TRP	1.5	1.5	1.5	1.9	2.0	1.9	1.5	2.2	1.2
TYR	3.6	3.4	3.4	3.7	5.1	4.9	4.2	5.5	2.7
VAL	7.0	5.6	5.8	3.8	13.2	12.5	16.1	12.3	4.2

3.3. Amino Acid Frequencies in Overall Proteins and Secondary Structural Elements

Frequencies of the amino acids are given in Table 2. Frequencies of amino acids were determined for overall protein, for secondary structure types (helix, sheet, and coil), and for subtypes of secondary structural elements (α-helix, 3_{10} – helix or first, parallel and anti-parallel strands of sheet), separately.

The most abundant amino acids in overall protein and overall helix structures are LEU and ALA residues. The most abundant residues in α-helix are LEU and ALA, while in 3_{10}-helix structure are LEU and ASP. The most abundant amino acids in sheet structure and in its subtypes are VAL and LEU residues. In parallel strand, there is also an abundance of ILE. In coil, the most abundant amino acids are GLY and PRO. Since the PRO residue is not abundant in helix and sheet structures, the abundance of it in coil is quite remarkable. However, this finding implies that PRO residue is excluded from the helix and sheet structures due to its inability to form backbone hydrogen bond.

The least abundant amino acids in overall protein, helix and α-helix structures are TRP and CYS residues. Besides those, MET is an another least abundant residue in 3_{10}-helix structure. Because PRO residue lacks of free amino group, it cannot form backbone hydrogen bond with carboxyl group of other amino acids. Therefore, it is not expected to be found in helix and sheet structures abundantly. However, it is noteworthy that PRO has been found 3.1 times more abundant is 3_{10}-helix structure. The least abundant amino acids in sheet structure are TRP, PRO and CYS. The least abundant residues in coil structure are CYS and TRP.

3.4. Abundance of Modified Residues

Of 1,419,498 amino acids, 4,192 are chemically modified and number of modified amino acids were presented in Table 3. While MET, ASN and CYS amino acids are the most modified residues, GLN, ILE and VAL residues have no modification.

Table 3. *Number of modified residues*

Amino Acid	#	Amino Acid	#
ALA	4	LEU	1
ARG	1	LYS	86
ASN	223	MET	3663
ASP	2	PHE	1
CYS	104	PRO	3
GLN	0	SER	27
GLU	22	THR	29
GLY	2	TRP	6
HIS	9	TYR	9
ILE	0	VAL	0

4. DISCUSSION

Frequencies of amino acids from nine studies (1-9) and this study tabulated in Table 4 for comparison. Because the frequencies were not given in quantitative values (given only in bar graphs), finding from other studies (10-12, 14-18) were not included in Table 4. Protein databases used by 1, 2, 3, 4, 6, 8, and 9 are UnitProtKB, NCBI+KEGG, PDB, PDB, SwissProt, OWL and SwissProt+PDB, respectively. Protein databases used by 5 and 7 were not specified. Amino acid frequencies in 4 were calculated from the *Table I* from Xia and Xie (9). While study 1 included only globular proteins, the other studies included various organisms/protein classes or they did not specify the protein class.

Table 4. *Comparison of frequencies of amino acids from different sources (%)*

	Nacar	1	2*	3	4	5	6	7	8	9SP	9PDB
ALA	8.3	7.8	11.1	8.3	11.3	8.2	7.6	7.4	7.5	7.9	7.7
ARG	5.1	4.7	4.1	4.7	4.9	5.2	5.2	4.2	5.2	5.4	4.9
ASN	4.3	4.1	5.0	4.8	3.6	4.4	4.4	4.4	4.6	4.1	4.6
ASP	6.0	5.1	5.0	5.9	4.9	5.3	5.3	5.9	5.2	5.4	5.8
CYS	1.4	1.6	0.4	1.5	1.5	1.1	1.6	3.3	1.8	1.5	1.7
GLN	3.7	3.8	5.9	3.7	3.8	3.6	3.9	3.7	4.1	4.0	4.0
GLU	6.4	7.1	5.9	6.1	6.5	6.5	6.5	5.8	6.3	6.7	6.7
GLY	7.3	6.1	16.3	7.9	5.5	6.9	6.9	7.4	7.1	7.0	7.2
HIS	2.5	2.3	1.9	2.2	2.2	2.1	2.2	2.9	2.2	2.3	2.4
ILE	5.5	5.5	3.5	5.5	6.1	6.8	5.9	3.8	5.5	5.9	5.6
LEU	9.2	10.8	5.7	8.4	9.8	10.1	9.5	7.6	9.1	9.7	8.7
LYS	5.4	5.4	2.1	5.8	5.9	6.0	6.0	7.2	5.8	5.9	6.4
MET	2.1	2.4	0.5	2.1	3.3	2.3	2.4	1.8	2.8	2.4	2.2
PHE	4.2	4.5	3.4	4.0	4.1	4.3	4.1	4.0	3.9	4.0	4.0
PRO	4.8	4.7	2.1	4.7	2.7	4.3	4.9	5.0	5.1	4.8	4.6
SER	6.1	7.7	11.8	6.1	5.2	6.5	7.1	8.1	7.4	6.8	6.2
THR	5.6	4.9	5.7	5.9	5.4	5.3	5.6	6.2	6.0	5.4	5.6
TRP	1.5	1.3	0.2	1.5	1.5	1.1	1.2	1.3	1.3	1.1	1.4
TYR	3.6	3.6	2.7	3.7	3.6	3.3	3.2	3.3	3.3	3.0	3.5
VAL	7.0	6.5	5.6	6.9	7.9	6.9	6.6	6.8	6.5	6.7	6.7

*1 – Tripathi, Tripathi, Gupta 2014, 2 – Moura, Savageau, Alves 2013, *ASN/ASP and GLN/GLU frequencies recalculated*

3 – Baud and Karlin 1999, 4 – Xia and Xie 2002

5 – Itzkovitz and Alon 20223, 6 – Varfolomeev, Uporov, Fedorov 2002

7 – King and Jukes 1969, 8 – Trinquier and Sanejouand 1998

3 – Baud and Karlin 1999, 8 – Trinquier and Sanejouand 1998

9 – Vacic, Uversky, Dunker, Lonardi 2007, SP: SwissProt, PDB: Protein Data Bank

Evaluating the Table 4 as a whole, it is observed that the frequencies of the ARG, ASN, ASP, GLU, HIS, PHE, THR, TYR, and VAL residues are nearly the same. The frequencies of remaining residues almost are the same except those all remaining residues of 2, ALA, GLY and PRO residues of 4, and CYS, ILE, LEU residues of 7.

Because study 1 based on globular proteins, its findings are comparable to this study. Despite the different data set sizes (study 1 included 557 peptides while Nacar (40) included 4,882 peptides), findings of these two studies are highly consistent, except for residues ASP, GLY, LEU and SER. The inconsistencies in frequencies for these residues are no more than 25%. Therefore, findings of this study contribute to the frequencies of ASP, GLY, LEU and SER amino acids in globular proteins.

The frequencies of amino acids in secondary structural elements of proteins from two studies (1, 9) and this study given in Table 5. Amino acid frequencies in 1 and 2 were calculated using data provided by Xia and Xie (9) and Baud and Karlin (1), respectively. Frequencies of residues in helix are almost completely consistent. But, in sheet and coil structures, inconsistency prevails. In sheet structure, the remarkable differences exist in frequencies of ILE, LEU and VAL residues. In the coil region of the peptide, most of the residues, except CYS, HIS, MET, PHE, TRP, TYR, and VAL, are conspicuous in regard of frequency changes, especially ALA, ASP, GLY, and PRO.

Table 5. *Comparison of amino acid frequencies of secondary structural elements from different sources*

	Overall (%)			Helix (%)			Sheet (%)			Coil (%)	
Amino Acid	Nacar	1	2	Nacar	1	2	Nacar	1	2	Nacar	1
ALA	8.3	11.3	8.3	10.6	11.2	11.7	6.5	6.8	4.2	6.4	46.4
ARG	5.1	4.9	4.7	5.7	5.6	5.7	4.7	4.1	2.8	4.6	2.0
ASN	4.3	3.6	4.8	4.0	3.9	3.8	2.5	3.2	1.9	5.8	3.7
ASP	6.0	4.9	5.9	6.0	5.4	5.3	3.3	4.0	2.0	8.1	4.8
CYS	1.4	1.5	1.5	1.2	1.3	1.1	1.7	2.0	1.3	1.3	1.0
GLN	3.7	3.8	3.7	4.5	4.3	4.7	2.9	3.1	1.9	3.3	1.8
GLU	6.4	6.5	6.1	8.0	7.7	8.6	4.5	4.8	2.9	5.6	3.3
GLY	7.3	5.5	7.9	4.9	5.0	4.0	4.9	6.1	3.3	12.3	7.6
HIS	2.5	2.2	2.2	2.4	2.1	2.0	2.5	2.4	1.5	2.6	1.3
ILE	5.5	6.1	5.5	5.1	5.2	5.6	9.4	8.3	6.3	3.1	1.7
LEU	9.2	9.8	8.4	11.0	10.5	11.1	10.4	9.2	6.3	5.9	3.4
LYS	5.4	5.9	5.8	6.0	6.6	6.6	4.2	4.9	3.0	5.6	3.2
MET	2.1	3.3	2.1	2.5	3.6	2.7	2.2	3.0	1.5	1.6	1.6
PHE	4.2	4.1	4.0	4.0	3.8	4.0	6.0	5.0	3.7	3.0	1.4
PRO	4.8	2.7	4.7	2.8	2.6	2.4	2.0	2.8	1.3	9.6	3.7
SER	6.1	5.2	6.1	5.9	5.1	5.0	5.3	5.7	3.3	7.0	4.3
THR	5.6	5.4	5.9	4.7	4.8	4.4	6.7	6.8	4.8	5.9	3.8
TRP	1.5	1.5	1.5	1.5	1.4	1.6	2.0	1.8	1.3	1.2	0.7
TYR	3.6	3.6	3.7	3.4	3.3	3.6	5.1	4.6	3.5	2.7	1.6
VAL	7.0	7.9	6.9	5.6	6.5	6.0	13.2	11.2	8.5	4.2	2.6

1 – Xia and Xie 2002

2 – Baud and Karlin 1999

Since the protein classes in the studies of 1 and 2 were not specified, these comparisons are valid to some extent. Despite this drawback, findings of this study are more reliable because of the comprehensive and qualified data set used in the study.

CYS and TRP amino acids are the least abundant residues in nearly all protein secondary structure. A biophysical explanation of this finding is probably based on functional and structural characteristics of the residues. The main function of CYS residue in protein is to establish disulfide bond between two CYS residues. This bond is an extremely

important biophysical determinant in stability of the peptide. However, the number of disulfide bonds in protein is very limited. Therefore, low frequency of the CYS in protein reflects this fact. TRP residue has an indole ring as side chain and this side chain remains very large compared to the side chains of the other amino acids. This spatial property of TRP cause difficulties in positioning of it in protein structure. This is the possible biophysical reason for the low frequency of TRP in the protein.

Despite there are few studies on the frequencies of amino acids in secondary structural elements of protein (1, 9), there is not any study on the frequencies of the residues in subtypes of secondary structural elements (i.e., α-, π-, 3_{10}-helix or first, parallel, anti-parallel strands of sheet structure). Therefore, residue frequencies in subtypes of secondary structural elements were determined for the first time in this study and presented in Table 2. Although the frequencies of most of the residues do not vary with subtype, it is worth discussing a few them. The frequency of PRO residue in 3_{10}-helix is 3,1 times higher than in α-helix. This remarkable increase is probably again related to its backbone hydrogen bond forming capability. 3_{10}-helix is a loose and short helix type. Its length generally spans several amino acids and backbone hydrogen bonds are formed between (n):(n+5) residues. Since the length is so short, most of the residues in 3_{10}-helix cannot form a backbone hydrogen bond due to the (n):(n+5) pattern restriction. Therefore, the reduction in the requirement of backbone hydrogen bond formation in 3_{10}-helix removes the biophysical barriers to PRO's presence in the helix and its frequency in 3_{10}-helix increases. The frequencies of ILE, LEU and VAL residues are higher in parallel strand than in first and anti-parallel strands. The frequency differences of the other residues are negligible. While the insights from the findings of this part are relatively minor, they are valuable for improving the understanding of the biophysical characteristics of the secondary structure of the protein. Besides, these findings may provide important information for secondary structure prediction algorithms. The differences in frequencies of these residues depending on subtypes can make them markers of subtypes and can be used in prediction algorithms.

5. CONCLUSION

The findings of this study are more conclusive than other studies due to the comprehensive and distinctive data set used in the study. The data set only includes globular proteins (excludes the extremophile proteins) with a similarity less than 25%. So, the results obtained from this study can eliminate the contradictions in amino acid frequencies in the scientific literature. In addition, the fact that the frequency variations of amino acids according to the subtypes of secondary structural elements were investigated for the first time in this study makes the findings of this study valuable in the field of research. Therefore, these findings may contribute the understanding of the structural features of protein secondary structure and to the improvement of the secondary structure prediction algorithms.

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Clinical and Experimental Health Sciences

The Assessment of the Association Between Systemic Diseases and Dental Findings

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ABSTRACT

Objective: In dentistry, systemic diseases are extremely important in terms of taking the required precautions for overall health and preventing complications before they emerge. Many clinicians are aware of the oral symptoms of systemic diseases; nevertheless, the major source of the problem must be addressed in order to limit the rate of misdiagnosis due to systemic origins and establish an appropriate treatment strategy. The purpose of this study is to investigate whether there is a relationship between systemic diseases and dental health.

Methods: The study included 200 individuals, 100 women and 100 men, all between the ages of 25 and 75, who had at least one systemic disease. The patients' medical records and panoramic radiography were examined. In the panoramic radiographs of the patients, the existence of existing teeth, caries, restorations, prostheses, and endodontic conditions were documented, taking into consideration the presence of systemic disorders, gender, and age of the patients.

Results: Caries rates were significantly higher in the 25-40 age group (43.3%) than in the 41-60 age group (20.7%) ($p<0.05$). There was no statistically significant difference between the incidence of caries, endodontic treatment and prosthesis according to the presence of diabetes mellitus ($p>0.05$). The prevalence of prosthesis was statistically significantly greater in patients with cardiovascular disease (64.3%) than in patients without cardiovascular disease (47.7%) ($p<0.05$).

Conclusion: According to the findings of our study, which confirm the link between oral and overall health, challenges with general health lead to several other adverse consequences, including worsening of dental health and related complications.

Keywords: Diagnosis, Oral; Dental Health Survey; Cardiovascular Diseases; Diabetes Mellitus

1. INTRODUCTION

Systemic diseases are crucial in dentistry since they allow dentists to take the necessary measures for overall health and to take measures before they occur. As a result, clinicians must evaluate the occurrence of systemic disorders, as well as the systemic and oral findings linked with these diseases, in order to limit the challenges and hazards of dental therapy (1, 2). Anamnesis is a basic element used in the pre-treatment evaluation of patients in dentistry, which is a part of human health; it allows the patient to learn about known systemic conditions, diseases that have been treated and/or are still being treated, drugs used, special diets, infectious disease risk, and conditions that require extra caution during dental treatment (2, 3). There are many systemic conditions that require modification of dental practice, such as cardiovascular diseases, renal diseases, pulmonary disorders, patients with immunosuppression, patients receiving chemotherapy-radiotherapy, endocrinal disorders, hematological disorders, infectious diseases (4).

Oral manifestations of systemic illnesses can be many. A thorough oral examination may show indicators of an underlying systemic illness, allowing for early diagnosis and treatment. Mucosal abnormalities, periodontal inflammation and bleeding, and the overall status of the teeth should all be evaluated (5). Systemic disorders can affect teeth, jaw bones, oral mucosa, and the temporomandibular joint. When the major source of the disease is a systemic origin, knowing the intraoral findings of many systemic disorders reduces the likelihood of misdiagnosis and is crucial in developing an effective treatment approach (6, 7).

Due to the obvious increase in life expectancy, the number of people seeking dental treatment for systemic disorders is on the rise. Oral and dental health is a significant issue that should not be viewed in isolation from overall health and has a direct impact on an individual's quality of life and comfort.

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There are clear links between the need for dental care and the impact of oral health on overall health. Systemic diseases, as well as systemic and oral findings linked with these diseases, should be identified in order to limit the problems and hazards that may arise during dental treatment, and measures should be taken accordingly (6-9).

Oral health is described as the absence of disease and diseases of the craniofacial complex, which includes the oral, dental, and cranial tissues. The American General Health Association's oral health report from 2000 underlined that oral health is more than just good teeth and should be examined as a component of overall health, and the link between dental and overall health has been established. In order to accomplish numerous essential human tasks including eating, tasting, speaking, kissing, and smiling, our oral health must be adequate (10). While systemic diseases can cause a variety of oral manifestations, the patient's overall health may decline in the long run as a result of the degradation of oral and dental health. The primary systemic diseases in which this association is usually recognized include cardiovascular system diseases and diabetes. Such individuals should be assessed more extensively in terms of disease progression, medicines taken, and other variables that might influence the treatment process (11, 1). According to Segura-Egea et al. (12), the total number of teeth in the mouth was lower in diabetics as a result of higher caries incidence and periodontal disease severity. Another study published in literature showed that those with cardiovascular illnesses (CVS) have tooth loss as a result of poor oral hygiene (13). Therefore, the purpose of this research is to reveal if there is a link between systemic diseases and dental results.

2. METHODS

The study protocol of this restrospective study was approved by Clinical Research Ethics Committee of Faculty of Medicine Marmara University on 02.07.2021 with protocol number 09.2021.814. The study group consisted of 100 randomly selected female and male individuals between the ages of 25 and 75 who applied to Department of Oral and Maxillofacial Radiology, Faculty of Dentistry, Marmara University for various reasons between June and December 2020 and had at least one systemic disease. It was made with the help of 200 patients. The patients' comprehensive anamnesis was reviewed, and their age, gender, and systemic disorders were noted. The patients' panoramic radiographs were scanned retrospectively. The patients' existing systemic disorders were investigated, and the frequency of the diseases was assessed.

2.1. Statistical Analysis

The IBM SPSS Statistics 22 (IBM SPSS, USA) software was used for statistical analysis for evaluating the study's findings. The parameters were tested for their fit to the normal distribution using the Kolmogorov-Smirnov and Shapiro Wilks tests, and it was established that they did not have a normal distribution. In addition to descriptive statistical methods (mean, standard deviation, frequency), Kruskal Wallis test was used for comparison of parameters across groups and Dunn's test of the group that caused the difference while analyzing the research data. For the comparison of parameters between two groups, the Mann Whitney U test was applied. Chi-Square test, Fisher's Exact Chi-Square test, Continuity (Yates) Correction and Fisher Freeman Halton Exact test were used to compare qualitative data. Significance was evaluated at the $p<0.05$ level.

3. RESULTS

The study included 200 participants (% 50 men and % 50 women) ranging in age from 25 to 75 years old (mean age 53.03±12.57 years). In the research, 30 cases (15%) were between the ages of 25 and 40, 116 cases (58%) were between the ages of 41 and 60, and 54 cases (27%) were between the ages of 61 and 75. The distribution of information on the systemic conditions of all patients is shown in Table 1.

The number of teeth in the instances varied from 0 to 32, with the mean number of teeth being 19.25 ±8.40 and the median number of teeth being 21. Caries is present in 51 instances (25.5%). The number of cavities ranges from 1 to 7, with an average of 1.47 ±0.9, and the median number of caries is 1. There are 122 persons who have received restoration (61%). The number of restored teeth varied from 1 to 16, with an average of 3.63 ±2.98, and a median of 3. Endodontic treatment was provided to 110 individuals (55%). The number of endodontically treated teeth varied from 1 to 8, with an average of 2.38 ±1.75 and a median of 2. In 114 (57 %) of the cases, a prosthesis is present. The number of teeth in the prosthesis ranges from 1 to 28, with an average of 8.36±5.61 and a median of 7 teeth (Table 2).

Women's allergy prevalence (17%) was statistically substantially greater than men's (6%) ($p <0.05$). The prevalence of thyroid-parathyroid disease in women (27%) was statistically greater than in males (9%) ($p<0.05$).

There is no statistically significant difference between men and women in terms of kidney diseases, diabetes, GIS diseases, cardiovascular system diseases, smoking-alcohol use, neurological diseases, cancer, psychiatric diseases, rheumatic diseases, liver diseases, and respiratory system diseases ($p>0.05$) (Table 3).

Diabetes mellitus (DM) incidence rates varied statistically significantly between age groups ($p<0.05$). The rate of DM (0%) was found to be considerably lower in the 25-40 age group than in the 41-60 age group (27.6 %) and 61-75 age group (33.3 %) ($p<0.05$). There was no statistically significant difference between the ages of 41-60 and 61-75 ($p>0.05$).

The incidence of cardiovascular system diseases (CVS) differs statistically significantly by age group ($p<0.05$). CVS rates were substantially lower in the 25-40 age group (23.3 %) than in the 41-60 age group (53.4 %) and 61-75 age group (79.6 %) ($p <0.05$). The 41-60 age group (53.4%) had a considerably lower rate than the 61-75 age group (79.6 %) ($p <0.05$).

Table 1. *Distribution of information on the systemic conditions of all patients*

	n	%
HEREDITARY FAMILIAL DISEASES	**4**	**2**
Familial Mediterranean Fever	4	2
ALLERGIES	**23**	**11.5**
Dental Materials Allergy	3	1.5
Medicine Allergy	12	6
Other Allergies	10	5
RENAL DISEASES	**3**	**1.5**
Chronic Kidney Disease	3	1.5
DIABETES MELLITUS	**50**	**25**
Diabetes Mellitus Type 1	0	0
Diabetes Mellitus Type 2	50	25
GIS DISEASES	**16**	**8**
Reflux	5	2.5
Gastritis	6	3
Crohn Diseaseı	2	1
Ulcer	3	1.5
CARDIOVASCULER DISEASES	**112**	**56**
Hypertension	87	43.5
Hypotension	2	1
Heart Attack	1	0.5
Bypass	6	3
Coronary Ater Disease	8	4
Coronary Infarct	1	0.5
Coronary Insufficiency	3	1.5
Stent Implantation	11	5.5
Angina Pectoris	3	1.5
Coronary Angiography	15	7.5
Arrhythmias	8	4
Hyperlipidemia	15	7.5
TOBACCO – ALCOHOL	**26**	**13**
SYNDROMES	**1**	**0.5**
Down Syndrome	1	0.5
HEMATOLOGIC DISEASES	**3**	**1.5**
Anemia	3	1.5
BLEEDING TENDENCY	**4**	**2**
CANCER	**12**	**6**
RADIOTHERAPY	**4**	**2**
CHEMOTHERAPY	**6**	**3**
ORGAN TRANSPLANTATION	**1**	**0.5**
PSYCHIATRIC DISEASES	**21**	**10.5**
AUTOIMMUNE DISEASES	**2**	**1**
Behçet Disease	2	1
LIVER DISEASES	**6**	**3**
Hepatitis	5	2.5
Cirrhosis	1	0.5
RESPIRATORY SYSTEM DISEASES	**24**	**12**
Asthma	18	9
Bronchitis	1	0.5
Chronic Obstructive Pulmonary Disease	4	2
Tbc	1	0.5
THYROID-PARATHYROID DISEASES	**36**	**18**
Goiter	4	2
Hashimoto's Thyroid	1	0.5
Hyperparathyroidism	2	1
Hyperthyroidism	6	3
Hypothyroidism	20	10
Thyroid Surgery	5	2.5

Table 2. *Distribution of dental findings of all patients*

		n (%)
Dental Caries	Present	51 (25.5)
	Absent	149 (74.5)
Restoration	Present	122 (61.0)
	Absent	78 (39.0)
Endodontic Treatment	Present	110 (55.0)
	Absent	90 (45.0)
Prothesis	Present	114 (57.0)
	Absent	86 (43.0)

Table 3. *Evaluation of diseases by gender*

	Men (n=100)	Women (n=100)	
	n (%)	n (%)	p
Allergies	6 (6)	17 (17)	0.027[1]*
Renal Disease	2 (2)	1 (1)	1.000[2]
Diabetes Mellitus	25 (25)	25 (25)	1.000[3]
GIS Diseases	6 (6)	10 (10)	0.434[1]
Cardiovasculer Diseases	57 (57)	55 (55)	0.776[3]
Tobacco-Drugs	17 (17)	9 (9)	0.141[1]
Neurological Diseases	7 (7)	3 (3)	0.330[1]
Cancer	7 (7)	5 (5)	0.766[1]
Psychiatric Diseases	9 (9)	12 (12)	0.645[1]
Rheumatic Diseases	4 (4)	7 (7)	0.535[1]
Liver Diseases	3 (3)	3 (3)	1.000[2]
Respiratory System Diseases	11 (11)	13 (13)	0.828[1]
Thyroid-Parathyroid Diseases	9 (9)	27 (27)	0.002[1]*

[1]*Continuity (yates) correction* [2]*Fisher's Exact Test* [3] *Chi-square test*
**p<0.05*

Table 4. *Evaluation of systemic diseases by age groups*

	25-40 years	41-60 years	61-75 years	
	n (%)	n (%)	n (%)	p
Allergies	5 (16.7)	12 (10.3)	6 (11.1)	0.623[1]
Renal Disease	0 (0)	2 (1.7)	1 (1.9)	1.000[2]
Diabetes Mellitus	0 (0)	32 (27.6)	18 (33.3)	0.002[1]*
GIS Diseases	0 (0)	13 (11.2)	3 (5.6)	0.110[2]
Cardiovasculer Diseases	7 (23.3)	62 (53.4)	43 (79.6)	0.000[1]*
Tobacco-Drugs	3 (10)	18 (15.5)	5 (9.3)	0.459[1]
Neurological Diseases	3 (10)	3 (2.6)	4 (7.4)	0.124[2]
Cancer	0 (0)	4 (3.4)	8 (14.8)	0.010[2]*
Psychiatric Diseases	3 (10)	15 (12.9)	3 (5.6)	0.343[1]
Rheumatic Diseases	2 (6.7)	5 (4.3)	4 (7.4)	0.610[2]
Liver Diseases	0 (0)	5 (4.3)	1 (1.9)	0.603[2]
Respiratory System Diseases	4 (13.3)	13 (11.2)	7 (13)	0.920[1]
Thyroid-Parathyroid Diseases	8 (26.7)	24 (20.7)	4 (7.4)	0.045[1]*

[1] *Chi-square test* *2Fisher's Freeman Halton Exact Test* **p<0.05*

There is a statistically significant difference in cancer incidence rates between age groups (p<0.05). Cancer rate (14.8%) in the 61-75 age group was found to be significantly higher than the 25-40 age (0%) and 41-60 age (3.4%) groups (p<0.05).

In terms of the incidence of thyroid parathyroid disease, there is a statistically significant difference between age groups (p<0.05). The thyroid ratio was found to be considerably lower in the 61-75 age group (7.4%) than in the 25-40 age group (26.7%) and the 41-60 age group (20.7%) (p<0.05).

There was no statistically significant difference between age groups in terms of the incidence of allergies, kidney diseases, GIS diseases, smoking-alcohol use, neurological diseases, psychiatric diseases, rheumatic diseases, liver diseases and respiratory system diseases (p>0.05) (Table 4).

In terms of caries incidence, there is a statistically significant difference between age groups (p<0.05). Caries rates were significantly higher in the 25-40 age group (43.3%) than in the 41-60 age group (20.7%) (p<0.05). Other age groups showed no significant differences (p>0.05).

In terms of the incidence of restorations, there is a statistically significant difference between age groups (p<0.05). The restoration rate in the 25-40 age group (93.3%) was substantially greater than the restoration rates in the 41-60 age group (59.5%) and the 61-75 age group (46.3%) (p<0.05). There was no statistically significant difference between the age groups of 41-60 and 61-75 (p>0.05). There is a statistically significant difference in the incidence of prosthesis between age groups (p<0.05).

The incidence of prosthesis usage was substantially lower in the 25-40 age group (30%) than in the 41-60 age group (60.3%) and the 61-75 age group (64.8%) (p<0.05).

Between age groups, there is a statistically significant difference in the number of teeth (p<0.05). The number of teeth in the 25-40 age group was substantially larger than the 41-60 age group and the 61-75 age group as a result of pairwise comparisons done to ascertain which age group the significance originated from (p <0.05). The 41-60 age group had a significantly higher number of teeth than the 61-75 age group (p<0.05) (Table 5).

Table 5. *Evaluation of dental findings by age and gender*

	Gender			Age			
	Male	Female	p	25-40	41-60	61-75	p
	N (%)	N (%)		N (%)	N (%)	N (%)	
Dental Caries n (%)	29 (29)	22 (22)	0.256[1]	13 (43.3)	24 (20.7)	14 (25.9)	0.040[1]*
Restoration n (%)	59 (59)	63 (63)	0.562[1]	28 (93.3)	69 (59.5)	25 (46.3)	0.000[1]*
Endodontic Treatment n (%)	51 (51)	59 (59)	0.256[1]	20 (66.7)	63 (54.3)	27 (50)	0.330[1]
Prothesis n (%)	54 (54)	60 (60)	0.391[1]	9 (30)	70 (60.3)	35 (64.8)	0.005[1]*
Tooth number Mean ±SD (median)	19.5±8.5 (21)	19.0±8.4 (21)	0.755[2]	26.4±3.6 (27)	19.8±7.6 (21)	14.0±8.6 (17)	0.000[3]*

[1] *Chi-square test* [2]*Mann Whitney U Test* [3]*Kruskal Wallis Test SD: standard deviation* *p<0.05*

Tablo 6. *Evaluation of dental findings according to the presence of diabetes mellitus and cardiovascular diseases*

	Diabetes Mellitus Cardiovascular Diseases					
	None (%)	Present (%)	p	None (%)	Present (%)	p
Dental Caries n (%)	39 (26)	12 (24)	0.925[1]	48 (24.4)	29 (25.9)	0.886[2]
Restoration n (%)	98 (65.3)	24 (48)	0.030[2]*	121 (61.4)	62 (55.4)	0.065[2]
Endodontic treatment n (%)	86 (57.3)	24 (48)	0.251[2]	110 (55.8)	58 (51.8)	0.303[2]
Prothesis n (%)	91 (60.7)	23 (46)	0.070[1]	112 (56.9)	72 (64.3)	0.019[2]*
Tooth Number Mean±SD (median)	19.9±8.2 (22)	17.1±8.8 (18)	0.036[3]*	19.2±8.4 (21)	17.9±8.4 (19)	0.006[3]*

[1]*Continuity (yates) correction* [2] *Chi-square test* [3]*Mann Whitney U Test SD: standard deviation*p<0.05*

The incidence of restoration in patients with diabetes (48%) was statistically significantly lower than in patients without diabetes (65.3%) ($p<0.05$) There was no statistically significant difference between the incidence of caries, endodontic treatment and prosthesis according to the presence of diabetes mellitus ($p>0.05$). The number of teeth of patients with diabetes was statistically significantly lower than those without diabetes ($p<0.05$) (Table 6). The prevalence of prosthesis was statistically significantly greater in patients with CVS disease (64.3%) than in patients without CVS disease (47.7%) ($p<0.05$). There was no statistically significant difference in the incidence of caries, restorations, or endodontic therapy when CVS disease was present ($p>0.05$). Patients with CVS disorders had significantly fewer teeth than those without ($p<0.05$) (Table 6). According to the existence of cancer, there was no statistically significant difference in the incidence of caries, restoration, endodontic treatment, and prosthesis ($p>0.05$). The number of teeth in cancer patients was statistically significantly less than in cancer-free cases ($p<0.05$**)**.

4. DISCUSSION

Systemic diseases are significant in dentistry practice because they require taking care for overall health and preventing issues before they emerge. To reduce the problems and risks that may develop during dental treatment, the incidence of systemic diseases, as well as systemic and oral findings connected to these diseases, should be recognized and precautions taken accordingly (3,5-7).

First and foremost, the anamnesis of the patients in our research was assessed, and their present systemic diseases were noted. According to the data, the most prevalent systemic illness group in our study was cardiovascular diseases, with a rate of 56%, while the rate of hypertension in this disease group was 43.5 %, and it was the most common systemic disease. In some studies, hypertension was examined within the context of cardiovascular illnesses, but in others, cardiovascular diseases and hypertension were evaluated as different disease groups, which influenced the incidence values (7-8). Despite conflicting findings in the literature, hypertension is the most frequent systemic disease. The most frequent systemic disease was found to be hypertension in the research of Maryam et al. (14), Şener et al. (8), Al-Bayaty et al. (3), and Hatipoğlu et al. (15). Diabetes mellitus was the second most prevalent systemic disease group in our analysis, while it was a less common condition in Kömerik et al. study's (16). Similar to our findings, Canger et al. (1) and Şener et al. (8) discovered it to be the second most prevalent systemic illness category. In diabetic patients, the dentist should evaluate local endothelial dysfunction and the risk of ischemia at the microvascular level, as well as the existence of atherosclerosis at the macrovascular level. According to Onat et al. (17)'s research of 3401 participants, diabetes mellitus affects roughly 11% of people aged 35 and older in Turkey, and it was more common in women. Diabetes mellitus was found in both men and women in our study, despite the fact that it was more frequent in the middle-aged group.

In our study, 11.5 % had allergies to any chemical, with women having the highest proportion. However, 5% of allergy sufferers did not specify which allergens they are sensitive to. Despite the fact that the amount of allergy patients varies among research, most publications have concentrated on medication allergies. While the rate of medication allergy was 7% in Radfar et al. (2)'s trial, it was only 6% in ours.

In our research, gastrointestinal system diseases were discovered in 8% of the participants. These are conditions that, depending on circumstances such as food choices and stress, can induce subjective problems at various degrees and necessitate medical intervention. The physician must be informed about the pharmacological effects of the active drug, especially when prescribing, in the presence of various pathologies, which do not generally require emergency intervention during dental treatment but might create difficulties.

In panoramic radiography, tooth groups can be seen as lower-upper, right-left, and anterior-posterior at the same time. According to Ahlqvist et al. (18), the use of panoramic radiography in dental epidemiological studies is reliable. Furthermore, using new panoramic systems, high-quality images may be acquired, and the evaluation of the tooth and periapical status in panoramic radiography is accurate. Panoramic radiographs were used for this research because of their consistency and capacity to analyze all regions of the mouth at once.

Oral and dental health is a significant aspect that has a direct impact on an individual's quality of life and comfort, and it should not be viewed in isolation from overall health. The necessity for dental care and the impact of oral health on overall health were shown to have significant correlations. Periodontal therapy is one of the most often required dental treatments, according to the findings of studies on the issue (19,20). We were unable to fully examine the periodontal tissues of the patients because our study was retrospective, thus we have no findings linked to the patients' periodontal problems. The most prevalent causes of tooth loss are dental caries and periodontal diseases. We believe that tooth loss caused by periodontal disease will necessitate prosthetic therapy, which will, in turn, have an unfavorable effect on the patient's overall health, although indirectly.

Caries become less common as you become older. The aging of the enamel, its maturation, and the removal of retention sites as a result of wear on the teeth, as well as the incapacity of bacterial plaques to form readily, are all factors that contribute to the inverse relationship between age and caries. The group of 61-75 years old had the lowest rate of endodontically treated teeth in our study. In light of this finding, it's worth noting that the number of teeth lost owing to caries and periodontal disorders in patients aged 60 and older was greater in past periods than in patients in the younger age groups.

It is self-evident that the patient's current tooth deficit will have a negative impact on his or her health. Since the patient is unable to properly perform chewing activities owing to a loss of teeth, the oral area, where digestion begins, will fail to accomplish this function, and the patient's overall health will suffer in the long term. We believe that tooth loss will necessitate the use of prosthetics.

The majority of diabetics experience dry mouth. The most significant impact of dry mouth is that it promotes the production of caries, burning in the mouth, loss of taste perception, and loss of tongue papillae. Despite the fact that several research have been conducted on the link between dental caries and diabetes, the topic remains contentious. Higher caries incidence in diabetic individuals; increased glucose in the gingival groove fluid and saliva, as well as a reduced salivary flow rate, have been linked to poor metabolic control in diabetic patients (21). There was no statistically significant difference in the number of decaying teeth in diabetic individuals, according to the findings of our study. Our results are likewise consistent with the findings of this investigation and support them. Marotta et al. (22), on the other hand, found no statistically significant difference between the average number of teeth in the mouths of diabetes patients and the control group.

Individuals with CVS illness have a higher than usual incidence of prosthesis, owing to the requirement for prosthesis due to the lesser number of teeth. In a research by Nawaz et al. (13), tooth loss owing to poor oral hygiene was detected in persons with CVS illness, and they concluded that compromised oral hygiene might worsen CVS further. Vanderlei et al. (23), on the other hand, associated this disease with tooth loss, in which high blood pressure caused by hypertension causes alveolar bone loss.

One of the limitations of the study is its small sample size. The findings and conclusions of the present study are exploratory, and further studies with larger sample sizes are needed to elucidate this topic. The purpose of this study was to determine the frequency of systemic diseases that dentists may encounter in patients, as well as the dental findings associated with these systemic diseases. It revealed the importance of the clinician recognizing systemic diseases, determining the oral and dental findings associated with these diseases, and identifying possible risk factors.

5. CONCLUSION

While systemic problems can express themselves in a number of ways in the mouth, the patient's overall health may suffer in the long term as a result of poor oral and dental health. Patients with diabetes have fewer teeth than those without diabetes, and the incidence of caries in patients with renal disease is higher than in patients without renal disease, according to our study. In order to avoid potential complications, it is critical that the systemic diseases of patients who visit the dentist have been under control. In the dentist clinic, blood pressure, blood sugar, and pulse levels may be measured quickly and easily. Abnormalities in these readings might also be a clinical indication of systemic disorders Patients who do not have regular medical check-ups or who do not take their prescriptions on a regular basis should have their dental procedures postponed, and the necessary medical doctor should be consulted before proceeding.

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Clinical and Experimental Health Sciences

Evaluation of Residual Root Canal Sealer Removal Efficacy of Different Irrigation Activation Techniques by Confocal Laser Microscopy Analysis

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ABSTRACT

Objective: The purpose of this study was to use confocal laser microscopy analysis to evaluate the effectiveness of conventional needle irrigation (CNI), EndoActivator (EA), and EDDY during endodontic retreatment.

Methods: This study included 45 maxillary incisor teeth with a single root and canal. Root canals were prepared with ProTaper Universal files (Dentsply Sirona, Ballaigues, Switzerland) and obturated with labeled sealer mixed with 0.1% Rhodamine B and gutta percha according to single cone techniques. Initial root canal filling material was removed using ProTaper Universal Retreatment files and F4 files. Teeth randomly were divided into 3 groups (n = 15) depending on the activation technique: CNI, EA (Dentsply, Tulsa Dental Specialties, Tulsa, OK), and EDDY (VDW, Munich, Germany). Confocal laser microscopy was used to evaluate the penetration area, depth, and percentage of the residual sealer in the apical, middle, and coronal sections after irrigation activation.

Results: In all sections, the EDDY group had a lower penetration area of residual sealer than the CNI group (P< .05). In comparison to the coronal section, the penetration percentage of the CNI and EA groups was lower in the apical section (P< .05). In the CNI group, the penetration depth was higher at the coronal section than at the apical and middle sections (P< .05), and it was higher at the coronal section than at the apical section.

Conclusion: Within the limitations of this study, none of the activation systems tested could completely remove the residual sealer. However, the lowest residual sealer was seen after using EA and EDDY.

Keywords: Confocal laser microscopy, EDDY, EndoActivator, residual sealer

1. INTRODUCTION

Inadequate disinfection and obturation of root canals may adversely affect the prognosis and lead to the failure of endodontic treatment (1). In cases where endodontic treatment fails, usually non-surgical retreatment is preferred as the first treatment option (2). The purpose of the retreatment procedure is to recover the health of the periapical tissues by disinfection, shaping, and obturation of the root canals after the complete elimination of residual filling material (3). It has been reported that if previous root canal filling material is not completely removed by the retreatment procedure, periapical inflammation and destruction of surrounding tissues may occur or may persist when present (4).

Irrigation of root canals is an essential part of endodontics (5). Because of the complex anatomy of the root canal, it is recommended to use various activation methods and devices to allow the irrigation solution to contact more surfaces of the canal and to increase antimicrobial activity (6). Although conventional needle irrigation (CNI) is a widely used irrigation technique, the irrigation solution cannot reach the 0-1.1 mm of the needle tip in this technique (7-9). Different irrigation systems have been developed to overcome this disadvantage. EndoActivator (EA; Dentsply, Tulsa Dental Specialties, Tulsa, OK) is a sonic device that uses 2-3 kHz frequencies for the activation of irrigation solutions. Irrigation solutions activated by this device have been reported to reach the root canal system with hydrodynamic

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activation, as well as morphological irregularities such as the lateral canal and apical delta (10). EA is a portable and cordless device with a battery-operated hand motor and a disposable flexible polymer tip of 3 different sizes (15/02, 25/04, and 35/04) (8, 11). EDDY (VDW, Munich, Germany) has been recently introduced and is another sonic device that has a tip made of flexible polyamide that is activated using an air-driven handpiece (5000-6000 Hz) (12).

Compared to scanning electron microscopy (SEM) and histological methods, the confocal laser scanning microscope (CLSM) has the benefit of providing detailed information on root canal sealer penetration under low magnification, such as X50-X100, with the use of a marker fluorescent dye (13). Root canal sealers marked with dyes, such as rhodamine, allow the sealer to be stimulated by certain wavelengths under the CLSM and transformed into a visible spectrum. Thus, the topographic characteristics of the root canal sealer can be evaluated (14). In addition, analyzing the samples at different depths and in a three-dimensional form makes CLSM more sensitive to intratubular penetration measurement than SEM (14). This study aimed to assess the effectiveness of CNI, EA, and EDDY in removing residual sealer using CLSM.

2. METHODS

The design of this study was approved by the Bolu Abant Izzet Baysal University Clinical Researches Ethics Committee (No: 2019/55 – date: 11.04.2019). Based on a previous study (13), the sample size was determined (G*Power 3.1 software; Heinrich Heine University, Dusseldorf, Germany). Forty-five maxillary incisor teeth with a single root/ canal that were extracted for orthodontic and periodontal reasons were included in this study. For each tooth, periapical radiographs were obtained from buccolingual and mesiodistal directions, and root canal anatomy was evaluated. Teeth completed root canal development and without calcification, fractures, resorption, and curvature were included. The teeth were stored at 4 °C in distilled water.

The access cavities were created using diamond round burs (Dentsply Maillefer, Ballaigues, Switzerland). The working length (WL) of the canals was determined to be 1 mm shorter than the length at which the # 10 K-file (Dentsply Maillefer) emerges from the apical foramen. The apical patency was controlled using the # 15 K-file (Dentsply Maillefer). Root canal preparation was carried out using ProTaper Universal (Dentsply Maillefer) rotary files up to size F3. The root canals were irrigated with 2 ml of 2.5% NaOCl (CanalPro; Coltene-Whaledent, Allstetten, Switzerland) after each file. After the preparation, the canals were irrigated with 10 ml 17% EDTA (CanalPro) and 10 ml 2.5% NaOCl, respectively, and dried with a paper point. Root canal sealer was given a 0.1% Rhodamine B addition for the CLSM examination (Batch 121K3688, RITC/Rhodamine B R6626 Sigma, St. Louis, MO, USA) (Dentsply De-Trey, Konstanz, Germany). Using AH Plus root canal sealer with 0.1% Rhodamine B added and ProTaper Universal F3 gutta-percha (Dentsply Maillefer), the root canals were filled using the single cone method. For 7 days, the teeth were maintained at 37 °C with 100% humidity to allow the root canal sealer to set. All teeth were retreatment with ProTaper Universal Retreatment Files (D1, D2, and D3) and a low-torque motor (VDW Silver; VDW) using the crown-down technique. In the cervical, middle, and apical sections, respectively, D1 (size 30/.09, 550 rpm, 200 g/cm torque), D2 (size 25/.08, 550 rpm, 200 g/cm torque), and D3 (size 20/.07 taper, 250 rpm, 150 g/cm torque) files were used. Root canals were irrigated with 2 ml of 2.5% NaOCl at each file change. The ProTaper Universal F4 file was used for the final apical preparation at WL. Each file was used in only three canals and then excluded from the study. Following the retreatment procedure, the samples were randomly divided 3 groups (n = 15) according to the final irrigation activation method:

CNI: The 30-gauge double-sided needle (Fanta Dental, Shanghai, China) was inserted into the canal at a length 1 mm shorter than the WL. The irrigation needle was moved back and forth, and the root canals were irrigated with 2.5% NaOCl in a 3 ml volume for 90 sec.

EA: In this group, the root canals were irrigated in 3 cycles with a 3 ml volume of 2.5% NaOCl irrigation solution for 30 sec (1 ml per 30 sec) at a 2 ml/min flow rate. After each irrigation cycle, a flexible polymer tip (25/.04) was inserted into the canal at a distance of 1 mm shorter than the WL and activated at 1000 rpm for 30 sec. with 2-3 mm vertical strokes.

EDDY: The irrigation activation procedure was the same as in the EA group. After each irrigation cycle, the polyamide tip (25/.04) was adapted to TA-200 (Micron, Tokyo, Japan) and activated at 6000 Hz. The EDDY tip was placed in the canal to be 1 mm shorter than the WL and activated for 30 sec.

1 mm thick, 3 horizontal sections were obtained from all teeth at 2, 4, and 6 mm levels under continuous water cooling using the microtome with a 0.3 mm diamond disc (Isomet, Buehler Ltd. Illinois, IL, USA) at 200 rpm. Each section was examined with CSLM (Fig. 1), and 3 parameters, including maximum penetration depth, maximum penetration area, and maximum penetration percentage, were calculated by using Image J (version 1.41; National Institutes of Health, Bethesda, MD) on images.

2.1. Statistical Analysis

The obtained data were analyzed with the statistical software package SPSS version 20 (IBM Corp., New York, NY, USA). Equality of variance and normal distribution were analyzed using the Shapiro-Wilk test. A one-way ANOVA and post-hoc Tukey's tests were used to analyze the data. The level of significance was set at $P< .05$.

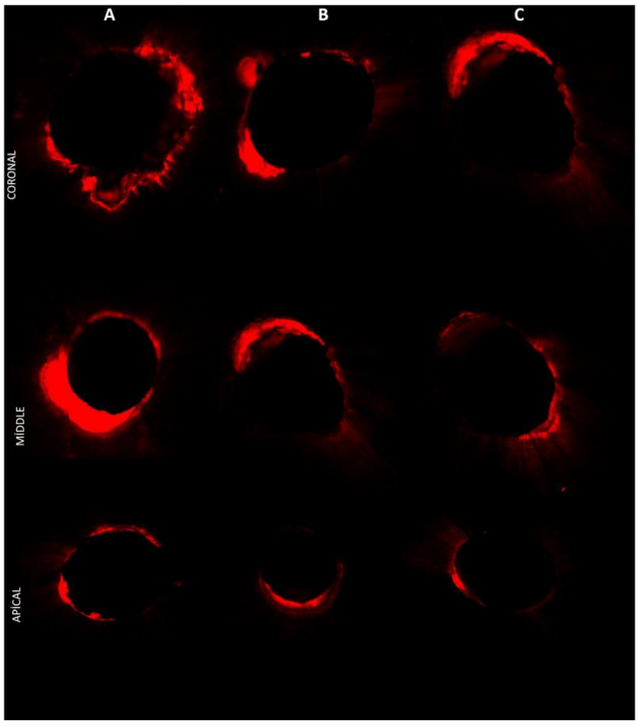

Figure 1. *CLSM images representative of residual sealer penetration in apical, middle, and coronal regions after irrigation activation. A; CNI group, B; EA group, C; EDDY group.*

3. RESULTS

The penetration area, depth, and percentage values of the residual root canal sealer in dentin tubules after retreatment are shown in Tables 1, 2, and 3. No difference was observed between the apical, middle, and coronal sections in all three groups in terms of the penetration areas (P> .05). The penetration area of residual sealer was lower in the EDDY group than in the CNI group at all sections (P< .05). There was no difference between EA and CNI, EA and EDDY groups in terms of penetration area in all sections (P> .05).

Table 1. *Mean penetration area (mm^2) and standard deviation of the residual sealer in each group after endodontic retreatment.*

	Apical	Middle	Coronal
CNI	3723.72±1423.54 ^{Aa}	4694.87±1065.47 ^{Aa}	5774.50±1800.34 ^{Aa}
EA	2474.68 ± 2192.56 ^{ABa}	2886.41±1943.78 ^{ABa}	3771.78±2263.92 ^{ABa}
EDDY	1102.37±8064.69 ^{Ba}	2049.65±1383.18 ^{Ba}	3168.09±1966.26 ^{Ba}

In the same column, different superscript uppercase letters indicate a statistically significant difference (P< .05).
In the same row, different superscript lowercase letters denote statistically significant difference (P< .05).

In the CNI group, the penetration depth of the residual sealer was higher at the coronal section than at the apical and middle sections (P< .05). In the EA group, there was no difference in the penetration depth of the residual sealer between all sections (P> .05). In the EDDY group, there was a significant difference between the apical section and the coronal section (P< .05). At all sections, the penetration depth of the residual sealer was statistically higher (P< .05) in the CNI group than in the EA and EDDY groups, but there was no difference between the EDDY and EA groups (P> .05).

Table 2. *Mean penetration depth (mm) and standard deviation of the residual sealer in each group after endodontic retreatment.*

	Apical	Middle	Coronal
CNI	0.489± 0.026 ^{Aa}	0.563±0.019 ^{Aa}	0.738±0.013 ^{Ab}
EA	0.289±0.080 ^{Ba}	0.299±0.011 ^{Ba}	0.256±0.080 ^{Ba}
EDDY	0.129±0.040 ^{Ba}	0.234±0.056 ^{Bab}	0.306±0.020 ^{Bb}

In the same column, different superscript uppercase letters indicate a statistically significant difference (P< .05).
In the same row, different superscript lowercase letters denote statistically significant difference (P< .05).

In the CNI group, the penetration percentage of the residual sealer was significantly lower at the apical section when compared to the coronal section (P< .05). In the EA group, the percentage of penetration at the apical section was significantly low than compared to the coronal section (P< .05). No significant differences were observed between the sections in the EDDY group in terms of the percentage of residual sealer. (P> .05). In all sections, the penetration percentage of the residual sealer was significantly higher (P< .05) in the CNI group compared to the EA and EDDY group, but there was no significant difference between the EDDY and EA groups (P> .05).

Table 3. *Percentage of residual sealer (%) by section in each group after endodontic retreatment.*

	Apical	Middle	Coronal
CNI	19.4±6.02 ^{Aa}	23.8±5.49 ^{Aab}	28.40±3.35 ^{Ab}
EA	11.46±4.37 ^{Ba}	15.06±4.47 ^{Bab}	17.53±4.42 ^{Bb}
EDDY	10.93±2.63 ^{Ba}	11.40±4.32 ^{Ba}	13.86±3.64 ^{Ba}

In the same column, different superscript uppercase letters indicate a statistically significant difference (P< .05).
In the same row, different superscript lowercase letters denote statistically significant difference (P< .05).

4. DISCUSSION

CLSM is an evaluation method that does not require a special procedure for the preparation of samples, such as SEM, and gives quantitative information. Fluorescent dyes are commonly used in CLSM analysis. In this study, Rhodamine B was used at a concentration of 0.1%. Studies in the literature have reported that low-dose Rhodamine B does not affect the properties of the paste (1-3).

In order to remove the residual sealer during the retreatment process, the preparation process should be performed as much as possible with larger files than the initial treatment. However, over instrumentation of the root canals should be avoided in this preparation process (2, 3). In view of this situation, in the present study, the file with a large size (F4)

from the initial preparation size (F3) was used for the final preparation of the retreatment.

In some studies, it was stated that the use of additional irrigation activation methods (sonic and ultrasonic) did not increase the removal of residual root canal filling materials significantly (15, 16). However, in other studies, it has been stated that passive ultrasonic irrigation provides more clean root canal walls and significantly reduces the amount of residual filling material (7, 17, 18). Grischke et al. (19) reported that passive ultrasonic irrigation (PUI) was more successful than EA, RinsEndo, and CanalBrush activation systems, and syringe irrigation in the removal of residual root canal filling materials. Also in the study, it was reported that EA was more effective than CanalBrush and had similar efficacy with manual irrigation. In the current study, the penetration depth and percentage of residual sealer were significantly lower in the EA and EDDY groups than the CNI group. In terms of the penetration area of residual sealer, only EDDY was found to be significantly superior to CNI. No significant difference was found between the EDDY and EA activation systems in all three parameters. Similar to the present study, Ugur Aydın et al. (20) compared the effect of EDDY, PUI, and CNI on the penetration of root canal sealer and reported that EDDY was superior to CNI in the apical part. However, Urban et al. (12) reported no difference between EDDY, EA, and PUI in removing debris and the smear layer. Differences between study results may be due to methodological differences, including activation system, activation time, sealer, and filling technique.

Although it was not statistically significant in our study, EDDY generally showed lower residual sealer penetration values than EA. In addition, in terms of the penetration area of the sealer, it was observed that EDDY was statistically more effective than CNI and that there was no significant difference between EA and CNI. Urban et al. (12) showed that PUI and EDDY were more effective in removing debris and the smear layer from the root canal compared to manual irrigation, and there was no difference between EA and manual irrigation. Researchers reported (12) that this result could be attributed to acoustic streaming and cavitation. The EA acts at 10000 cpm, or approximately 167 Hz, whereas the EDDY acts at 6000 Hz. As a result, it may be assumed that the irrigant flow rate will be significantly reduced and that the sonic instrument oscillation patterns may be different.

In the present study, while there was no difference in the penetration depth of the residual sealer between all sections in the EA group, the percentage of penetration was found to be significantly lower in the apical section compared to the coronal section. We think that the reason for this inconsistency is that the penetration percentage is more significant since the penetration depth of the residual sealer is measured only from a certain point. This is a limitation due to the nature of this study.

In the results of our study, there was no difference among the sections in any group in terms of the penetration area of residual sealer. In the groups where the difference between the sections was determined in terms of penetration depth and percentage, it was observed that sealer penetration of the apical section was mostly less than the coronal section. This may be due to low initial sealer penetration in the apical region. Because the number and length of the dentin tubules in the apical section is usually less than the coronal section. In addition, due to the occurrence of more tubular sclerosis in the apical section, penetration of the sealer during root canal filling is less in the apical section than in the middle and coronal sections (21, 22).

5. CONCLUSION

In the present study, none of the irrigation methods was able to completely eliminate the residual root canal sealer after retreatment. However, since EDDY and EA were more successful than traditional irrigation methods, they can be used as an activating method to effectively remove residual sealer for endodontic retreatment.

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Clinical and Experimental Health Sciences

Determinants of Self-Rated Health for Adults in Türkiye

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ABSTRACT

Objective: Self-rated health as an important health outcome is affected by several factors. It is of great importance to investigate the determinants of self-rated health of individuals in order to obtain better results regarding public health. The purpose of this study was to determine the factors that affect the self-rated health of adults in Türkiye.

Methods: Logistic regression analysis was performed to analyze data from the TurkStat's 2014 Health Survey, with 19,129 people. The independent variables were related to socio-economic characteristics, health problems, lifestyle, and utilisation of healthcare services, while the dependent variable was self-rated health.

Results: It was found that younger people, men, and people with higher educational and income levels rated their health status better. The health status of individuals with chronic diseases, mental disorders, sleeping problems and those who did not have a normal range body mass index also rated their health status as poor. However, the self-rated health of people who had no inpatient treatment in the last 12 months and those who took no prescription medicine in the last two weeks was good.

Conclusion: This study provided the identification of the most advantaged and disadvantaged groups through determining the factors affecting the health status of adults in Türkiye. To improve the unfavourable condition of disadvantaged individuals, more specific interventions need to be designed and implemented.

Keywords: Self-rated health, health status, health inequalities, Türkiye

1. INTRODUCTION

The advances in technology and medicine occurring recently have led to considerable improvement in the quality of health of societies across the world. However, socio-economic inequalities in the field of healthcare still persist in some segments (1). Analysing socio-economic status and self-rated health (SRH) together is often a strategy used to evaluate these inequalities (2). Besides being a determinant of morbidity and mortality, the notion of SRH is also a subjective and single-item health assessment scale that is commonly utilised in epidemiological studies all over the world (1-8).

Scales including simple questions have been used since the 1950s in sociological studies to provide indications in assessing the health status of people. Researchers have observed that the scale of SRH has provided better indications than objective health indicators, such as diagnosis by a physician or a biological specimen analysis (9-11). Besides being a tool that makes it possible to collect data in a simple and cost-effective way, the SRH provides an inclusive picture of one's health condition and is acknowledged as an important indicator in itself (12). SRH has been integrated as an indicator into the programme 'Health for All' designed by the World Health Organization; it is also a part of the SF-36 survey used in studies on health (8).

SRH as an important health outcome that is affected by several factors (13). It is of great importance to investigate the determinants of SRH of individuals in order to obtain better results regarding the health status of people (14). International research has shown that self-rated health can vary depending on one's socio-demographic characteristics, health problems, lifestyle, and utilisation of healthcare services (5,13,15,16).

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While developed countries widely make use of SRH to research trends and inequalities in public health, limited research is undertaken on this subject in developing countries (1,17-19), such as Türkiye. Identifying disadvantaged groups through research on factors affecting the health status of the population can greatly improve the general health status of society in Türkiye. The purpose of this study was to determine the factors that affect the SRH of the adult population in Türkiye. In this context, the study aimed to provide insight into whether factors such as socio-demographic characteristics, health problems, lifestyle, and utilisation of healthcare services have an effect on the SRH of individuals.

2. METHODS

2.1. Study Design, Sources of Data and Participants

This study used the dataset of the 'Health Survey 2014' that has been regularly undertaken by the Turkish Statistical Institute (TurkStat) every two years since 2008 in order to paint a general health portrait of the population in the country. Micro datasets were obtained with official permission from TurkStat. These studies are cross-sectional and data are collected based on reporting. In health researches, a sample representing Türkiye is formed by cluster sampling in the first stage and systematic sampling in the second stage. In this survey, where researchers recruited 26.075 people representing the overall population in the country, different questionnaire forms specifically designed for households, adults, and children were used. In line with the purpose of this study, 19.129 adults over 15 years of age were included in the study. This study was carried out over existing data and it does not require any human/animal subjects to acquire an ethics approval.

2.2. Variables of the Study

2.2.1. Dependent Variable

The evaluation of SRH, which is the dependent variable of this study, is based on the responses to the question 'How would you describe your general health condition?' A 5-point Likert type scale with responses scored from 1=Very good to 5=Very poor was used to elicit the responses. The SRH variable was used to form two groups, in line with similar studies available in the literature (1,5), by combining the categories of 'very good', 'good', and 'moderate' into a group with the title 'good health status' and the categories of 'poor' and 'very poor' into another one entitled 'poor health status'.

2.2.2. Independent Variables

The variables of age (1,14,16,20), gender (1,14,16,20), educational level (14,16,20), and income level (1,14,16,20) were identified in the relevant literature as variables believed to affect the health status of people; these were integrated into the present study as socio-demographic variables. The variable of age was evaluated in seven categories, and variables of educational level and income level in four categories.

The variables evaluated in the category of health problems were chronic diseases (5,13,15-17,21) and mental disorders (1,5). Individuals who expressed having suffered from one of the 19 chronic diseases in the last 12 months were categorised as 'having' a chronic disease, and the individuals who expressed that they were low-spirited, depressed, and desperate, or felt themselves as worthless and bad were categorised as 'having' a mental disorder.

The category of lifestyle addressed the variables sleep problems (5,13) and body mass index (BMI) (1,2,5,17). The variable of sleep problem was evaluated on the basis of the question 'Did you have difficulties falling asleep or in sleeping/ the problem of excessive sleepiness in the last two weeks?'. BMI, on the other hand, was examined on the basis of the calculations of body height and weight of the participants in four categories as underweight (<18.49 kg/m), normal range (18.5-24.99 kg/m), overweight (25-29.99 kg/m), and obese (>30 kg/m).

Last, the variable of utilisation of health services included hospitalisation (5,21,22) and utilisation of a prescription drug (5). The response given to the questions 'Have you been hospitalised at least once in the last 12 months?' was used to analyse the variable of hospitalisation, and that given to the question 'Have you taken any drug prescribed to you in the last two weeks?' for the analysis of the variable of prescription drug utilisation.

2.3. Statistical Analysis

For the analysis of the study data, besides descriptive statistics, simple and multiple (backward stepwise) logistic regression analysis was used to examine the determinants of health status. Simple logistic regression analysis was used to determine the variables to be included in the multiple regression analysis. Before using logistic regression analysis, the goodness-of-fit test of Hosmer – Lemeshow was utilised. The package of SPSS 21.0 was used for the statistical analysis, with an alpha level of 0.05 for statistical tests.

3. RESULTS

Table 1 presents the descriptive statistics for the study participants, according to which 56.5% of the participants were under 45 years of age, 54.4% were women, 53.9% were primary or secondary school graduates, and 51% had an income between 0-1550 Turkish Lira (TL). Regarding health problems, 62.4% expressed having a chronic disease, 48.7% a mental disorder, and 36.4% a sleep problem. With respect to calculated body mass index, 39.9% of the participants were categorised in the group with normal range BMI. The evaluation with respect to the utilisation of health services showed that 12.2% of the participants had been hospitalised at least once in the last 12 months, and that 37.2% had taken a drug prescribed by a physician in the last two weeks. To the

question about their overall SRH, 58.3% reported having a good health status.

Table 1. *Descriptive Statistics*

Variables		*n*	%
Age	*15-24*	3388	17.7
	25-34	3661	19.1
	35-44	3768	19.7
	45-54	3332	17.4
	55-64	2555	13.4
	65-74	1498	7.8
	75+	927	4.8
Gender	*Women*	10408	54.4
	Men	8721	45.6
Educational Level	*No Education*	2849	14.9
	Primary School	10317	53.9
	High School and/ or Two-Year Degree	4247	22.2
	Undergraduate and/or Graduate Degree	1716	9.0
Income Level	*0-1550 TL**	9753	51.0
	1551-2170 TL	3115	16.3
	2171-3180 TL	3274	17.1
	≥ 3181 TL	2987	15.6
Chronic Disease	*Have*	11936	62.4
	Not Have	7193	37.6
Mental Disorder	*Have*	9307	48.7
	Not Have	9822	51.3
Sleep Problem	*Have*	6957	36.4
	Not Have	12172	63.6
Body Mass Index	*Underweight*	734	3.8
	Normal Range	7635	39.9
	Overweight	6632	34.7
	Obese	4128	21.6
Hospitalisation	*Yes*	2332	12.2
	No	16797	87.8
Utilisation of Prescription Drug	*Yes*	7125	37.2
	No	12004	62.8
Health Status	*Good*	11157	58.3
	Bad	7972	41.7
Total		**19129**	**100**

** TL= Turkish Lira*

Table 2 shows the results of the simple and multiple logistic regression analyses with the variables that affect the SRH of adults. The results of the simple logistic regression analysis demonstrated that there was a statistically significant relationship between all independent variables and SRH. The sufficiency and goodness-for-fit of the multiple logistic regression model created according to the results of the logistic regression analysis showed that the model has an explanatory power of 0.454 (Nagelkerke R^2). The Hosmer-Lemeshow statistics indicated that the model fits the data ($p \geq .05$), with a model classification accuracy percentage of 77.5%.

According to the results of the multiple logistic regression analysis, men, younger people, those with higher levels of education and income, people having no chronic diseases, mental disorders and sleep problems, those with normal range BMI, people who had not been hospitalised in the last 12 months, and those who had not taken a drug prescribed by a physician in the last two weeks rated their health status as good. The analysis shows that when compared with participants over 75 years of age, participants in the age group of 15-24 years had 6.89 times better health status. The same rate was found to be 3.68 in the age group of 25-34 years; 2.41 in the age group of 35-44 years; 1.69 in the age group of 45-54 years; and 1.45 in the age group of 55-64 years compared with those over 75 years. Women had 1.23 times better health status than men. Examining the results broken down by educational level, individuals with higher educational levels had better SRH (Table 2).

Similarly, when compared with participants who had received no education, primary or secondary school graduates, and high school graduates and/or two-year degree, participants who had under – or postgraduate degree were respectively found to have 3.03 times (1/0.33), 2.08 (1/0.48) times, and 1.43 times (1/0.70) better SRH. Examining the results regarding income levels, higher income was linked to better self-rated health. In this respect, the comparison between participants with an income over 3181 TL and those having a lower income level showed that the participants with an income over 3181 TL had respectively 1.52 (1/0.66) times, 1.25 (1/0.80) times, and 1.11 (1/0.90) times better SRH than the participants with an income lower than 1550 TL, an income between 1551 and 2170 TL, and an income between 2171 and 3180 TL (Table 2).

Table 2 also presents the results for the variables related to health problems, lifestyle, and utilisation of health services. The results show that participants who expressed having no chronic diseases had 4.06 times better health status than those who had one; those who have no mental disorder has 1.77 times better health status than those who suffered from mental disorders; and those who had no sleep problem, has 1.73 times better health status than those who suffer from such a problem. The results concerning BMI showed that participants had normal range BMI and overweight BMI had 1.31 times and 1.28 times better health status respectively, when compared to the group with obese BMI. Another result indicates that participants who were not hospitalised once in the last 12 months had 1.67 (1/0.60) times better health status than those who were, and those who did not take a prescription drug in the last two weeks had 2.13 (1/0.47) times better health status than the participants who took one.

Table 2. *Determinants of Self Rated Health: Results of Simple and Multiple Logistic Regression Analysis*

Variables		Model 1: Univariate Analysis			Model 2: Multivariate Analysis		
		β (SE)	OR	%95 CI	*β (SE)*	OR	%95 CI
Socio-Demographic Characteristic	**Age**						
	15-24	3.24 (0.10)	25.40*	21.02-30.69	1.93 (0.11)	6.89*	5.53-8.56
	25-34	2.52 (0.10)	12.39*	10.36-14.81	1.30 (0.11)	3.68*	3.00-4.52
	35-44	1.91 (0.09)	6.73*	5.65-8.02	0.88 (0.10)	2.41*	1.97-2.95
	45-54	1.32 (0.09)	3.75*	3.15-4.48	0.53 (0.10)	1.69*	1.38-2.07
	55-64	0.95 (0.09)	2.59*	2.16-3.10	0.38 (0.10)	1.45*	1.19-1.78
	65-74	0.42 (0.10)	1.52*	1.24-1.85	0.17 (0.11)	1.19	0.95-1.48
	75+	Reference	1.00			1.00	
	Gender						
	Men	0.63 (0.03)	1.87*	1.77-1.99	0.21 (0.04)	1.23*	1.14-1.33
	Women	Reference	1.00			1.00	
	Education						
	No Education	-2.39 (0.07)	0.09*	0.08-0.11	-1.10 (0.10)	0.33*	0.28-0.40
	Primary School	-1.22 (0.07)	0.29*	0.26-0.33	-0.74 (0.08)	0.48*	0.41-0.56
	High School and/or Two-Year Degree	-0.36 (0.07)	0.70*	0.61-0.80	-0.35 (0.08)	0.70*	0.60-0.83
	Undergraduate and/or Graduate Degree	Reference	1.00			1.00	
	Income						
	*0-1550 TL***	-0.92 (0.05)	0.40*	0.37-0.44	-0.41 (0.06)	0.66*	0.59-0.75
	1551-2170 TL	-0.52 (0.06)	0.60*	0.54-0.66	-0.23 (0.07)	0.80*	0.70-0.91
	2171-3180 TL	-0.27 (0.06)	0.76*	0.68-085	-0.11 (0.07)	0.90	0.78-1.03
	≥ 3181 TL	Reference	1.00			1.00	
Health Problem	**Chronic Disease**						
	Not Have	2.21 (0.04)	9.12*	8.44-9.85	1.40 (0.04)	4.06*	3.72-4.42
	Have	Reference	1.00			1.00	
	Mental Problem						
	Not Have	1.12 (0.03)	3.08*	2.90-3.27	0.57 (0.04)	1.77*	1.63-1.92
	Have	Reference	1.00			1.00	
Life Style	**Sleep Problem**						
	Not Have	1.26 (0.03)	3.52*	3.31-3.75	0.55 (0.04)	1.73*	1.59-1.88
	Have	Reference	1.00			1.00	
	BMI						
	Underweight	1.24 (0.09)	3.47*	2.93-4.12	-0.11 (0.11)	0.90	0.72-1.12
	Normal Range	1.11 (0.04)	3.04*	2.81-3.29	0.27 (0.05)	1.31*	1.19-1.45
	Overweight	0.65 (0.04)	1.92*	1.77-2.08	0.25 (0.05)	1.28*	1.17-1.41
	Obese	Reference	1.00			1.00	
Utilisation	**Hospitalisation**						
	Yes	-1.02 (0.05)	0.36*	0.33-0.40	-0.52 (0.06)	0.60*	0.53-0.67
	No	Reference	1.00			1.00	
	Prescription Drug						
	Yes	-1.50 (0. 03)	0.22*	0.21-0.24	-0.76 (0.04)	0.47*	0.44-0.51
	No	Reference	1.00			1.00	
					Nagelkerke R^2=0.454; **Hosmer and Lemeshow**: 6.955; ***p*** **=** 0.541; **Accurate Classification Percentage** = 77.5%		

**p < 0.05 ** TL= Turkish Lira*

4. DISCUSSION

This study was undertaken to determine the factors related to the categories of socio-demographic characteristics, health problems, lifestyle, and utilisation of health services, which affect the SRH of adult individuals. This study confirmed that age, gender, educational level, income level, chronic diseases, mental disorders, sleep problems, body mass

index, hospitalisation in the last 12 months, and utilisation of a prescription drug in the last two weeks are important determinants affecting one's SRH.

The results based on the variables in the category of socio-demographic characteristics indicated that younger individuals, men, and those with higher educational and income levels are associated with better health status ratings. This finding is consistent with those observed in studies available in the literature (1,14,16,20,23,24).

Looking at the findings from the present study and other similar studies in the literature with focus on similar reasons, we believe that older people have poorer health status due to their health condition being more vulnerable to diseases and decline in vigour with the passing of time. The findings in previous research indicating overall poorer SRH of women may be attributed to their unfavourable biological characteristics (higher vulnerability to diseases) and their social roles. As is the case all over the world including Türkiye, even though women participate in the labour market to an ever-increasing extent (25), the responsibility for domestic tasks and household chores still lies with women. The fact that women have to deal with more than one job at the same time causes them to be more stressed, tired, and therefore more ill. It is the conventional wisdom in medical sociology and social epidemiology that women live longer than men but experience more morbidity (26), thus poorer SRH. As a matter of fact, in a study conducted with the participation of 9668 people aged 18 and over in China, it was found that women were less likely to report good SRH (1).

Research shows that the higher the educational level, the better is the SRH. This may be explained by the fact that higher educational level leads to higher health literacy, which in turn, leads to more awareness of health in people. However, it is known that higher education level is also associated with factors that are associated with better health, such as higher income and better working conditions (27). It is also a known fact that individuals with a better education level have more skills to access better tools and information to improve their health (27,28) and exhibit healthier behaviors (29,30,31). The association of income level with a better health status, on the other hand, can be explained with the financial support which income provides with respect to having better access to healthcare services. Higher household income does not only facilitate access to healthcare services, but it also enables them to afford more expensive services.

The study found, based on the variables regarding the category of health problems, that people with chronic diseases and those suffering from mental disorders had poorer SRH. The findings of the study are consistent with those of previous research (1,5,13,15-17,21). That people with a chronic disease or a mental disorder tend to rate their health status as poorer as compared to others may be a natural result of the health problems they experience.

The findings concerning the variables in the lifestyle category showed that people with sleep problems and those not having normal range BMI (underweight or obese) had poorer SRH. Healthy sleep is critical for all individuals, as it supports the general health of the person by leading to excitement and joy, which provides high energy, a very good mood and the ability to do daily tasks during the day (32). It is also an important need in terms of meeting the physical and spiritual needs of people. Therefore, it is an expected finding that individuals with poor sleep quality or who have difficulty falling asleep evaluate their health status as poor. As a matter of fact, it was found that people with sleep problems in Spain rated their health status as poor (5), and in a study conducted in Greece, poor sleep quality was found to be associated with poor health status ratings (13). Underweight and overweight are also associated with poor SRH (1). In developing countries, overweight prevalence is increasing while underweight prevalence is also still high. Both underweight and overweight are related to increased risk of non-communicable diseases, reduced well-being and quality of life (33). Thus, it is also crucial to avoid underweight, not only overweight.

The results regarding the variables in the category of healthcare service utilisation showed that people who were not hospitalised in the last 12 months and those who did not take a prescription drug in the last two weeks rated their health status as good. This finding also matches those observed in previous research (5,21,22). Especially in certain age groups, as the utilization of health services increases, the anxiety about the future increases and this anxiety reflects negatively on the perceived health status of individuals (34,35).

5. CONCLUSION

The present study showed that the most disadvantaged group in terms of SRH included people of advanced age, women, those with lower educational and income levels, people with a chronic disease, mental disorders and sleeping problems, individuals who do not have normal range BMI, people who have received impatient treatment in the last 12 months, and those who have taken prescription medicine in the last two weeks. To improve the unfavourable condition of these people, some interventions can be recommended.

With a focus on the predisposition of elderly people to rate their health status as poor, it is recommended to extend the scope of services intended for these people, with the necessary precautions to ease access to these services. Besides, it is of particular importance, specifically due to the ever-increasing older population in Türkiye, to also give priority to planning programmes for the services aiming at the elderly population in the country. Further, given the vulnerability of women to diseases, their biological characteristics, their social role in the family, and their responsibilities in relation to the general health of their family, the study also recommends that healthcare services aimed at women should be expanded, with necessary measures taken to ease access to such services. Another way to improve the health status of women would be to improve the health literacy of women, with special focus on preventive health services. Women may

benefit from trainings, courses, and seminars to be organised to this end. Considering the finding indicating that people with lower education and income levels tend to rate their health status as poor, it becomes clear that there is a need to give priority to social and economic projects to address inequalities in education and distribution of income across the country.

In view of the association of chronic diseases with poor health ratings and the need of continuous treatment for such diseases, community-based projects aimed at improving the people's perception of health status and symptom management can be considered as another area of intervention. Because the biological reasons underlying mental disorders or sleep problems cannot yet be described in concrete terms, such disorders are not traditionally considered a health problem among people; despite this fact, these problems have been found to be important determinants with respect to health status ratings. The study recommends, in this respect, taking measures to change people's perceptions towards these problems by enhancing their knowledge. People not having normal range BMI constitute another disadvantaged group with respect to SRH. Improving easily accessible training and follow-up programmes relating to nutritional habits and encouraging physical activity is recommended for this group.

Factors affecting health status can vary from one group to another (residents in rural/urban areas, women/men, younger/older populations, etc.). Taking this fact as a starting point, the study further recommends that future research should be undertaken with the participation of samples representative of different groups.

As the limited number of studies undertaken in Türkiye has only investigated health status determinants with a focus on the population in certain areas, the strength of the present study is that it investigated the phenomenon with a sample that is representative of the whole population in the country. Besides, another strength of the study was that it was the first to investigate the community-based SRH in Türkiye in such a comprehensive manner. Besides its strengths, the findings of this study are also subject to some limitations, of which the most important is that the study variables are limited with those originating from the data source.

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Clinical and Experimental Health Sciences

The opinions of postgraduate nursing students about evidence-based practice: A qualitative study

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ABSTRACT

Objective: This study was conducted for the purpose of exploring the opinions and experiences of postgraduate nursing students regarding evidence-based practice and its use in the clinical setting in Turkey.

Methods: A qualitative study design was employed. Data in this research study were collected through Semi-structured questions were asked face-to-face in focus group discussions.

Results: The findings of the study set forth the individual and organizational barriers that faced postgraduate nursing students in their efforts to implement evidence-based practices. The main themes of the study were highlighted as: perceptions regarding evidence-based practice, the impact on the group cared for, challenges faced in the implementation, institutional support in applying practices. Subthemes were data verification, useful and reliable application, difficulties in communicating with colleagues, traditional views, troubles with team collaboration, the indifference of young colleagues, insufficient resources, and the need for a mentor in the workplace.

Conclusion: The students in their undergraduate programs, and elective courses on EBP should be added to the curriculum. In order to eliminate organizational barriers, institutions should establish EBP committee and mentoring system.

Keywords: Evidence-based practice, nursing students, qualitative research

1. INTRODUCTION

Evidence-based practice (EBP) refers to the problem-solving process of accessing research results on patient care, assessing these results and making decisions based on this knowledge (1). The World Health Organization stresses that healthcare services must rely on the best research evidence (2-4). The International Council of Nurses (ICN) and research of recent years emphatically point to the knowledge that EBP yields positive results in nursing, provides the best available knowledge needed for patient care, improves healthcare outcomes, increases quality, and achieves standardization and professional satisfaction among nurses (5-8). Advanced communications and technology have contributed to increased expectations regarding healthcare services among the general public and patients alike and professional nursing has made EBP a requirement (8).

The quality of patient care will be enhanced if nursing students can learn about EBP in their training and integrate this into the clinical setting under the guidance of sample applications (9,10).

It is reported in the literature that nurses have positive attitudes about evidence-based care but use EBP only to a limited extent (7,11,12). Postgraduate students of nursing and graduate nurses seem to experience problems in transitioning from their theoretical knowledge to practical applications (10,13).

It has been found that the limited use of EBP is a result of individual and organizational factors. Many of the barriers are regarded as minor but it is emphasized that they still make processes difficult and complex. Barriers stemming from the individual are cited in the literature as not having the time to research and read resources, having difficulty in making changes in the clinical setting, and relying on old knowledge, experiences and social interaction. Organization barriers are said to be personnel shortages and resource inadequacy and the absence of persons that can take on leadership roles (5-9).

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The issue of EBP has come to the fore in the world for the last two decades. It is important that graduate school curricula incorporate EBP processes in both clinical practice and academic courses. Numerous quantitative studies have been conducted on EBP (14-16).

Postgraduate students constitute an important group in terms of their potential to become leaders in the clinical setting. It is therefore necessary that the problems experienced with implementing EBP in the clinic are identified in depth. The aim of this study was to investigate for the purpose of exploring the opinions of postgraduate nursing students about evidence-based practices.

2. METHODS

2.1. Research Design

This was a qualitative study using personal in-depth interviews as the data collection method. A qualitative study differs from conventional/quantitative studies with respect to study questions, goals, sample selection, data collection and analysis methods. The data collected in qualitative research cannot be reduced to numerical findings as in quantitative studies. The main purpose is to provide the reader with a descriptive and realistic presentation of the topic at hand. For this, it is important that the data collected is detailed and in-depth and that they directly reflect the views and experiences of the individuals participating as accurately as possible (17-20). We chose this method as an appropriate way of identifying views and experiences regarding EBP. Attention was paid to the consolidated criteria for reporting qualitative research (COREQ) in the reporting of all phases of the research (21).

2.2. Participants and Setting

The purposive sampling method was used in our study. The participants consisted of postgraduate students studying in a master's program in the nursing department of a foundation university in the city of Istanbul/Türkiye. Twenty-two currently enrolled postgraduate students were invited to participate in the research. Nine of the students did not wish to participate in the study, citing problems with time. The participants were not informed about the research prior to the study. The study was conducted with the 13 students who agreed to participate (Table 1). The criteria for the students who would be in the focus group discussions were consenting to participate and being a postgraduate student at the same university. Semi-structured interview questions and exploratory queries were used in the interview. The interview was conducted by means of the face-to-face interview method.

Purposive sampling method was used in this study. This sampling method is within the general qualitative research tradition. Purposeful sampling allows the study of situations that are thought to have rich information. In this sense, purposive sampling is useful in many cases in discovering and explaining facts and events.

Among the purposive sampling methods, in homogeneous method interviews, groups of 6-8 people can be interviewed. Qualitative research is flexible in nature. This study is a graduate education in nursing department. It was aimed to get more information and opinions by taking all of the students who saw it.

2.3. Data Collection

This qualitative study was carried out in February 2019 with focus group interviews with the nursing students in Türkiye. The interviews were carried out by three researchers and one observer in a classroom of the university where the research took place. Two of the researchers had Ph.D. degrees and were working at the university as teaching staff; they had an average of twenty-two years of experience. The other researcher was a graduate student in the field and had ten years of field experience. All of the researchers were women. All of the students were familiar with one of the researchers because they had attended the researcher's master's course. A mutually convenient time was arranged for the interviewing. The students provided their written consent before starting the interview the permission of the participants. Data continued to be collected until the saturation point (18). The session took about 120 minutes. The interview took place in one session at a classroom at the university. No one was present during the interview at the classroom except participants and researchers. The students were asked four questions to describe their personal information (age, gender, the clinic they worked in, and how many years of experience they had). Semi-structured and open-ended questions were posed on the students' views about how evidence-based practices functioned in the clinical setting. The following four questions were included in the individual in-depth interview form:

1.What do you understand from evidence-based practices in nursing?

2. What do you understand from evidence?

3. What do you experience about evidence-based practices in nursing?

4. Which opportunities do you think you have in the clinical field about evidence-based practices?

2.4. Data Analysis

The discussions were recorded on a sound recording device. Later, these recordings were transcribed. The transcription took about two days. The following processes took place in creating the themes and sub-themes for the research. One of the researchers transcribed all of the data collected on the sound recording device using the Microsoft Office Word program 2013 and numbering the data from 1 to 30.

The other two researchers read and checked the transcription of the entire data set against the recordings to make sure that the text produced was accurate. After the checking, the final form of the entire data set was encoded separately by two researchers specialized in their field, each unaware of the other. About 22-25 codes were generated for the data sets that both researchers had separated into parts. Both researchers combined the codes to create themes, thus reducing the number of codes to 20.

Both researchers grouped the codes they had created under 4-8 themes. The researchers shared the themes they had created and made comparisons of both the themes and the reasons for creating them.

The comparisons resulted in the creation of common themes that both researchers found appropriate.

The opinion of a fourth expert outside of the study team was enlisted to ensure the reliability of the encoding and the themes. The graduate students participating in the study were called upon to offer their views on the themes and sub-themes. All of the participants affirmed the themes.

2.5. Ethical Considerations

The Marmara University Health Sciences Institute Ethics Committee approved the implementation of the study. (January 14, 2019-23). The students that would be participating in the research were informed of the purpose of the scientific study and about the fact that the data obtained would not be shared with third parties outside of the requirements of the research, after which their written consent was obtained. In the analysis of the data, the participants were referred to by a code containing a letter and a number instead of their names.

2.6. Limitations

This study is limited in its results because it was conducted with a small group in a single university with a single session allotted to data collection. There is a need for similar studies conducted in different institutions with different groups of nurses. The sample group of this study is postgraduate students.

3. RESULTS

Twelve of the students were women; their mean age was 26.8. Nine had been working in the profession for 1-3 years and most worked on the night shift (Table 1). In the final analysis, the study worked with 4 main and 8 sub-themes. The main themes were perceptions regarding evidence-based practices, the impact on the group cared for, challenges faced in the implementation, institutional support in applying the practices. Sub-themes were data verification, useful and reliable application, difficulties in communicating with colleagues, traditional views, troubles with team collaboration, the indifference of young colleagues, insufficient resources and the need for a mentor in the workplace (Table 2).

Table 1. *Participants characteristics.*

Characteristic	Number of Participants
Gender	
Male	1
Female	12
Age range	
23-26	7
27-30	5
31-34	1
Work period	
1-3 years	9
4 and over Not working	3 1
Work pattern	
Shift	9
Day Time	4

Table 2. *Main and sub-themes*

Main theme	Sub-theme
1.Evidence-based practice perception	Validated information
2. Effect on care group	Useful and reliable application
3. Difficulties in application	Difficulty in communicating with colleagues Difficulty in team collaboration traditional judgment Disinterest of young colleagues Lack of resources
4. Institutional support in accomplishing the application	The need for mentors at workplace

3.1. Theme 1. Evidence-Based Practice Perception

The participants answered the question, “What do you understand from the term ‘evidence?” by explaining their own definitions. It was found that the participants’ views on EBP were positive. All of the definitions offered were in accord with each other. None of the participants’ definitions was contrary to the consensus.

3.1.1. Verified Data

It was seen that in defining the word “evidence,” the participants focused on the objective principles of science, as was seen in their responses regarding matters such as verification of results, proven knowledge, reaching the same conclusions at every try, and being sure of information. Most of the participants stated that “evidence” meant verified information.

It’s what we’re sure of It has to be the entirety of objective knowledge. We can get the same accurate result when we apply anything that has been proven (Age,22). *I think it means the whole of everything that is done to prove to others that*

there is something missing (Age,21). *It means getting the same result from every similar study or experiment* (Age,23). *It's to find out a benefit and loss relationship by trial and error* (Age ,21).

3.2. Theme 2. Impact on Group Cared For

"What do you understand from the expression 'evidence-based practices' in nursing?" To this, the participants agreed on the response "reliable and useful practices." No other definition was encountered in the group.

3.2.1. Useful and Reliable Practices

Things that have been tried on people and proved, leaving no question mark in the mind (Age ,21). *Clarity closes to precision, and even more accurate because of its precision. It suggests the principle of doing no harm to the patient. Everybody always doing the same thing* (Age ,20).

It will lead to standardizing care and treatment. It represents security for both the patient and us (Age,21). *There needs to be knowledge generated by persons specialized in the field. It's something that leaves no question mark in either the patient's, the nurse's, or the doctor's mind* (Age,21). *It brings the risk of making a mistake down to a minimum* (Age,22). *I can say that it's a piece of olid evidence. I can say that this is proof for me, so I think it's a very good thing from this aspect* (Age,22). *We can save time* (Age, 21).

3. 3. Theme 3. Challenges in Practice

To the question, "Which barriers do you run up against in the hospital regarding evidence-based practice?" the participants said that they ran into difficulties with implementing EBP, both as an institution and individually. They stated further that they could not make the connection between EBP and patient care, they had not seen any example of evidence-based practice, they experienced problems of communication with colleagues, friends were more prone to adopting traditional views, they had incompatibilities in the team, the professional qualifications of their young colleagues was lacking, they did not know a foreign language, they could not access the right resources at the right time and they were unable to participate in scientific congresses.

3.3.1. Difficulties in Communicating with Colleagues.

I can't say that these are implemented in our chest surgery unit. This is sometimes because of our colleagues, sometimes because of the doctor, and sometimes the patient. Our colleagues are never open to the current and new. Our younger colleagues are unenthusiastic. They are closed off to new knowledge, they don't want to try anything new (Age,21). *I work in pediatrics. I can't say with one-hundred percent certainty that we use evidence-based practices. Implementing different practices causes lapses in communication* (Age,20).

3.3.2. Challenges with Teamwork

Sometimes the team is against it. There are some that show resistance. Practices can change even from doctor to doctor. Sometimes a doctor sets up some working principle and insists that it should be followed, and we are unfortunately forced to do what they say (Age,21). *They can put you on the blacklist. It's as if they think that I'm not there to do research but to prove myself* (Age,21). *We go out on our internship rounds. To learn as much as we can from the nurses there. Sometimes there can be two nurses present. One will tell you to do something one way, the other will want you to do it differently* (Age,23).

3.3.3. Traditional Views

Patients usually want you to practice and continue to practice traditional methods. They can be closed to new things (Age,20). *There can be a disruption in communication with patients. That's when they don't allow the procedure and refuse the treatment* (Age,20). *Some physical conditions, sometimes very different things can be barriers. The team may not want it; they can resist* (Age, 21). *They consider it like teaching an old dog new tricks* (Age 20).

3.3.4. Our Younger Colleagues Have No Interest.

They're an unhappy group. They're a group that doesn't even want to come to work. They don't like what they're doing. Where I work, there's a lot of disinterests. Unfortunately, that's how I see it. The new generation doesn't think about what they must do (Age,21). *They don't know what research entails* (Age, 21).

They think that I'm not there to do research but to prove myself. At the end of the day, we're all professionals. This is a profession that's risen above the high school level and requires a college degree now. Because of this, it's only logical that we work with evidence-based practices but when we go out on the field, our relations are more on a master-apprentice level (Age ,21).

3.3.5. Insufficient Resources

I think I'm having difficulties with doing and choosing the right thing. For example, when I do research in a reference work written in English, I can only go halfway and then I get bored and leave it. If only my English was better, if I could read better, or if there more resources in Turkish and I could easily access them… (Age,22). *Our workload is too much; I think this type of work leaves no room for research-based practices* (Age,21). *I didn't know how I could attend congresses* (Age,23).

3.4. Theme 4: Institutional Support in Launching Practices

To the question, "What kind of support do you get from the hospital regarding evidence-based practice?" the participants said that nurses in charge of quality control at the hospital

could provide in-house training, that they were eager to learn about current practices, that they had the opportunity to work with colleagues at a higher educational standing in the same setting, and that an opportunity could be provided to them so they could organize. They also said that mentors in the clinical setting would be needed in the process of introducing EBP. The participants of our study explained that setting up an EBP committee would play an important role in implementing practices, assuring us that they would be acting on this need to have this study started at their own hospitals.

3.4.1. The Need for Mentors in the Workplace

There are qualified nurses at all hospitals now. These qualified nurses could be encouraged to follow up on current developments, share these with the team, and form an EBP committee (Age, 21). *If I had learned about EBP in my undergraduate program, I would have liked to follow up on current knowledge. In other words, I would have preferred to follow up on the latest practices, not traditional methods* (Age,21). *We have trouble organizing as a professional group. We should increase our organizing efforts and form a communication network* (Age,21).

4. DISCUSSION

In this study, we found that the participants used the word "evidence" as validated knowledge and knowledge that is based on reason. On the other hand, the participants did not feel the need to integrate the values and preferences of the patient in this definition. This suggests that more emphasis must be given in nurses training to the "individuality, culture and preferences of the patient." At the same time, it is our belief that if nurses can focus on the patient's preferences, culture and needs in their EBP, this would also raise the quality of nursing practices, creating a positive effect by turning simple techniques into a holistic approach toward patients. Our participants defined EBP in nursing as safe applications that were beneficial both to the patient and to the nurse. In another study conducted with nursing students, among the students answering the question, "What are your aims in finding evidence?" only 10% said that it was to use these practices in nursing care (5).

The participants in this study did not mention anything about how EBP could improve healthcare outcomes or how they could be used to bring down costs. This finding suggests that although the participants thought evidence-based practices to be useful, there is a need for interventions to strengthen their beliefs in the benefits EBP holds for patient care. Scott et al. (22) stresses that it is essential that nurses know exactly what evidence-based practice encompasses. Fairbrother et al. (2015) found higher educational level, lower emotional exhaustion and higher relational job satisfaction were found to be the best predictors of EBP skill level (23). Yilmaz et al. (2018), in their study with nurses, found that attitudes toward evidence-based nursing were low, while Durmuş et al. (2017) found these levels to be average (15,24). This suggests that nurses either do not have sufficient knowledge or the necessary skills to appreciate the process of EBP and what this can achieve or they have not internalized these concepts. It has been discovered that nurses experience various challenges in implementing evidence-based practice in the clinical setting. These challenges are both institutional and individual. In the literature, individual challenges have been identified as not being able to allot time to research, having difficulty with interpreting research results, having trouble accessing resources and not knowing enough English. Institutionally, the difficulties have been reported as the fact that nurses and other healthcare personnel show resistance and are not open to new ideas and there is a shortage of supportive mentors (5,25,26). The participants in this study similarly stated that the most common problems they faced in adopting and implementing EBP in the clinical setting had to do with other members of the health team. It is clear in the light of this that for EBP to be adopted and implemented in the clinic, support and mentorship need to be provided so that the importance of EBP and its integration into clinical practice is fully understood.

Our study showed that the participants relied on the experience of their colleagues rather than on current knowledge in the literature in managing the care process. Gerrish et al. (2008) and Ozga et al. (2019) found that nurses regard the use of research results as worthless but clinical experiences more valuable in the decision-making process. (27). Huang et al. (2017) have asserted that lack of knowledge of a foreign language is a major obstacle in implementing practices due to the inability to access, read and understand resources (28). Similarly, Tas et al. (2018) found that 57.4% of undergraduate students did not read nursing journals and 45.2% did not participate in scientific research (29). Yılmaz et al. (2018) reported that when nurses working at a university hospital participated in scientific congresses and read professional publications, this had a positive impact on their attitudes toward EBP (15).

The participants in this study pointed to the difficulties they faced because of the general disinterested attitude of their younger and newly hired colleagues toward EBP. They also stated that more experienced colleagues showed resistance in putting new knowledge and techniques into practice. In a study by Melnyk et al. (2012), nurses expressed the opinion that not only institutional, but administrative and educational opportunities should be provided in order to achieve the clinical integration of EBP (5,30). Wallis (2), Fisher et al. (31) recommend that clinical mentors be given the task of acting as a locomotive force in bringing about the implementation of EBP. Similarly, the participants in this study expressed their belief that the implementation of EBP would require that an EBP committee be organized at the hospitals, the topic of EBP be given priority in in-house programs and that university academics provide mentorship in setting up the appropriate systems. Also, the participants revealed that this study had raised their awareness about EBP. The participants said that they welcomed taking responsibility in the clinical setting

in solving the problems related to EBP, and they stated that they would be talking to their administrators about setting up an "evidence-based practices committee."

5. CONCLUSION

Our study indicated that graduate nursing students worked in clinical settings where there was no policy or practices governing the use of EBP and that the adoption of EBP was hindered by individual, institutional and educational barriers. In order to ensure the adoption of EBP, these barriers need to be identified so that effective solutions can be developed. Although the students attitudes toward EBP were generally positive, it was found that the most common individual challenge they faced in implementing EBP was their foreign language deficiency. This deficiency makes it harder to keep up with current developments. In light of the results obtained, we recommend the following:

- Administrators, nurses, academics and other healthcare providers responsible for patient services should conduct studies to understand and eliminate the barriers standing in the way of implementing evidence-based practices.
- Attention must be devoted to improving the foreign language competencies of students in their undergraduate programs, and elective courses on EBP should be added to the curriculum. In the context of eliminating organizational obstacles, holding evidence-based interactive training workshops as part of nurses' training, encouraging institutions to set up committees and mentorships to create a culture of EBP, increasing in-house training opportunities, supporting congresses and other scientific efforts, engaging in workload planning and increasing collaboration at the universities may all be effective methods of action.

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Clinical and Experimental Health Sciences

Awareness of Infectious Disease Risks and Vaccination Behaviors Among Health Professionals

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ABSTRACT

Objective: This study aims to evaluate the awareness of infectious disease risks and vaccination behaviors of health professionals.

Methods: This descriptive and cross-sectional study was conducted who worked at a research and training hospital, met the inclusion criteria and agreed to participate. Personal Information Form and Communicable Diseases Risk Awareness Protection Scale (CDRAPS) were used for data collection. The study was completed with 208 health professionals actively working at the hospital. Personal information form and communicable diseases risk awareness and protection scale were collected.

Results: In this study, 62% of the participants have had influenza before and 85.1% were vaccinated with at least one of the adult vaccines. 10.6%were formerly vaccinated with pneumococcal vaccine and half of them were vaccinated during the COVID-19. 16.8% of the participants, who were not formerly vaccinated with influenza vaccine, stated their intentions to receive vaccine, 90% decided during the pandemic. Mean CDRAPS score was 155.70±15.47. There was statistically significant relationship between the knowledge of the participants on adult vaccines and the mean scores obtained from the CDRAPS (p=.004) and between the decision to receive influenza vaccination and the CDRAPS scores (p=.047).

Conclusion: There was a statistically significant relationship between knowledge on adult vaccines, plans to receive influenza vaccination and the scores obtained from the CDRAPS.

Keywords: Vaccine, vaccination, COVID-19, infectious disease, health professionals.

1. INTRODUCTION

Infectious diseases are disorders caused by microorganisms, which may result in morbidity, mortality, pandemics, and consequent economic and social problems, including, anxiety and panic among the population, overcrowding in health institutions, and high economic burden (1). Globalization, rapid urbanization, public transportation, climate change and global warming facilitated the spread of infectious agents throughout the world (2). Despite the advances in controlling infectious diseases, they are still among the crucial public health problems since they can be easily transmitted via contact with infected people or contaminated water and food products at health centers or outside (3). Knowledge, attitudes, and beliefs among the population are as important as health systems and technologies to control infectious diseases. Appropriate attitudes and behaviors to prevent the occurrence and transmission of these diseases have important individual and social benefits (4). Consequently, individual awareness about infectious diseases and protective behaviors are vital to protect personal well-being and prevent the transmission of these diseases (1).

Immunization is the leading method of protection against infectious diseases. Immunization with vaccination is the most effective and the cheapest method of protection (5). No methods other than vaccination have far-reaching returns in the struggle against infectious diseases (6). World Health Organization (WHO) reported that global vaccination programs prevent 2-3 million deaths every year and may save 1.5 million people every year if the target vaccination levels may be reached (7). Although infectious diseases are mostly considered as a reason for mortality among developing countries, COVID-19 disease, which turned into a global pandemic in a short time and was responsible for hundreds of thousands of deaths as of April 2020, shows that infectious diseases will be a problem for all countries in the near future (8).

Following the outbreak of the COVID-19 pandemic, most of the health professionals without former intentions for vaccination applied to health centers for vaccination. Due to these reasons, analysis of the awareness of infectious disease risks among health professionals and their protective

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behaviors, including vaccination, are crucial to take measures to prevent the spread of infectious diseases and prepare education programs on this public health problem. This study aims to analyze the awareness of infectious disease risks and vaccination behaviors of health professionals.

2. METHOD

2.1. Ethical Considerations

Prior to the study, we obtained permission from the Republic of Turkey Ministry of Health COVID-19 Scientific Research Commission and Acıbadem University and Acıbadem Healthcare Institutions Medical Research Ethics Committee (ATADEK) (17/09/2020-20/16).

Written informed consent of the participants that agreed to participate was obtained. The research was conducted in accordance with the Declaration of Helsinki.

2.2. Study Design

The descriptive and cross sectional study was conducted to determine the awareness of infectious disease risks and vaccination behaviors of health professionals.

2.3. Participants

All health professionals, including physicians, nurses, medical assistants, and technicians, who had been working at a research and training hospital in İstanbul Provincial Directorate of Health Süreyyapaşa Chest Diseases and Thoracic Surgery Training and Research Hospital between September and December 2020, constituted the universe of the study. Sample of the study comprised 208 health professionals, who agreed to take part in the study and met the inclusion criteria. Being above the age of 18 years and actively working at the hospital constituted the inclusion criteria. Participants, who did not complete all the questions in data collection instruments, were excluded from the study. Cochran's formula for unknown population sample size was used to calculate the sample size of the study. According to this, the minimum sample was calculated at 200 people for P = .50 and q = 0.50, with 5% error (d = 0.05) in the confidence interval range of 95% (α = .05) (9). Considering that there may be some dropouts from the study, it was decided to include 208 people in the study.

2.4. Data Collection

Personal Information Form and Communicable Diseases Risk Awareness and Protection Scale (CDRAPS) were used for data collection. The form and the scale were completed by the participants in about 15 minutes. The data were collected by the researchers through the face-to face. The form and the scale were distributed to the participants and asked to fill them out.

2.5. Instruments

Personal Information Form

This form was prepared by the researchers by using the existing studies in the literature and was composed of two parts with 26 questions in total (10-16). The first part asked 6 questions on personal characteristics (age, martial status, sex, educational level, and occupation etc.) of the participants whereas the second part had 20 questions on vaccination attitudes and behaviors. After the questions were created by the researchers according to the literature, expert opinions were taken from five people and their final form was given in line with the suggestions.

Communicable Diseases Risk Awareness and Protection Scale (CDRAPS)

CDRAPS was developed by Ener (2020) to measure the risk awareness and the levels of protection, and the validity and the reliability of the scale has been confirmed. The scale had 36 items that were scored on a five-point Likert scale. Answers to the items on risk awareness ranged between 'strongly disagree' (1 point) to 'strongly agree' (5 points) whereas the items on protective behaviors ranged between 'never' (1 point) to 'always' (5 point). No items were reverse scored. The scale had six factors, namely 'common life risk awareness' (items 1, 2, 3, 4, 5, 14, 15, 16 and 17), 'personal protection awareness' (items 6, 7, 8, 18, 19, 20, 21 and 22), 'protective behaviors' (items 24, 25, 26, 27, 28, 29, 30 and 31), 'hand washing behaviors' (items 23, 32 and 36), ' social protection awareness' (items 9, 10, 11 and 12), and 'personal contact awareness' (items 13, 33, 34 and 35). Total score was calculated by summing the scores obtained from each item and higher scores indicated higher risk awareness and protective behaviors. Cronbach's alpha of the original scale was 0.91 (10). Cronbach's alpha in our scale was also 0.91, indicating that the scale was a reliable instrument for the sample.

2.6. Data Analysis

Statistical analyzes were reported using the SPSS version 26.0 statistical software. Frequency, mean, and standard deviation were used as descriptive statistics. Independent Samples t test, Mann-Whitney U test and Kruskal-Walli's test were used to analyze the difference between mean scores of the continuous variables. The value of $p<0.05$ was accepted to be statistically significant.

3. RESULTS

The mean age and length of professional experience of the 208 participants were 32.39±9.38 and 9.66±9.37 years, respectively. Of the participants who participated in the study 44.2% were physicians (n=92), 39.4% were nurses (n=82), 6.3% were medical assistants (n=13) and 10.1% were medical technicians (n=21). Of the participants who participated in the study 75% were female (n=156) and 50.5% were married (n=105). 4.3% had high school degree (n=9), 9.1% had associate degree (n=19), 63.5% had bachelor's degree (n=132) and 23.1% had master's or doctoral degree (n=48) (Table 1).

***Table 1**. Sociodemographic and vaccination characteristics of the participants*

Variables	n	%
Martial Satatus		
Single	103	49.5
Married	105	50.5
Sex		
Female	156	75.0
Male	52	25.0
Occupation		
Physician	92	44.2
Nurse	82	39.4
Medical assistant	13	6.3
Medical technician	21	10.1
Educational Status		
High school degree	9	4.3
Associate degree	19	9.1
Bachelor's degree	132	63.5
Master's or doctoral degree	48	23.1
Previous diseases		
İnfluenza	129	62.0
Pneumonia	19	9.1
Hepatitis B	5	2.4
Hepatitis A	10	4.8
Diphtheria –Tetanus	1	.5
Meningitis	1	.5
Type of adult vaccines*		
Influenza vaccine	61	29.3
Pneumococcal vaccine	17	8.2
Hepatitis B vaccine	120	57.7
Hepatitis A vaccine	43	20.7
Diphtheria – Tetanus vaccine	86	41.3
Meningitis vaccine	6	2.9
Reasons for vaccination*		
Recommended by a physician	95	45.7
Believed in and relied on vaccines	107	51.4
Positive news on TV and media	178	85.6
Knowledge on adult vaccination		
Excellent	18	8.7
Sufficient	130	62.5
Insufficient	60	28.8
Reasons for not being vaccinated		
Negative news on TV and media	1	.5
Did not believe in the protective potential of vaccines	7	3.4
Fear from side effects	9	4.3
Believed that vaccines might be allergic	6	2.9
Did not know the vaccination schedule	6	2.9
Did not know how to access vaccination	2	1.0
Opinions on Influenza vaccine*		
Protects only the children	1	.5
May protect the adults and prevent the disease	60	28.8
Maintains a mild course of disease	147	70.7
Is not necessary for adults	27	13.0
No opinion	14	6.7
Vaccination due to travels or going abroad		
Yes	14	6.7
No	194	93.3

**more than one option can be ticked*

Table 1 showed the characteristics of the participants about vaccines and vaccination. Of the participants who participated in the study 62% had influenza previously (n=129). Of the participants who participated in the study 85.1% were vaccinated with at least one of the adult vaccines (n=177) and 57.7% were vaccinated with hepatitis B vaccine (n=120). Of the participants who participated in the study 51.9% were vaccinated in a state or a research and training hospital (n=108).

Of the participants who participated in the study, 10.6% (n=22) were vaccinated with the pneumococcal vaccine (n=22) and half of them were vaccinated during the COVID-19 pandemic. Of the participants who participated in the study 89.4% (n=186) were not formerly vaccinated with pneumococcal vaccine but 18.3% (n=34) expressed their intentions to be vaccinated. In this study, 82.4% (n=28) of the participants that planned to receive pneumococcal vaccine stated that they decided to receive the vaccine during the COVID-19 pandemic (Table 2).

Of the participants who participated in the study, 42.8% (n=89) were formerly vaccinated with influenza vaccine and 92.1% (n=82) were vaccinated before the COVID-19 pandemic. In this study, 57.2% (n=119) were not formerly vaccinated with influenza vaccine but 16.8% (n=20) expressed their intentions to be vaccinated. 90% (n=18) of participants that planned to be vaccinated with influenza vaccine stated that they decided to receive the vaccine during the pandemic (Table 2).

Table 3 showed the mean scores obtained from the CDRAPS and its subscales. Mean CDRAPS score of the participants was 155.701±15.474. Mean scores obtained from the common life risk awareness, personal protection awareness and protective behaviors subscales were 37.043±5.483, 34.783±3.871 and 34.711±4.515, respectively. Besides, mean scores obtained from the hand washing behaviors, social protection awareness and personal contact awareness subscales of the CDRAPS were 14.019±1.427, 16.649±2.470 and 18.495±2.470, respectively.

Table 4 showed the findings on the distributions of vaccination characteristics of the participants and the scores obtained from the CDRAPS. There was no statistically significant relationship among healthcare professionals in terms of CDRAPS scores ($p > .05$). We found a statistically significant relationship between the knowledge of the participants on adult vaccines and the mean scores obtained from the CDRAPS ($p < .01$). Besides, there was statistically significant relationship between the decision to receive influenza vaccination and the CDRAPS scores ($p < .05$). However, there was no significant relationship between the mean CDRAPS scores and other vaccination characteristics.

Table 2. *Vaccination with pneumococcal and influenza vaccines before or during the COVID-19 Pandemic*

	n	%
Formerly vaccinated with pneumococcal vaccine		
Yes	22	10.6
No	186	89.4
If vaccinated, time of pneumococcal vaccination (n=22)		
Before the COVID-19 pandemic	11	50
During the COVID-19 pandemic	11	50
If not vaccinated, planned to receive pneumococcal vaccine (n=186)		
Yes	34	18.3
No	152	81.7
If planned to receive pneumococcal vaccination, intended time (n=34)		
Before the COVID-19 pandemic	6	17.6
During the COVID-19 pandemic	28	82.4
If vaccinated, time of influenza vaccination (n=89)		
Before the COVID-19 pandemic	82	92.1
During the COVID-19 pandemic	7	7.9
If not vaccinated, planned to receive influenza vaccine (n=119)		
Yes	20	16.8
No	99	83.2
If planned to receive influenza vaccination, intended time (n=20)		
Before the COVID-19 pandemic	2	10
During the COVID-19 pandemic	18	90

Table 3. *Scores obtained from the CDRAPS and its subscales*

	Min (min*)	Max (max**)	Mean	Standard Deviation
Common life risk awareness	21(9)	45 (45)	37.043	5.483
Personal protection awareness	20 (8)	40 (40)	34.783	3.871
Protective behaviors	15 (8)	40 (40)	34.711	4.515
Hand washing behaviors	5 (3)	15 (15)	14.019	1.427
Social protection awareness	8 (4)	20 (20)	16.649	2.470
Personal contact awareness	7 (4)	20 (20)	18.495	1.956
Total	79 (36)	180 (180)	155.701	15.474

**Minimum score to be obtained from the subscale and the CDRAPS; ** Maximum score to be obtained from the subscale and the CDRAPS.*

Table 4. *CDRAPS scores and vaccination characteristics*

	Mean	SD	p	Statistical value
Occupation				
Physician	157.043	1.511	.306	3.615[2]
Nurse	155.597	1.920		
Medical assistant	154.154	3.061		
Medical technician	151.190	3.035		
Vaccinated with vaccines other than childhood vaccines				
Yes	155.807	15.033	.814	.235[1]
No	155.096	18.048		
Knowledge on adult vaccination				
Excellent	151.611	14.649	**.004***	10.980[2]
Sufficient	158.100	15.694		
Insufficient	151.733	14.351		
Attitudes towards influenza vaccine				
Regularly vaccinated every year	162.583	16.483	.329	3.434[2]
Vaccinated irregularly	156.736	13.963		
Never vaccinated	154.735	14.631		
Did not have any information but could have been vaccinated if s/he knew	139.666	52.880		
Formerly vaccinated with pneumococcal vaccine				
Yes	153.545	16.271	.442	-.768[3]
No	155.957	15.402		
If vaccinated, time of pneumococcal vaccination				
Before the COVID-19 pandemic	148.454	16.439	.237	-1.183[3]
During the COVID-19 pandemic	158.636	15.121		
If not vaccinated, planned to receive pneumococcal vaccine				
Yes	157.823	13.347	.416	.815[1]
No	155.427	15.921		
If planned to receive pneumococcal vaccination, intended time				
Before the COVID-19 pandemic	154.000	10.899	.587	-.543[3]
During the COVID-19 pandemic	158.642	13.848		
Formerly vaccinated with influenza vaccine				
Yes	155.852	16.454	.905	.120[1]
No	155.591	14.783		
If vaccinated, time of influenza vaccination				
Before the COVID-19 pandemic	156.719	16.848	.191	-1.308[3]
During the COVID-19 pandemic	151.500	7.259		
If not vaccinated, planned to receive influenza vaccine				
Yes	160.636	16.831	**.047**	-1.985[3]
No	153.305	15.586		
If planned to receive influenza vaccination, intended time				
Before the COVID-19 pandemic	160.000	18.681	.886	-.144[3]
During the COVID-19 pandemic	160.736	17.077		

*[1] Independent Samples t test, 2 Kruskall-Wallis (KW), 3 Mann-Whitney U test (Z) * p<0.05*

4. DISCUSSION

Vaccination of the health professionals constitutes an important step of public health. Effective vaccination programs may not only protect the health professionals but also reduce the prevalence of nosocomial infections (11,12). Routine vaccination programs and developments in

infection control measures resulted with a 98% decrease in the prevalence of hepatitis B among the health professionals (12). A study reported that with the increase in COVID-19 vaccination, it resulted in a 90% decrease in intective cases (13). Another study noted that vaccination of five health professionals prevented a disease such as influenza and vaccination of eight health professionals prevented a death. Besides, influenza vaccination of healthcare workers has been shown to protect hospitalized patients, including bone marrow transplant recipients (14). In this study, 85% of the participants were vaccinated with at least one of the adult vaccines and more than half of the participants were vaccinated with hepatitis B vaccine. However, the percentage of participants that received other adult vaccines, including influenza and pneumococcal vaccines, was relatively low. Influenza vaccination among health professionals in the existing studies ranged from 2.1% to 82% (15). Despite all efforts, influenza vaccination among health professionals in developed countries was 52% (16). A study on Spanish health professionals reported that influenza vaccination among health professionals was 29.5% (17). Another study reported that health professionals did not have sufficient knowledge of pneumococcal vaccine and did not recommend pneumococcal vaccine to their patients compared to other adult vaccines (18). Reasons behind the behaviors of health professionals to refrain from vaccination included concerns about side effects, forgetting, doubts about the efficiency of vaccines and the belief that exposure to diseases helps protection (19, 20). Therefore, a working environment that encourages knowledge on and positive attitudes towards vaccination may help the health professionals to develop positive attitudes towards vaccination.

Among the participants that were not vaccinated, the percentages of health professionals that planned to receive pneumococcal and influenza vaccines in our study were 18.3% (n=34) and 16.8% (n=20), respectively. 82.4% (n=28) of the participants that decided to receive pneumococcal vaccine and 90% (n=18) of those that would receive influenza vaccine expressed that they changed their minds during the COVID-19 pandemic. The recent COVID-19 pandemic clearly showed the importance of vaccination. Importance of vaccines and immunization become clear during the periods of epidemics and pandemics, such as the current COVID-19 pandemic (21). Besides, higher vaccination rates among the health professionals have been observed during the pandemics. A study conducted in Italy reported that physicians were more likely to recommend vaccination to their patients during the times of pandemics (22). The study of Hidiroğlu et al. (2010) found that vaccination rate during the H1N1 pandemic was 27.2% among the health professionals (23). Our finding on the high percentage of participants that decided on vaccination during the COVID-19 pandemic indicates that the awareness on the importance of vaccination during the pandemic increased. Therefore, we may expect an increasing rate of vaccination among the health professionals during the COVID-19 pandemic. This finding of our study is similar to the literature. In a meta-analysis reviewing twenty-three articles, it was stated that COVID-19 vaccination intention was high at 73.3% worldwide (24). Another meta-analysis reported that the COVID-19 pandemic has increased vaccination rates worldwide (25).

High scores obtained by the participants from the CDRAPS indicate a high level of awareness of infectious disease risks among the health professionals and their inclination to perform protective behaviors. Existing studies suggested that lack of information on vaccines and the diseases that may be prevented with vaccination resulted in reluctance to vaccination. People with insufficient knowledge on vaccines and vaccination may reject to be vaccinated even if vaccination was for free. In this sense, knowledge of vaccination may have positive effects on the attitudes towards immunization services (17, 26). Since the increase in knowledge of vaccination resulted with a consequent awareness of the importance of vaccination, various institutions, including the WHO and the Ministry of Health, attempted to increase awareness of vaccination. Higher level of awareness of infectious diseases and vaccination behavior among the participants of our study may be influenced by these attempts.

Limitations of this study are twofold. Firstly, the study was conducted on health professionals, who were busy with delivering healthcare to the patients during the COVID-19 pandemic. Consequently, the number of participants was limited. Secondly, the sample was not randomly chosen but all health professionals that agreed to participate were included to the study.

5. CONCLUSION

Increasing the rate of vaccination among the health professionals is vital to maintain the well-being of health professionals and to preventing the transmission of infectious diseases from health professionals to patients. Participants of our study were vaccinated with at least one of the adult vaccines. Besides, most of the participants were vaccinated with hepatitis B vaccine and the rate of pneumococcal and influenza vaccination was relatively low. Furthermore, CDRAPS scores were higher for the participants that had sufficient knowledge of adult vaccination and that planned to receive influenza vaccination. Therefore, health professionals might be periodically informed about the vaccines and the diseases that may be prevented with vaccination. Besides, their immunization status might be periodically followed up by their institutions and vaccination might be recorded.

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Clinical and Experimental Health Sciences

The Turkish Validity and Reliability of the Adolescent Dysmenorrhea Self-Care Scale

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ABSTRACT

Objective: Dysmenorrhea is a common health problem that negatively affects daily life and academic skills when not managed well. This study was conducted to examine the Turkish validity and reliability of the Adolescent Dysmenorrhea Self-Care Scale.

Methods: This methodological study was conducted in 7 high schools in Bursa, Turkey. The research was carried out with 1041 high school students who met the participation criteria. The Adolescent Dysmenorrhea Self-Care Scale was adapted to Turkish by using translation-back translation method. The validity of the scale was evaluated with the content validity index, confirmatory factor analysis, similarity, and discriminant validity coefficients. The reliability was evaluated by item-total correlation, internal consistency coefficient, and test-retest analysis.

Results: According to the evaluations of the 10 experts whose opinions were taken for the Content Validity, it is determined that each item scored above 3 (3.52-4.00), and the mean score was 3.92 ± 0.14 and the content validity index was .98. Cronbach's alpha reliability coefficient was found .96. Confirmatory factor analysis fit indexes and similarity and discriminant validity coefficients showed that the scale has sufficient compatibility with the theoretical six-factor structure. Item total correlations of subdimensions were .20-.68 and Cronbach's alpha coefficients were .77-.65. Test-retest correlations of the Adolescent Dysmenorrhea Self-Care Scale and its subdimensions were found .87-.99.

Conclusion: Turkish Adolescent Dysmenorrhea Self-Care Scale is a valid and reliable data collection tool consisting of 40 items and six subdimensions.

Keywords: Dysmenorrhea, Orem's self-care theory, reliability and validity, self-care

1. INTRODUCTION

Dysmenorrhea is a common problem among young girls that negatively affects daily life and academic success when it is not managed well. The number of women with dysmenorrhea has tripled in the last 30 years, and the prevalence of dysmenorrhea in the society varies between 20% and 90% (1,2,3).

Dysmenorrhea is considered the normal nature of the female sex in most cultures (2). Adolescents knowledge, attitudes, values, and beliefs about reproductive and sexual health are influenced by the culture in which they live (4). In this direction, the adolescence period is the key point for improving women's health.

It is important for adolescents to be aware of the abnormal symptoms of dysmenorrhea. In this period, there is increase in adolescents desire to be independent in meeting their self-care needs, the risk of exposure to stress, and their cognitive and emotional development needs. Therefore, self-care training is very important in the management of dysmenorrhea (5).

Orem's theory of self-care deficit offers the most comprehensive approach to the importance of self-care for humans (6). Self-care according to Orem; self-care needs include actions to improve health, self-care management, disease prevention, and coping with disease symptoms (7). Self-care for dysmenorrhea usually consists of physical (8), pharmacological (9), non – pharmacological (10) and psychological strategies that adolescents self-administer with the advice of family (5) or friends without medical supports (11). Research has shown that adolescent girls rarely seek medical treatment for the treatment of dysmenorrhea

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but prefer self-care instead (2,11,12). However, self-care practices in adolescents are not at the desired level (13).

Turkish validity and reliability studies have been carried out in the literature regarding the content of dysmenorrhea; the Menstrual Attitude Questionnaire (14), the Menstrual Symptom Questionnaire (15), Menstrual Distress Questionnaire (16), Functional and Emotional Measure of Dysmenorrhea (17) and the Effects of Dysmenorrhea Scale (17) scales are used. With these scales, premenstrual syndrome or dysmenorrhea symptoms and attitudes are determined, but self-care skills for dysmenorrhea cannot be evaluated in all dimensions (13). One of these scales, the Effects of Dysmenorrhea Scale, and the Adolescent Dysmenorrhea Self-Care Scale (ADSCS), whose validity and reliability we have studied it includes the way individuals express their feelings about dysmenorrhea, how external factor controls are provided for dysmenorrhea symptoms, and what forms of coping practices for pain are determined. ADSCS the information sources used by the individual on self-care are questioned, the resources for seeking help for self-care are determined in relation to the information seeking dimension, and the cognitive and psychological factors of the individuals self-control for dysmenorrhea are questioned.

Self-care strategies in studies focus on self-care practices and cannot be evaluated holistically in terms of external support and regulation of negative emotions (12). It is unclear which self-care strategies adolescents use, the effectiveness of these strategies and which information sources they refer to in determining strategy (1). At the same time, it is necessary to increase the awareness of adolescents about the psychological aspect of self-care and to ensure their evaluation.

Research based on large study populations is needed to determine the use of self-care strategies in adolescents to gain an in-depth understanding of dysmenorrhea self-care strategies (12). In order to provide the necessary support to young people with dysmenorrhea problems, and to develop and evaluate self-care programs, firstly, self-care behaviors should be evaluated with a valid and reliable measurement instrument (18). It is thought that this measurement tool, which combines cognitive and psychological factors, will contribute to the literature by evaluating the sub-dimensions of searching for knowledge, expression of emotions, seeking assistance, control over external factors, coping practices and self-control being of dysmenorrhea self-care (6). Thus, effective and reliable self-care practices specific to the individual will be developed.

The aim of this study is to adapt the ADSCS, which was developed by Hsieh et al., (19) to provide a comprehensive assessment of the self-care behaviors of adolescents, into Turkish, and to make its validity and reliability analysis.

2. METHODS

2.1. Ethical Considerations

Written permission was obtained from Ching-Hsing Hsieh via e-mail for the use of the ADSCS. Before the research, ethical approval was received from the Marmara University Institute of Health Sciences Ethics Committee (06.02.2017-63), and the official permission was obtained from the Directorate of National Education. Permission for the research was obtained from the institutions where the research was conducted and from the parents. In the test-retest phase, information security was provided by assigning personal codes to the participants.

2.2. Design and Sample

This methodological study was conducted between 15.04.2017-15.05.2017 with female students (7 high schools, N = 1200) studying in all high schools in a district of Bursa. The criteria for participation in the study were having primary dysmenorrhea and no chronic disease that caused dysmenorrhea. Primary dysmenorrhea was assessed by the criteria of (1) the occurrence of the pain from time to time or in every menstrual cycle, (2) being in the waist, groin, or abdomen, (3) starting on the day before menstruation or the first day of menstruation, (4) to disappear within 48-72 hours after the onset of menstruation (20,21).

1056 students with primary dysmenorrhea filled the ADSCS. 15 scales that were filled incompletely were excluded from the analysis (N=1041). The power analysis of Statistica 10 package program for sample size showed that N = 1041 is sufficient for Confirmatory Factor Analysis (CFA).

The pilot test was conducted with 131 female students by selecting a random class from each level (high school 1,2,3,4).

The re-test was conducted with 100 female students.

2.3. Instrument

2.3.1. The Adolescent Dysmenorrhea Self-Care Scale (ADSCS)

It was developed by Hsieh et al. (2004) to determine the self-care experiences of adolescent young girls for dysmenorrhea. The scale consists of 6-likert type (0 = totally disagree, 1 = 20% agree, 2 = 40% agree, 3 = 60% agree, 4 = 80% agree, 5 = 100% agree) 40 questions. Six factors that are divided into two parts in terms of theory as externally oriented behavior and internally oriented behavior, have been defined. Externally oriented subscales are searching for knowledge, expression of emotions, seeking assistance and control over external factors, and internally oriented subscales are resource utilization and self-control being. Cronbach alpha reliability coefficient was found .89 for the whole scale, resource utilization .73, seeking assistance .80, control over external factors .65, self – control being .62, expression of emotions .77, searching for knowledge .76, for the subscales (19).

As the ADSCS score increases, self-care behaviors increase.

2.4. Procedures

Data was collected from students based on self-report and data collection took about 20 minutes.

2.5. Data Analysis

For the content validity, content validity index analysis; for the construct validity, similarity and discriminant validity analysis along with confirmatory factor analysis were performed. The data were analyzed using the Social Sciences Statistics Package Version 24 and Lisrel 9.2 program for Windows. Stats tool package excel file was used for calculations related to similarity and discriminant validities (22).

Since item scores were ordinal data, CFA was performed using the Diagonally Weighted Least Square (DWLS) estimation method on asymptotic covariances. In CFA, firstly the items were associated with primary level factors in Model 1 as suggested by Hsieh et al. (19) and this theoretical structure defined as Model 1. In addition, Model 2 based on the hypothesis that sub-factors are independent features and Model 3, which predicts that sub-factors can be combined under a single basic factor, were examined within the scope of the test of equivalent models. Also, in Model 4, the results of the theoretical structure (structural model) in which six primary level factors are represented under two secondary level factors were evaluated.

The reliability coefficients of the measurements were examined using Cronbach, composite reliability, and stratified alpha methods. Composite reliability is one of the methods of determining reliability based on internal consistency of a multidimensional scale. As a result of the composite reliability confirmatory factor analysis, it is calculated with factor loads and error rates. The acceptable value of composite reliability .70 is specified as (23). Cronbach alpha internal consistency reliability coefficient, the lower limit of reliability real data on scores obtained from multidimensional tests. The truth about scores from multidimensional tests was stated that the reliability measure could be better determined by the stratified alpha coefficient (24). The total test scores obtained from multidimensional tests such as ADSCS reliability were compared using the stratified alpha coefficient and Cronbach's alpha.

Pearson correlation analysis was performed for test-retest reliability. Statistical significance was accepted as $p<.05$.

2.6. Instrument Adaptation

For language adaptation, the ADSCS's English form was translated into Turkish by two language experts. Two Turkish forms were examined by researchers and created as a single form. Later, the Turkish ADSCS was retranslated into English by two different linguists. The back-translated scales were examined by the researchers and converted into a single form (25). English back-translation and the ADSCS English form were compared. Original and back-translation forms were examined, and it was determined that there was no difference in meaning between the two forms.

2.7. Content Validity

After the translation process, opinions of 10 experts who were experts in the fields of gynecology and obstetrics, child and public health nursing were received for the content validity of the scale. The Content Validity Index (CVI) was calculated to evaluate scope validity (26). Experts were asked to evaluate the suitability and comprehensibility of each item in the scale by scoring 1-4 according to the content validity index (CVI) [*1 point: Not suitable-4 points: Very suitable*].

With the suggestion of the experts, the word 'pain' was used instead of the word 'cramp' in the scale, and minor corrections that did not change the meaning were made in the items 10, 12, 13, 17, 34, 36 and 38.

2.8. Pilot Test

During the pilot test with 131 students selected from each level (high school 1,2,3,4), the students were asked whether there were any items that were not understood. Only some questions about acupuncture were asked, and an explanation was made by the researcher and an explanation was added to the 34th question accordingly. Additionally, minor changes that did not change the meaning were made to the items 20, 27, 36, and 39.

3. RESULTS

3.1. Descriptive Characteristics

The average age of the students was 16.52 ± 1.08 (min:14, max:19) and 31.5% were tenth grade students. It was found that 49.2% of the students 'occasionally' experienced and 50.8% of them 'always' experienced menstrual pain (Table 1).

Table 1. *Individual and dysmenorrhea features of students (n:1041)*

Variables	n	%
Grade		
9	216	20.8
10	328	31.5
11	276	26.5
12	221	21.2
Dysmenorrhea		
Occasionally	512	49.2
Always	529	50.8

3.2. Content Validity

According to the expert evaluations, it was determined that each item received more than 3 points (3.52-4.00), the mean

score was 3.92 ± 0.14 and the content validity index (CVI) was .98.

3.3. Pilot Test

When the pilot test data were evaluated (n = 131), the item-total scores of all items were >.20, the Cronbach Alpha internal consistency coefficient of the scale was .88, and the Cronbach Alpha internal consistency coefficient of the sub-dimensions were .60-.76.

3.4. Construct Validity

3.4.1. Confirmatory Factor Analysis (CFA)

In the study conducted by Hsieh et al., (19) it was suggested that the ADSCS theoretically consists of six primary factors; four of these primary factors (searching for knowledge, expression of emotions, seeking assistance, and control over external factors) are externally oriented behaviors and two of these primary factors (coping practices and self-control being) are internally oriented behaviors. In the CFA, as suggested by Hsieh et al., the theoretical structure (measurement model) created in this direction by associating the items with the primary level factors is given in the Model 1 line in Table 2. In addition, the results of the three different models are presented in Table 2.

Table 2. *Model tests and comparisons*

Measurement Models				Structural Model
	Model 1 (Theorotical)	Model 2 (Six Unrelated Factors)	Model 3 (Single Factor)	Model 4 (Two Factors)
Satorra Bentler x^2_{df}	$4895.22_{(725)}$	$6559.57_{(740)}$	$8347.97_{(740)}$	$4973.94_{(733)}$
$\frac{x^2}{sd}$	6.75	8.86	11.28	6.78
CFI	.90	.86	.82	.90
RMSEA (90% CI)	.07 (.07-.08)	.09 (.09-.09)	.10 (.10-.10)	.08 (.07-.08)
SRMR	.09	.18	.11	.09
Δx^2_{sd}		$1.664.35_{(15)}$	$3452.75_{(15)}$	

CFI: comparative fit index, RMSEA: root mean square error of approximation, SRMR:standardized root mean square residual; Satorra Bentler: Satorra-Bentler is a widely used statistical method in the field of structural equation modeling (SEM). Satorra-Bentler is used to test the model fit when observed variables are not normally distributed; Satorra Bentler x^2_{df}: Satorra Bentler Scaled Chi-Square (degree of freedom), sd:standard deviation

It was determined that Model 1 in Table 2 has better coefficients of agreement and disagreement than Models 2 and 3. Except for chi-square / sd and Standardized Root Mean Square Residual (SRMR), it was observed that the coefficients for Model 1 were close to the recommended cut-off values. The coefficient of agreement of the equivalent models fell far behind the theoretical model.

3.4.2. Theoretical Model Similarity and Discriminant Validity

The coefficients regarding the similarity and discriminant validities of the theoretical model are given in Table 3 and the composite reliability coefficients (CR) of the sub-dimensions were found to be higher than .70. However, average variance extracted (AVE) values of the other factors, other than searching for knowledge, are <.50. maximum squared variance (MSV) values are smaller than AVE for self-control being and searching for knowledge but larger than AVE for the other dimensions. AVE value is smaller than average shared square variance (ASV) only for control over external factors. Also, the square root of the average variance extracted (√AVE) for self-control being and searching for knowledge are larger than their correlations with the other factors.

In Table 4, the standard regression weights of the dimensions are between .76 and .80 for searching for knowledge; .45 and .75 for expression of emotions; .58 and .81 for seeking assistance; .26 and .68 for control over external factors; .25 and .77 for coping practices and .37 and .92 for self-control being.

Table 3. *The ADSCS theoretical model (model 1) similarity and discriminant validity coefficients*

Variables	CR	AVE	MSV	ASV	1	2	3	4	5	6
Coping practices	.85	.30	.52	.26	**.55**					
Seeking assistance	.78	.48	.67	.34	.54	**.69**				
Control over external factors	.70	.27	.52	.30	.72	.62	**.52**			
Self-control being	.79	.45	.01	.00	-.10	.03	.04	**.67**		
Expression of emotions	.81	.43	.67	.35	.57	.82	.65	-.03	**.65**	
Searching for knowledge	.87	.62	.37	.26	.42	.61	.39	.10	.58	**.79**

ADSCS: Adolescent Dysmenorrhea Self-Care Scale, CR:composite reliability, AVE:average variance extracted, MSV:maximum squared variance, ASV:average shared square variance

3.5. Reliability

Cronbach and Stratified alpha coefficients calculated (24) for the ADSCS's four

and two dimensional models are given in Table 5. The Cronbach alpha reliability coefficients of the sub-dimensions of the scale were found between .65 and .77 and the stratified alpha reliability coefficient was found .96. Item total correlations are between .20 and .68. The mean score of the ADSCS was determined to be 136.54 ± 30.33. When

item means were analyzed, it was found that the lowest mean was in the searching for knowledge sub-dimension and the highest one was in the expression of emotion sub-dimension (Table 5).

The test-retest correlation of the scale examined through Pearson correlation analysis was.98, and the correlation values of its sub-dimensions were .98 for searching for knowledge, .99 for expression of emotion, .97 seeking assistance, .94 for control over external factors, .97 for coping practices, and .87 for self-control being.

Table 4. *The ADSCS item – factor loadings*

Factor	Item	Load	Factor	Item	Load
Searching for knowledge	m1	.80	Coping practices	m22	.51
	m2	.80		m23	.48
	m3	.79		m24	.25
	m4	.76		m25	.42
Expression of emotions	m5	.45		m26	.50
	m6	.67		m27	.46
	m7	.66		m28	.64
	m8	.63		m29	.53
	m9	.72		m30	.64
	m10	.75		m31	.62
Seeking assistance	m11	.81		m32	.68
	m12	.75		m33	.50
	m13	.58		m34	.45
	m14	.61		m35	.77
Control over external factors	m15	.47	Self-control being	m36	.51
	m16	.26		m37	.92
	m17	.44		m38	.81
	m18	.45		m39	.60
	m19	.64		m40	.37
	m20	.55			
	m21	.68			

Table 5. *The ADSCS reliability coefficients and average values (N=1041)*

Factors	Cronbach Alfa	Stratified Alpha	Item – Total Correlations	Mean	Ss
Coping practices	.77		.20 to .52	50.45	13.13
Seeking assistance	.72		.47 to .60	11.45	5.43
Control over external factors	.65		.26 to .42	24.55	7.63
Self-control being	.71		.31 to .64	17.41	6.60
Expression of emotions	.76		.33 to .58	22.18	7.36
Searching for knowledge	.76		.41 to .68	10.49	5.21
Total		.96		136.54	30.33

Stratified Alpha: Reliability test used in multidimensional scales.

4. DISCUSSION

In this study, data obtained from Turkish adolescent girls showed that the Turkish ADSCS is a valid and reliable tool. The scale can be used to evaluate dysmenorrhea self-care of young Turkish-speaking girls.

4.1. Discussion of confirmatory factor analysis results

In the CFA in this study, firstly, the six-factor theoretical structure (Model 1) in which items are related to primary level factors (externally oriented behavior and internally oriented behavior) was examined as suggested by Hsieh et al (19). When the Model 2, Model 3 and Model 4 results were evaluated later, it was determined that Model 1 had better coefficients of agreement and disagreement than Models 2 and 3. Kline (27) finds it sufficient to give Comparative Fit Index (CFI), Root Mean Square Error of Approximation (RMSEA) and SRMR coefficients of agreement and disagreement in evaluating the relationships between model-based covariances and observed covariances. Hair et al. (28) suggest that chi-square value will be found statistically significant even if the models with more than 250 people and more than 30 items fit well, yet, they suggest it is sufficient for the CFI to be greater than .90, and SRMR is less than .08 and the RMSEA is less than .07. In addition, the chi-square / sd coefficient is desired to be 1-5. Accordingly, in our study, apart from chi-square / sd and SRMR, it is observed that the coefficients related to the theoretical model are close to the cut-off values recommended by Hair et al. (28). The high rate of chi-square / sd is related to the sample size of the study. Because chi-square is calculated with the chi-square = $(N - 1)*F_{min}$ formula. F_{min} (minimum fit function) is defined as the degree of discrepancy between the covariances (S) obtained from the model and the covariances (S) obtained from the data set. Due to this equation, no matter how small F_{min} is, chi-square statistics increase depending on the sample size as it is multiplied by the sample size. Since this analysis is based on data from 1041 people, the chi-square / sd coefficient was not taken into consideration in the evaluation of the models.

SRMR is defined as the standardized difference between the observed and predicted correlations (29). Marsh and Balla (30) state that SRMR is sensitive to sample size and therefore should not be used. Kenny (31) states that the SRMR coefficient is positively biased and this bias increases in small N and low sd studies. Also, Kenny (31) states that a model with high overall coefficient of agreement and parameter estimation might not be a valid, correctly defined model whereas a model with false marking, poor discriminant validity, or Heywood cases may have high coefficients of agreement. Crowley and Fan (32) state that evaluating goodness-of-fit does not have a golden rule, and since each reflects a different aspect of model fit, model fit should be evaluated based on a series of indexes. Based on this idea, since most of the indices show acceptable fit, the fit of the theoretical model is assumed high enough. The coefficient of agreement of the equivalent models is far behind the theoretical model.

4.2. Discussion of similarity and discriminant validity coefficient results

The similarity validity (do the items show a certain structure?) is examined depending on the standard regression weights >.50, the CR >.70 and the AVE >.50 for each factor. The discriminant validity (are the factors independent, separate features?) is evaluated with the square of the greatest structural covariances (MSV is MSV <AVE), the average of the common structural covariance squares (ASV, ASV <AVE) and the correlation of factor √AVE with other factors being greater than the absolute value of itself (33). In the study, it was determined that the item – factor loadings were mostly >.50. When the similarity validity results are examined, it can be suggested that the similarity validity is provided to a great extent for the dimensions of seeking assistance, self-control being, expression of emotions, and searching for knowledge. When the findings are examined in terms of discriminant validity, it is seen that the measurements obtained from the searching for knowledge and self-control being dimensions meet all the criteria. Although ASV values of all factors are smaller than AVE values, except for the control over external factors, the fact that the MSV values of the other dimensions (Coping practices, seeking assistance, control over external factors, and expression of emotions) are larger and that the correlation of √AVE with the other factors for each factor is less than the absolute value indicates that their discriminant validities are weak. Jöreskog (34) proposes three strategies for structural model applications: absolute and confirmatory, model development and model comparison. Within the scope of the scale adaptation study, this study adopted confirmatory and model comparison strategies. The confirmatory strategy analyzes the creation of a single model and the fit of the model to the data. The original structure is considered to be supported when the model produces results that fit the data. In this context, the original model is compatible with the data obtained from the Turkish sample. In comparison, the power of different models to explain the relationships between data on theoretical or conceptual basis is compared. In the study, the coefficients of the original model for divergent validity were low. Hair et al (35) stated that if a factor is better explained by items that do not belong to it, or if the factors are highly correlated, there will be a discriminant validity problem because when the factors are highly correlated, they share some of their predictive power in the items. The single factor model tried in the study is related to the issue of divergent validity. If the data contains the problems stated by Hair et al. (35) then the single factor model should explain the relationships between the data as good as the original model. Model comparison showed that the multi-factor model had better explanatory power than the single-factor model the relationships. At this stage, including the solution of factor or item deletion or creating composite variables from highly correlated ones to solve the problem of divergent validity, the model development strategy which starts with a certain model and involves experimenting until the most suitable model for the data and interpretation is obtained was not used. This is because model development is used to test hypotheses, results vary depending on the sample, and possibly have different non-theoretical factors than the original.

4.3. Discussing the reliability results

In order for a scale to be accepted as reliable, the Cronbach Alpha value generally accepted greater than .60, and as the value approaches 1, the reliability level of the scale increases (36). In the original study, the Cronbach alpha reliability coefficient was found .89 for the whole scale, and for the subscales resource utilization .73, seeking assistance .80, control over external factors .65, self – control being .62, expression of emotions .77, searching for knowledge .76 (19). In the study of Yeh et al. (37), the Cronbach alpha reliability coefficient was .90 and in the study of Wong et al. (38) .92. In this study, Cronbach alpha reliability coefficient was found .96 and for the subscales Cronbach alpha coefficient was determined, coping practices .77, seeking assistance .72, control over external factors .65, self – control being .71, expression of emotions .76, searching for knowledge .76. These results show that the reliability of the Turkish ADSCS is quite high and its sub-dimensions have sufficient reliability. Item-total correlations in the original scale ranges from .30 to .92 (19). In the result of our research, item total correlations are between .20 and .68. Clark and Watson (39) suggest that the mean of item – total correlations should be in the range of .15 to .20 for scales measuring large features and between .40 and .50 for narrower ones. Streiner and Norman (40) stated that it is acceptable to have item total correlations >.25 in a scale or sub-dimensions. In this framework, the item-total correlations of the Turkish ADSCS are sufficient (39).

The test-retest correlation value of >.40 to evaluate the temporal invariance of the score obtained from the scale is considered acceptable (40). In this study, the test-retest total correlation performed at two-week intervals was .98, and the reliability of the correlations of the sub-dimensions were remarkably high .87-.99.

4.4. Discussion of average scale scores

When the studies conducted with the ADSCS were analyzed, it was found that the total average score was 150.24 ± 30.94 and above in the study of Yeh et al. (37) and 124 ± 31.6 in the study of Wong et al. (38). In our study, the total score of the ADSCS was 136.54 ± 30.33, similar to the previous study results. Despite the differences in pain as a subjective expression, the variability of cultural characteristics and the number of universes used, it is thought that the similarity between the results of the study shows the effectiveness of the measurement tool. This similarity and the fact that the range of scores determined in the sample groups is above the mean score of the scale shows that adolescents living in different regions prefer self-care practices in the management of dysmenorrhea.

4.5. Implications for Practice

The Turkish ADSCS can be used to detect deficiencies by evaluating self-care skills for dysmenorrhea of Turkish-speaking adolescents and young people in Turkey and other countries. The effects of self-care improvement programs to be applied for adolescents with problems can be evaluated with the ADSCS. A valid and reliable pretest-posttest evaluation can be done with the ADSCS in interventional researches applied to increase dysmenorrhea self-care.

4.6. Study Limitations

This study has several limitations. The fact that the data is based on self-report and the recall of past self-care experiences may affect data quality. At the same time, the fact that the data is based on self-report may create a social acceptability bias in adolescents.

5. CONCLUSION

This research showed that the Turkish ADSCS is a valid and reliable tool and has six sub-dimensions consistent with the original scale. With this scale, self-care for dysmenorrhea can be evaluated in young girls, and adolescents with problematic or inadequate dysmenorrhea self-care behaviors can be identified and appropriate interventions can be made afterwards.

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Research idea: ES, AE
Design of the study: ES, AE
Acquisition of data for the study: ES
Analysis of data for the study: ES, AE
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Appendix

ADÖLESAN DİSMENORE ÖZBAKIM ÖLÇEĞİ

Alt Ölçek 1: Bilgi Arama	Hiç katılmıyorum (0)	%20 katılıyorum (1)	%40 katılıyorum (2)	%60 katılıyorum (3)	%80 katılıyorum (4)	%100 katılıyorum (5)
Adet ağrısını azaltmak amacıyla uygulanabilecek yöntemleri çevremdeki insanlara sorarım (örneğin: aileye, öğretmenlere, sınıf arkadaşlarına, sağlık çalışanlarına).						
Adet ağrısı ile ilgili televizyon haberleri, kitaplar veya dergilerle ilgilenirim.						
Kitap ve dergilerde adet ağrısı ile ilgili olan araştırma sonuçlarına ve makalelere bakarım.						
Adet ağrısı ile ilgili bilgi almak için interneti kullanırım.						
Alt Ölçek 2: Duyguların İfadesi						
Adet dönemlerimde hangi bölgelerimden rahatsız olabileceğimi net olarak bilirim.						
Adet ağrım olduğunda, suratım asılır ve kendimi iyi hissetmediğimi çevremdeki insanlara söylerim.						
Karnımı tutar ve kendimi iyi hissetmediğimi söylerim.						
Arkadaşlarıma adet dönemimdeki ağrı deneyimlerimi anlatırım.						
Aileme adet ağrısı çektiğimi söylerim.						
Adet ağrısı sorunumla ilgili çevremdeki insanlarla (örneğin: ailem, öğretmenlerim, sınıf arkadaşlarım, sağlık görevlileri) konuşurum.						
Alt Ölçek 3: Yardım Arama						
Adet ağrım olduğunda ailemden yardım isterim.						
Okulda adet dönemi ağrı sorunları yaşadığımda bunun üstesinden gelebilmek için arkadaşlarımdan yardım isterim. (Örneğin revire kadar bana eşlik etmesi gibi)						
Okulda adet dönemi ağrı sorunlarımın üstesinden gelebilmek için okul hemşiresinden yardım isterim (…okulda hemşire olsaydı yardım isterdim).						
Okulda adet dönemi ağrısorunlarımın üstesinden gelebilmek için öğretmenlerimden yardım isterim.						
Alt Ölçek 4: Dış faktörlerin kontrolü						
Adet ağrım olduğunda evdeysem, uyurum.						
Adet ağrım olduğunda, ağrıyı hafifletmek için müzik dinlerim.						
Adet ağrısı yaşadığımda dışarıda yapılan aktivitelere katılmaktan kaçınırım.						
Evdeyken adet ağrım olduğunda kendimi daha rahat hissetmek için odayı havalandırırım.						
Evdeyken adet ağrım olduğunda kendimi daha rahat hissetmek için odanın ısı derecesini ayarlarım.						
Adet ağrım okulda olduğunda beden eğitimi derslerine girmem.						
Adet ağrım olduğunda okuldan izin alarak evde dinlenirim.						
Alt Ölçek 5: Başetme uygulamaları						
Adet ağrım olduğunda rahat kıyafetler giyerim. (Örneğin; rahat tişört veya pantolon)						
Adet ağrım olduğunda daha çok ılık su içerim.						
Adet ağrım olduğunda daha çok çikolata veya tatlı yiyecekler yerim.						
Adet ağrım olduğunda yoğun egzersiz yapmaktan kaçınırım.						
Adet ağrım olduğunda sıcak suyla duş alırım.						
Adet ağrım olduğunda soğuk, buzlu yiyecekler yemem.						
Adet ağrım olduğunda ağrıyan bölgeye masaj yaparım.						
Adet ağrım olduğunda ağrıyan bölgeye vurur veya hafifçe sıvazlarım.						
Adet ağrım olduğunda sıcak tutacak giysiler giyerim.						
Adet ağrım olduğunda karnımın alt kısmına sıcak su torbası veya diğer ısıtıcı cihazları koyarım.						
Adet ağrım olduğunda ağrı kesici ilaçlar kullanırım.						
Adet ağrım olduğunda bitkisel ilaçlar kullanırım.						
Adet ağrımın üstesinden gelebilmek için akupunktur* noktalarına masaj uygularım.						
Adet ağrısı yaşadığımda eczaneden ağrı kesici ilaçlar satın alırım.						
Alt Ölçek 6: Öz Kontrol						
Adet ağrım olduğunda bir şey yapmam geçene kadar dayanmaya çalışırım.						
Adet ağrım olduğunda kendi kendime bunun normal olduğunu söylerim.						
Adet ağrım olduğunda kendi kendime bunun hayatın gerçeği olduğunu söylerim.						
Adet ağrım olduğunda kendi kendime çok fazla ağrım olmadığını söylerim.						
Adet ağrım olduğunda bir şeylerle meşgul olmaya çalışırım.						

Clinical and Experimental Health Sciences

Evaluating the Effect of Jointly Administering Synthetic Graft and Ankaferd Blood Stopper for Bone Recovery in Created Bone Deformities in Diabetic Rats

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ABSTRACT

Objective: In the present paper, it was purposed to examine the late impacts of bone tissue recoveries in combination with synthetic grafts and Ankaferd Blood Stopper (ABS), which are effective in bone wound healing in diabetic rats.

Methods: A total of 64 Wistar albino male rats with diabetes were studied. A bone deformity was generated in the calvarium of diabetic rats. These diabetic rats are divided into 4 different groups. Only saline was applied to the bone defect in group 1, Beta-tricalcium phosphate (β-TCP) graft was administered to Group 2, ABS solution to Group 3, (β-TCP+ABS) were administered to group 4. Eight rats from each group were sacrificed on the 28th day and another 8 rats on the 56th day. Immunohistochemical, histopathological, and Dual Energy X-ray Absorptiometry (DEXA) analyzes of the obtained samples were made.

Results: In histopathological measurements, osteoblastic activity and bone regeneration were considerably higher in the group treated with group 2 and group 4 when compared to the control group on the 28th and 56th day ($p< .05$). Western blotting findings showed that the osteopontin (OP) and osteonectin (ON) expression at 28th day increased dramatically in the treated with group 4. DEXA analyzes revealed that BMC values in groups treated with group 2, group 3 and group 4 on 28th day were considerably higher than the control group ($p< .05$).

Conclusion: We can believe and conclude that ABS in combination with a β-TCP bone graft will produce more successful outcomes on wound healing and formation of new bone in diabetic rats.

Keywords: Bone Regeneration, Beta-tricalcium phosphate, Dual-Energy X-Ray Absorptiometry

1. INTRODUCTION

The periodontal disorder is an infectious disease characterized by periodontal tissue and bone loss that develops with bacterial chronic inflammation of tissues that make up the periodontium (1). The etiologic factor of periodontal disease is the pathogen bacteria found in microbial dental plaque and oral cavity. Bacteria are not alone responsible for the formation of periodontal disease. In the development of periodontal disease, the inflammation in the tissue may result in changes in bone morphology and deterioration of bone tissue (1,2). However, a lot of graft materials are used in periodontal defects. The purpose of bone grafting applications is to increase bone volume (3). Studies are still being carried out to achieve the desired effect in these applications. However, in spite of these studies, an ideal graft material has not yet been found to allow the lost tissues to return to their original state and fill the bone defects completely (3,4). β-TCP being a synthetic alloplast is a reliable and biocompatible graft material with osteoconductive and bioactive. It plays a role as a biological filler that is partially resorbed, mechanically clinging to the bone and enables the graft and bone to replace. However, β-TCP is a poor graft due to its inappropriate porosity, small grain and dissolution in a 6-weeks period (4,6).

Although the primary cause of periodontal diseases is a microbial dental plaque, it is accepted that existing systemic diseases increase the risk of periodontal disease by affecting the severity and prognosis of the disease. Diabetes mellitus (DM) is one of the most common systemic diseases (7,8). It has been reported that DM changes bone characteristics and has negative effects on fracture healing in both human and animal studies (9). Diabetes reduces osteoblastic activity and bone mineralization. In addition, microvascular complications of diabetes and therefore decreased blood flow increases bone fragility (10).

ABS (Ankaferd Pharmaceuticals Cosmetic Co., Ltd., Istanbul, Turkey) is the first herbal content used in Turkish medical sector as a haemostatic material containing Glycrhiza Glabra, Vitis Vinifera, Alphina Officinarum's dry leaf extracts, Urtica dioica's dry root extract, Thymus vulgaris' (thyme) dried herb extracts (11). ABS produces a protein network that supports erythrocyte aggregation. In addition, ABS is revealed to have an impact on bone healing at early periods (12). As a second

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characteristic, anti-inflammatory and antioxidant effect of ABS has been reported. The effect on the bone in the late period is not definitively determined (13).

There is limited number of studies evaluating effects of ABS on late-term diabetic bone tissue healing. Thus, the purpose of this research was to evaluate the impacts of jointly applying β-TCP and ABS as anti-bleeding agents on the recovery of diabetic bone tissue by histopathological, immunohistochemical and radiological methods.

2. METHODS

This study received an approval from Dicle University Experimental Animals Ethics Committee (No: 2015/13 – Date:18.02.2015). A total of 64 4-month-old Wistar male Albino diabetic rats, weighted around 300 g, were utilized.

2.1. Inducing Diabetes in Rats

0.294 g sodium citrate dihydrate was taken, and its volume was completed to 100 ml with pure water and, its pH was stabilized to 4.5 by hydrochloric acid (HCl). STZ (Streptococin®, Sigma-Aldrich, China) was prepared by sodium citrate buffer. A one shot of 50 mg/kg STZ solution was given to 64 rats to initiate diabetes by intraperitoneal injection. 3 days after STZ injection, glucose measurements were performed in the glucometer device (Optima®, Hsinchu, Tayvan) by taking blood samples from the tail vein of the rats by fasting for 12 h, if the fasting blood glucose was 140 mg/dl or higher, it was considered diabetic.

2.2. Surgical Method and Experiment Grouping

Experimental diabetic animals were left hungry 12 hours before surgery. The rats were applied 10% ketamine HCl and 2% xylazine HCl anesthesia. They were incised in the coronal midline of head skin via a no-15 surgical lancet, the heads were freely positioned. Later, the frontal bones were unveiled via a periost elevator. Following this procedure, a full-thickness bone incision was made in the midline circularly with a 7 mm diameter trephen burs (Trephine®, Turkey). After the operation, the skin incision was mainly covered with 3/0 silk suture for prophylactic purposes. Right after the surgery, one shot of 50 mg/kg antibiotic (Betamoxla®, Turkey) was administered into the muscles of each rat.

Diabetic rats used in our study were planned for 28th day and 56th day. Group 1**:** Saline was performed on bone defects of 16 diabetic rats. Group 2: 0.125 cc β-TCP (KeraOS®, Spain) graft with 0.25-1 mm particle was put into the deformities of 16 rats. Group 3: 0.125 cc ABS was administered to the deformities of 16 rats Group 4: 0.125 cc (β-TCP graft + ABS) was implanted into the bone deformities of 16 diabetic rats. In experimental diabetic animals, euthanasia was performed on 64 rats. Rats were anesthetized through sodium thiopental (Pentalyn Sodyum®, Turkey) whose mortal dose is injected as 60 mg/kg.

2.3. Preparing Sections for Histological and Immunohistochemical Examination

The calvarium bone was taken in an annular form with round burs to coat the deformed area. The samples were put in 10% formaldehyde solution. After completely fixing the samples, they were washed for 12 hours under water. Later, they were kept at the gradually concentration alcohol for dehydration during 12 hours. After that, they were embedded in the paraffin blocks. After making 5 µm sections via a microtome (Rotatory Microtome, Germany) from paraffin blocks, it was stained through Hematoxylin-Eosin (H-E).

Paraffin Sections were put on poly-L-lysine covered slides after the sections were kept in the xylenes for 2 x 5 minutes. Then, they were placed the EDTA (Ethylene-diamine-tetraacetic acid) solution to dissolve the bone tissue. All following processes were performed in this incubation vessel. Then, the Osteonectin and osteopontin antibodies (mouse monoclonal, 1/200, Santa Cruz, CA) were administered to the sections. Main antibody was dropped and kept for 1 hour. After biotinized dropping, secondary antibody (Histostain-Plus Kit, Carlsbad, CA) became consistent with the primary antibody. AEC (Aminoetil Carbazole) was dropped as chromogen. Antigen-antibody reaction was prevented by washing with distilled water. At the last stage, sections for blind evaluation were evaluated by photomicroscope (Nikon Eclipse i50, Japan) immunohistopathologically.

2.4. Western Blotting

The calvarium tissues frosted in liquid nitrogen were pulverized in porcelain mortar. 50 mg of powdered placenta tissue was maintained in 250 µl RIPA lysis solution including a blend of protease inhibitor in frost for 1 hour. Total cellular protein concentration was computed by the BCA Kit (Pierce, Thermo scientific). Protein samples were constituted in 1xSDS loading solution (2% SDS, 5% glycerol, 0.01% bromophenol blue, 8% DL-ditiyotretol) and electrophoresis was conducted in SDS running solution (2.4 mM Tris, 19.2 mM Glycin, 0.01% SDS) for 1 hour at 200 V. Removed proteins were moved from SDS-PAGE to PVDF membrane within transfer solution at 100 V for 1 hour. Following washing, the membrane was applied with horseradish peroxidase conjugated secondary antibodies at a dilution rate of 1/ 10000 for 1 hour at room temperature. Protein bands were visualized using ECL (increased chemiluminescent reagent) chemical (BioRad). Photos were taken using BioRad ChemiDoc™ MP tool.

2.5. Radiological Examination

Bone deformities of rats were measured with DEXA. Bone Mineral Amount (BMC) (gr) and Bone Mineral Density (BMD) (gr/cm2) calculations of all rats were carried out via the DEXA (Hologic®, USA) tool. But, solely, the measurements of the head was examined.

2.6. Statistical Analysis

The effect size was 2.56 and the α value was set at .05. The power of this experimental animal study was calculated to be 95%. The sample size was calculated in a computer program (G*Power, ver; 3.1.9.2, Kiel, Germany). In our study, statistical analyses of data were done with SPSS (IBM® Ver: 21.0, USA) statistical program. Mann Whitney U test was employed to make a comparison for the data with non-normal distributions for two groups, and the Kruskal Wallis test was employed for the two groups. Mann Whitney U test, as corrected by Bonferroni, was also employed to compare the groups. Wilcoxon test was employed for intra group comparison. p< .05 was considered as statistically important in all the tests.

3. RESULTS

3.1. Histological Findings

On the 28th day of group 1, intense vascularization and increased fibrous tissue were observed in the defect area. Vacuolar structures and few osteoblastic cells were witnessed in areas where bone trabeculae developed (Fig. 1A). On day 56 of group 1, dilatation and hemorrhage in blood vessels, inflammatory cell infiltration and an increase in fibrous tissue were observed (Fig. 1E). On the 28th day of group 2, reduced vascularity and diffused mononuclear cells in the outside area of the defect site were observable. We saw that the bone trabeculae where the defect rings are located was prominent. In addition, the osteocyte cells came to the forefront with the increase of osteoblastic activity (Fig. 1B). On the 56th day of group 2, an increase in the osteoblastic activity around the graft and prominent bone trabeculae with enhanced osteocyte formation was clearly observed. Hemorrhage was seen in blood vessels with inflammatory cells in the form of clusters (Fig. 1F). On the 28th day of group 3, small increases in vascularization and inflammatory cells were observed, and osteoblastic cells began to stand out in small trabecular parts where islet-like bone fragments were observed (Fig. 1C). On the 56th day of group 3, dilatation in blood vessels was seen, inflammatory cells were placed in clusters, and osteoblastic activity began in small bone trabeculae as wrapped around the graft (Fig. 1G). On the 28th day of group 4, a rise in collagen fibers and a decline in inflammatory cells in the deformed zone were seen, and the development of osteoblastic activity and osteocytes showed some increase. The bone trabeculae expanded and the osteocytes within the lacunae became evident (Fig. 1D). On the 56th day of group 4, there was a parallel distribution of collagen fibers along with hemorrhage and inflammatory cell density. Osteoblastic activity and osteocyte organization were clearly observed in the expanding bone trabeculae. New bone formation began to take shape (Fig. 1H).

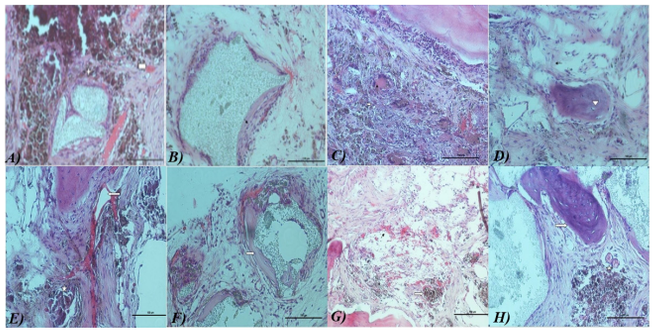

Figure 1. *Hematoxylin-eosin stained sections of the groups. A) Vascularization (stellate) and osteoblastic cells (white arrow) are shown on a histopathologic section taken at 28th day. B) Osteocyte cells (thin black arrow) at 28th day. C) A histopathologic section taken at 28th day showed vascularization and inflammatory cells (white arrow) and islet-shaped bone fragments (black thin arrow). D) The histopathologic section taken at 28th day showed collagen fibers (thin black arrow) and osteocytes within lacunae (triangle). E) On the histopathologic section taken at day 56, dilated blood vessels (thick white arrow) and inflammatory cells (stellate) are shown. F) A histopathologic section taken at 56th day demonstrates enhanced bone trabeculae (thick white arrow) developing osteocytes. G) Histopathological examination taken at 56th day revealed inflammatory cells (thick white arrow) and the onset of osteoblastic activity (thin black arrow). H) On the histopathologic section taken at day 56, inflammatory cells (stellate) and prominent osteocytes are shown (thick white arrow). Hematoxylin-Eosin staining Bar 100μm.*

3.2. Immunohistochemical Findings

On day 28 of group 1, negative osteopontin expression was observed in osteoblasts (Fig. 2A). On the 56th day of group 1, osteopontin expression was demonstrated in osteoblast cells between inflammatory cells outside the graft sites (Fig. 2E). On day 28 of group 2, positive osteopontin expression was observed in osteoblast cells in the periphery of bone trabeculae (Fig. 2B). On the 56th day of group 2, osteoblast cells in the bone grafts demonstrated positive osteopontin expression in bone trabeculae in the graft region, and osteocyte cells initiated to form new bone structure (Fig. 2F). On day 28 of group 3, osteopontin positive expression was seen in tiny bone trabeculae between inflammatory cells (Fig. 2C). On day 56 of group 3, weak osteopontin expression in osteoblast cells outside the bone trabeculae in the graft site and positive osteopontin expression among inflammatory cells were observed (Fig. 2G). Positive osteopontin expression in osteoblast cells in flat bone fragments at the graft site was demonstrated on the 28th day of group 4 (Fig. 2D). On the 56th day of group 4, osteopontin positive expression was seen in osteoblasts outside the bone trabeculae of different sizes except for graft sites, and osteopontin positive cells were observed in connective tissue cells (Fig. 2H).

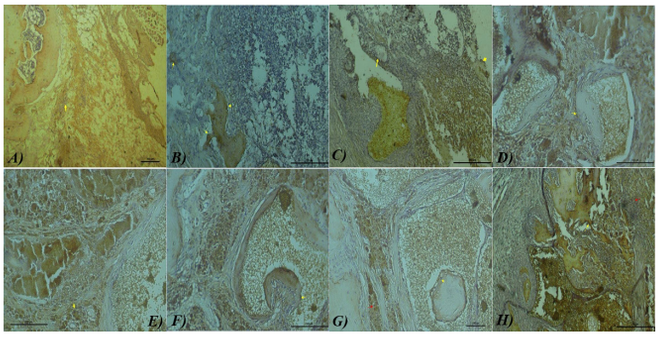

Figure 2. *Photomicroscope view of sections prepared with osteopontin antibody. A) Negative osteopontin expression in osteoblasts (yellow arrow) on the immunohistochemical section on day 28. B) The osteoblast cells in the periphery of the immunohistochemical section on day 28 were positive for osteopontin expression (yellow arrow). C) Osteopontin expression in bone trabeculae positive (yellow arrow) on the immunohistochemical cut at day 28. D) Positive osteopontin expression in osteoblast cells (yellow arrow) in the immunohistochemical section taken on 28th day. E) Osteopontin expression of osteoblast cells (yellow arrow) in the immunohistochemical section taken on day 56. F) On the 56th day, osteoblast cells marked positive osteopontin expression (yellow arrow). G) An immunohistochemical cross-section taken on day 56, osteoblast cells showed weak osteopontin expression (yellow arrow), and inflammatory cells showed positive osteopontin expression (red arrow). H) At day 56, positive osteopontin expression in bone osteoblast cells (yellow arrow) and positive osteopontin expression in osteoblast cells of connective tissue (red arrow). Osteopontin immunostaining Bar 100µm.*

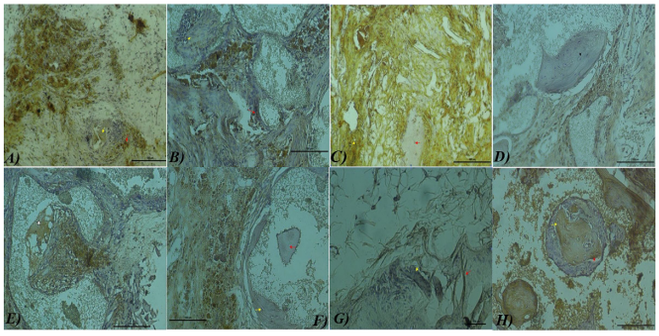

Figure 3. *Photomicroscope view of sections prepared with osteonectin antibody. A) The osteonectin expression in the connective tissue cells is positive (red arrow) and the osteocyte expression in osteocyte cells of bone is negative (yellow arrow) in the immunohistochemical section on day 28. B) Positive osteonectin expression in the connecting tissue cells (red arrow) and positive osteonectin expression in the osteocytes in bone (yellow arrow) are shown in the immunohistochemical section on day 28. C) Positive osteonectin expression (yellow arrow) in osteocytes and negative osteonectin expression in bone trabeculae (red arrow) are shown through immunohistochemical section taken on the 28th day. D) Osteonectin expression in osteocytes in the immunohistochemical section on day 28 (thin black arrow). E) Negative osteonectin expression (stellate) in osteocytes in the immunohistochemical section on day 56. F) Positive osteonectin expression (yellow arrow) in osteocytes and negative osteonectin expression (red arrow) in osteocyte cells in bone within the graft in the immunohistochemical section at day 56. G) Weak osteonectin expression in osteocytes (yellow arrow) and positive osteonectin expression in collagen bands (red arrow) immunohistochemical section taken on day 56. H) Positive osteonectin expression in osteocytes in bone (yellow arrow) and positive osteonectin expression in bone matrix (red arrow) at the immunohistochemical section on day 56. Osteonectin immunostaining Bar 100µm.*

On day 28 of group 1, positive expression was seen in the connective tissue cells in the collagenous fiber spaces among the inflammatory cells, while osteocytes in the small bone fragments showed an osteonectin-negative reaction (Fig. 3A). On day 56 of group 1, osteocyte cells did not develop in bone trabeculae within the graft site, and osteonectin expression was negative (Fig. 3E). On the 28th day of group 2, osteonectin-positive expression was less in certain connective tissue cells between the collagen fibers around the graft sites, while there was the osteonectin-positive reaction in a small number of osteocytes in bone trabeculae (Fig. 3B). On the 56th day of group 2, bone trabeculae began to mature within the connective tissue outside the graft site and positive osteonectin expression in osteocytes was observed. Negative expression was also detected in osteocytes in trabeculae within the graft site (Fig. 3F). On day 28 of group 3, positive osteonectin expression in osteocytes in some bone trabeculae was observed, while negative expression was also determined in certain trabeculae (Fig. 3C). On the 56th day of group 3, weak osteonectin expression in the osteocytes of the small bone trabeculae and positive osteonectin expression in the thickened collagen bands were seen (Fig. 3G). Osteonectin expression was demonstrated in osteocytes in bone trabeculae on the 28th day of group 4 (Fig. 3D). On day 56 of group 4, osteonectin expression was positive in a few osteocytes in the bone trabeculae placed between connective tissue cells in the graft site, while osteonectin expression in the bone matrix was positive (Fig. 3H).

3.3. Western Blotting Findings

The expression level of OPN and ON in the calvarium tissue rose drastically in β – TCP and ABS+ β – TCP groups on day 28. Twenty µg total protein was run on a gel. Anti-osteonectin and anti-β-actin antibodies were employed in Western Blotting method, and β-actin was utilized for load control (Fig. 4).

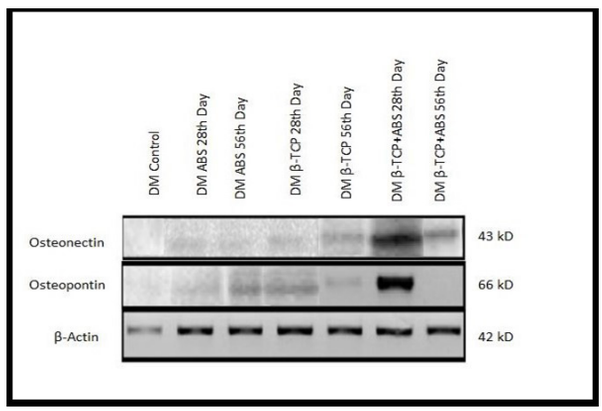

Figure 4. *Osteopontin and osteonectin expression in calvarium tissue rose dramatically in β-TCP and ABS+ β-TCP groups on day 28. DM: diabetes mellitus, β-TCP: beta-tricalcium phosphate, ABS: ankaferd blood stopper, kD: kilodalton.*

3.4. Histopathological Findings

The bone specimens taken on the 28th and 56th days from calvariums of diabetic rats were histopathologically examined, and significant difference was identified when the values of osteoblastic activity, inflammatory cell infiltration, vascular dilatation and hemorrhage and new bone formation were compared between groups (p< .001). Post hoc test was carried out on the 28th day of diabetic rats. There was statistically significant difference in terms of osteoblastic activity and new bone formation scores between group 1 and group 2, group 1 and group 4, group 2 and group 3, and group 3 and group 4. There was also significant difference in terms of inflammatory cell infiltration, vascular dilatation and hemorrhage scores between group 1 and group 2, group 1 and group 3, and group 1 and group 4. Post hoc test was applied to diabetic groups on the 56th day. There was significant difference in terms of osteoblastic activity and new bone formation scores between group 1 and group 2, group 1 and group 3, and group 1 and group 4 (Table 1).

Table 1. *Comparing histopathological values between groups on days 28 and 56 of rats with diabetes*

Groups		Group 1 (mean±S)	Group 2 (mean±SD)	Group 3 (mean±SD)	Group 4 (mean±SD)	p	p_{1-2}	p_{1-3}	p_{1-4}	p_{2-3}	p_{2-4}	p_{3-4}
Osteoblastic activity	28. Day	1.00±0.53	2.63±0.52	1.18±0.54	2.63±0.52	< .001**	.001**	.078	.001**	.001**	1.000	.001**
	56. Day	1.00±0.54	2.75±0.46	2.13±0.35	2.75±0.46	< .001**	.001**	.001**	.001**	.015*	1.000	.015*
Inflammatory cell infiltration	28. Day	3.63±0.52	1.38±0.52	2.88±0.64	1.25±0.46	< .001**	< .001**	.028*	< .001**	.001**	.602	.001**
	56. Day	2.88±0.64	1.13±0.36	200±0.54	1.13±0.64	< .001**	.001**	.013*	.001**	.004*	.945	.013*
Vessel dilatation and hemorrhage	28. Day	3.13±0.64	2.00±0.54	1.50±0.54	1.38±0.52	< .001**	.004*	.001**	.001**	.085	.036*	.626
	56. Day	3.38±0.52	1.00±0.54	1,.75±0.46	1.25±0.46	< .001**	< .001**	< .001**	< .001**	.013*	.332	.053
New bone formation	28. Day	1.13±0.36	2,.50±0.54	1.38±0.52	3.38±0.52	< .001**	.001**	.264	< .001**	.003*	.010*	< .001**
	56. Day	0.75±0.46	2.75±0.46	2.25±0.46	2.75±0.46	< .001**	< .001**	< .001**	.001**	.053	.880	.082

*SD: standard deviation, *p≤ .005: significant, ** p≤ .001: very significant, p: Kruskal Wallis Test; p1-2, p1-3, p1-4, p2-3, p2-4 and p3-4:Mann Whitney U Test*

Table 2. *Comparing BMC and BMD values between groups of diabetic rats*

		Group 1 (mean±SD)	Group 2 (mean±SD)	Group 3 (mean±SD)	Group 4 (mean±SD)	p	p_{1-2}	p_{1-3}	p_{1-4}	p_{2-3}	p_{2-4}	p_{3-4}
BMC	Beginning	2.25±0.09	2.28±0.10T	2.26±0.08	2.30±0.07	.800	-	-	-	-	-	-
	28. Day	1.97±0.20	2.65±0.17	2.75±0.12	2.89±033	.021*	.021*	.021*	.020*	.663	.146	.375
	56. Day	2.61±0.21	2.78±0.26	2.81±0.13	2.76±0.15	.442	-	-	-	-	-	-
BMD	Beginning	0.30±0.01	0.31±0.01	0.29±0.03	0.30±0.03	.708	-	-	-	-	-	-
	28. Day	0.30±0.04	0.31±0.01	0.31±0.01	0.33±0.03	.301	-	-	-	-	-	-
	56. Day	0.31±0.03	0.32±0.01	0.32±0.01	0.33±0.01	.560	-	-	-	-	-	-

*BMC: bone mineral amount, BMD: bone mineral density, SD: standard deviation, *p< .05: significant, p: Kruskal Wallis Test, p1-2, p1-3, p1-4, p2-3, p2-4 and p3-4: Mann Whitney U Test..*

3.5. DEXA Findings

The BMC and BMD values were measured in the bone defect in the calvarium of diabetic rats on the 28th day. Comparison of the mean BMC and BMD values between groups showed that 28th day BMC values were statistically significant (p= .021)**.** Post hoc test was performed on diabetic rats on the 28th day. Significant difference was seen in the BMC values between group 1 and group 2, group 1 and group 3, and group 1 and group 4 (Table 2).

We conducted an intragroup comparison of diabetic rats. No significant difference was found in the BMC and BMD values between the baseline days (28th day) and between days 28 and 56 of the rats. In Group 1, significant difference was found in the BMD values between 28th and 56th days (p=.021) (Table 3).

Table 3. *Comparing intragroup BMC and BMD values of diabetic rats*

		Beginning (mean±SD)	28. Day (mean±SD)	56. Day (mean±SD)	p_{0-28}	p_{0-56}	p_{28-56}
Group 1	BMC	2.25±0.09	1.97±0.20	2.61±0.21	.144	.068	.021*
	BMD	0.30±0.01	0.30±0.04	0.31±0.03	.713	.414	.375
Group 2	BMC	2.28±0.10	2.76±0.20	2.78±0.26	.068	.068	.189
	BMD	0.31±0.01	0.32±0.01	0.32±0.01	1.000	.180	.129
Group 3	BMC	2.26±0.08	2.75±0.12	2.81±0.13	.068	.068	.772
	BMD	0.29±0.03	0.31±0.01	0.32±0.01	.144	.144	.369
Group 4	BMC	2.30±0.07	2.89±0.33	2.76±0.15	.068	.068	.561
	BMD	0.30±0.03	0.33±0.03	0.33±0.01	.144	.144	1.000

*BMC: bone mineral amount, BMD: bone mineral density, SD: standard deviation, *p< .05: significant, p0-28 and p0-56: Wilcoxon Test, p28-56: Mann Whitney U Test.*

4. DISCUSSION

The main purpose of using these materials is to accelerate the healing process in bone tissue, restoring alveolar bone, gaining clinical attachment, decreasing pocket depth, and achieving desired bone healing in the regeneration zone (14).

Synthetic TCP and HA grafts are biocompatible, therefore, are widely used instead of autogenous bone grafts due to their osteoconductive properties. β-TCP is resorbed at the earliest 4 weeks on radiographs and SEM. After using β-TCP; new bone formation differs depending on the material combination, pore size, geometry and particle structure (15).

Diabetes is known to be a high-risk group for bacterial infections such as periodontitis, and the relationship between periodontitis and diabetes has been a topic of many research for years. Even periodontal diseases have been recognized as complications of diabetes (7,16). Studies show that diabetes affects the wound healing during the formation of the bone. It has been reported that diabetes reduces the production of osteoblasts, osteoids, and osteocalcin in bone tissue and destabilizes bone formation and destruction (17).

Experimental diabetes animal models can be created by chemical, viral and surgical methods. By administering a single dose of STZ chemically, diabetes can be formed by creating partial damage to pancreatic β cells. Junod et al. (18) reported that their STZ administration dose is 25-100 mg/kg. According to the literature, there is no difference in the diabetes effect between 55-65 mg/kg STZ doses. In addition, the general metabolism of diabetes in STZ-induced diabetic rats is similar to that of DM in humans (19). In the present study, diabetic rats were formed with a single dose of 50 mg/kg STZ.

There are some studies evaluating antibacterial, antimicrobial and antifungal efficacy besides the hemostatic effect of ABS (20). Studies have also been performed to examine the impact of healing bone and soft tissue scars (21,22). Bulut et al. (23) found that in studies evaluating the effect of ABS on bone healing in diabetic rats, forming new bone in the diabetic group was considerably reduced on both 7th and 14th day when compared to the control group. It was observed that the amount of forming new bone in the diabetic group treated with ABS was greater than in the control group.

In our study, the difference between ß-TCP (ß-TCP +ABS) group and the control group was statistically important when comparing the histological scores between the groups in diabetic rats on the 28th day. The control group was compatible with the findings of Alpan et al. (24). The fact that there is no statistically significant difference in new bone formation between ABS and the control group is compatible with the researches of Bulut et al. (23). Although ABS does not increase osteoblastic activity and new bone formation on day 28th there is a positive effect on the reduction of inflammation, vascular dilatation, and hemorrhage during bone healing. ABS was found to be highly effective in combination with ß-TCP, although it was not effective by itself in bone formation.

In our study, the difference between ß-TCP (ß-TCP +ABS) group and the control group was statistically important when comparing the histological scores between the groups in diabetic rats on the 56th day. But, the control group was contrary to Alpan et al. (24). ß-TCP and ABS have been found to have a favorable impact on bone healing in the late period, but the effect of ß-TCP on the bone was found to be higher than that of ABS. It may be due to the short-term resorption of the clot formed by the ABS in the defect area.

In our study, there was an increase in new bone formation on the 28th and 56th days when ß-TCP and ABS were applied separately compared to the control group. However, it was observed that this increase was higher in the ß-TCP group than in the ABS group. However, using ß-TCP and ABS together had a synergistic effect in forming new bone. The results of our study by Tanik et al. (22) are consistent with the findings of his study in rats nondiabetic. The reason for this can be explained by the fact that the ß-TCP graft remains in the defect area for a long time without resorption, and ABS is resorbed in the defect area quickly.

OPN enables the production and differentiation of different levels of osteoblastic cells in the bone and is secreted from many tissue cells such as wound healing areas (25). ON is

used to show the deep layers of osteoid and is thought to be effective in the mineralization of the bone matrix (26). In our study, a rise was found in OPN and ON expressions of diabetic rats on day 28th. Expression of OPN and ON were found considerably higher in β-TCP +ABS groups at day 28th. This may be due to high osteoblastic activity or the positive effect of ABS on bone wound healing with bone graft.

DEXA is frequently used in the diagnosis of osteoporotic patients. This method measures bone mineral density and quantity by the amount of absorption of photons at different energy levels[27]. Barou et al. [28] studied bone loss and changes in osteoporotic rats and utilized high-resolution three-dimensional microcomputer tomography (3D-μCT), DEXA and histomorphometric analysis. DEXA and 3D-μCT detected the bone loss earlier than histomorphometry on day 13. In another study where the density of bone trabeculae in rats was measured by DEXA, histomorphometry and computed tomography (CT), the DEXA and CT findings were found significantly different from the histometry findings; that's why DEXA and CT make cortical and cancellous bone measurements, histomorphometry only makes cancellous bone measurements [27]. For this reason, in the present study, DEXA was used to evaluate both early cortical and cancellous bone healing. In the study of AboElAsrar et al. (29) investigating the insulin-dependent impacts of cross-talk and growth factor-1 cytokines in Type 1 DM patients, DEXA analysis was performed using follow-up records of 10 months, so no significance was found between the BMC and BMD values of whole body and waist region among well controlled and uncontrolled diabetic patients. In a study by Duartae et al. (30) evaluating DM-related bone diseases, the DEXA analysis at day 120 did not show a significant in BMD values in the femur and metadiaphyseal region.

Studies that are similar to our study were taken as reference because of the lack of similar work. Only BMC values at 28th day were found significantly higher in comparison of BMC and BMD values. BMD findings of the present study support the results of AboElAsrar et al. (29) and Duartae et al. (30). In addition, it is compatible with the study of AboElAsrar et al. (29) in terms of BMC value at day 56th. Findings of BMC on the 28th day of the present study are inconsistent with AboElAsrar et al. (29). It may result from the shorter duration of 4-week of the present study than AboElAsrar et al. (29).

In our study, the diabetic rats of all experimental groups on the 28th day were significantly higher in BMC values than control groups. This is because the graft and ABS used in the early period of diabetes may have a slight effect. The BMD values on the 28th and 56th day is consistent with the literature. This may be because DM decreased bone density due to decreased bone trabeculae.

No statistically important divergence was found for BMC and BMD values between the baseline to 28th and the baseline to 56th day. The reason for not having a significant difference may be that the adverse effects of diabetes on bone delayed wound healing, the graft material was late resorbed or the amount of ABS was inadequate. There was a high degree of difference in BMC values only in the control group on the 28th and 56th day of the diabetic rats. These results are important to us in showing that diabetes has an adverse effect on bone regeneration.

As a limitation of our study, it is stated in the literature that the results of studies with a longer term of 6 months on bone regeneration treatments will be more successful. However, since it would be difficult for many rats to survive due to the side effects of diabetes in long-term bone regeneration studies in diabetic rats, we had to make plans to finish our study earlier.

5. CONCLUSION

ABS has an effect on wound healing and new bone formation in diabetic groups. However, we think that the use of ABS with a bone graft, especially in bone recovery, will yield more positive outcomes. Thus, further studies should be done in this field.

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Clinical and Experimental Health Sciences

Effects of SPARC and Possible Receptors on Colon Cancer Cell Line

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ABSTRACT

Objective: The aim of this study was to observe the apoptotic/cytotoxic effects of exogenous SPARC on colon cancer cell line HT-29, then to investigate the function of stabilin-1 and integrin αvβ3, which are possible receptors for SPARC in colon cancer cells and to determine the quantitation of their receptor numbers.

Methods: Appropriate doses of exogenous SPARC and it's inhibitor, cilengitide added to HT-29 cell line were determined by xCELLigence Real-Time Cell Analysis system, SPARC-mediated caspase 3 expressions were measured. Using the RT-PCR system, gene expression levels of SPARC, stabilin-1 and integrin αvβ3 receptors (silenced/nonsilenced with cilengitide) were detected then the numbers of receptors per cell were quantitated by flow cytometry.

Results: IC50 value of SPARC was determined as 4.57 µg/mL and IC50 value of cilengitide was determined as 50 nM. 5 µg/mL exogenous SPARC caused increased apoptosis in the HT-29 line. Significant increase in gene expression of integrin αvβ3 receptor was observed in the group incubated with 5 µg/mL SPARC, contrarily, the addition of cilengitide decreased gene expressions. The integrin αvβ3 receptor numbers increased approximately 2-fold with SPARC compared to the control. No significant changes were observed in the gene expression and receptor numbers of stabilin-1.

Conclusion: Exogenous SPARC was shown to reduce proliferation and induce apoptosis in colon cancer cells. Integrin αvβ3 is thought to be the possible receptor mediating SPARC in colon cancer cells. Quantification of surface receptors per cell, which we think we have done first, can be considered as a marker in the follow-up of anticancer treatments.

Keywords: SPARC, cilengitide, colon cancer, stabilin-1, integrin αvβ3

1. INTRODUCTION

SPARC is a human protein that is encoded by the SPARC gene. It is also known as osteonectin or basement membrane protein 40 (BM-40). Osteonectin is a 40 kDa, acidic, cysteine-rich glycoprotein, which has a single polypeptide chain consisting of three structural domains. The first domain is the calcium binding region (low affinity) located at the N-terminal end and consists of 52 amino acids. The second domain is the cysteine-rich (containing 10 cysteine residues) FS-like (FS: Follostatin) region and consists of 85 amino acids (Asn53-Pro137). The third domain is the EC-binding (EC: extracellular calcium) site. The last domain, the C-terminal end, consists of 149 amino acids (Cys138-Ile286) and has high calcium binding affinity. This region inhibits cell division, proliferation, and adhesion while matrix methyloproteinases induce communication between the cell and the matrix (1-3).

SPARC is involved in wound healing, cell migration and angiogenesis by regulating interactions and communications between cells. While it may also exert anti-angiogenic activity; the role of osteonectin in colorectal cancer is controversial (4). Colorectal cancer is the third most prevalent diagnosed cancer in men and the second in women known worldwide (5) and is a group of diseases with complex genetic and epigenetic features (4). A study, investigating the relationship between the hypermethylation of CpG islands and SPARC in colon cancer tissues, indicated that SPARC expression was downregulated in colorectal cancer due to abnormal methylation of CpG islands in the promoter region (6). It has

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been concluded that methylation of CpG islands in colon cancer is suppressed in the presence of SPARC (6).

In advanced cancers, a large number of stabilin-1 positive macrophages associated with poor prognosis have been observed, revealing the effect and importance of the overexpression of this receptor on macrophages in carcinogenesis (7).

Cell surface receptors, integrins, are the most important family of cell adhesion receptors which regulate the interaction between cells and extracellular matrix (ECM) proteins. The integrin family consists of at least 24 sub-subunits; 18α and 8β subunits connected by non-covalent bonds (8).

Originally called the vitronectin receptor, αvβ3 integrin is one of the most complex receptors that binds at least 21 different extracellular matrix proteins, including osteopontin, laminin, fibrinogen, thrombospondin, thrombin, and von Willebrand factor (9,10). With its non-covalent heterodimer structure consisting of 170 kDa αV / CD51 and 93 kDa β3 / CD61 subgroups, this integrin is widely expressed in smooth muscle cells (SMC), myofibroblasts, osteoclasts, endothelial cells (EC), monocytes, and platelets (9, 11).

As integrins are the main regulators of communication between cells and with the microenvironment, they play important roles in various cellular processes such as cell migration, differentiation, and survival (12, 13). Therefore, integrins have been targeted for cancer therapy in recent years, and RGD-containing peptides have been developed and synthesized to directly inhibit this receptor (14, 15). The cilengitide molecule, the cyclic Arg-Gly-Glu (RGD) pentapeptide, is an integrin inhibitor. There are several different integrin subtypes that recognize and bind to RGD. Cilengitide has been shown to act as an inhibitor in angiogenesis and induces apoptosis through inhibition of the interaction between integrins and ECM ligands (16, 17). Cilengitide has been found to affect the adhesion between integrin αvβ3 and its ligands and induce apoptosis in cells expressing integrin αvβ3 and αvβ5 with *in-vitro* studies (16).

Herein, we aimed to determine the apoptotic and cytotoxic effects of exogenous SPARC protein on colon cancer cell line HT-29 cells. Furthermore, we investigated the functions of stabilin-1 and integrin αvβ3 (by inhibition with the relevant peptide), possible receptors mediating effects of SPARC in colon cancer. These two receptors selected among many receptors will help us explain the mechanism of action of SPARC protein in colon cancer cells, and the measurement of the number of receptors per cell will guide other studies as a new marker that can be used in cancer treatment.

2. METHODS

2.1. Cell Line

The colon cancer cell line, HT-29 was used in this study. Cells were cultured in DMEM medium containing high glucose supplemented with 10% heat inactivated fetal bovine serum and 100 IU/mL penicillin/streptomycin solution. The cells were cultured at 37°C in a tissue culture incubator containing 5% CO_2 under humid environment, passaging once cells were reached 80% confluency by trypsin-EDTA solution.

2.2. Cell Lysis and Total Protein Determination

For protein isolation, cells were washed with cold phosphate buffered saline solution (PBS) followed by the addition of the lysis buffer and the cells were scraped off. Homogenization was achieved by sonicating cells for 2 minutes with an ultrasonic homogenizer. Proteins were separated from cell membrane residues by centrifugation at +4°C and, 14000 rpm for 10 minutes. Protein concentration was measured by the Bradford method (18).

2.3. Cytotoxicity Test

Cytotoxicity was determined by xCELLigence real-time cell analysis system (xCELLigence, Roche, CA, USA), which measures the changes in electrical impedance created by cellular adhesion to the cell cultureware in which the cells are seeded (19). As the cells divide, the micro electrodes record the the impedance alterations. The system records the electrical changes simultaneously, so that quantitative values about the proliferation of cells are obtained. For evaluating cytotoxicity, $1x10^4$ cells were seeded as triplicates and incubated for 24 hours to allow adherence. Fresh culture media containing SPARC protein at concentrations of 2.5 µg/mL, 1 µg/mL, 0.5 µg/mL and 0.1 µg/mL was added in wells, and its effect on cellular viability was examined for 72 hours, followed by calculation of the IC_{50} value. Cytotoxicity of cilengitide was also evaluated in a similar manner; cells were treated with cilengitide at concentrations of 250 nM, 125 nM, 50 nM, 25 nM and 5 nM for 48 hours and the IC_{50} value was calculated.

2.4. Reverse Transcription-Polymerase Chain Reaction (RT-PCR)

The isolation kit (Cat no: 12183025, Ambion/by Life Technologies, MA, USA) was used to isolate mRNA. Using Nanodrop technology (Epoch, Biotech Ins., CA, USA), the quantity and purity of RNA were measured spectrophotometrically. The High Capacity cDNA Reverse Transcription Kit (Cat no: 4374966, ThermoFisher, MA, USA) was used to reverse transcribe RNA according to the manufacturer's procedure. Real-time polymerase chain reaction (qPCR, Biorad, CA, USA) was used to determine gene expression levels using TaqMan Gene Expression Assays (Invitrogen/Life Technologies, Carlsbad, CA, USA). Hs00234160_m1 (SPARC), Hs01109068_m1 (STAB), Hs01547673_m1 (integrin subunit alpha 5), Hs01105177_m1 (integrin subunit beta 3), and Hs0275899_g1 (GAPDH), all of which are human genes encoding osteonectin, stabilin 1, integrin subunit alpha 5, and integrin subunit beta 3 binding protein. As a housekeeping gene, GAPDH was employed.

2.5. Caspase-3 Activity

Caspase-3 activity was determined by Millipore Colorometric Activity Test Kit (Cat no: APT 165, Sigma-Aldrich, MA, USA) according to the manufacturer's instructions. Total protein samples were collected and their concentrations were measured as given in the section "Cell Lysis and Total Protein Determination." Protein samples were incubated with the reagent for 2 hours at 37°C in the dark, followed by measuring the absorbance of pNA (p-nitroaniline) released at the end of the reaction at 405 nm using a microplate reader at the 0, 1st and the 2nd hours as triplicates, while untreated cells were used as control. Results were expressed as caspase activity (pNA/ protein).

2.6. Flow Cytometry Analysis

Qifikit (fluorometric bead solution for quantitation) kit (Cat no:K0078, DAKO, Agilent, CA, USA) was used for receptor quantification. 100 µL of both set-up and calibration beads were transferred to separate flow cytometry tubes, washed once with PBS w/o Ca^{2+} and Mg^{2+} containing 0.1% sodium azide by centrifuging at 1800 rpm for 5 minutes. Supernatants were discarded, tubes were vortexed and beads were labelled with 10 µL fluorescein isothiocyanide (FITC) secondary antibody (1/50 diluted in PBS) by incubating for 1 hour at +4°C. HT-29 cells were detached by trypsinization, cells were pelleted by centrifuging at 1800 rpm for 5 minutes and counted by JuLi Br&Fl Station (NanoEnTek, South Korea). Cell concentration was adjusted to $2x10^5$ per mL. Cells were labelled with anti-stabilin-1 (1:500/tube) and anti-integrin αvβ3 (7 µL/tube) receptor antibodies by incubating cells at +4°C. All measurements were performed as triplicates. Tubes were read with Beckman Coulter CytoFLEX flow cytometry system (IN, USA), and analyses were performed with CytExpert software.

For receptor quantification, bead population was gated from the Side Scatter/Forward Scatter dot plot. Mean fluorescence intensities (MFI) regarding each bead population were obtained according to the respective histogram plot. For calculating stabilin-1 and integrin αvβ3 receptors on HT-29 cells, quantitative antibody binding capacity (ABC) was calculated according to samples' respective MFI values; the measured MFI value for each sample was compared with the respective bead MFI value which is the closest. Receptor numbers (BAE) of unstained control cells were calculated in the same manner, and specific antibody binding capacity (SABC), the quantitative antibody numbers, were calculated for each group by subtracting the ABC values of the samples.

2.7. Statistical Analysis

GraphPad Prism5 software was used to perform the statistical analysis. Statistical analysis was performed using one-way ANOVA (pairwise comparison test: Tukey) and two-way ANOVA test (pairwise comparison test: Bonferroni) with significances set at *ns: p>0.05,* *p < 0.05, ** p < 0.01 and *** p < 0.001 as indicated.

3. RESULTS

3.1. Cytotoxicity Analysis by xCELLigence System

IC_{50} values for SPARC and cilengitide was calculated according to the data obtained from xCELLigence real-time cell analysis systems; and IC_{50} values of these two molecules were determined as $4.57463x10^{-6}$ g/mL (Figure 1) and 50 nM (Figure 2), respectively.

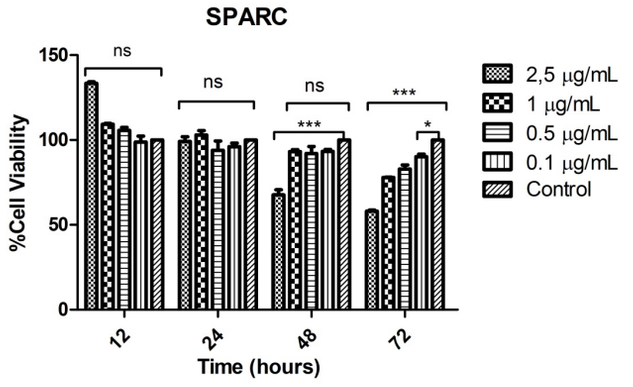

Figure 1**. IC_{50} values for SPARC protein on HT-29 cells (p<0.001, *p<0.05, ns:p>0.05, relative to control)*

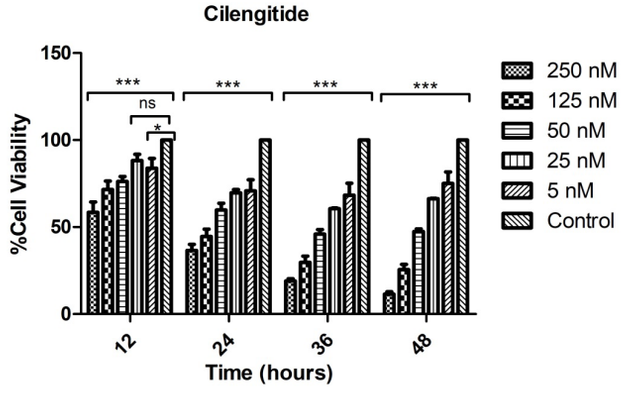

Figure 2**. IC_{50} values for cilengitide molecule on HT-29 cells (p<0.001, *p<0.05, ns:p>0.05, relative to control)*

3.2. Caspase-3 Levels

Caspase-3 enzyme activity in HT-29 cells was measured after incubating cells with either 5µg/mL SPARC or 5µg/mL SPARC and cilengitide simultaneously for two hours while untreated cells were used as control (Figure 3). 5 µg/mL SPARC significantly increased caspase-3 activity in all timepoints in comparison to the control group (p<0.001) while combinatorial treatment with SPARC and cilengitide significantly decreased active caspase-3 levels (p<0.001), indicating exogenous SPARC is a promoter of caspase-3

mediated apoptosis, and its pharmacological inhibition reverses the apoptotic effect.

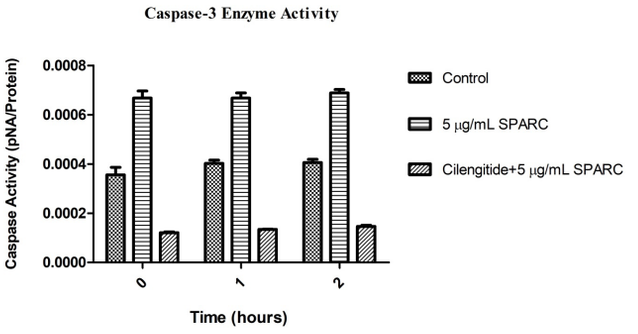

***Figure 3**. Caspase-3 activity results measured at 0, 1 and 2 hours in groups incubated with substances for 24 hours (p<0.001)*

3.3. RT-PCR Results

According to Figure 4a, when the gene expressions of SPARC, stabilin-1 and integrins in HT-29 cells were examined, it was determined that the base expressions of SPARC and integrins were quite low, but the expressions of stabilin-1 were higher. When 5µg/mL SPARC was added externally to the same cells, statistically significant differences were observed between SPARC, integrin αv and β3 expressions compared to the control ($p<0.001$, $p<0.001$ respectively). At the level of stabilin-1, there was no statistically significant difference ($p>0.05$).

As seen from the previous Figure 4a, the initial expressions of integrin αv and β3 were quite low and increased significantly with 5 µg/mL SPARC. However, when the integrin inhibitor cilengitide was added to the HT-29 cell lines first and then treated with 5 µg/mL SPARC, the gene expressions of both integrins were significantly reduced ($p<0.001$ and $p<0.01$, respectively) (Figure 4b).

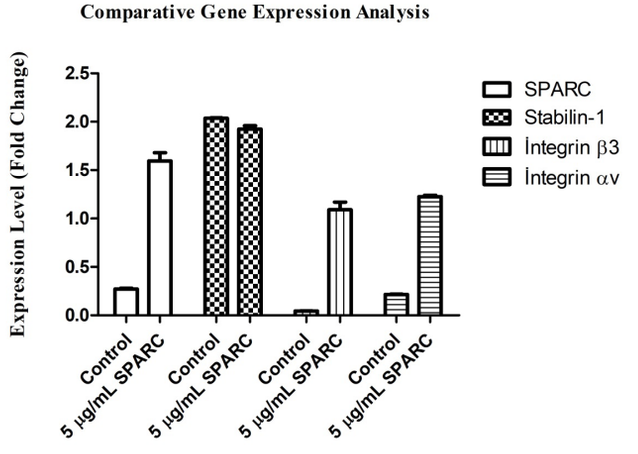

***Figure 4a**. Expression levels of SPARC, stabilin-1, integrin αv and β3 genes in HT-29 cells of control (base) and with 5µg/mL SPARC (terms of fold increase)*

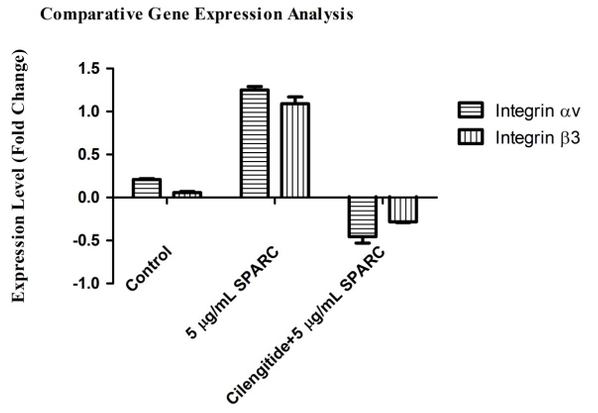

***Figure 4b**. Expression levels of integrin αv and β3 genes in HT-29 cells with 5µg/mL SPARC and cilengitide+5µg/mL SPARC (terms of fold increase)*

3.4. Flow Cytometry Results

The mean number of integrin αvβ3 receptors in the HT-29 cell line in the control group was 34203±9790/cell while addition of 5 µg/mL SPARC increased integrin αvβ3 receptor count to 74496±1665/cell, leading to a significant difference ($p<0.01$). However, cilengitide addition decreased integrin αvβ3 receptor count to 25731±12442, which is significantly lower than both control and 5 µg/mL SPARC groups ($p<0.01$) (Figure 6). When considering stabilin-1 receptor count; no significant difference between control and 5 µg/mL SPARC groups was observed ($p>0.05$) (Figure 7). The histogram plot, indicating five different bead population is given in Figure 5.

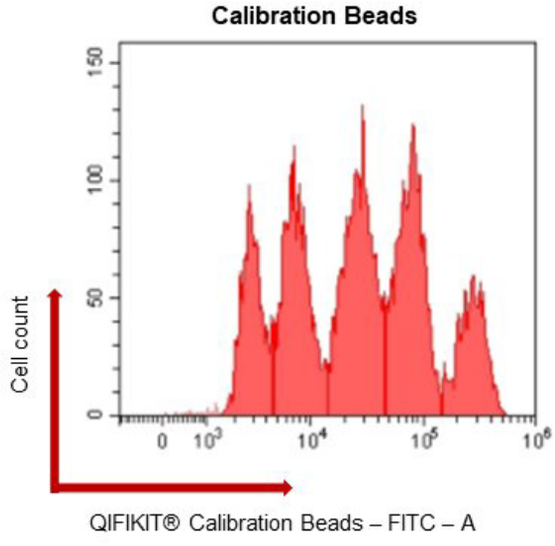

***Figure 5**. Histogram of calibrator beads used to determine quantitative receptor numbers*

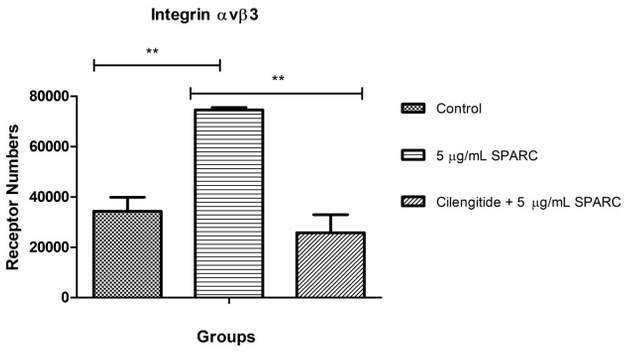

***Figure 6**. Integrin αvβ3 receptors numbers in different groups (**p<0.01)*

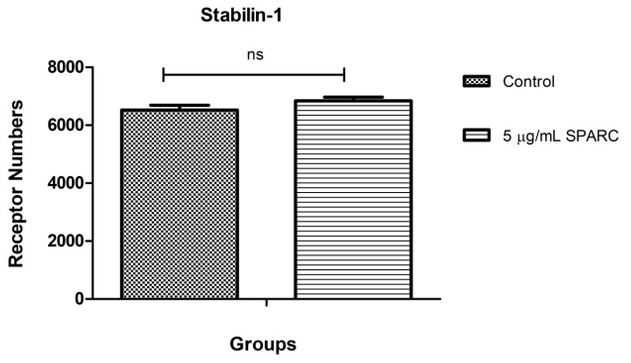

***Figure 7**. Stabilin-1 receptor numbers different groups (ns: p>0.05)*

4. DISCUSSION

In this study, firstly we determined SPARC protein, integrin αvβ3, and stabilin-1 levels in HT-29 cells, a colon cancer cell line. Then, the effects of exogenous SPARC protein at different concentrations on its possible receptors integrin αvβ3 and stabilin-1 in colon cancer cells were investigated. During this research, the cilengitide molecule, an integrin inhibitor, was used.

In recent studies, it has been found that SPARC has many roles on important molecular mechanisms in malignancies, including tumor microenvironment, apoptosis, and is effective in the regulation of tumor growth/invasion.

The findings suggest that SPARC may perform a tissue-specific function in cancer development, with SPARC being downregulated in some cancers and upregulated in other cancers. Studies in lines with colon cancer show that downregulation of SPARC is associated with aberrant methylation, particularly in the promoter region, thus suppressing methylation of CpG islands in the presence of SPARC. Despite the fact that most studies in people with colon cancer show that high SPARC expression is associated with a better disease outcome and low expression is associated with a worse prognosis, studies on SPARC expression and its relationship with pathological features in this cancer are extremely rare, and its prognostic value is still debated.

Our first findings by western blot analysis show that, in agreement with the literature, SPARC expression is greatly reduced in colon cancer lines (data not shown), these values are more clearly demonstrated as gene levels measured by RT-PCR in our study (as observed in Fig4a).

There is very little research in the literature on the interactions between tumor cell and exogenous SPARC. According to a study conducted on a prostate cancer cell line, it has been shown that the externally added SPARC protein causes inhibition in cell proliferation dose dependently (20, 21). Other study on the SPARC protein has shown that increased expression in neuroblastoma cells suppresses the proliferation of cells.

In the light of the information from the literature, in our xCELLigence system results, it was observed that SPARC suppressed cell proliferation at increasing concentrations that we added externally, and the most effective dose was found to be IC_{50}: 4.57 μg/mL (Figure 1).

In SKOV3 cells (The ovarian carcinoma cell line), the addition of 5 μg/mL and 20 μg/mL SPARC reduced the number of living cells to approximately 68% and 54% of the control respectively (22).

In the literature, it is seen that SPARC has different behaviors related to apoptosis in different cancer types. In a study on melanoma cells, an increase in the activity of caspase enzymes was observed after SPARC knockdown, and in another study on human ovarian cancer, high expression of SPARC protein was shown to indicate a poor prognosis mediated by apoptosis (23, 24). Our results with the apoptotic enzyme caspase-3 activity support our xCELLigence system results. We observed that the enzyme activity increased in the groups to which SPARC was added. In a study in colorectal cancer cell lines, SPARC addition during chemotherapy treatment further potentiated apoptosis (25).

Paralelly, according to our results, the increase of SPARC in the colon cancer cells induced apoptosis. Unlike other studies, when we used cilengitide, an integrin inhibitor, the apoptotic effect of SPARC was reduced. This case seems to be very supportive for the next step data in which we investigated the possible receptors of SPARC in colon cancer cells in our study.

The cell surface receptors of SPARC remain unclear, however, it can be said to act as a competitor of ligand-receptor interactions. In two separate studies, it was determined that integrin β4 controls the SPARC protein to stimulate invasion in breast cancer, whereas SPARC induces invasion in melanoma via a collagen I/α2β1 integrin pathway (26, 27).

In our study, two receptors were preferred according to the literature to elucidate the interaction of the exogenous SPARC protein, which showed antiproliferative effect on colon cancer cell line, with possible receptors. These were selected as integrin αvβ3 and stabilin-1 (7, 21).

According to our RT-PCR observations (Figure 4b), the baseline values of the subunits of the integrin αvβ3 receptor in colon cancer lines were quite low, as were the SPARC protein values. However, stabilin-1 receptor expressions, which are known to be found in macrophages and some endothelial cells, were found to be quite high (28). Moreover, exogenous SPARC protein significantly increased the expression of integrin αvβ3 subunits, while stabilin-1 receptor expressions remained at the same levels as control ($p>0.05$).

SPARC has been demonstrated to decrease adhesion, invasion, and cell proliferation in human ovarian cancer cells by lowering the surface localization and/or aggregation of αv, β1, β3 and β5 integrins, according to a study (28-30).

Since SPARC affects integrin interaction in many different cell types and this interaction is known to be cell type dependent, we determined that with increasing SPARC concentration (5 µg/mL), integrin response in colon cancer cells increased both at the gene level and quantitatively in terms of receptor numbers (base αvβ3 receptor numbers: 34203±9790/per cell, after 5 µg/mL SPARC, αvβ3 receptor numbers: 74496±1665/ per cell).

Incubation of colon cancer cells with the integrin inhibitor cilengitide first, significantly reduced integrin levels (to less than the control values) by inhibiting binding of receptor antibodies (Fig4b, Fig6, Table 1) (after 5 µg/mL SPARC, integrine αvβ3 receptor numbers: 74496±1665/ per cell and; after cilengitide+5µg/mL SPARC, αvβ3 receptor numbers: 25731±12442/ per cell).

Our RT-PCR and flow cytometric findings show that the possible receptor of SPARC in colon cancer is likely to be integrin αvβ3 receptor and intracellular transport is mostly via this receptor (integrin αvβ3 receptor), but the same is not true for stabilin-1. Even more to the best of our knowledge, in this study, we determined the number of integrin receptors per cell, especially in colon cancer cells, for the first time in the literature.

Table 1. *Receptor numbers of Integrin αvβ3 and Stabilin-1*

	İntegrin αvβ3	Stabilin-1
Control	34203±9790/cell	6525± 287.6/cell
5 µg/mL SPARC	74496±1665 /cell	6845± 220.3/cell
Cilengitide+5 µg/mL SPARC	25731±12442 /cell	-

5. CONCLUSION

In this study, we showed that exogenous SPARC can reduce proliferation and induce apoptosis in colon cancer cells. Although different signaling pathways mediate these tumor suppressor effects, they are likely dependent on SPARC binding to its cell surface receptor. The interaction of SPARC and its putative receptor may also contribute to the tissue- and cell-specific biological functions of SPARC in different normal and cancerous cells. According to our results, this possible receptor integrin αvβ3 may be in colon cancer cells, and the use of inhibitors strongly supports this interpretation. Quantification of receptors on the cancer cell surface can be considered as a marker in the follow-up of anticancer treatments. However, further studies are needed in this regard.

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Design of the study: DÖ, ÖBÖ
Acquisition of data for the study: DM
Analysis of data for the study: DM, TŞ, BA
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Drafting the manuscript: DÖ, DM
Revising it critically for important intellectual content: DÖ, ÖBÖ, GYD
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Clinical and Experimental Health Sciences

Evaluation of Emotional State and Mediterranean Diet Adherence During the COVID-19 Pandemic: Butterfly Effect

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ABSTRACT

Objective: The COVID-19 disease, which is caused by the SARS-CoV-2 infectious agent, is the last member of infectious diseases. Factors such as uncertainties about the prognosis of the disease, insufficient control measures, lack of effective therapeutic mechanisms can cause negative effects on individuals' daily living activities and mental health. In this study, it was aimed to evaluate the mood changes and Mediterranean diet adherence of individuals during the COVID-19 pandemic.

Methods: The study was carried out between November 2020 and January 2021 with individuals over 18 years of age. The data required for the research were collected with an online questionnaire. The COVID-19 Phobia Scale (C19P-S) and the Coronavirus Anxiety Scale (CAS) were used to assess the emotional state of individuals. The Mediterranean Diet Adherence Screener (MEDAS) was used to examine the adherence to the Mediterranean diet.

Results: A total of 6609 individuals between the ages of 18-70 participated in the study and 70% of the participants are female. The median score obtained from the C19P-S in female individuals was found to be significantly higher than in male subjects ($p<0.001$). A positive relationship was shown between the Mediterranean diet adherence and the COVID-19 Phobia Scale ($p<0.001$).

Conclusion: The COVID-19 related phobia and fear may have positively affected the adherence to the Mediterranean diet of individuals as a coping strategy with this pandemic period.

Keywords: COVID-19 pandemic, Emotional state, Mediterranean diet, Phobia, Anxiety

1. INTRODUCTION

Throughout history, infectious diseases have had devastating effects on societies. Especially in recent years, globalization has facilitated the spread of pathological agents, leading to worldwide pandemics. Considering the last two decades of infectious diseases, it is known that coronavirus-related diseases (SARS and MERS) have emerged and these diseases have caused great damage to public health systems. The COVID-19 disease, which is caused by the SARS-CoV-2 infectious agent, is the last member of these pandemics (1). COVID-19, which was identified in China at the end of 2019, is a disease with a high spread potential and its incidence is increasing exponentially. Factors such as uncertainties about the prognosis of the disease, insufficient control measures, lack of effective therapeutic mechanisms, public health measures that violate personal freedoms and economic burden can cause negative effects on individuals' daily living activities and mental health (2, 3). Psychologists and mental health experts predict that the pandemic will adversely affect the mental health of the population globally, with increases in depression, suicide and self-harm cases (4, 5).

Although the determinants of mental health are complex, one of the most important factors is nutrition. Epidemiological data emphasise the relationship between nutrition and mental health, but don't ensure sufficient information about causality or mechanisms (6). Especially the Mediterranean diet can be recommended as a therapeutic approach strategy to control the inflammatory and prothrombotic process during the COVID-19 pandemic (7). It is known that the Mediterranean diet can also have positive effects on mental health (8). In terms of nutritional pattern, the Mediterranean diet is low in saturated fat and animal protein; rich in antioxidants, fiber and monounsaturated fatty acids and provides an adequate balance of omega-6/omega-3 fatty acids. That's why the Mediterranean Diet is currently recognized as one of the healthiest eating patterns worldwide (9). In this study, it was aimed to examine the emotional changes and Mediterranean diet adherence of individuals during the COVID-19 pandemic.

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2. METHODS

2.1. Data Collection

Research data were collected with an online questionnaire (Google Form) applied to individuals over the age of 18 between November 2020 and January 2021, through snowball sampling, including samples from different cities of Turkey. As a result of the power analysis using the Minitab program; A total of at least 5088 samples were found to be sufficient with 80% power and 5% margin of error. The questionnaire includes demographic information, statement of health (disease state diagnosed by the physician, COVID-19 diagnosis status) and anthropometric measurements (body weight, height), COVID-19 Phobia Scale (C19P-S) and the Coronavirus Anxiety Scale (CAS), and the 14-item Mediterranean Diet Adherence Screener (MEDAS). Ethical approval was obtained from the Gazi University Ethics Committee as a result of the meeting decision dated 16.02.2021 and numbered 03, with the research code 2021-212. Written informed consent was obtained from all subjects.

2.2. Mediterranean Diet Adherence Screener (MEDAS)

In the study, the Mediterranean Diet Adherence Screener (MEDAS) was used to evaluate the participants' adherence to the Mediterranean diet. The Mediterranean Diet Adherence Screener was developed by Martinez-Gonzalez et al (10). The scale consists of 14 questions in total, those who answer "Yes" to the questions get 1 point, and those who answer "No" get 0 points. The highest score that can be obtained from the scale is 14. The total score obtained from the scale is evaluated as ≤5 (low adherence), 6-9 (moderate adherence), and ≥10 (high adherence) (10). Turkish validation and reliability of Mediterranean Diet Adherence Screener was made by Pehlivanoğlu et al (11).

2.3. COVID-19 Phobia Scale (C19P-S)

Developed by Arpacı et al. (12), COVID-19 Phobia Scale isas a 20-item self-report tool that addresses the phobia diagnostic criteria of the DSM-V. Initial tests performed in relation to the scale show that the scale has construct, convergent and discriminant validity, and internal consistency reliability. However, further testing of the scale is needed. Items on a five-point Likert-type scale is graded between "strongly disagree" and "strongly agree". The score obtained from the scale can vary between 20 and 100. A higher score on the scale shows a greater phobia (12).

2.4. Coronavirus Anxiety Scale (CAS)

Coronavirus Anxiety Scale (CAS) was developed by Sherman Lee (13) to determine possible dysfunctional anxiety related to the COVID-19 pandemic. The scale consists of 5 questions, the answers to the scale are determined as "Never (0)", "Rarely, less than one or two days (1)", "A few days (2)", "More than 7 days (4)" and "Almost every day in the last two weeks (5)". Coronavirus Anxiety Scale distinguishes between individuals with and without dysfunctional anxiety using a cut-off score of ≥ 9 (13). Turkish validation and reliability of Coronavirus Anxiety Scale was made by Evren et al. (14).

2.5. Statistical Analysis

The data obtained from the questionnaires were analyzed with appropriate statistical methods using the SPSS 22.0 program (15). Descriptive values are shown as number (n), percent (%), arithmetic mean ($\bar{x}$), and standard deviation (ss). Pearson chi-square test was used to compare categorical variables. The conformity of the variables to normality was examined by visual (histogram and probability graphs) and analytical methods (Kolmogorov-Smirnov/Shapiro-Wilk tests). Mann-Whitney U test and Kruskal-Wallis test were used to compare data that did not have normal distribution. Multivariate regression analysis was performed to predict the Mediterranean diet adherence by using age, body mass index (BMI), CAS and C19P-S. Statistical significance level was determined as $p<0.05$.

3. RESULTS

This study, whose data were collected using an online questionnaire, was carried out with 6609 individuals between the ages of 18-70. Approximately 70% of the individuals participating in the study are female (69.8%). When the demographic data were analyzed, it was found that the median age of male individuals was significantly higher than female individuals ($p<0.001$). According to education level, more than 70% of male and female individuals are university graduates and this rate is significantly higher for females than for males ($p<0.001$). According to the working status, the ratio of working male individuals (43.8%) was found to be significantly higher than female individuals (23.1%) ($p<0.001$). The median BMI in male individuals was significantly higher than female individuals ($p<0.001$). However, the BMI value for individuals in both groups can be classified as normal according to the World Health Organization cut-off values (16). Data on demographic and health information are given in Table 1.

The Mediterranean diet adherence profile of the individuals is shown in Figure 1. Of the participants, 61% are in the group that moderately adheres to the Mediterranean diet. More than half of the individuals (57.8%) use only olive oil in their meals. While the rate of those who consume two servings or more of vegetables a day is close to 70%, the rate of those consuming three servings or more of fruit per day remained at 25%. The rate of those who consume less than one serving of red meat and processed meat products, butter and sugar-sweetened beverages per day constitutes 75%. However, according to the Mediterranean diet adherence profile, the consumption rate of three servings or more of legumes (34.1%), fish and seafood (15%) and nuts (30.8%) per week is low.

Table 1. *Demographic characteristics, health data and anthropometric measurements of individuals*

Demographic and health characteristics	Male (n=1995)		Female (n=4614)		Z/X²	p
	n	%	n	%		
Age (year)						
Median [Interquartile range (IQR)]	23.0 [9.0]		22.0 [8.0]		-6.150	<0.001*
Range	52.0		52.0			
Min-max	18-70		18-70			
Educational status						
Literate	15	0.8	43	0.9	22.611	<0.001†
Primary school	36	1.8	139	3.0		
Secondary school	56	2.8	141	3.1		
High school	350	17.5	718	15.6		
University	1431	71.7	3405	73.8		
Postgraduate	107	5.4	168	3.6		
Working status						
Working	873	43.8	1067	23.1	285.958	<0.001†
Not working	1122	56.2	3547	76.9		
Marital status						
Married	1505	75.4	1160	25.1	0.250	0.617†
Single	490	24.6	3454	74.9		
Living place						
Urban	1587	79.5	3620	78.5	3.381	0.184†
Rural	407	20.5	994	21.5		
Chronic disease diagnosis status						
Yes	215	10.8	795	17.2	44.800	<0.001†
No	1780	89.2	3819	82.8		
COVID-19 diagnosis status						
Yes	226	11.3	476	10.3	1.502	0.220†
No	1769	88.7	4138	89.7		
Anthropometric measurements						
Height (cm)						
Median [Interquartile range (IQR)]	178 [8]		164 [8]		-56.550	<0.001*
Range	67		43			
Min-max	140-207		145-188			
Body weight (kg)						
Median [Interquartile range (IQR)]	77 [16]		59 [14]		-46.336	<0.001*
Range	117		131			
Min-max	43-160		37-168			
BMI (kg/m²)						
Median [Interquartile range (IQR)]	24.4 [4.68]		21.8 [5.08]		-23.602	<0.001*
Range	35.8		48.1			
Min-max	16.2-52.0		14.4-62.5			

**Mann-Whitney U test*
†Pearson Chi-square test

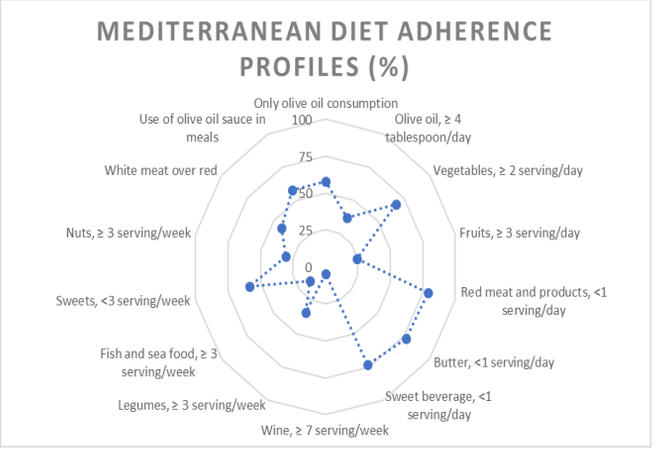

Figure 1*. Radar graph of Mediterranean diet adherence profile*

Table 2 shows the distribution of Mediterranean Diet Adherence Screener, Coronavirus Anxiety Scale, and COVID-19 Phobia Scale classification by sex. According to the Mediterranean diet adherence scale classification, 52.8% of male individuals and 64.6% of female individuals show moderate adherence. The ratio of females who moderately and highly adherence to the Mediterranean diet is significantly higher than that of males (p<0.001). Similarly, the median score of the C19P-S in females was found to be significantly higher than in male subjects (p<0.001).

In Table 3, the relationship between the Mediterranean Diet Adherence Screener classification, the CAS, and the C19P-S was evaluated. The median score of C19P-S of individuals with low adherence to the Mediterranean diet was significantly lower than those with moderate adherence (p<0.001). Likewise, the C19P-S median score of individuals with low adherence was significantly lower than those with high adherence (p<0.001).

In the study, multivariate regression analysis was performed to predict the Mediterranean diet adherence by using age, BMI, CAS and C19P-S variables. In the first model of multivariate regression analysis, only the CAS and C19P-S variables were used; in the second model, age and BMI variables were also included. As a result of the analysis, significant results were obtained for both models. For Model 1, F (2, 6606) =28.46, p<0.001, and the C19P-S positively and significantly predict adherence to the Mediterranean diet. For Model 2, F (4, 6604)= 23.07, p<0.001, and although C19P-S and age predicted adherence to Mediterranean diet positively and significantly; BMI predicted negatively and significantly (Table 4).

Table 2. *Distribution of Mediterranean Diet Adherence Screener, Coronavirus Anxiety Scale, and COVID-19 Phobia Scale classification by sex*

	Male (n=1995)		Female (n=4614)		Z/X^2	p
	n	%	n	%		
Mediterranean Diet Adherence Screener classification						
≤5 (low adherence)	774	38.8	1214	26.3	104.548	<0.001*
6-9 (moderate adherence)	1054	52.8	2980	64.6		
≥10 (high adherence)	167	8.4	420	9.1		
Coronavirus Anxiety Scale (CAS) classification						
<9 (normal)	1935	97	4504	97.6	2.160	0.142*
≥9 (dysfunctional anxiety)	60	3	110	2.4		
COVID-19 Phobia Scale (CP19-S)						
Median [Interquartile range (IQR)]	46 [15]		49 [15]		-12.328	<0.001†
Range	57		62			
Min-max	25-82		27-89			

**Pearson Chi-square test*
†Mann-Whitney U test

Table 3. *Relationship between Mediterranean Diet Adherence Screener classification, Coronavirus Anxiety Scale and COVID-19 Phobia Scale*

	Mediterranean Diet Adherence Screener classification						X^2	p
	Low adherence		Medium adherence		High adherence			
Coronavirus Anxiety Scale (CAS) classification								
<9 (normal)	1937	97.4	3938	97.6	564	96.1	4.840	0.089 *
≥9 (dysfunctional anxiety)	51	2.6	96	2.4	23	3.9		
COVID-19 Phobia Scale (CP19-S)								
Median [Interquartile range (IQR)]	47 [15]		49 [14]		50 [16]		41.225	<0,001†
Range	58		59		62			
Min-max	25-83		27-86		27-89			

** Pearson Chi-Square test*
† Kruskal-Wallis test
(According to Post Hoc Bonferroni correction, there is a significant difference between low adherence and medium adherence; low adherence and high adherence.)

Table 4. *Multiple regression analysis of independent variables affecting adherence to the Mediterranean diet*

Variables	Non-standard coefficients		Standard coefficients	R	R^2	t	p
	B	Standard error	Beta				
Model 1							
Constant	5.585	0.137	-			40.859	<0.001
COVID-19 Phobia Scale (CP19-S)	0.021	0.003	0.100	0.008	0.009	7.413	<0.001
Coronavirus Anxiety Scale (CAS)	-0.022	0.012	-0.026			-1.897	0.058
Model 2							
Constant	5.647	0.200	-			28.288	<0.001
COVID-19 Phobia Scale (CP19-S)	0.020	0.003	0.096			7.127	<0001
Coronavirus Anxiety Scale (CAS)	-0.021	0.012	-0.024	0.013	0.014	-1.805	0.071
Age	0.018	0.003	0.082			5.913	<0.001
Body Mass Index (BMI)	-0.022	0.007	-0.042			-3.021	0.003

4. DISCUSSION

In this study, which aims to evaluate the mood changes of individuals and their adaptation to the Mediterranean diet during the Covid-19 pandemic, it was concluded that older age, low body mass index and high COVID-19 phobia significantly affect the adaptation to the Mediterranean diet.

COVID-19 pandemic is associated with a variety of mental and psychological complications and it is a condition that negatively affects health and social systems, with a high rate of contamination, morbidity and mortality (7, 17). Although there is no diet to prevent coronavirus and there are limited clinical nutrition protocols for COVID-19 patients, the Mediterranean diet is recommended as a healthy diet model to control inflammation and thrombosis accompanying complications (18). In an ecological study conducted in Spain and 23 member countries of the Organization for Economic Cooperation and Development (OECD), adherence to the Mediterranean diet was shown to be negatively associated with COVID-19 cases and COVID-19 death rates (19). Similarly, in another study, the risk of developing COVID-19 was found to be significantly lower in individuals with moderate adherence to the Mediterranean diet (20). Therefore, adherence to the Mediterranean diet may be promising for conditions predisposing to COVID-19 infection and COVID-19 complications during the pandemic period (7). In studies examining the change in dietary habits of individuals during the pandemic period, it has been reported that individuals generally adhere to the Mediterranean diet moderately (21-23). In our study, it was found that 61% of individuals moderately adhered to the Mediterranean diet.

In addition, the proportion of females who moderately and highly adhered to the Mediterranean diet is significantly higher than males (p<0.001). Similar to our study, in a study conducted with the data obtained from the COVIDiet study, it was reported that the participants with a higher adherence to the Mediterranean diet were mostly female (88.8%) and had a higher education level (66.3%) (24). However, there are also studies showing that men's adherence to the Mediterranean diet is significantly higher than that of women (25, 26). In our study, the reason for the higher rate of adherence to the Mediterranean diet in female individuals may be the significantly higher education level of females than males (p<0.001). In addition, the fact that the ratio of working male individuals is significantly higher than female individuals (p<0.001) may be a factor affecting food consumption outside and reducing adherence to the Mediterranean diet. Alternatively, the fact that the rate of female individuals diagnosed with chronic disease is higher than male individuals are (p<0.001) may be related to a healthier change in eating habits of female individuals after the diagnosis of the disease.

Although the whole world acted quickly and in cooperation to control the spread of the virus that caused the COVID-19 pandemic, it was not easy to control this situation, which adversely affected human life around the world. Thus, this process not only affected all activities, but also had a tremendous negative impact on the mental health of individuals. In a study evaluating depression, anxiety, stress and sleep problems in the first seven months of the COVID-19 pandemic, the prevalence of depression was recorded as 20%, anxiety 35% and stress 53% (27). Similarly, other studies have reported anxiety and depression symptoms and self-reported stress as common psychological responses to the COVID-19 pandemic (28-30). However, in our study, 97.4% of individuals were classified as normal according to Coronavirus Anxiety Scale. When the mean scores from the C19P-S were evaluated, it was shown that female individuals (50.4±10.63) had a significantly higher mean score than male individuals (46.9±9.94) (p<0.001). A higher level of COVID-19 phobia in women has also been reported in previous similar studies in Turkey (31, 32) and Iran (33). In this process, there may be many factors that can affect the mental health of individuals. Living alone, low education level, living place (urban or rural area), previous medical condition, poor sleep quality, previous events that may increase the stress load can be counted among these factors (34). The fact that 75% of female individuals were single and higher rate of disease diagnosed in females than males in our study may explain the higher mean score obtained from the C19P-S in females.

Another remarkable result we obtained from the study is that the factors affecting the adherence of individuals to the Mediterranean diet are age, BMI and CP19-S according to the multiple regression analysis model. The mean score from the CP19-S was significantly higher in patients with high adherence to the Mediterranean diet screener (50.6±10.98) than those with low adherence (48.2±10.67). Unlike our study, in an international cross-sectional study conducted online, the majority of participants (54%) reported at least mild anxiety during the pandemic, while 25% reported moderate or severe anxiety. In addition, in the logistic regression analysis model, which includes individuals with at least moderate anxiety, after controlling for age, gender and other variables, Mediterranean diet score reduced the rate of increased anxiety (OR 0.92, 95% CI 0.89–0.95, p < 0.0001) (35). Similarly, individuals with high adherence to the Mediterranean diet were found to have lower rates of depression, anxiety, and psychological distress than those with low adherence (36). In addition, other studies reported a positive relationship between anxiety level and consumption of sugar-sweetened beverage (p=0.013), fast food (p<0.001) and pastry food (p<0.001) during the COVID-19 pandemic period (37), and food with high energy content increased consumption was found (38). There is also a study showing that an increase in COVID-19 phobia is associated with a decrease in eating awareness (39). The reason for finding different results from the literature in our study may be that the increased level of phobia and fear against COVID-19 has led individuals to adopt healthier eating habits. In addition, with the increase in the time spent at home in this period, adaptive coping strategies with COVID-19 such as more fruit and vegetable consumption, insecurity against out-of-home food consumption, and increase in home food consumption (38, 40) are associated with the fact that individuals' dietary habits has become more suitable for Mediterranean diet.

The strength of the research is that the results are generalizable because the number of participants is high and it includes different cities in Turkey. However, due to the fact that the study was performed online, cross-checking could not be achieved by taking the food consumption records of the individuals as well as the scale results in the evaluation of compliance with the Mediterranean diet.

5. CONCLUSION

Consequently, in this study aiming to evaluate the emotional change and Mediterranean diet adherence of individuals during the COVID-19 pandemic period, it was found that the Mediterranean diet adherence rate of female individuals was higher than male individuals. While the majority of individuals are classified as normal according to the CAS, according to the C19P-S, the mean score of female individuals from the scale was found to be significantly higher than that of male individuals. In addition, the mean scores of the CP19-S were found to be significantly higher in those with a high adherence rate for the Mediterranean diet. This situation has been evaluated as a coping strategy compatible with this process, as the fear and phobia of the coronavirus lead individuals to healthier eating habits. The Mediterranean diet is a healthy eating model that has protective features from many diseases. In this period, when emotional state changes and mental health is negatively affected, adherence to Mediterranean diet is important in getting through the pandemic period in a healthier way both physically and mentally.

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Acquisition of data for the study: BA, GA, TK, NT
Analysis of data for the study: BA, GA, TK, NT
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Drafting the manuscript: BA, GA
Revising it critically for important intellectual content: BA, GA, TK, NT
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Clinical and Experimental Health Sciences

Effect of Different Remineralization Agents on Artificial Caries Lesion: An in-vitro Study

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ABSTRACT

Objective: Remineralization technologies have been shown to arrest or reverse early carious lesions. This study aimed to evaluate and compare the effect of different agents on enamel remineralization in-vitro.

Methods: Thirty-five enamel blocks were prepared and divided into the following groups: Group 1; CaGp and Xylitol-containing gel (R.O.C.S Medical Mineral Gel), Group 2; 1100 ppm NaF and 10% CPP-ACP toothpaste (MI Paste One), Group 3; 0.45% SnF2-1150 ppm F toothpaste (Enamelon), Group 4; Positive Control-1450 ppm NaF toothpaste (Colgate Total) and Group 5; Negative Control (deionized water). Microhardness was measured at baseline, after demineralization, and after respective treatments for different treatment groups using a digital Micro Vickers Hardness Tester. For producing demineralized lesions, samples were stored in acidic hydroxyethylcellulose (HEC, pH=4.8) for three days. The Paired Sample t-test, one-way ANOVA, and Tukey were used to compare data and SMH recovery (%SMHR) calculated among treatments.

Results: The mean baseline surface microhardness value was statistically non-significant between the groups (p=.378). CaGP and Xylitol-containing gel demonstrated having the most protective effect against demineralization. The surface remineralization potential of 1100 ppm NaF and 10% CPP-ACP containing novel toothpaste (MI Paste One) was almost similar to the positive control (1450 ppm Fluoride toothpaste) group (p>.05).

Conclusions: All treatment groups showed remineralization after respective treatments and these agents can be used as an effective preventive measure for pediatric patients.

Keywords: Remineralization, CaGP and Xylitol, Fluoride Toothpaste, Surface Microhardness, CPP-ACP

1. INTRODUCTION

Dental caries is a chronic multifactorial process and develops as a result of the combination of many genetic, diet, environmental, and lifestyle factors. Dental caries prevalence decrease has been declared however it remains one of the most common preventable non-communicable diseases worldwide. In recent years dental caries prevention has been the primary purpose of dental health care (1, 2).

The development of the caries process begins with the disruption of the balance between demineralization and remineralization (3). Minerals must be reabsorbed into carious areas to enhance remineralization (4). Enamel surface remineralization therapies can arrest the progression of early caries lesions. The minimally invasive dentistry approach focuses on the management of the condition that causes dental caries, restoring only when necessary, reducing the evolution of dental caries, and preventing the progression of caries lesions (5).

Recently developed remineralization technologies aim to prevent caries formation and treat at the initial term. Over the years, it has been generally accepted to recommend using fluoride-containing agents to prevent the demineralization of dental tissues (6). Regular toothbrushing with fluoridated toothpaste is the most common form of dental caries prevention (7). Sodium fluoride (NaF), a form of fluoride commonly used in oral health care products, significantly reduces the frequency of dental caries. SnF_2 (stannous fluoride), another form of fluoride, is used in dental care products and was indicated to have a suppressive effect against microbial biofilm by inhibiting the glycolysis of aciduric bacteria (8). Toothpaste containing SnF_2 has also proven effective in remineralizing early caries lesions (9).

Even though the remineralization effect of fluoride is generally considered successful, different enamel remineralization

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therapies have been suggested for personal and professional practice (10). Casein phosphopeptide–amorphous calcium phosphate (CPP-ACP) is a bioactive agent, obtained from milk protein, and has been shown to support remineralizing efficacy of superficial enamel lesions in various studies (11, 12). CPP-ACP can enhance the activity of fluoride-based remineralization. Co-administration of CPP-ACP and fluoride has been demonstrated to have synergistic remineralizing action and antibacterial activity against cariogenic bacteria (13). MI Paste One (1100ppm NaF and 10% CPP-ACP toothpaste, GC America Inc, USA) is a toothpaste that combines the advantages of fluoride and CPP-ACP.

Calcium glycerophosphate (CaGP) and Xylitol-containing gel (R.O.C.S Medical Mineral Gel, DRC Group, Moscow, Russia) show remineralizing effect by increasing the mineral content on dental enamel has been reported (14). CaGP demonstrates a preventive action against demineralization by enhancing the resistance of hydroxyapatite crystals (15). Xylitol is a sugar alcohol and provides an alternative to sugar. It has been declared in the studies that the replacement of dietary sugar (sucrose) in chewing gums or sweets with Xylitol can decrease caries formation by increasing saliva production and reduction of cariogenic bacteria (16, 17).

Microhardness tests are easy, quick methods and are often used to investigate the physical structure of materials (18). Data from several studies have identified that enhanced remineralization is associated with increased enamel surface microhardness (19). Enhancing levels of microhardness of the enamel surface is dependent on the improvement of remineralization of the caries lesions has been stated (19). Featherstone et al. (20) investigated the artificial caries lesions by microhardness tests and found that mineral alterations in tooth structure as a result of remineralization and demineralization could be evaluated as microhardness change.

Many studies evaluated comparing CPP-ACP complex (21, 22) CaGP containing gel (15, 23) and conventional agents (24, 25) on caries remineralization with different results. Thus the main aim of this in vitro study is to demonstrate the remineralization activity of various agents and compare between groups using a digital Micro Vickers Hardness Tester.

Three null hypotheses were proposed: (1) the use of CaGP and Xylitol-containing gel will result in no significant difference in the enamel remineralization compared with 1450 ppm F toothpaste (positive control) (2) the use of CPP-ACP and 1100 ppm NaF containing toothpaste will result in no significant difference in the enamel remineralization compared with 1450 ppm F toothpaste (positive control) (3) the use of 0.45% SnF_2-1150 ppm F toothpaste will result in no significant difference in the enamel remineralization compared with 1450 ppm F toothpaste (positive control).

2. METHODS

2.1. Study Design

This study was approved by Marmara University, Faculty of Dentistry Ethics Committee (Approval number and date:2020-398/ 01.06.2020). The study was conducted following the Declaration of Helsinki.

The number of enamel samples in each group was evaluated as 7 specimens per group based on a previous study (G*power version 3.1.9.6, α=0.05, 1−β=0.80, effect size: 1.767) (26).

2.2. Preparation of Enamel Blocks

Twenty-eight freshly extracted permanent human molars were collected. Specimens were ultrasonically cleaned and removed from deposits/stains and sterilized following Occupational Safety and Health Administration recommendations (27). The infrared light transillumination (DIAGNOcam 2170U, Kavo, Biberach, Germany) was used to exclude the possibility of enamel cracks, decalcification of enamel, white spot lesions (WSLs), and extraction damage. After exclusion, the crowns of all teeth were cut from the roots at the cementoenamel junction and split mesiodistally into two halves using an ISOMET Low-Speed Saw cutting machine (Buehler, Lake Bluff, IL, USA).

The resultant 35 specimens were randomly distributed into five groups (n=7 specimens in each group) as follows: Group 1; CaGP+magnesium chloride ($MgCl_2$)+10% Xylitol (R.O.C.S. Medical Minerals Gel®), Group 2; 1100 ppm NaF and 10% CPP-ACP toothpaste (MI Paste One®), Group 3; 0.45% SnF_2-1150 ppm F toothpaste (Enamelon®), Group 4; Positive Control-1450 ppm NaF toothpaste (Colgate Total®), Group 5; Negative Control (deionized water). Group distribution and details are shown in Table 1. Enamel blocks (3 mm x 3 mm x 2 mm) were prepared from the labial surfaces. After embedding samples in epoxy resin, the superficial surface of the enamel was polished with water-cooled carborundum discs and 1200-grit waterproof silicon carbide paper (Amico), thereby removing about 200 µm of enamel (28).

2.3. Experimental Design

For producing demineralized lesions, samples were stored in acidic hydroxyethylcellulose (HEC, pH 4.8) for three days, following the procedure of Amaechi et al. (29). The solution was prepared by adding 100 mmol/l sodium hydroxide to 100 mmol/l lactic acids (pH value of 4.5). Following demineralization, surface microhardness measurements were made using the Vickers Hardness Number (VHN) indenter. The samples were immersed in one part agent and three parts artificial saliva (9 g: 27 ml) using a laboratory stand mixer until homogeneous. After the mixture was prepared, different remineralizing agents were applied to the enamel surface of each group with the applicator brush and left for 2 minutes (30, 31).

Artificial saliva was used in all treatment regimens and compositions were as follows: Magnesium chloride hexahydrate – ($MgCl_2 6H_2O$, 0.148 mmol/L), Dibasic potassium phosphate (K_2HPO_4, 4.59 mmol/L), Potassium phosphate monobasic (KH_2PO_4, 2.38 mmol/L) KCl (Potassium chloride, 8.39 mmol/l), calcium lactate (1.76 mmol/l), fluoride (0.05 ppm), sodium carboxymethyl cellulose (2.25 mmol/l), methyl-4-hydroxybenzoate ($HOC_6H_4CO_2CH_3$, 13.14 mmol/l). Solution pH was adjusted to 7.2 (32).

After each incubation, enamel specimens were carefully washed with sterile water to remove residual acid. The enamel specimens were then stored in filtered distilled water throughout the study. Surface microhardness was evaluated in 5 groups using the Vickers Hardness Test after treatment, and a comparative analysis was made.

Table 1. *Distribution of groups with respective active ingredients*

Groups	Agents	Active ingredients	Company
1	R.O.C.S (Remineralizing Oral Care Systems) Medical Minerals Gel®	Calcium glycerophosphate (CaGP) + Magnesium chloride (MgCl2) + 10% Xylitol	DRC Group, Moscow, Russia
2	MI Paste One®	1100ppm Sodium Fluoride (NaF) + 10% Casein phosphopeptides-amorphous calcium phosphate (CPP-ACP)	GC America Inc, USA
3	Enamelon® Fluoride Toothpaste	1150 ppm (0.45%) Stannous Fluoride (SnF_2) +Amorphous Calcium Phosphate (ACP)	Premier, USA
4	Positive Control (Colgate Total®)	1450 ppm (0.32%) Sodium Fluoride (NaF)	Colgate,USA
5	Negative Control	Deionized water	

2.4. Surface Microhardness Analysis

A digital Micro Vickers Hardness Tester (Wilson Wolpert Europe BV, 401 MVD, Netherland) fitted with a Vickers diamond and 200 Newton load was used to make indentations in the enamel surface. The loaded diamond was allowed to rest on the surface for 15 seconds at three different points, each 1 mm apart, and the mean value was saved as VHN.

The mean values of all three measurements of the midline surface at the three steps (baseline, after demineralization, after respective treatments). The percentage of surface microhardness recovery (%SMHR) was evaluated for each group using the following formula (33).

$$\%SMHR = 100 \times \frac{\text{SMH after remineralization} - \text{SMH after demineralization}}{\text{SMH baseline} - \text{SMH after demineralization}}$$

2.5. Statistical Analysis

Data is analyzed using SPSS 20.0 software. The Paired Sample t-test is used to compare surface microhardness before and after the treatments and the percent SMH recovery (%SMHR) calculated among treatments. One-way Analysis of variance (ANOVA) and Tukey was used for comparing data. The significant level (p) was set at .05.

3. RESULTS

A total of 50 specimens were initially demineralized, of which 35 specimens were selected for this study as described above. VHN for the baseline of all groups were compared, and there was no statistically significant difference found between groups (p=.378) (Table 2). After respective treatments, VHN remineralization of all the treatment groups had comparatively increased when compared to VHN demineralization (p< .05 for all groups) (Table 3).

Table 2. *Mean VHN for both groups measured at baseline*

	Remineralization agents	VHN baseline Mean±SD	p
Group 1	CaGP+Xylitol	374.91±5.65	.378
Group 2	CPP-ACP+1100 ppm NaF	373,91±6.55	
Group 3	1150 ppm SnF_2+ACP	363.70±8.92	
Group 4	1450 ppm NaF (positive control)	359.48±7.20	
Group5	Deionized water (negative control)	375.519±7.11	

VHN: Vickers Hardness Number, CaGP: Calcium glycerophosphate, CPP-ACP: Casein phosphopeptides-amorphous calcium phosphate, NaF: Sodium Fluoride, SnF_2: Stannous Fluoride, ACP: Amorphous Calcium Phosphate p<.05, One-way ANOVA*

Table 3. *The comparison of VHN after demineralization and remineralization of each treatment group*

	Groups	VHN demineralization	VHN remineralization	p
Group 1	CaGP+Xylitol	272.77±15.04	313.67±7.23	<.001*
Group 2	CPP-ACP +1100 ppm NaF	269.17±11.35	299.37±6.77	<.001*
Group 3	1150 ppm SnF_2+ ACP	242.49±8.04	267.81±5.36	<.001*
Group 4	1450 ppm NaF (Positive control)	252.40±6.40	279.95±5.74	<.001*
Group 5	Deionized water (Negative control)	259.06±6.44	271.89±5.51	<.002*

VHN: Vickers Hardness Number, CaGP: Calcium glycerophosphate, CPP-ACP: Casein phosphopeptides-amorphous calcium phosphate, NaF: Sodium Fluoride, SnF_2: Stannous Fluoride, ACP: Amorphous Calcium Phosphate
** p<.05, The Paired Sample t-test*

The mean %SMHR was found statistically different among groups (p<.001) (Table 4). The mean (±SD) %SMHR of groups were as follows: Group 1: 38.63 (±10.56), Group 2: 27.48 (±10.34), Group 3: 20.48 (±7.37), Group 4: 22.89 (±13.18) and Group 5: 11.35 (±7.38). The pairwise comparison of the percentage surface microhardness recovery (%SMHR) of treatment groups was shown in Table 5. There were significant differences between intergroup comparisons, but not between Group 2 (CPP-ACP+1100 ppm NaF) and Group 4 (1450 ppm NaF, positive control).

Table 4. *Mean and standard deviations (SD) of percentage surface microhardness recovery (%SMHR) for all groups*

	Microhardness Groups	SMHR (%) Mean±SD
Group 1	CaGP+Xylitol	38.63±10.56
Group 2	CPP-ACP+1100 ppm NaF	27.48±10.34
Group 3	1150 ppm SnF_2+ACP	20.48±7.37
Group 4	1450 ppm NaF (positive control)	22.89±13.18
Group 5	Deionized water (negative control)	11.35±7.38
p		<.001*

*CaGP: Calcium glycerophosphate, CPP-ACP: Casein phosphopeptides-amorphous calcium phosphate, NaF: Sodium Fluoride, SnF_2: Stannous Fluoride, ACP: Amorphous Calcium Phosphate, *p< .05, One-way ANOVA*

Table 5. *The pairwise comparison of percentage surface microhardness recovery (%SMHR) of treatment groups*

	Group 2 (CPP-ACP + 1100 ppm NaF)	Group 3 (1150 ppm SnF_2 + ACP)	Group 4 Positive control (1450 ppm NaF)	Group 5 Negative control (Deionized water)
Group 1 (CaGP and Xylitol)	.006*	< .001*	< .001*	< .001*
Group 2 (CPP-ACP+1100 ppm NaF)		< .001*	.95	< .001*
Group 3 (1150 ppm SnF_2+ACP)			< .001*	.030*
Group 4 Positive control (1450 ppm NaF)				< .001*

CaGP: Calcium glycerophosphate, CPP-ACP: Casein phosphopeptides-amorphous calcium phosphate,
*NaF: Sodium Fluoride, SnF_2: Stannous Fluoride, ACP: Amorphous Calcium Phosphate,*p< .05, ANOVA, Tukey HS*

The first hypothesis of the present study, (1) the use of CaGP and Xylitol-containing gel will result in no significant difference in the enamel remineralization compared with 1450 ppm F toothpaste, have to be rejected. Second null hypothesis (2) the use of CPP-ACP and 1100 ppm NaF containing toothpaste will result in no significant difference in the enamel remineralization compared with 1450 ppm F toothpaste, accepted. The third null hypothesis (3), the use of 0.45% SnF_2-1150 ppm F toothpaste will result in no significant difference in the enamel remineralization compared with 1450 ppm F toothpaste, has to be rejected.

4. DISCUSSION

Remineralizing agents can preserve caries emergence or intercept the worsening of enamel demineralization, and this could be provided by boosting calcium phosphate levels in the environment (28). In our study, we use demineralization and remineralization solutions to imitate the caries formation and anti-caries process (29,34). To produce demineralized lesions, we chose acidic hydroxyethylcellulose (HEC), and the pH was adjusted to 4.8. Specimens were stored in this solution for three days, based on the study protocol conducted by Amaechi et al. (29). Artificial saliva could promote remineralization on early enamel caries lesions, according to research by Huang et al. (35). In the present study, we used artificial saliva to prepare a respective remineralizing treatment to apply enamel sample surfaces. Microhardness changes are directly related to mineral alteration in the subsurface lesion (36). Kielbassa et al. (37) reported a significant association between microhardness and the mineral ratio of caries lesions. Surface microhardness values are a commonly used technique for assessing the level of mineralization and vary according to the level of calcium content in enamel specimens (38,39). We used the Vickers microhardness test in our study to evaluate demineralized and remineralized enamel samples since it has been used in many studies and is a rapid and non-damaging method (33, 39). Multiple variables might change the explication of surface microhardness values, such as the setting of samples, exposed angle, and the region picked. We considered these factors and measured each enamel midline surface at three different times. No statistically significant difference was observed in baseline surface microhardness values between all groups (p> .05).

Calcium glycerophosphate and Xylitol-containing agent showed remineralizing and antibacterial efficacy in some studies (15, 40). In the present study, Group 1 (CaGP and Xylitol-containing gel) showed the highest %SMHR. Sezer and Kargul declared that CaGP containing agent has remineralization potential on hypomineralized enamel in their in-vivo study, on the other hand when comparing with the casein phosphopeptide amorphous calcium fluoride phosphate (CPP-ACFP) there were no statistically significant differences observed (15). Another in-vitro study reported that there was no significant difference between the CPP-ACP, CPP-ACFP, and CaGP+MgCl2+Xylitol groups in terms of the amount of advancing in the %SMHR (23).

Stannous fluoride has a protective effect against dental plaque and dentin hypersensitivity (9). SnF_2-containing toothpaste has been shown more effective in reversing early carious lesions compared with conventional NaF-containing toothpaste (41). In the present study, SnF_2 toothpaste showed a lower %SMHR than the 1450 ppm F toothpaste group and of the CPP-ACP and 1100 ppm NaF group. Likewise our study results, another study reported SnF_2 toothpaste showed

inferior efficiency against 1100 ppm fluoridated toothpaste (42).

CPP-ACP has demonstrated its impact on the significant recovery of initial enamel lesions in-vivo and in-vitro (43, 44, 45). According to our study results, CPP-ACP and 1100 ppm NaF toothpaste (Group 2) showed higher remineralization against 1150 ppm SnF_2 (0.45%) + ACP (group 3) and negative control (Group 5). However, there was no significant difference between the 1450 ppm NaF toothpaste-positive control (Group 4). Similar to our results, Vashisht et al. (46) they were reported that CPP-ACP indicated significant remineralization against the control group (deionized water). CPP-ACP promotes the stabilization of calcium and phosphate ions and mineral deposition in an acidic environment for enhanced buffering. In some studies, CPP-ACP has shown superior efficacy to fluoride in caries management (38, 39). By contrast, in 2016 Rirattanapong et al. (47) reported that 500 ppm F toothpaste had a superior remineralization efficiency than CPP-APP.

Some researchers have shown that CPP-ACP 's remineralization capacity is equal to or lesser than fluoride (33, 48). In an in-vitro study, Gonçalves et al. in 2021 declared that CPP-ACP+F paste promoted a superior efficacy compared with 1100 ppm F toothpaste (21). We designed an in-vitro study; there were inadequate calcium and phosphate ions values to promote remineralization; thus, it could be a reason for the different results between studies. In the current study CPP-ACP and 1100 ppm NaF toothpaste (Group 2) did not show additional surface remineralization against positive control (Group 4). A possible explanation for these results may be; that CPP attaches to apatite crystal on the enamel surface and preserves ionic diffusion to allow minerals to penetrate deeper; thus, remineralization occurs in both the superficial layer and body of the lesion. Similar to our study results, Vyavhare et al. (49) found that the CPP-ACP group and NaF (1000 ppm) group had the same %SMHR, and there was no significant difference between them.

Our study limitations were; that in-vitro conditions could not simulate the complete demineralization/remineralization cycle in the oral cavity, and the artificial saliva and artificial caries lesions did not contain bacteria as in the actual environment. The results of this investigation show that CaGP+Xylitol has the best effectiveness in remineralizing enamel lesions. CPP-ACP and 1100 ppm NaF toothpaste showed similar results as the positive control group.

5. CONCLUSION

It could be considered that CaGp and Xylitol-containing gel and CPP-ACP and 1100 ppm NaF toothpaste can be used in preventive dentistry as an alternative to conventional fluoride toothpastes and a new approach to reducing the risk of caries. Further randomized controlled trials should be focused on different groups to confirm the accuracy of long-term effects, administration patterns, and dosage for these agents to be widely used in preventive and therapeutic applications.

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Design of the study: EAS and BK
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Analysis of data for the study: EAS and APG
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Clinical and Experimental Health Sciences

Do Sexual Myths Affect Menopause Attitudes and Symptoms?

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ABSTRACT

Objective: Sexual myths are common in some societies. Menopause is one of the important processes in a woman's life and many changes are seen during this period. Many factors play a role in these changes, menopausal attitudes and symptoms, and one of them is sexual myths. In this study, it was aimed to determine the relationship between having sexual myths and menopausal attitudes and symptoms in postmenopausal women.

Methods: The study had a descriptive analytical design and the study sample included 424 postmenopausal women. Data were gathered with personal characteristics form and the self-report tools Sexual Myths Scale, Menopausal Attitude Assessment Scale and Menopause Rating Scale.

Results: The mean age of the women was 57.12±5.60 years and the mean duration of menopause was 10.58±7.02 years. The mean score on Sexual Myths Scale was 92.28±17.80, the mean score on Menopausal Attitude Assessment Scale was 27.86±8.06 and the mean score on Menopause Rating Scale was 17.11±9.43. There was not a relation between the score on Sexual Myths Scale and the mean score on Menopausal Attitude Assessment Scale (r=-0.067, *p=0.168*), but there was a significant, positive weak relation between the mean score on Sexual Myths Scale and the mean score on Menopause Rating Scale (r=0.125, *p=0.010*).

Conclusions: The postmenopausal women had a fairly high level of sexual myths. Sexual myths had a significant, positive relation with menopausal symptoms and their severity, though the relation was weak. Also, sexual myths had no relation with attitudes to menopause.

Keywords: Menopause, attitude, sexuality, sexual myths, symptom

1. INTRODUCTION

Women receive information about sexuality from their family, their social environment and the media. However, the information they obtained may not be accurate. The society transfers prejudices created over time to new generations (1-3). Inaccurate information and false ideas transferred from generation to generation are called myths (1,3).

Sexual myths are strong, false beliefs adopted without sufficient evidence and spreading through verbal transmission from person to person (4). Sexual myths adopted by women are affected by many factors (duration of education, family type, marriage type, sources of information, first sexual experience, pornographic material and network of friends etc.) (5,6) and can affect many aspects of life (sexual life, life satisfaction, marriage, social life, quality of life and physical/psychological disorders etc.) (7,8).

Menopause, one of the important stages of women's life, many problems are experienced due to hormonal, physical and emotional changes due to estrogen deficiency. In women in this period, vasomotor symptoms such as hot flashes, sweating, headache; cardiovascular symptoms due to changes in the blood lipid picture and increased risk of vascular disease; atrophic manifestations such as vaginal dryness, itching, dyspareunia, dry skin and wrinkling; musculoskeletal problems such as susceptibility to osteoporosis; psychological changes such as irritability and depressive symptoms may occur (9-13). The attitude of women towards menopause is the sum of biological, psychological, social and cultural factors. Many factors such as individual characteristics, tradition, ethnicity, the meaning that women and society ascribe to menopause, the life philosophy of women, the value that society gives to women,

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the role of women in society and the meaning of women's fertility for themselves and the society in which they live have an important effect (10,12). Symptoms arising from these changes in menopause and attitudes to menopause are affected by many factors and create an impact on many aspects of lives of women (9-10). One of these aspects is sexual life (14,15). There are studies in which the quality of sexual life and sexual dysfunctions were questioned in postmenopausal women (14,16-18), but no study was found that questioned the status of believing in sexual myths and the periodical differences in believing in sexual myths in women in this age group. In the specific screening, no research was found that examined the relationship between believing in sexual myths and menopausal attitudes and symptoms in postmenopausal women. Therefore, the current study was performed to reveal the relation between having sexual myths and menopausal symptoms and attitudes to menopause in postmenopausal women. To this aim, answers to the following questions were sought:

- Do postmenopausal women have sexual myths?
- Is there a relation between having sexual myths and attitudes to menopause in postmenopausal women?
- Is there a relation between having sexual myths and menopausal symptoms and their severity in postmenopausal women?

Knowing the relation between sexual myths and attitudes of women to menopause and the severity of menopausal symptoms can guide nurses and health professionals while offering care and treatment. Besides, it can help to incorporate new information to education programs about sexual and reproductive health.

2. METHODS

2.1. Research Design and Sample

This is a descriptive and analytical study. The study population comprised 4361 women aged 40-64 years and living in a town with a population of 90.000 in the north of Turkey (19). The sampling method whose universe is known (n= N. t2. pq/ y2. (N-1) + t2. Pq) was used to determine the sample. Accordingly, considering the 95% confidence interval (d=0.05), t= 1.96, p=0.5, q=0.5, the number of women included in the sample was calculated as at least 354 (20). Inclusion criteria were receiving care from two family health care centers in the center of the town, having amenorrhea lasting over one year, being literate, being able to speak and understand Turkish or speaking and understanding Turkish and not having a cognitive or mental problem.

2.2. Data Collection

Data were collected from 424 women fulfilling the inclusion criteria and presenting to the family health centers between January and May 2018. Data collection was performed in a room available in the family health centers to prevent any distractions. A personal characteristics form was completed at face to face interviews. The other data collection tools utilized were self-report scales and included Sexual Myths Scale, Menopausal Attitude Assessment Scale and Menopause Rating Scale. It took about 15 minutes for each woman to fill in the data collection tools.

2.3. Data Collection Tools

A personal characteristics form, and the self-report tools Sexual Myths Scale (SMS), Menopausal Attitude Assessment Scale (MAAS), and Menopause Rating Scale (MRS) were employed to gather data.

Personal Characteristics Form: The personal characteristics form was prepared by the researchers (5-7,12,16) and composed of questions about sociodemographic features and menopausal status.

Sexual Myths Scale (SMS): SMS which was developed by Golbası et al. (2016) to determine sexual myths of women in Turkish culture (21), was utilized to collect data about sexual myths of the participants. The scale has been used in several prior studies (22-24). SMS includes eight subscales and 28 items. It is a five-point Likert scale, and "1" corresponds to completely disagree and "5" corresponds to completely agree. Points for all the items are added to obtain the total score for the scale. The lowest and the highest scores for the scale were 28 and 140 respectively. High scores indicate a high number of sexual myths. Cronbach's alpha reliability coefficient of the scale is 0.91 (21). The cronbach's alpha reliability coefficient of the scale in this study was found to be 0.87.

Menopausal Attitude Assessment Scale (MAAS): MAAS was created by Koyuncu et al. (2015) to evaluate attitudes of Turkish women to menopause and includes four subscales and 13 items. It is a five-point Likert scale and "0" corresponds to completely disagree and "4" corresponds to completely agree. The negative items 1, 2, 3, 4, 5, 10, 12 and 13 are scored in the reverse order. The lowest and highest scores for the scale are 0 and 52 respectively. High scores for the subscales indicate a positive attitude to menopause. The cronbach's alpha reliability of the scale is 0.74 (25). Cronbach's alpha reliability coefficient in this study was found to be 0.63.

Menopause Rating Scale (MRS): MRS was developed by Schneider, Heinemann et al. in 1992 and the validity and reliability of its Turkish version were tested by Can Gurkan in 2005. The scale is composed of three subscales and 11 items. It is a five-point Likert scale and "0" corresponds to none and "4" corresponds to very severe. The lowest and the highest scores for the scale were 0 and 44 respectively. High scores indicate an increase in the severity of menopausal complaints. The cronbach's alpha reliability coefficient of the scale is 0.84 (26). In this study, the Cronbach's alpha reliability coefficient of the scale was 0.87.

2.4. Statistical Analysis

Data were analyzed with the Statistical Package for Social Science 24 (SPSS Inc., IL, USA). Descriptive statistics were used to analyze the data about general characteristics of the participants. Kolmogorov Smirnov test was utilized to determine whether the data had a normal distribution. Since the scores on SMS, MAAS and MRS were not normally distributed, Spearman's correlation analysis was made to determine the relations between the total scores for these scales. The statistical significance was set at $p<0,05$.

2.5. Ethical Approval

Ethical approval was obtained from the ethical board of Aydın Adnan Menderes University Health Sciences Faculty (Date: 27.09.2017; Approval number: 2017/42) and written permission was obtained from Zonguldak Health Directorate (date:16.01.2018, approval number: E.992). The women were provided with both oral and written information about the study and their oral informed consent was taken. Permission was requested from the researchers developing the self-report tools utilized for data collection.

3. RESULTS

The mean age of the women was 57.12±5.60 years. The mean age at the last menstruation was 46.54±5.35 years and the mean duration of menopause was 10.58±7.02 years. Of all the women included in the study, 53.3% were primary school graduates, 76.4% were married, 89.2% had a nuclear family, 85.4% were unemployed and 74.5% thought their family income was sufficient (Table 1).

Table 1. *Sociodemographic features of the postmenopausal women (N=424)*

Sociodemographic features	n	%
Education		
Literate	143	33.7
Primary school	226	53.3
Secondary school	24	5.7
High school	24	5.7
University	7	1.7
Marital status		
Married	324	76.4
Single/Widowed/Divorced	100	23.6
Family type		
Nuclear family	378	89.2
Extended family	46	10.8
Paid employment status		
Unemployed	362	85.4
Retired	36	8.5
Employed	26	6.1
Perceived monthly family income		
Sufficient (income equal to expenses)	316	74.5
High (income higher than expenses)	56	13.2
Insufficient (income lower than expenses)	52	12.3

Eighty-four percent of the women reported having natural menopause. All of the women had at least one menopausal complaint and 32.5% reported that they consulted a doctor about their menopausal complaints. About one-fourth of the women (26.2%) noted that they received education/information about menopause and more than half of them (57.7%) received it from a doctor. The vast majority (93.6%) of the women participating in the study stated that they did not receive information about sexuality or sexual health. Very few women (n=27) received information about sexuality and sexual health and their most important source of information was audio-visual and written sources (n=7) (Table 2).

Table 2. *The distribution of menopause – and sexuality-related features of the postmenopausal women (N=424)*

Menopause – and Sexuality-Related Features	n	%
Type of menopause		
Natural	356	84.0
Surgical	63	14.9
Medical treatment	5	1.2
Presence of menopausal complaints		
Yes	424	100.0
No	0	0.0
Seeing a doctor for menopausal complaints		
No	286	67.5
Yes	138	32.5
Receiving education/information about menopause		
No	313	73.8
Yes	111	26.2
Sources of information about menopause (n=111)		
Doctors	64	57.7
Nurses or midwives	23	20.7
Friends, neighbors and relatives	14	12.6
Audio-visual and written sources	10	9.0
Receiving information about sexuality and sexual health		
No	397	93.6
Yes	27	6.4
Sources of information about sexuality and sexual health (n=27)		
Audio-visual and written sources	7	25.9
Nurses or midwives	6	22.2
Parents/spouses	6	22.2
Doctors	5	18.6
Friends, neighbors and relatives	3	11.1

The mean score of the women on SMS was 92.28±17.80. As the scores on SMS increase, so the beliefs about sexual myths. It can be suggested that the women believe a quite high number of sexual myths. The mean score on MAAS was 27.86±8.06. The mean score on MRS was 17.11±9.43. High scores on MRS indicate a higher severity of menopausal symptoms (Table 3).

There was not a significant relation between the mean score on SMS and the mean scores on MAAS (r= – 0,067, $p=0,168$). However, the mean score on SMS had a significant, positive

weak relation with the mean score on MRS (r=0,125, *p=0,010*) (Table 4).

Table 3. *The mean scores of the postmenopausal women on SMS, MAAS and MRS (N=424)*

Scales	Scores		Reference Scores
	Mean±SD	min-max scores	
SMS	92.28±17.80	28-133	28-140
MAAS	27.86±8.06	7-51	0-52
MRS	17.11±9.43	1-44	0-44

Tablo 4. *The relationship between the mean scores of SMS, MAAS and MRS in postmenopausal women (N=424)*

Scales	SMS	
	r	*p*
MAAS	-0.067	*0.168*
MRS	0.125	*0.010*

4. DISCUSSION

This study was directed towards examining the relation of sexual myths with attitudes to menopause and menopausal symptoms in a sample of 424 postmenopausal women. Data obtained to seek answers to the research questions revealed that sexual myths were common among the women. Sexual myths had a significant, positive correlation with menopausal symptoms and their severity, but the correlation was weak. Besides, sexual myths had no relationship with attitudes to menopause.

The mean score of the women on SMS was 92.28±17.80. This shows that the women believed a fairly high number of sexual myths, which is consistent with the findings reported by other studies in Turkey (6,8,27-30). The high rate of the sexual myths shown by other studies including samples with different demographic characteristics reveals that misbeliefs about sexuality are common in Turkish society. In a study by Sasanpour, Azh and Alipour (2020) including women with similar socioeconomic status to the women in the current study, education about sexuality was found to have a positive influence on sexuality-related beliefs (31). In the current study, inadequate knowledge about sexuality and receiving this knowledge from unreliable sources might have increased the participants' sexual myths.

Based on the mean score on MAAS, the women were found to have a positive attitude towards menopause, which is compatible with the findings from other studies about attitudes of postmenopausal women to menopause (32-39). The MAAS (34.50±11.30) score of women in the study of Yagmur and Akturk (2021) was higher than in our study (39). The fact that the mean MAAS score of the women in this study was lower than the study of Yagmur and Akturk (2021), may be due to the low level of information about menopause in the women in our study. In the study of Gumusay and Erbil (2019), it was determined that seven out of ten women had a negative attitude towards menopause (36.06±7.34) (40). The fact that the women in our study had more positive menopause attitudes than the women in Gumusay and Erbil (2019)'s study may be due to the older age of the women in our research group. The postmenopausal women in Barth Olofsson and Collins's (2000) study in Sweden and the women in Kowalcek et al.'s (2005) study in Germany and Papua New Guinea displayed a more positive attitude towards menopause than the women in the present study (33,35). This can be attributed to the fact that perceptions about menopause vary from culture to culture (lack of a risk of becoming pregnant and having a better social status).

The women in the present study had a quite low score on MRS, utilized to determine menopausal symptoms and their severity. This indicates that the number and severity of menopausal symptoms experienced by the women were not high, which is congruent with the literature (38,41). In Kowalcek et al.'s (2005) study, the severity of menopausal symptoms in postmenopausal women was higher than that found in the current study (35). This may be that most of the women in the current study had menopause lasting more than ten years and could be accustomed to menopausal symptoms and that the severity of their symptoms decreased.

The present study also focused on the relation of sexual myths with menopausal symptoms and attitudes to menopause. Having sexual myths was found to have no relation with attitudes to menopause in postmenopausal women. However, the quality of sexual life is associated with positive attitudes to menopause (42,43). In the present study, it was surprising that although the women strongly believed sexual myths, they had a neutral attitude to menopause and few menopausal symptoms with low severity. Alirezaei et al., (2020) found that negative attitudes of the postmenopausal women to sexuality had an unfavorable effect on their attitudes to menopause, which is not consistent with the results of the present study (16). This difference can be explained by the older age and a lower education level of the women in the present study and their inability to receive information/education about sexuality.

In the current study, sexual myths of the postmenopausal women had a relation with menopausal symptoms and their severity, though the relation was very weak. Sexual life was found to have a relation with menopausal symptoms in samples having similar sociodemographic features to the sample of the present study (17,44,45).

5. CONCLUSION

Sexual myths had a significant, positive but poor relationship with menopausal symptoms and their severity and had no association with attitudes to menopause. Besides, the women received information about sexuality from the media rather than from health professionals. They might have refrained from talking to health professionals about sexuality. Therefore, health professionals especially nurses, who spend more time with patients while giving care and treatment, should provide women from all age groups with

information about sexuality or sexual health and education programs about sexual health and encourage them to talk about sexuality related issues comfortably. The education programs arranged by nurses can eliminate misbeliefs of the women about sexuality. This is the first study to reveal the relation of sexual myths with menopausal symptoms and attitudes to menopause. Further studies about this issue can provide guidance for education and counseling about sexual health to be offered to women.

Study limitations

The women included in the study might have considered the questions about sexuality as violation of their privacy and might not have answered them completely and properly. Also, the results of the study are based on data from self-report tools. On the other hand, to our knowledge, this is the first study to focus on the relation between sexual myths and menopausal symptoms and attitudes to menopause. Therefore, the study provided new knowledge about the issue and contributed to the relevant literature. However, since there have not been any studies on the relation between sexual myths and menopausal symptoms and attitudes to menopause, the results of the study could not be compared with evidence from the available literature. This might have decreased the strength of the discussion of the study results.

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Clinical and Experimental Health Sciences

Is Allergic Rhinitis Associated with Enuresis Nocturna in Childhood?

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ABSTRACT

Objektive: Allergic rhinitis is the most common form of non-infectious rhinitis and is manifested with the symptoms of nasal congestion, sneezing, nasal discharge, and itching. Primary enuresis nocturna is involuntary urination while asleep after five years, at which bladder control usually begins. Our objective was to evaluate the relationship between allergic rhinitis and primary enuresis nocturna in childhood in this research.

Methods: We included 300 children with allergic rhinitis and 300 control cases between the age of 5 and 17 years. With allergic rhinitis cases, age, gender, body mass index percentile, other allergic disorders, allergic rhinitis diagnosis age, allergic rhinitis severity and distribution, presence of primary enuresis nocturna, and presence of primary enuresis nocturna in the family were recorded. Subjects with disorders causing enuresis were excluded from the study.

Results: The mean age in the allergic rhinitis and control groups was 9.6 ± 3.4 vs. 10.0 ± 3.1 years, respectively (p=0.15). There was no statistical difference between the two groups in terms of gender, age, body mass index percentile, and primary enuresis nocturna presence in the family (p=0.29, p=0.15, p=0.46, p=0.17; respectively). Primary enuresis nocturna was significantly higher in allergic rhinitis cases (p=0.02). Primary enuresis nocturna was significantly higher in boys than in girls (p=0.007). There is a significant difference in age and gender between those with and without primary enuresis nocturna in allergic rhinitis groups (p=0.001, p=0.01, respectively)

Conclusion: We conclude that as allergic rhinitis increases the incidence of primary enuresis nocturna and worsens the quality of life, its treatment should not be neglected.

Keywords: allergic rhinitis, primary enuresis nocturna, childhood, quality of life

1. INTRODUCTION

Allergic rhinitis (AR) is the most common form of non-infectious rhinitis and is accompanied by symptoms including nasal congestion, sneezing, nasal discharge and itching (1). The frequency of allergic rhinitis in Turkey has been reported as 11.8% to 36.4% (2). The prevalence of AR increases parallel to age. The prevalence of AR was found to be 5% at the age of four and increased up to 14% in the upcoming eight years (3). Sleep disorders at different levels are seen due to nasal congestion in AR, which is thought to be amongst the risk factors for obstructive sleep apnea (4).

Primary enuresis nocturna (PEN) is involuntary urination while asleep in children over five years of age at which bladder control develops (5). The incidence rate of PEN varies across cultures and decreases with age (5,6). Genetic factors, detrusor activity, psycho-behavioral problems, circadian rhythm of vasopressin, and sleeping disorders are emphasized for nocturnal enuresis pathogenesis; the underlying cause of PEN is unknown (7).

In this research, we aimed to determine the relationship between primary enuresis nocturna and allergic rhinitis.

2. METHODS

2.1. Study Design and Ethical Approval

This prospective cross-sectional study was approved by Celal Bayar University School of Medicine Institutional Review Board (30/05/2018-20.478.486).

2.2. Study Population

We enrolled 5-17 year old subjects who applied to the pediatric allergy outpatient clinic for the first time between July 2018 and June 2019 and had never received regular treatment for AR. Allergic rhinitis was diagnosed according to ARIA (Allergic Rhinitis and its Impact on Asthma) guidelines with

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recurrent sneezing, nasal drainage, nasal itching and, nasal obstruction (8). A control group was formed with cases aged 5-17 who applied to pediatrics outpatient clinics for healthy child follow-up. Exclusion criteria; having adenotonsillar hypertrophy, being diagnosed with obstructive sleep apnea syndrome, having an irregular sleeping pattern and school/ work activities associated with AR, having neurological and metabolic syndrome, having an anatomical disorder causing upper airway obstruction, having a urinary system disorder.

2.3. Data Collection

Informed consent forms were obtained from all cases included in the study. With allergic rhinitis subjects, age, gender, body mass index (BMI) percentile, presence of other allergic disorders, allergic rhinitis diagnosis age, allergic rhinitis severity and distribution, total Ig-E, eosinophil count, skin prick test, presence of PEN, and PEN presence in the family were recorded. Control subjects' age, gender, BMI percentile, presence of PEN, and PEN presence in the family were recorded in the control group. The body mass index percentile was calculated using reference values for Turkish children (9).

2.4. Skin Prick Test

Skin prick test performed according to The European Academy of Allergy and Clinical Immunology (EAACI) recommendations (10). Skin prick tests applied with standardized solutions with Dermatophagoides Mix, Canis Familiaris, Felis Domesticus, Olea Europaea, Plantago Lanceso, Grasses Mix, Alternaria tenuis and Cockroach (Allergopharma, Germany / ALK, Denmark). Positive and negatives (saline) control was applied (histamine 10 mg/ml Allergopharma).

2.5. Statistical analysis

The data obtained were analyzed by SPSS for Windows v18 (Chicago, IL). Gender, age, BMI percentile, and allergic rhinitis data were analyzed by calculating the mean, standard deviation, and percentages. While analyzing the enuresis nocturna data, the data showing normal distribution were analyzed with the Student – T test, the data not showing the normal distribution were analyzed with the Mann Whitney U test, and the categorical data were analyzed with the Pearson Chi Square test.Statistical significance was defined as $p < 0.05$.

3. RESULTS

3.1. Sociodemographic Characteristics

The mean age of 300 AR cases was 9.6 ± 3.4 years, control cases' mean age was 10.0 ± 3.1 years (p=0.15). Of the patients with AR, 51% (n=153) cases were male, whereas of the subjects in the control group, 46% (n=143) cases were male (p=0.29). The mean BMI percentile in the rhinitis group was 37.9% ± 18.7% and the mean BMI percentile in the control group was 39.1% ± 20.1% (p=0.46). Primary enuresis nocturna was detected 19.6% (n=59) in the allergic rhinitis group, and 12.3% (n=37) in the control group and PEN was significantly higher in the AR group (p=0.02). There was no difference between the groups in terms of the presence of PEN in the family (p=0.17). (Table 1)

Table 1. *Socio-demographic characteristics of the allergic rhinitis group and the control group*

	With Allergic Rhinitis (n=300)	Control Group (n=300)	p=
Boys *	153 (51)	139 (46)	0.29 ***
Age (year)	9.6 (3.4)	10.0 (3.1)	0.15 ****
BMI Per (%) **	37.9 (18.7)	39.1 (20.1)	0.46 ****
With Primary enuresis Nocturna *	59 (20)	12 (37)	0.02 ***
Precense of Primary enuresis Nocturna in Family *	28 (9)	18 (6)	0.17 ***

** Expressed as n (%)** Expressed as mean (standard deviation); *** Chi square test; **** Student t test*

3.2. Primary Enuresis Nocturna Outcomes

The mean age of 600 cases was 9.8 ± 3.3 years, and PEN incidence was 16% (n=96). The mean age of the group with PEN was 8.4 ± 2.7 years, and the mean age of the group without PEN was 10.0 ± 3.1 years (p=0.001). Primary enuresis nocturna was 29.8% (n=59) in boys, 6.1% (n=37) in girls, and was significantly higher in boys than girls (p=0.007). There was no difference between groups with and without PEN in terms of BMI percentile and PEN presence in the family (p=0.19, p=0.88; respectively) (Table 2).

Table 2. *Sociodemographic Characteristics of With and Without Primary Enuresis Nocturna (PEN) Cases*

		With PEN	Without PEN	p=	OR
Age (years) *		8.4 (2.7)	10.1 (3.3)	0.001 ***	
Sex **	***Boys***	59 (20)	233 (80)	0.007 ****	1.86
	Girls	37 (12)	271 (88)		
BMI pers (%) *		36.0 (38.6)	38.9 (19.7)	0.19 *****	
Have Allergic Rhinitis *	***Yes***	59 (20)	241 (80)	0.02 ****	1.74
	No	37 (12)	263 (88)		
PEN in Family *	***Yes***	7 (15)	39 (85)	0.88 ****	
	No	89 (16)	465 (84)		

** Expressed as mean (standard deviation); ** Expressed as n (%) ; *** Student t test; **** Chi square test; ***** Mann Whitney-U test; OR: Odds Ratio*

3.3. Allergic Rhinitis Outcomes

In the AR group, the mean age with PEN was 8.3 ± 2.6 years, and the mean age of without enuresis nocturna was 9.9 ± 3.5 years (p=0.001). Primary enuresis nocturna incidence in the AR group was significantly higher in boys (13% vs. 6%) (p=0.01). There was no significant difference in PEN according to the allergic rhinitis severity score (p=0.19), but PEN was higher in the persistent AR group according to the AR distribution score, although it was not statistically significant (OR=0.58; p=0.08). The most common additional atopic diseases were 9% (n=27) asthma, 3.6% (n=11) atopic dermatitis and 2.3% (n=7) food allergy. Body mass index percentile, presence of PEN in the family, age of AR onset, additional atopic disease, AR distribution, and AR severity were not significantly different between the groups with and without enuresis (p=0.31, p=0.46, p=0.19, p=0.43, p=0.08, p=0.19; respectively) (Table 3).

Table 3. *Characteristics of Cases With and Without Primary Enuresis Nocturna (PEN) in Allergic Rhinitis (AR) Cases*

		With PEN (n=59)	Without PEN (n=241)	p=	OR
Age *		8.3 (2.6)	9.9 (3,5)	0.001 ***	
Sex **	*Boys*	39 (25)	114 (75)	0.01 ****	2.17
	Girls	20 (14)	127 (86)		
BMI Per (%) *		35.7 (18.9)	38.4 (18.6)	0.31 ***	
PEN in Family *	*Yes*	7	21	0.46 ****	
AR Diagnosis Age **	*< 5 years*	36 (23)	124 (77)	0.19 ****	
	> 5 years	23 (16)	117 (84)		
Additional Atopic Disease **	*Yes*	7 (14)	42 (86)	0.43 ****	
AR Distribution **	*Intermittant*	37 (17)	179 (83)	0.08 ****	0.58
	Persistant	22 (26)	62 (74)		
AR Severity **	*Mild*	48 (21)	181 (79)	0.19 ****	
	Moderate	7 (12)	51 (88)		
	Severe	4 (30)	9 (70)		

** Expressed as mean (standard deviation); ** Expressed as n (%) ; *** Student t test; **** Chi square test; OR: Odds Ratio*

4. DISCUSSION

Allergic Rhinitis is the most common chronic respiratory tract disease seen in childhood and for children, is reported to be the most common chronic disease in developed countries (8,11). Allergic rhinitis is classified as the seasonal/intermittent type in which the symptoms develop in the pollen season and last no more than four days a week or less than four weeks in total, and the persistent/perennial type in which the symptoms occur year-round and last more than four days a week or more than four weeks (4). Moreover, based on the severity of the symptoms, it is classified as the mild type in which both sleeping and daily school and working activities are regular and the severe type in which both sleeping and daily school and working activities are disturbed (4,12). Our study cases generally were of the mild, intermittent type having no allergic rhinitis-related sleeping disorders with regular school and work activities.

Primary enuresis nocturna is one of the most common urinary system disorders in childhood (13). The incidence rate of PEN is 3.8-18% in the literatüre (14). This broad difference is thought to be stemming from the differences between the criteria such as cultural, racial, environmental features, variable case definition, and age group adopted in studies (15). The incidence rate of PEN among 600 cases in our study was 16% conforming with the literature.

It is reported that nocturnal enuresis is more common among boys than girls (16), which is thought to be due to the fact that PEN is related to developmental maturity, and girls develop more rapidly than boys do (17). However, there is no gender-based difference at all (18). As a result of our research, PEN was found more frequently in boys both in all cases and in cases with AR.

The pathophysiology of nocturnal enuresis is not clear yet; however, some publications indicate that causes of sleep disorder also increase PEN (19,20). It is believed that disorders causing upper respiratory tract obstructions are often related to enuresis in the mechanism of which the brain natriuretic peptide secreted from cardiac atrial walls that are dilated due to the increased negative intrathoracic pressure in case of upper respiratory tract obstructions increases the sodium and water excretion and inhibits the renin-angiotensin-aldosterone system and vasopressin release (21,22). In another mechanism associated with nocturnal enuresis, sleeping respiratory disorder reduces the responses to the awakening stimuli, and recurrent short-term sleep and awakening caused by upper respiratory tract obstruction lead to an increase in the response threshold given to such stimuli as a full bladder and/or the contraction of the detrusor muscle (23). In this study, the incidence rate of PEN was significantly higher in the cases who had allergic rhinitis.

There are publications in the literature suggesting a relationship between allergic diseases and NE (24-26). Primary enuresis nocturna is associative for those with known bronchial asthma, allergic sensitization confirmed through skin tests, and a family history of PEN (27). In the pathogenesis of PEN associated with allergic reactions, some allergens cause a decrease in the functional bladder capacity, smooth muscle contractions, and bladder inflammation, and thus detrusor instability, which leads to enuresis (28). According to another theory, allergic reactions triggered by allergens may lead to smooth muscle contractions in the bladder, and the bladder hypersensitivity or allergic bladder inflammation may cause the functional bladder capacity to decrease (28). In our study, we believe that allergic reactions increase PEN incidence through various mechanisms in the

urinary system because the incidence rate of PEN differs significantly between the control group and the group with allergic rhinitis.

The limitations of our study are that we could not reach the sufficient number of cases in both the allergic rhinitis group and the non-allergic rhinitis group. Moreover; In order to understand the relationship between allergic rhinitis and primary enuresis nocturna, whether cytokines and mediators that play a role in the pathophysiology of allergic rhinitis affect the bladder muscles; or not being able to understand whether it causes enuresis nocturna by stimulating diuresis hormonally are our limitations.

5. CONCLUSION

The frequency of PEN was significantly higher in children with allergic rhinitis. PEN and allergic rhinitis impair the quality of life. Allergic rhinitis further impairs the quality of life by increasing the frequency of PEN. Thus, allergic rhinitis is a disease that should be treated in order to increase the quality of life and reduce comorbidities.

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Author Contributions:

Research idea: AY, OY,HY
Design of the study: AY,OY
Acquisition of data for the study: AY,OY,HY
Analysis of data for the study: AY
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Clinical and Experimental Health Sciences

Turkish Adaptation of Nursing Students Competence Instrument

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ABSTRACT

Objective: The study was conducted to examine the validity and reliability of the Nursing Students Competence Instrument, which was developed in order to evaluate the competences of nursing students, for the Turkish society.

Methods: This methodological study was conducted with the participation of 224 third and fourth-year nursing students studying at a state university in Turkey. Identifying Information Form and Nursing Students Competence Instrument were used for data collection. In order to determine the validity of the scale, linguistic content validity analysis and confirmatory factor analysis were performed. In order to identify the reliability of the scale, test-retest and Cronbach's alpha analysis were used.

Results: In line with the validity and reliability analyses of the scale, it was determined that the model was compatible according to fit indices of confirmatory factor analysis (X^2/sd= 2.42, RMSEA= 0.08, SRMR= 0.07). The Cronbach's alpha coefficient of the scale was determined to be 0.96, and the Cronbach's alpha coefficients of the subscales varied between 0.92 and 0.94.

Conclusion: It was determined that the Turkish adaptation of the "Nursing Students Competence Instrument" was a valid and reliable scale for measuring the competence levels of nursing students. It is recommended to administer the scale to larger samples and to plan interorganizational comparative studies in order to identify nursing students' competences.

Keywords: Validity, reliability, nursing, nursing students, competence.

1. INTRODUCTION

As a result of developments in the fields of science, technology, and medicine, fast developments are also experienced in healthcare services. The use of various therapy methods, widespread use of technological equipment in healthcare, and the increase in patients' awareness and expectations have brought the need for nurses who are experts in their field and have scientific knowledge, skills, and competences (1-3). As Masters (4) stated in her book titled "Nursing Theories", the competences expected from nurses are helping roles, teaching-guiding functions, function of diagnosing and following up the patient, effective management of fast developing situations, managing and monitoring applications and regimens related to the therapy, ensuring and monitoring the quality of healthcare practices, and the role of organizing and working. Nursing education should aim at getting the students to gain these competences and transfer them to their practices (5).

Regarding the studies conducted, in their study, Theisen and Sandau (3) demonstrated that newly graduated nurses felt themselves incompetent in the fields of communication, leadership, organization and management skills, critical thinking, and stress, while Karahan et al (6) determined in their study that nursing students felt themselves incompetent in terms of basic nursing practices. In the study conducted by Berkow et al (7), it was determined that only 10% of manager nurses believed that newly graduated nurses could provide safe and effective nursing care.

Nurses who graduate without attaining the competence at the targeted level during their education experience stress in healthcare, and this situation decreases quality of care and patient satisfaction (8-10). Nursing students need to gain appropriate knowledge and skills in order to adequately prepare for their nursing roles (11). Therefore, it is important to integrate active learning strategies (12-15) into nursing curricula in order to educate nurses in this direction. Also, it

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is necessary to develop valid and reliable assessment tools which will evaluate competences of nursing students.

In our country, there is no assessment tool that will evaluate nursing students' pre-graduation and post-graduation competences in nursing education, which consists of clinical and theoretical dimensions. Accordingly, the present study aimed to adapt the "Nursing Students Competence Instrument" to Turkish language.

2. METHODS

2.1. Study Desing and Aim

The study is methodological research conducted in order to test the validity and reliability of the Nursing Students Competence Instrument for Turkish (NSCI) nursing students.

2.2. Settings and Samples

The population of the study consisted of third – and fourth-year undergraduate students studying at the nursing department of a state university in the fall semester of the 2018-2019 (September-December) academic year (n=432). In studies on scales, it is recommended to include threefold, fourfold or more participants for each scale item (16). Accordingly, the study sample was determined to be over ten times the number of items in the 27-item scale. No sampling method was used in the study. All students, except those who were absent on the days when the application was performed and those who refused to participate in the study, were included in the study (n=224). The first application of the Nursing Students Competence Instrument was performed with 224 students who volunteered to participate in the study, and the second application performed for test-retest three weeks later was completed with the participation of 70 (31%) students.

2.3. Measurement

In the study, Identifying Information Form created by the researchers following the literature review and the Nursing Students Competence Instrument were used as data collection tools.

Identifying Information Form

The form prepared by the researchers in line with the literature consists of 10 questions inquiring about sociodemographic characteristics of the nursing students (2,6).

Nursing Students Competence Instrument

The scale was developed by Lin et al (17). The scale consists of four subscales and 27 items that measure the nursing students' competences. Students are asked to score themselves between 1 and 10 on the scale in terms of competences. The scale's Cronbach's alpha coefficient is 0.96. The minimum and maximum scores to be obtained from the scale are 27 and 270, respectively. A high score obtained from the scale indicates a high level of competence. The subscales of the scale are integrating care abilities (items 1-10), leading humanity concerns (items 11-16), advancing career talents (items 17-23), and dealing with tension (items 24-27).

2.4. Linguistic Equivalence

In analyzing linguistic validity of the scale, back-translation method was used. Firstly, the scale was translated to Turkish by the experts who were competent both in Turkish and English. By comparing the translated forms, the most suitable translation for each item was determined, and the scale items were gathered in one form. Then, the scale was translated back to its original language, i.e. English, by three different experts. After the form translated to English was compared with the original scale form, appropriate English expressions were adopted. The authors who developed the scale were contacted and their opinions were obtained. As a result of the comparisons, the Turkish version of the scale was finalized.

2.5. Cultural Adaptation and Content Validity

After the linguistic equivalence study of the scale, the scale was presented to the opinions of 10 faculty members who had different specialties in nursing (principles of nursing, public health nursing, teaching in nursing) in order for them to evaluate it in terms of content validity. Suitability of the scale items were scored by the experts (1=Not suitable, 2=Suitable to some extent (item needs to be revised), 3=Quite suitable (suitable, but small changes are needed), 4=Very suitable). Content validity was calculated as 0.94 by using Davis technique, and this value shows that the scale is appropriate in terms of content validity (18,19).

2.6. Face Validity

By making necessary arrangements following the expert opinions, a pilot study was performed with the participation of 24 students with similar characteristics. A pilot study was conducted to determine the readability and comprehensibility of scale items, identify areas that respondents did not understand, and determine average response times. The scale items were perceived as understandable by the respondents. Average response time was 15 minutes. The data obtained from these students were not included in the study.

2.7. Confirmatory Factor Analysis

In order to evaluate the adequacy of the sample size, Kaiser-Meyer-Olkin (KMO) and Bartlett test were performed. As a result of these tests, KMO value was found to be 0.95, X^2=5920.31 p< 0.01. In addition, confirmatory factor analysis (CFA) was performed in order to evaluate construct validity. For CFA, the multiple fit indices of Chi-Square Goodness,

Goodness of Fit Index (GFI), Adjusted Goodness of Fit Index (AGFI), Comparative Fit Index (CFI), Normed Fit Index (NFI), Standardized Root Mean Square Residuals (SRMR), and Root Mean Square Error of Approximation (RMSEA) were examined (16).

2.8. Reliability Analysis

For the reliability of the scale, test-retest, the Cronbach alpha (α) internal consistency coefficient, the Spearman-Brown and Guttman Split-Half reliability coefficient, Upper-Lower %27 mean scores and item total correlation were examined.

Test-retest reliability, was examined to determine scale consistency in measurements. The Cronbach alpha, Spearman-Brown and Gutmann Split-Half coefficient, Upper-Lower %27 mean scores are measure of the uniformity of the scale items. A high alpha factor of the scale indicates that the items are consistent. The item-total-score correlation describes the relationship between the score obtained from an item and the total score. A high positive correlation between the item and the total indicates that the items measure similar behaviors. A correlation value of at least 0.20 is recommended (16).

2.9. Statistical Analysis

In the analysis of the data, IBM SPSS V23 software was used. The descriptive values of the data were presented as number, percentage, mean, and standard deviation. In the validity and reliability of the Nursing Students Competence Instrument, Cronbach's alpha coefficient, Spearman Brown and Guttman coefficient, Upper-Lower %27 mean scores, item-total score correlations, test-retest and confirmatory factor fit indices were examined. Within the scope of reliability analysis, and internal consistency were examined. In the test-retest analysis, the students were asked to write their school numbers on data collection tools, and the second application was performed three weeks later. In the evaluation of the data, significance level was accepted as $p< 0.05$.

2.10. Ethical Considerations

Permission for the Turkish validity and reliability study of the Nursing Students Competence scale was obtained through e-mail from Lin et al, who developed the scale. In addition, ethics committee approval was taken from the ethical board of the university where study was conducted (Approval number: 2018/157, Date: 30.07.2018). Official permission was also taken from the institution where the study was conducted. The voluntary participants were informed about the purpose and process of the study. Verbal and written consent of the participants were taken as well.

3. RESULTS

When examining the results of the study, 79.5% of the participating students were female, 47.3% were third year students, and their mean age was 21.57± 0.99. The percentage of the students who willingly chose nursing department was 72.3, and academic score average of the students were found to be 2.97± 0.28 (Table 1.).

Table 1. *Descriptive characteristics of the students (n=224)*

Descriptive Characteristics	n	%
Gender		
Female	178	79.5
Male	46	20.5
Class		
3.class	106	47.3
4.class	118	52.7
Choosing nursing department		
Willingly	162	72.3
Not willingly	62	27.7
Age	x̄±SD= 21.57±0.99	
Academic score average	x̄±SD= 2.97±0.28	

x̄: Mean, SD: Standard Deviation

3.1. Confirmatory Factor Analysis Results

When the model fit indices were examined, X^2/sd (2.42), RMSEA (0.08), SRMR (0.07), NFI (0.87), CFI (0.92), GFI (0.79) and AGFI (0.74) values were found (Table 2).

Table 2. *Confirmatory factor fit indices for the Nursing Students Competence Instrument*

Fit Measure	Acceptable Value	Value	Evaluation
X^2/sd	<3= Perfect fit	2.42	Perfect fit
RMSEA	<0.08= Good fit	0.08	Good fit
SRMR	<0.08= Good fit	0.07	Good fit
NFI	>0.90=Good fit	0.87	Moderate fit
CFI	>0.90=Good fit	0.92	Good fit
GFI	>0.90=Good fit	0.79	Weak fit
AGFI	>0.90=Good fit	0.74	Weak fit

X^2/sd: Chi-square/standard deviation, RMSEA: Root Mean Square Error of Approximation, SRMR: Standardized Root Mean Square Residuals, NFI: Normed Fit Index, CFI: Comparative Fit Index, GFI: Goodness of Fit Index, AGFI: Adjusted Goodness of Fit Index

The standardized solution for the path diagram of the conceptual model is presented in Figure 1 (Figure 1).

Item analysis of the Nursing Students Competence Instrument is presented in Table 3. In this context, it was determined that the scale item score average of the nursing students was high. The total correlation values of the items ranged between 0.60 and 0.90 and the mean scores of the items were between 6.27±2.31 and 8.37±1.59 (Table 3).

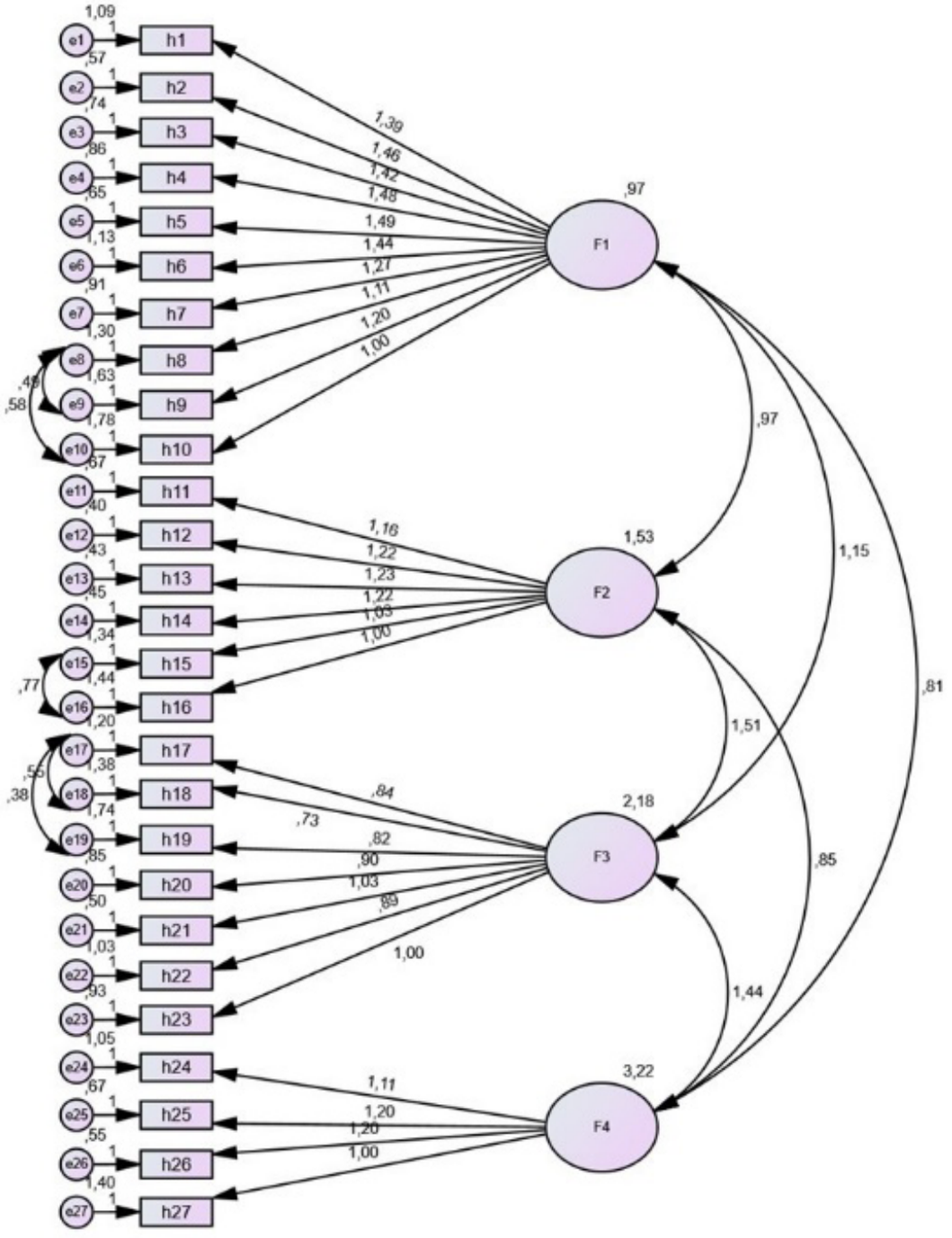

Figure 1. *Path diagram of the confirmatory factor analysis*

3.2. Reliability Analysis Results

Cronbach's alpha coefficient of the Nursing Students Competence Instrument was found as 0.96. When Cronbach's alpha coefficients of the subscales were examined, it was seen that they varied between 0.92-0.94. These values suggest that the Nursing Students Competence Instrument and its subscales were reliable. Test-retest coefficient was found greater than 0.76, so the internal consistency of the scale is very good. Also values the mean of the upper 27% and lower 27% by t-test was found to be positive. (Table 3).

Spearman and Guttmann values were calculated as confidence coefficients. The results close to 1 was obtained (Table 4). The correlations between the NSCI total score and subscale scores are presented in Table 5. A positive and significant correlation was found between NSCI total score and its subscales ($p< 0.01$). In addition, it was determined that there was a positive and significant relationship between the subscales of integrating care abilities, leading humanity concerns, advancing career talents, and dealing with tension (Table 5).

Table 3. *Nursing Students Competence Instrument item analysis and comparisons of Upper-Lower %27 mean scores (n=224)*

Scale items	x̄±SD	Item-Total Correlation	Cronbach's Alpha (α) when the item is removed	X̄		t
				Upper %27	Lower %27	
1.In patient care, I can use the required basic biomedical science and general clinical skills together.	6.47±1.72	0.74	0.28	6.51	6.43	56.06
2.I can holistically evaluate and analyze health problems of the patient and the underlying factors.	6.74±1.62	0.83	0.28	6.78	6.70	62.04
3.I can use my diagnosis skills while providing care to the patient in the clinic.	6.96±1.64	0.80	0.28	7.00	6.93	63.33
4.I can analyze a suspicious case and evaluate all probabilities related to the situation.	6.56±1.73	0.80	0.28	6.60	6.52	56.74
5.I can use integrative nursing care in patient care.	7.00±1.68	0.83	0.28	7.03	6.96	62.33
6.While examining a clinical case, I can apply conceptual mapping.	6.34±1.77	0.75	0.28	6.38	6.30	53.41
7.I can be aware of the roles and functions of the nurse in patient care.	7.66±1.57	0.79	0.28	7.70	7.62	72.79
8.I can exchange ideas with others on the health status of the patient.	8.09±1.61	0.71	0.28	8.13	8.05	75.17
9.I can analyze clinical cases in detail and show personal interest for the improvement of the patient's health status.	7.51±1.74	0.69	0.28	7.55	7.47	64.39
10.I can distinguish between the roles of the nurses and other healthcare professionals.	8.14±1.66	0.60	0.28	8.18	8.10	73.24
11.I can show general care behaviors to my peers.	7.69±1.66	0.80	0.28	7.73	7.65	69.28
12.I can display professional care behaviors to my patients.	7.83±1.64	0.87	0.28	7.87	7.80	71.39
13.I can show my general care behaviors to my peers.	7.76±1.65	0.87	0.28	7.80	7.72	70.11
14.I can evaluate my general care behaviors towards my patients.	7.76±1.65	0.87	0.28	7.80	7.72	70.11
15.I value professional care behaviors.	8.32±1.72	0.76	0.28	8.41	8.33	72.61
16.I can display responsibility towards professional roles and ensure nursing ethics.	8.15±1.72	0.74	0.28	8.19	8.11	70.66
17.I can research and use information independently.	7.81±1.67	0.77	0.28	7.85	7.77	69.78
18.I value all types of learning activities.	8.37±1.59	0.69	0.28	8.41	8.34	78.66
19. I trust myself in terms of offering new ideas for others' hypotheses.	7.53±1.79	0.68	0.28	7.57	7.48	62.82
20.I can develop effective communication channels in groups.	7.53±1.62	0.76	0.28	7.56	7.49	69.54
21.I can use communication skills in collaboration and fulfill my care duty.	7.67±1.68	0.83	0.28	7.71	7.63	68.32
22.I can resort to group dynamics in solving learning problems.	7.55±1.66	0.74	0.28	7.59	7.51	68.10
23.I can do my job professionally and effectively.	7.99±1.76	0.77	0.28	8.03	7.95	67.60
24.I have the necessary methods to cope with stress.	6.45±2.23	0.86	0.28	6.50	6.40	43.15
25.I can manage my emotions when under stress.	6.27±2.31	0.89	0.28	6.32	6.21	40.63
26.I can analyze stressors and look for solutions.	6.46±2.28	0.90	0.28	6.51	6.41	42.35
27.I can notice all types of stressors in the learning process.	6.53±2.15	0.81	0.28	6.58	6.48	45.38

x̄: Mean, SD: Standard Deviation, t: One sample t test

Table 4. *Findings for Confidence Analyzes (n=224)*

Scale and subscales	Number of items	Cronbach's Alpha	Test-retest	Spearman Brown Coefficient	Guttman Coefficient
Nursing Students Competence Instrument	27	0.96	0.80	0.87	0.87
Integrating care abilities	10	0.94	0.78	0.87	0.87
Leading humanity concerns	6	0.94	0.79	0.90	0.90
Advancing career talents	7	0.92	0.81	0.89	0.88
Dealing with tension	4	0.94	0.76	0.95	0.95

Table 5. *Correlations between the Nursing Students Competence Instrument total score and subscale scores (n=224)*

	NSCI	Integrating care abilities	Leading humanity concerns	Advancing career talents	Dealing with tension
	r; p	r; p	r; p	r; p	r; p
NSCI	1				
Integrating care abilities	0.922; <0.001	1			
Leading humanity concerns	0.883; <0.001	0.801; <0.001	1		
Advancing career talents	0.915; <0.001	0.781; <0.001	0.798; <0.001	1	
Dealing with tension	0.659; <0.001	0.448; <0.001	0.379; <0.001	0.525; <0.001	1

NSCI: Nursing Students Competence Instrument, r: Correlation coefficient, p: Significance level (<0.05)

4. DISCUSSION

Nursing education planned in two dimensions as theoretical and practical aims to get the students to gain competences in various fields in line with the changing student profile, learning needs, and care expectations (20). However, there is no valid and reliable measurement tool for evaluating the students' professional competences on the national scale, and thus it is not possible to systematically examine the competences of students, especially those in the final years of their undergraduate studies. In the present study, it was determined that the Turkish adaptation of the Nursing Students Competence Instrument met the validity and reliability criteria at an acceptable level. In line with the recommended international scale adaptation procedures, linguistic and content validity of the scale was ensured in the first place (21,22).

In calculating content validity index, Davis technique was employed, and the value was found as 0.94. This value indicates that the scale has a comprehensible linguistic structure and content (18).

4.1. Confirmatory Factor Analysis

In evaluating construct validity, which is one of the criteria for testing validity, factor analysis was used. Prior to the factor analysis, the sufficiency of the sample size was evaluated with KMO and Bartlett tests, as a result of which sample size was found to be sufficient. In the evaluation of model data fit, Chi-square value was divided by freedom degree, and x^2/sd value was determined as 2.42. This value shows that model data fit was good (16). Similarly, in the scale development study conducted by Lin et al (17), KMO sampling sufficiency was found as 0.92, Bartlett's test as $x^2_{(351)} = 4576.85$ and statistically significant with $p< 0.01$. It is stated that it is more appropriate to evaluate other fit indices together (23). CFA conducted in this direction showed that the scale was acceptable with RMSEA (good fit), SRMR (good fit), CFI (good fit), NFI (moderate fit) and it was confirmed that the scale consisted of 27 items and 4 subscales as the original scale (Table 2). Also, it was determined that GFI and AGFI were weak fit indices. These values are thought to be affected by the sample size. Therefore, the chi-square value, which is an alternative to these values, was taken as a reference in the evaluation of the model fit. Moreover, the factor loads between the subscales of the scale and the scale items were determined to be between 0.40-3.22 and above 0.30, and no item was removed from the scale (16).

4.2. Reliability Analysis

Reliability, which is defined as the consistency between different measurements of the same variable, was determined through Cronbach's alpha coefficient. The proximity of this coefficient to 1 represents high internal consistency of the items (21). In the study, the Cronbach's alpha coefficient was 0.96. When the Cronbach's alpha coefficients of the subscales were examined, it was determined that it varied between 0.92-0.94. Similarly, in the scale development study they conducted, Lin et al (17) reported that the Cronbach's alpha coefficient of the total scale score was 0.96, and that the subscale coefficients ranged between 0.87 and 0.94. Also values the mean of the upper 27% and lower 27% by t-test was found to be positive. If the mean values for the upper 27% and lower 27% by t-test are positive, it means that the item measures the characteristic that the test measures.

Another analysis used in order to test reliability is test-retest analysis. In the literature, it is stated that in test-retest method, applying retest to 25-50% of the individuals who participated in the first measurement would be enough for calculating reliability (24). In the present study, three weeks after the data collection, 31% of the study sample were contacted and test-retest was applied. While the scale total test-retest value was calculated as 0.80, it was determined that test-retest values of the subscales varied between 0.76-0.81. As the test-retest values of the scale and its subscales were found to be in the range of 0.75-1.00, it can be stated that the scale's consistency in temporal process was very good (25). Also the Spearman and Guttman scores were found close to 1.00, indicating high internal consistency.

5. CONCLUSION

As a result of the study, it was determined that NSCI Turkish version is a valid and reliable measurement tool for the evaluation nursing students' competences. It is recommended that studies should be conducted on larger samples by using this scale. Also, as different methods and techniques are used in educational institutions, studies that compare interinstitutional competences of nursing students should be planned. Using NSCI in these studies to be planned will offer an opportunity to reveal nursing education program outcomes through an objective and reliable measurement tool.

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Acquisition of data for the study: MÇ, AA
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Clinical and Experimental Health Sciences

Evaluation of Laboratory Results with Data from Bio-Speedy Respiratory Panel 2 in Nasopharyngeal Swab Specimens of COVID-19-Suspected Patients Having PCR(-) Results

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ABSTRACT

Objective: The distinction between COVID-19 and other respiratory infections can be difficult during the flu and winter seasons. The aim of this study is to detect bacterial/viral microorganisms in nasopharyngeal swab samples and to evaluate routine laboratory results of patients with PCR (-) but suspected covid 19.

Methods: Between 1 July 2021 and 31 December 2021, 78 patients who were hospitalized and followed up in the suspected Covid service were included in the study. The patients were divided into two groups as those with and without growth on the respiratory panel. Laboratory, demographic and radiological data were compared between groups.

Results: C-reactive protein (CRP) and ferritin levels were found to be statistically significantly higher in the group with growth on the respiratory panel compared to the group without growth (p= .05, p= .041, respectively). Reproduction was detected in nasopharyngeal swab samples taken in 56.4% of the patients. More than half of the patients were radiologically defined as CO-RADS 3.

Conclusion: It should not be forgotten that other respiratory viral and bacterial infections that mimic the COVID-19 clinic are also commonly observed during this period.

Keywords: SARS CoV-2, nasopharyngeal/throat swab, PCR, flu season

1 .INTRODUCTION

The cause of a serious respiratory disease epidemic that occurred in Wuhan City of China in December 2019 is the new type of coronavirus named as "novel coronavirus-2019" by the World Health Organization (WHO) (1,2). The coronaviruses that can cause disease in humans are as follows: SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2), MERS-CoV, SARS-CoV HCoV-NL63, HCoV-OC43, HCoV-HKU1, and HCoV 229E (3,4). Intense symptoms associated with SARS-CoV-2 infection are shortness of breath, cough, fatigue, fever and muscle aches. (5-7). Similar symptoms can be seen in infections associated with different viruses such as influenza A, influenza B, parainfluenza A, parainfluenza B, and RSV. The distinction between COVID-19 and other respiratory viral infections can be difficult during the flu and winter seasons (8). The clinical course of most COVID-19 patients is mild. However, it may have a more severe course, especially in elderly people and those with comorbidities such as coronary artery disease (CAD), chronic obstructive pulmonary disease (COPD), hypertension (HT), and diabetes mellitus (DM) (2,9).

Early identification of the SARS CoV-2 virus is very important to prevent the progression of clinical pathologies caused by this virus. Polymerase chain reaction (PCR) is a laboratory method with high sensitivity and specificity used for rapid detection of some viral and bacterial microorganisms (10,11). In viruses other than SARS CoV-2, clinical pictures such as upper respiratory tract infection, pneumonia, and unilateral or bilateral ground-glass and patchy consolidated lesion-like radiological appearance can be observed. Therefore, many 'suspicious' cases have been reported with computed tomography (CT) images of the thorax according to the CO-RADS classification, showing the clinical features of COVID-19 with RT-PCR (-) (12).

Our aim in this study is to detect bacterial/viral microorganisms in nasopharyngeal swab samples and to evaluate routine laboratory results of patients with PCR (-) but suspected covid 19, especially during the flu season.

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2. METHODS

Ethical approval was obtained from the Faculty of Medicine of Harran University (Approval number: 22/01/07, Approval date: 10.01.2022) and all participants gave their written informed consents.

Between 1 July 2021 and 31 December 2021, 100 patients who were hospitalized and followed up in the suspected Covid service were analyzed retrospectively. All patients over 18 years of age, who had a COVID 19 PCR test (-), whose Chest Computer Tomography (CT) report was interpreted as CO-RADS 3, CO-RADS 4 or CO-RADS 5, and patients whose respiratory panel was studied with nasopharyngeal swap were included in the study. Patients under 18 years of age, whose Chest CT report was interpreted as CO-RADS 1 and CO-RADS 2, and patients whose respiratory panel was not studied with nasopharyngeal swap were excluded from the study. According to these criteria, 10 patients who did not have nasopharyngeal swabs, 8 patients whose registered laboratory data could not be accessed, and 4 patients who did not have Chest CT images and reports were excluded from the study (Figure 1). Information such as patients' age, sex, comorbidity status as well as hemogram, biochemistry and coagulation results were obtained from all patients on the first day of hospitalization. Chest CT reports and nasopharyngeal swab results were obtained from the recorded data.

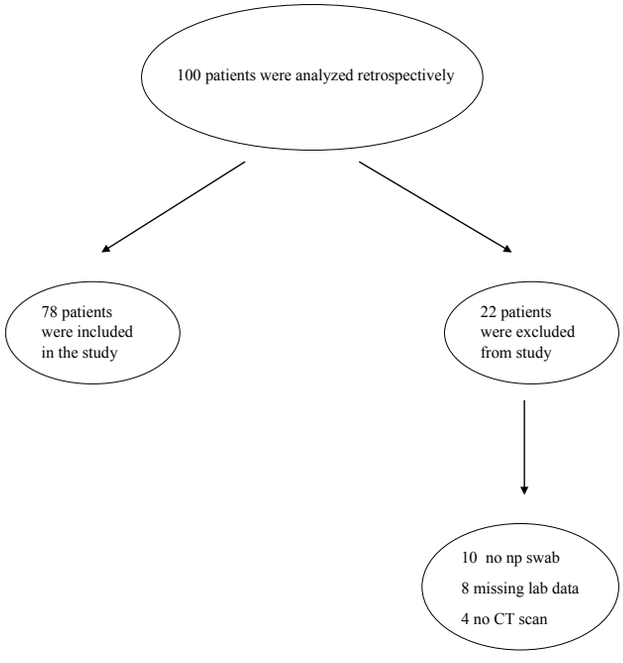

Figure 1. *Distribution of patients*

Chest CT images of the patients were reported by experienced radiologists according to the CO-RADS classification. CO-RADS is a classification developed by the Dutch Society of Radiology to determine the level of suspicion for COVID-19 pneumonia and is consistent with the consensus statement recommended by the RNSA (13,14). The classification was defined in 5 levels: Normal: 1 **negative**; not atypical for COVID-19 signs of infection: 2 Low (***atypical***); consistent with COVID-19 and other infections: 3 Equivocal/unsure (***indeterminate-lower likelihood***); Suspicious for COVID-19: 4 High (***indeterminate –high likelihoo***d); Typical for COVID-19: 5 Very high (***typical***).

Nasopharyngeal swab samples were taken from all patients by experienced healthcare personnel under sterile conditions. Bio-speedy Respiratory RT-qPCR MX-24S Panel is used for evaluation of nasopharyngeal swap samples (Table 1). It is used for rapid and accurate diagnosis of 24 respiratory tract pathogens (as viral and bacterial agents) from clinical samples with multiplex polymerase chain reaction. The kit is applied to nucleic acid isolates obtained from nasopharyngeal swab, bronchoalveolar lavage, oropharyngeal swab and nasopharyngeal aspirate samples. Rapid diagnosis with the kit is performed by one-step reverse transcription (RT) and real-time PCR (qPCR / RT-qPCR) targeting genomic RNA and DNA regions specific to the target agent. LoD values were determined as copies/mL. Transport is provided with a vNat Transfer tube (BS-NA513s-100 or Bio-Speedy vNat Transfer Tube BS-NA-513-100). Swab samples were collected using dacron or polyester swabs. The samples were transported to the laboratory and stored at 2-8°C. The RT-qPCR Application Protocol has been programmed by the manufacturer and is run on the appropriate Bio-Rad CFX96 device. Program t includes the steps of reverse transcription, Pre-incubation and Growth (denaturation, binding-elongation and fluorescence reading). Test results are interpreted by microbiologists according to the shape of the amplification curves.

The patients were divided into two groups as those with and without growth on the respiratory panel. Laboratory, demographic and radiological data were compared between groups. In addition, microorganisms grown in the respiratory panel were compared based on the radiological classification. Nasopharyngeal swab respiratory panel results of all patients included in the study were expressed as numerical and percentage values.

2.1. Statistical Analysis

SPSS (version 22.0) was used for all analyses. The Kolmogorov-Smirnov test was used to determine whether the data were normally distributed. Continuous measurements are presented as mean ± standard deviation (SD) if normally distributed, median (25-75 quartile range) if not, and categorical variables are presented as numbers (%). Student's t test was used to compare normally distributed data, and Mann-Whitney U test was used to compare not normally distributed data. Categorical data were expressed in numbers (percentage) and compared using the Chi-square test. A p value of < 0.05 was considered statistically significant.

Table 1. *Bio-speedy respiratory rt-qpcr mx-24s panel targets*

Sars-Cov-2-Nucleocapsid gene	Legionella pneumophila	Human Coronavirus 229E	Parainfluenza 1
Influenza-B	Mycoplasma pneumoniae	Human Coronavirus NL63	Parainfluenza 2
Influenza-A	Chlamydophila pneumonia	HumanCoronavirus HKU1	Parainfluenza 3
Human Bocavirus	Haemophilus influenzae	Human Coronavirus OC43	Parainfluenza 4
Human Parechovirus	Bordetella pertussis	Respiratory syncytial virus A	H.Metapneumovirus
H.Enterovirus/Human Rhinovirus Set 2	Streptococcus pneumoniae	Respiratory syncytial virus B	H.Enterovirus/Human Rhinovirus Set 1adenovirus

Table 2. *Comparison of demographic and comorbidity data in the between groups*

	Respiratory panel reproduction (-) (n=34)	**Respiratory panel reproduction** (+) (n=44)	p
Age, years	68.0 (54.7-78.0)	69.5 (52.0-78.0)	0.876
Gender, f/m	18/16	22/22	0.797
DM, %	7 (20.5)	12 (27.2)	0.495
HT, %	11 (32.3)	10 (22.7)	0.342
CAD, %	15 (44.1)	13 (29.5)	0.183
SVD, %	5 (14.7)	6 (13.6)	0.893
CKF, %	3 (8.8)	6 (13.6)	0.530
COPD, %	5 (14.7)	9 (20.4)	0.546
Malignancy, %	1 (2.9)	1 (2.3)	0.853

DM, diabetes mellitus; HT, hypertension; CAD, coronary arterial diseases; SVD, cerebrovascular diseases; CKF, chronic kidney failure; COPD, chronic obstructive pulmonary disease.

Table 3. *Laboratory and radiological data of patients*

	Respiratory panel reproduction (-) (n=34)	**Respiratory panel reproduction (+)** (n=44)	p
Glucose, mg/dL	143.5 (120.5-239.0)	138.5 (110.0-178.7)	0.182
Urea, mg/dl	48.6 (33.0-76.8)	47.5 (30.5-70.5)	0.668
Creatine, mg/ dL	0.9 (0.7-1.4)	0.9 (0.6-1.1)	0.203
Albumin, g/ dL	3.5 (2.7-4.0)	3.2 (2.6-3.9)	0.212
AST, U/L	20.0 (10.0-35.6)	13.5 (8.9-26.7)	0.173
ALT, U/L	25.5 (17.5-35.5)	22.0 (13.1-35.7)	0.316
T.bilirubin, mg/ dL	0.5 (0.3-0.8)	0.4 (0.2-0.6)	0.185
Sodium, mg/ dL	137.7 ± 4.7	139.0 ± 7.4	0.372
Potassium, mg/ dL	4.5 ± 0.8	4.3 ± 0.6	0.395
Calcium, mg/ dL	8.3 ± 0.7	8.2 ± 0.6	0.666
LDH, U/L	223.0 (184.2-339.5)	194.0 (167.7-426.7)	0.692
CRP, mg/dl	103.0 (34.7-220.7)	64.5 (116.7-426.7)	**0.05**
WBC, x10^3/mL	10.8 (8.0-14.3)	9.9 (6.0-14.8)	0.261
Neutrophil, x10^3/mL	8.7 (6.3-11.4)	8.5 (4.2-11.8)	0.288
Lymphocite, x10^3/mL	(0.6-1.4)	1.0 (0.6-1.9)	0.653
Platelet, x10^3/mL	228.5 (190.0-289.2)	228.0 (165.2-287.7)	0.860
MPV, fL	10.5 ± 0.9	10.2 ± 1.1	0.201
MCV, fL	87.4 ± 8.3	87.8 ± 11.5	0.862
RDW, %	15.2 ± 2.7	15.5 ± 2.1	0.586
Procalcitonin, ng/ml	0.2 (0.06-0.8)	0.1 (0.01-0.5)	0.612
Ferritin, mg/L	323.0 (92.0-496.0)	452.0 (180.5-857.0)	**0.041**
D-dimer, ng/ mL	0.8 (0.5-2.2)	1.9 (0.6-4.1)	0.109
CORADS, % *Uncertain (corads 3)* *High suspect (corads 4)* *Very high suspicious (corads 5)*	 16 (47) 9 (26.5) 9 (26.5)	 27 (61.3) 11 (25) 6 (13.6)	0.306

ALT*, alanine aminotransferase;* ***AST****, aspartate aminotransferase;* ***LDH****, lactat dehydrogenase;* ***CRP****, C-reactive protein;* ***WBC****, white blood cell;* ***MCV****, mean corpuscular volume;* ***MPV****, mean platelet volume;* ***RDW****, red cell distribution width;* ***CO-RADS****, COVID-19 Reporting and Data System.*

Table 4. *Respiratory panel results of nasopharyngeal swab samples*

RESPIRATORY PANEL	PATIENTS (n=78)
Reproductive *Non-reproductive*	44 (56.4) 34 (43.6)
SARS CoV 2, %	3 (3.8)
Influenza A, %	8 (10.3)
Coronavirus HKU1, %	1 (1.3)
Coronavirus OC43, %	3 (3.8)
Parainfluenza, %	8 (10.3)
Haemophilus influenzae, %	15 (19.2)
Streptococcus pneumonia, %	16 (20.5)
Respiratory syncytial virus, %	4 (5.1)
Pseudomonas spp, %	1 (1.3)
Rhinovirus, %	2 (2.6)

3. RESULTS

A total of 78 patients consisting of 40 women and 38 men were included in the study. Demographic and comorbidity data between the groups were compared in Table 2. There was no significant difference between the two groups in terms of age and sex. The most common comorbidities were coronary arterial disease (CAD), hypertension (HT) and diabetes mellitus (DM), respectively.

Table 3 shows the laboratory and radiological data of two groups. C-reactive protein (CRP) and ferritin levels were found to be statistically significantly higher in the group with growth on the respiratory panel compared to the group without growth (p= .05, p= .041, respectively). More than half of the patients were radiologically defined as CO-RADS 3.

SARS CoV-2 was detected in the second nasopharyngeal swab sample in three patients (3.8%). Reproduction was detected in nasopharyngeal swab samples taken in 56.4% of the patients. In the remaining patients, the most frequently detected microorganisms were *streptococcus pneumonia* (20.5%), *hemophilus influenza* (19.2%), *parainfluenza* (10.3%) and *influenza A* (10.3%), respectively (Table 4).

Nasopharyngeal swab results are compared according to the radiological classification of the patients. There was growth in nasopharyngeal swab samples at a rate of 62.7% in the CO-RADS 3 (n=43) group, 55% in the CO-RADS 4 (n=20) group, and 40% in the CO-RADS 5 (n=15) group. SARS CoV-2 growth occurred in only one patient in each of the three groups. Among the microorganisms that grow in all groups, the most frequently detected *Streptococcus pneumonia*, *Hemophilus influenzae*, *İnfluenza A*, respectively.

4. DISCUSSION

In this study, different viral and bacterial microorganisms were determined in repeated PCR examinations conducted via nasopharyngeal swabs from COVID-19 infection-suspected patients with PCR (-) results during the flu season.

The clinical picture of the COVID-19 infection, which has caused a pandemic all over the world for about two years, can imitate those of different viral and bacterial infections, especially during the seasonal flu period. Usually during the winter months, the variation patterns peak in most of the viruses, causing epidemics (15). The best-known respiratory viruses are *parainfluenza 1, parainfluenza 2, parainfluenza 3, adenovirus, rhinovirus, influenza A, influenza B, respiratory syncytial virus, coronavirus, human metapneumovirus,* and *human bocavirus* (16,17). Furthermore, viral infections can damage the respiratory epithelium, facilitating the development of bacterial infections. Some laboratory parameters play a role in the clinical diagnosis and follow-up of many viral and bacterial infections, including SARS CoV-2 (2,18,19). CRP and ferritin are the most important acute phase reactants. Ferritin and CRP levels are increased in acute or chronic inflammatory diseases characterized by tissue damage and repair (20). In many studies, it has been shown that there is a significant increase in serum ferritin and CRP levels in bacterial and viral infections (21-23). CRP and ferritin levels were found to be significantly higher in our patients with growth on the respiratory panel. Also, many studies have shown that patients with viral and bacterial pneumonia have common comorbidities, similar to patient with COVID-19. The most common comorbidities are HT, DM, Congestive heart failure (CHF) and COPD (24-26). Similarly, in our study, the most common comorbidities in patients with growth on the respiratory panel were CAD, HT, and DM.

Nasopharyngeal swab RT-PCR result is used as the gold standard method in the diagnosis of COVID-19 disease. Although the sensitivity of the RT-PCR test is 89% and the specificity is 100%, the test result may be (-) due to different reasons (delays before arrival at the laboratory or poor storage conditions, lack of standardization for sample collection, use of insufficiently validated assays, insufficient viral samples and load, presence of mutations that escape PCR inhibitors etc.) (27). In one study, it was emphasized that up to 54% of COVID-19 patients had a false-negative RT-PCR result at baseline, and therefore, RT-PCR tests should be repeated in these patients with suspected SARS-CoV-2 infection (28). In our study, SARS CoV-2 was detected only in 3.8% (n=3) of the patients in the PCR analysis of the nasopharyngeal swab samples taken for the second time. We can say that the reason for this low value in our study is the infections caused by other respiratory viral and bacterial factors related to the winter and flu seasons.

The most common symptoms in SARS-CoV-2 pneumonia are shortness of breath, cough, fatigue, fever and muscle aches (5-7). While the most common symptoms associated with bacterial pneumonias are cough, sputum, and chest pain; In viral pneumonias, fever, myalgia, cough and shortness of breath are more prominent (29). Both bacterial and viral microorganisms were grown in the nasopharyngeal swap swab samples of the patients in our study. The most frequently observed ones were *Streptococcus pneumonia*, *Hemophilus influenzae*, *Influenza A* and *Parainfluenza 3*, respectively.

Similar to literature data, the main symptoms seen in study patients were fever, cough, dyspnea, and myalgia.

Chest CT is a routinely used test for the diagnosis of pneumonia. Therefore, it has been stated that it can also be used for the diagnosis of COVID-19 (30). The most common CT abnormalities observed in COVID-19 patients are ground-glass opacities (GGO), consolidation, and interlobular septal thickening (31,32). While some studies stated that COVID-19 patients had high and/or very high probability radiological findings in Chest CT scans, other studies emphasized that they might have similar radiological findings with different viral pneumonias, and therefore Chest CT scans were considered to have low specificity (33). Therefore, in this period when we are in the process of pandemic, nasopharyngeal/throat swab examination should be performed in patients with Chest CT that is radiologically interpreted as CO-RADS ≥ 3 during the flu season and winter months (34). In our study, different viral and bacterial microorganisms other than SARS CoV-2 were detected in the nasopharyngeal/throat swab sample of more than half of the patients. Chest CT of these patients commonly had GGO and consolidation appearances.

In this study conducted during the pandemic, we showed that lower respiratory tract infection caused by different viral and bacterial agents is similar to COVID-19 infection in terms of both radiological, clinical and laboratory features.

There are some limitations of our study. In this single-center and retrospective study, the absence of cases under the age of 18, the insufficiency of long-term follow-up information of the patients and the lack of recorded information about the time between the onset of symptoms and the application of the respiratory panel test are the limitations of our study.

5. CONCLUSION

Especially during the flu season, the nasopharyngeal swab respiratory panel test is an important diagnostic test recommended for COVID-19-suspected patients with a PCR (-) result. It should not be forgotten that other respiratory tract viral and bacterial infections are also commonly observed during this period.

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Author Contributions:
Research idea: IH, LK
Design of the study: IH
Acquisition of data for the study: IH, FG
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Clinical and Experimental Health Sciences

Evaluation of the Incidental Prevalence of Soft Tissue Calcifications in the Neck Region with Cone Beam Computed Tomography

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ABSTRACT

Objective: The aim of this study was to retrospectively evaluate the incidental prevalence of heterotopic soft tissue calcifications in the neck region, on Cone Beam Computed Tomography (CBCT) images, and their relationship with age and sex.

Methods: A total of 6620 CBCT images were examined. CBCT images of 503 patients aged between 20 and 86 years were included in the study. Patients were grouped into five age groups: 20-30 (N = 132), 31-40 (N = 68), 41-50 (N = 92), 51-60 (N = 104), and 61 and above (N = 107). The images were assessed according to the presence of lymph node calcification, carotid artery calcification, thyroid cartilage, and triticeous cartilage calcification in the neck region. Descriptive statistics, crosstabs, and chi-square tests were used for data analysis. The significance level was set to 0.05

Results: At least one calcification was detected in 372 (73.9%) patients. The difference between the prevalence of each calcification according to age groups was statistically significant. A significant relationship was found between the presence of carotid artery calcification and the sex variable ($p < .05$). The presence of other calcifications did not show statistically significant differences associated with sex ($p > .05$).

Conclusion: The results of this study showed a significant amount of soft tissue calcification in the neck region. The most common calcifications were thyroid cartilage calcifications, and the least common calcification was lymph node calcification.

Keywords: Soft tissue, calcification, neck, cone beam computed tomography

1. INTRODUCTION

Calcium salts are usually stored in the skeleton of the body. These salts are called heterotopic calcification if they are deposited in soft tissue without any organization (1). Calcifications don't show any important symptoms. These are usually detected incidentally during radiographic examination (2). The structures with the most common calcification tendency in the head and neck region are laryngeal cartilages (thyroid, cricoid, and arytenoid cartilages), vertebrae, arteries (carotid artery), and thyroid gland (3). These soft tissue calcifications are generally considered physiological, but sometimes serious diseases may play a role in the etiology of the calcification or calcification may be a manifestation of the disease. Therefore, medical consultation and examination may be required for differential diagnosis. (4). Lymph node calcifications are an indication of specially active or previous tuberculosis, infectious and non-infectious agents such as sarcoidosis, rheumatoid arthritis, fungal infections, sclerosis, neoplastic metastases, cat scratch disease, Bacille Calmette-Guérin vaccine, and chronic inflammation after radiotherapy in lymphomas (2). Carotid artery calcifications are the most important cause of ischemic stroke (5). Calcified thyroid cartilage may affect neurovascular structures, for example, compress the recurrent laryngeal nerve, and bilateral nerve injury may cause difficulty in breathing and loss of ability to speak (aphonia) (6).

Cone Beam Computed Tomography (CBCT) is the preferred three-dimensional imaging method for oral and maxillofacial radiology in recent years (7). Dentists and dentomaxillofacial radiologists usually evaluate only the area of request on the CBCT image. However, various incidental findings such as soft tissue calcifications are ignored by dentists and dentomaxillofacial radiologists because soft tissue resolution of CBCT is not sufficient (4). Whereas even soft tissue calcifications with a volume of 1 mm^3 can be visualized by CBCT (5). Therefore, research on soft tissue calcifications has increased in recent years on CBCT images (4,5,8-14). In the

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literature, studies on this subject have generally evaluated these calcifications alone or a few together. In this study, unlike other studies, all soft tissue calcifications in the neck region were examined.

The aim of this study was to retrospectively evaluate the prevalence of lymph node, thyroid cartilage, triticeous cartilage, and carotid artery calcifications, and their relationship with age and sex.

2. METHODS

2.1. Ethical Considerations

Ethics approval was obtained from the Gazi University Clinical Research Ethics Committee before starting the study (Research No: 2018-192 Ankara, Turkey). This study was conducted in accordance with the principles defined in the Helsinki Declaration, including all revisions.

2.2. Study Design

In this study, 6620 CBCT images in the archive of Gazi University Faculty of Dentistry, Department of Dentomaxillofacial Radiology were examined. The images obtained from the maxillofacial region, with a Field of View (FOV) 16.0 cm × 9.2 cm, or only the mandible region (FOV: 16.0 cm × 5.2 cm) including the neck region were selected. In total, the CBCT images of 503 patients have been included in the study. CBCT images of the patients who had a history of maxillofacial trauma or operation, images with various artifacts (motion artifact, metal artifact, noise artifact, etc.) affecting the image quality, calcifications smaller than 1 mm^3, and undifferentiated calcifications were excluded from the study. CBCT images were obtained by Planmeca ProMax 3D Mid (Planmeca, Helsinki, Finland), the device using parameters of 90 kVp, 10 mA, total scanning time of 13,519 seconds, and 400 µm voxel size. The images were assessed as the presence of lymph node calcification (Fig. 1, Fig. 2), carotid artery calcification (Fig. 3), thyroid cartilage (Fig. 4), and triticeous cartilage calcification (Fig. 5) (1,15). The prevalence of these calcifications was evaluated. In addition, it was examined whether the relevant calcifications were seen on the right or left, unilateral or bilateral, and the relationship between these calcifications and age, and sex were evaluated. All radiographic evaluations were performed by a specialist dentomaxillofacial radiologist (N.G.I) with five years of experience in Gazi University Faculty of Dentistry, Department of Dentomaxillofacial Radiology. Images were examined in sagittal, coronal, and axial sections. All evaluations were performed on a 24-inch Philips medical monitor with NVIDIA Quadro FX 380 graphics card and 1920×1080 pixel resolution by using the Romexis 4.6.2.R. program (Planmeca Oy, Helsinki, Finland) and in a quiet room with subdued ambient lighting, approximately 50 cm away from the screen.

2.3. Statistical Analysis

The data were categorized and statistically analyzed by using SPSS program version 21.0 (SPSS Inc., Chicago, USA) with descriptive statistics, crosstabs, and chi-square tests. The significance level was set to 0.05.

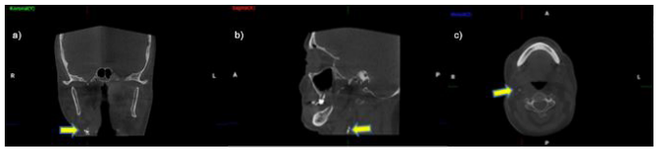

Figure 1. *Cervical lymph node calcification in coronal (a), sagittal (b), and axial sections (c) of CBCT images*

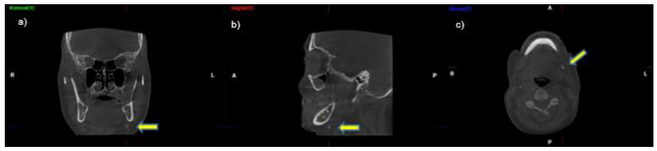

Figure 2*. Submandibular lymph node calcification in coronal (a), sagittal (b), and axial sections (c) of CBCT images*

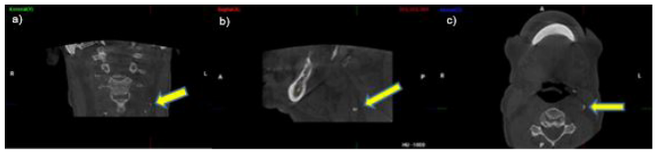

Figure 3. *Carotid artery calcification in coronal (a), sagittal (b), and axial sections (c) of CBCT images*

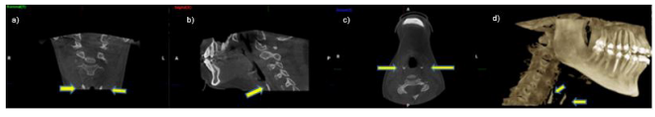

Figure 4*. Thyroid cartilage calcification in coronal (a), sagittal (b), axial sections (c), and 3D reconstruction (d) of CBCT images*

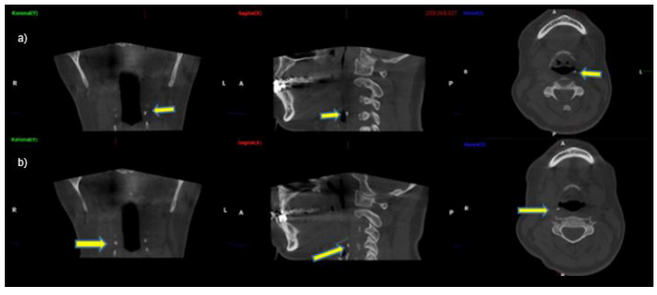

Figure 5*. Triticeous cartilage calcification in coronal, sagittal, and axial sections a) left, b) right of CBCT images*

3. RESULTS

CBCT images of a total of 503 patients aged between 20 and 86 years (mean age ± standard deviation: 45.51±16.05),

consisting of 287 females (57.1%) and 216 males (42.9%) were examined in the study. At least one calcification (lymph node, carotid artery, thyroid, and/or triticeous cartilage) was detected in 372 (73.9%) patients with a mean age of 48.39±14.87.

Patients were grouped as five age groups: 20-30 years (N = 132), 31-40 years (N = 68), 41-50 years (N = 92), 51-60 years (N = 104), and 61 and above years (N = 107) for data analysis. The difference between the prevalence of each calcification according to age groups was statistically significant. The prevalence of lymph node calcification and carotid artery calcification increased in old age. For example, lymph node calcification was more common in patients older than 61 years (17.8%, N = 19) than in patients younger than 40 years (1.5%, N = 1). Thyroid cartilage calcification was less common in patients between the ages of 20-30 years compared to other age groups, for instance, thyroid cartilage calcification was less common in patients aged 20-30 years (39.4%, N = 52) than in patients over 40 years (69.6%, N = 64). Detailed information about the distribution of calcifications according to age groups is given in Table 1. The proportion of patients with lymph node calcification was 6.8% (N = 34), 15.1% (N = 76) with carotid artery calcification, 62.6% (N = 315) with thyroid cartilage calcification, and 21.3% (N = 107) with triticeous cartilage calcification. A statistically significant relationship was found between the presence of carotid artery calcification and the sex (p< .05), and this calcification was more common in males (21.3%, N=46) than in females (10.5%, N=30). There was no statistically significant relationship between the presence of other calcifications (lymph node calcification, thyroid cartilage calcification, and triticeous cartilage calcification) and sex (p> .05) (Table 2). Regarding the affected side (left or right); the most common calcifications were found in right thyroid cartilages (77.7%, N=289) followed by left thyroid cartilages (76.3%, N=284). Left (1.9%, N=7) and right (1.6%, N=6) submandibular lymph nodes had the less common calcifications (Table 3).

Regarding the symmetry (unilateral or bilateral) of the calcifications, 194 (36.3%) were unilateral, and 341 (63.7%) were bilateral. For the calcifications of the thyroid cartilage, submandibular, and cervical lymph nodes, statistically significant differences (p< .05) were found between each of their symmetry prevalence (Table 4). When the prevalence and relationship of each calcification with other calcifications were compared, lymph node calcification-carotid artery calcification, thyroid cartilage calcification-carotid artery calcification, triticeous cartilage calcification-carotid artery calcification, and triticeous calcification – thyroid cartilage calcification were found to be statistically significant (p< .05) (Table 5).

Table 1. *The crosstabs and relationships between age groups and calcifications*

Type of the calcification	Presence of the calcification		20-30	31-40	41-50	51-60	61 ≤	Total	Chi-square	P value
Lymph node calcification	Presence	N	4	1	3	7	19	34	28.251	0.00*
		%	3.0	1.5	3.3	6.7	17.8	6.8		
	Absence	N	128	67	89	97	88	469		
		%	97.0	98.5	96.7	93.3	82.2	93.2		
Carotid artery calcification	Presence	N	7	3	13	13	40	76	57.972	0.00*
		%	5.3	4.4	14.1	12.5	37.4	15.1		
	Absence	N	125	65	79	91	67	427		
		%	94.7	95.6	85.9	87.5	62.6	84.9		
Thyroid cartilage calcification	Presence	N	52	49	64	78	72	315	42.713	0.00*
		%	39.4	72.1	69.6	75.0	67.3	62.6		
	Absence	N	80	19	28	26	35	188		
		%	60.6	27.9	30.4	25.0	32.7	37.4		
Triticeous cartilage calcification	Presence	N	9	23	24	24	27	107	25.342	0.00*
		%	6.8	33.8	26.1	23.1	25.2	21.3		
	Absence	N	123	45	68	80	80	396		
		%	93.2	66.2	73.9	76.9	74.8	78.7		

**p< 0.05 statistically significant*

Table 2. *The crosstabs and relationships between gender and calcifications*

Type of the calcification	Presence of the calcification		Female	Male	Total	Chi-square	P Value
Lymph node calcification	Presence	N	16	18	34	1.488	0.223
		%	5.6	8.3	6.8		
	Absence	N	271	198	469		
		%	94.4	91.7	93.2		
Carotid artery calcification	Presence	N	30	46	76	11.298	0.001*
		%	10.5	21.3	15.1		
	Absence	N	257	170	427		
		%	89.5	78.7	84.9		
Thyroid cartilage calcification	Presence	N	184	131	315	0.632	0.427
		%	64.1	60.6	62.6		
	Absence	N	103	85	188		
		%	35.9	39.4	37.4		
Triticeous cartilage calcification	Presence	N	61	46	107	0.000	0.991
		%	21.3	21.3	21.3		
	Absence	N	226	170	396		
		%	78.7	78.7	78.7		

**p< 0.05 statistically significant*

Table 3. *Frequency of calcification according to localization*

Variables	Absence		Presence	
	n	%	n	%
Submandibular lymph node right	366	98.4	6	1.6
Submandibular lymph node left	365	98.1	7	1.9
Servikal lymph node right	358	96.2	14	3.8
Servikal lymph node left	360	96.8	12	3.2
Carotid artery right	315	84.7	57	15.3
Carotid artery left	319	85.8	53	14.2
Thyroid cartilage right	83	22.3	289	77.7
Thyroid cartilage left	88	23.7	284	76.3
Triticeous cartilage right	296	79.6	76	20.4
Triticeous cartilage left	294	79.0	78	21.0

Table 4. *Comparisons between unilateral prevalence and bilateral prevalence of calcifications*

Variables	Unilateral		Bilateral			
	n	%	n	%	χ^2	P
Submandibular lymph node calcification	11	91.7	1	8.3	8.33**	0.004
Cervical lymph node calcification	24	96.0	1	4.0	21.16**	0.000
Carotid artery calcification	42	55.3	34	44.7	0.84	0.359
Thyroid cartilage calcification	57	18.1	258	81.9	128.26**	0.000
Triticeous cartilage calcification	60	56.1	47	43.9	1.58	0.209

** *p< 0.01*

Table 5. *The crosstabs and relationships between each calcification and the others*

			Carotid artery calcification				
			Absence	Presence	Total	χ^2	p
Lymph node calcification	Absence	n	408	61	469	23.92**	0.000
		%	81.1	12.1	93.2		
	Presence	n	19	15	34		
		%	3.8	3.0	6.8		
	Total	n	427	76	503		
		%	84.9	15.1	100.0		
Thyroid cartilage calcification	Absence	n	149	39	188	7.43**	0.006
		%	29.6	7.8	37.4		
	Presence	n	278	37	315		
		%	55.3	7.4	62.6		
	Total	n	427	76	503		
		%	84.9	15.1	100.0		
Triticeous cartilage calcification	Absence	n	328	68	396	6.17*	0.013
		%	65.2	13.5	78.7		
	Presence	n	99	8	107		
		%	19.7	1.6	21.3		
	Total	n	427	76	503		
		%	84.9	15.1	100.0		
			Lymph node calcification				
Thyroid cartilage calcification	Absence	n	174	14	188	0.23	0.635
		%	34.6	2.8	37.4		
	Presence	n	295	20	315		
		%	58.6	4.0	62.6		
	Total	n	469	34	503		
		%	93.2	6.8	100.0		
Triticeous cartilage calcification	Absence	n	368	28	396	0.29	0.593
		%	73.2	5.6	78.7		
	Presence	n	101	6	107		
		%	20.1	1.2	21.3		
	Total	n	469	34	503		
		%	93.2	6.8	100.0		
			Thyroid cartilage calcification				
Triticeous cartilage calcification	Absence	n	175	221	396	36.95**	0.000
		%	34.8	43.9	78.7		
	Presence	n	13	94	107		
		%	2.6	18.7	21.3		
	Total	n	188	315	503		
		%	37.4	62.6	100.0		

** p<0.05; ** p<0.01*

4. DISCUSSION

Soft tissue calcifications are usually detected incidentally during radiographic examination by dentists and dentomaxillofacial radiologists (2). These soft tissue calcifications are generally considered physiological and may have little clinical significance (4). Although these soft tissue calcifications are generally considered physiological and have little clinical significance, some cases may be life-threatening (8,16). The radiographic appearance of this condition may help to make the correct diagnosis of the patient. Previous studies have reported that the prevalence of soft tissue calcifications for the head and neck region ranges from 12.92% to 62.6%. (4,8,10-14). Due to the relatively high prevalence of the condition, the prevalence of soft tissue calcification in the head and neck region on CBCT images should be determined, thereby raising the awareness of dentists about early diagnosis. (17). In addition, the CBCT-receiving dentist or dentomaxillofacial radiologist is responsible for identifying every finding in the field of imaging because accurate documentation is indispensable in a medical profession. The American Academy of Oral and Maxillofacial Radiology (AAOMR) and the European Academy of Dentomaxillofacial Radiology (EADMFR) have identified the need for the careful examination of all CBCT images within the FOV (4).

The results of this study showed that 73.9% of patients had at least one soft tissue calcification in the neck region. This result indicates that calcifications are quite common. To the best of our knowledge, there are a limited number of studies in the literature about soft tissue calcifications including the neck region evaluated with CBCT (4,8,10-14). However, these studies evaluate both head and neck regions and show prevalence differences ranging from 12.92% to 62.6% in general. This may be due to different age groups, populations, assessment methods (such as evaluation of different incidental findings, including soft tissue calcifications), evaluation of different soft tissue calcifications in the evaluated anatomical regions, and differences in sample size.

Lymph node calcifications in the neck are rare (18). The prevalence of lymph node calcification was found to be 6.8% in the present study. The mean age of the patients with lymph node calcification was 57 years, and the most common age was 61 years. There are few studies on the prevalence of lymph node calcification (8,18-20). Missias et al. (8) reported only one lymph node calcification on 626 CBCT images. Eisenkraft and Som found a 1% prevalence of calcified lymph nodes on 2300 Computed Tomography (CT) images (19). It has been reported that the prevalence of lymph node calcification in the neck region was 1-7% in tuberculosis patients (20). Barghan et al. (18) found that the prevalence of lymph node calcification in CBCT was 0.77%, and the mean age of their study group was 64 years.

Three-dimensional CBCT technology allows us to see the exact location of these calcifications that may be indicative of subclinical atherosclerosis (11). In the literature, several imaging methods were used for the detection of carotid artery calcifications. In most of the studies, panoramic radiographs were used to determine the prevalence of carotid artery calcification, and it has been reported as in the range of 0.43%-29% (21-23). The prevalence of carotid artery calcification was found as 22.9% in CT (16). In CBCT studies, the prevalence of carotid artery calcification was found to be 1.5-10.41% (3,7,10,13,17,22,23). In the present study, the prevalence of carotid artery calcification was 15.1%. This result was slightly higher than in previous reports (4,8,11,13,14,18,24,25). The reason for this situation may be due to the difference in the study sample, age range, and section thicknesses used in CBCT in the previous studies. Its unilateral prevalence was 55.3%, and its bilateral was 44.7% in this study. Damaskos et al. (12) indicated that the unilateral prevalence of carotid artery calcification was higher (56.95%) than bilateral ones (43.05%) in the CBCT study. In another study, using CT images, bilateral carotid artery calcification was more common than unilateral (24). The prevalence of unilateral carotid artery calcification was higher than bilateral in previous studies (12,25) compatible with the present study. Previous studies have emphasized that it was most common at the age of 60 years (4,10,12,25). In the present study, carotid artery calcification was seen at most 61 years of age in accordance with previously published articles (4,10,12,25). On the other hand, Özdede et al. (4) found that carotid artery calcification was seen in 11.6% of females and 4.4% of males, with a statistically significant difference. In the present study, the presence of carotid artery calcification was higher in males (21.3%) than females (10.5%) in accordance with Togan et al. (25). Differences between these carotid artery calcification studies and this study may be due to different factors such as the number of populations in the study, the difference in imaging method used (CT or CBCT or panoramic), section thicknesses, and evaluation criteria.

The thyroid cartilage is the largest of laryngeal cartilages. In humans, thyroid cartilage calcification usually occurs after the end of adolescence period (6). There are few studies on the prevalence of thyroid cartilage calcification on CBCT (8,11,18,26). In those studies, the prevalence of thyroid / triticeous cartilage calcifications was found to be 3.9% – 59.3% (8,11,18,26). Nandita Shenoy et al. (6) evaluated the calcification frequency of thyroid and cricoid cartilage on lateral cephalometric radiographs and reported it as 28%. In another lateral cephalometric radiographic study, the prevalence of thyroid cartilage calcification was 26.2%, more common in males (36%) than in females (19%), and was most common in individuals over 40 years of age (27). Calcification for thyroid cartilage starts in the early 20s and then spreads to cartilage with aging. This process is markedly different between individuals over time. In addition, this process has gender differences in the action of hormones (28). In the present study, the prevalence of thyroid cartilage calcification was 62.6%. The relationship between age groups and the presence of thyroid cartilage calcification was statistically significant, and the most common age group was 51-60 years old. It was more common in females (64.1%) than in males (60.6%). These prevalences were relatively higher than in previous studies. This may be due to the imaging method (CBCT or lateral cephalograms) and the number of samples and the difference in the male-female distribution in the samples.

There are few studies in the literature investigating triticeous cartilage calcification in different populations with different methods (8,29,30). Alqahtani et al. (29) and Hatley et al. (30) also reported triticeous cartilage calcification on computed tomography images of the neck. In these studies (29,30), the prevalence of triticeous cartilage calcification was 12% – 65% on CT. Alqahtani et al. (29) also reported that 37% of triticeous cartilage calcifications were unilateral and 63% bilateral. In studies using panoramic radiography images, the prevalence of triticeous cartilage calcification was reported as 8.6%-10.6% (31,32). In this study, the prevalence of triticeous cartilage calcification was 21.3%, the unilateral prevalence of triticeous cartilage calcification was 56.1% and the bilateral prevalence was 43.6%. Contrary to the previous study (29), the reason why the prevalence of unilateral triteceous cartilage was higher than bilateral in this study may be due to the different imaging devices used, the evaluation of different populations, and the evaluation of different age ranges. The prevalence of triticeous cartilage is stated not to be related to age in the literature (8,29). Unlike the ones published in this study, the relationship between age groups and the presence of triticeous cartilage calcification was found to

be statistically significant; triticeous cartilage calcification was found about 5 times more in patients over 30 years of age compared to patients between 20-30 years of age. There was no statistically significant relationship between triticeous cartilage calcification and sex. In the panoramic radiographic study performed by Aoun and Nasseh (32), the prevalence of triticeous cartilage calcification was 58.5% in females and 42.5% in males. In the study of Missias et al. (8), the prevalence was 6.6% in females and 6.8% in males. These previous studies reported no statistically significant relationship between triticeous cartilage calcification and sex, so our study also supported this finding.

This study had some limitations. Our study was a retrospective radiographic study, thus the possible systemic diseases and complaints of the patients were not known. In addition, due to the absence of a soft tissue window in CBCT, calcifications were difficult to localize, and these calcifications were not confirmed.

5. CONCLUSION

In the study, the prevalence of soft tissue calcifications in the neck region on CBCT images was investigated. The most common calcifications were thyroid cartilage calcifications, and the least common calcifications were lymph node calcifications. The results of this study showed a significant amount of soft tissue calcification in the neck region. Dentomaxillofacial radiologists or dentists should examine the CBCT image as a whole, and keep in mind that there may be soft tissue calcifications in the neck region, and patients should be medically consulted or followed up when necessary.

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Design of the study: NGİ, İP, MT
Acquisition of data for the study: NGİ
Analysis of data for the study: NGİ, İP, MT
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Clinical and Experimental Health Sciences

Effect of Nurses' Autonomy Levels and Problem-Solving Skills on Job Satisfaction

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ABSTRACT

Objective: This study aimed to determine the effect of nurses' autonomy levels and problem-solving skills on job satisfaction and was conducted in public, private and university hospital between July and September 2021.

Methods: This descriptive and relational – screening study was conducted with 278 nurses. Data were collected through the Socio-demographic Form, the Problem-Solving Inventory, the Sociotrophy-Autonomy Scale, and the Minnesota Job Satisfaction Questionnaire (MJSQ). Data were analyzed using SPSS 26 and AMOS 24 programs using numbers, percentages, minimum/maximum values, means, and standard deviation values.

Results: Modern mediation analysis findings performed using the Bootstrap method showed that the indirect effect of the autonomy score on the overall satisfaction score with the mediation of the problem-solving skills was significant ($p < .05$).

Conclusion: Problem-solving skills were found to have a partial mediating role in the effect of nurses' autonomy levels on their job satisfaction.

Keywords: Autonomy, problem-solving skills, nursing, job satisfaction, structural equation

1. INTRODUCTION

Autonomy is defined as an individual's ability to protect and increase his/her rights and independence (1). While the developments in the health services with the developing technology have enabled many innovations as well as multidisciplinary work, they have also caused some changes in workers' attitudes and expectations. These changes have brought the need for considering workers' not only abilities but also values, preferences, and expectations in the work environments with a hierarchical structure (2). Developing the autonomy of individuals is possible only by respecting it. Autonomy is an intangible and complicated concept indicating individuals' conscious and independent decision-making capacity to reach desired outcomes (3,4). Beside individual autonomy, professional autonomy is defined as the ability to control the scope, management, and pacing of the things to be done to initiate and conclude a movement or a series of events (5). Autonomy is the freedom of an individual's making voluntary and binding decisions and taking action on these decisions (6). Nurses' actions such as saving the patient's life in emergency cases, coordinating care, enhancing patient safety, and preventing medical errors are also indicators of professional nurse autonomy. In all the stages of health services, nurses' fulfilling their independent roles without limitations is highly important. In addition, autonomy, taking appropriate decisions for the solutions to the problems encountered during nursing practices, and problem-solving skills are considered to be among the top factors affecting the quality of care (7). Right decisions taken in these kinds of cases, by revealing nurses' knowledge and skills, could enhance experiencing a sense of accomplishment (8). Nurses' job satisfaction is also considered to be affected by the feelings of accomplishment. Job satisfaction is defined using the qualities such as workers' love and positive and negative attitudes about their job, meeting their needs in the workplace, and securing justice in the workplace (9). Factors enhancing job satisfaction and professional development were found to be positively associated with autonomy (10). Enhancing nursing skills with full autonomy is among the factors that affect job satisfaction the most (11-14). While lack of nurses' job descriptions despite the heavy workload has negative effects on their job satisfaction (15), job satisfaction levels were reported to be higher in nurses working in institutions that provide nurses with opportunities to improve their knowledge and skills and include clear role definitions (16). However, the literature in our country was found to include no studies that investigated the effect of

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nurses' autonomy levels and problem-solving skills on their job satisfaction. Therefore, this study aims to determine the effect of nurses' autonomy levels and problem-solving skills on job satisfaction.

Research Questions

- Nurses' autonomy levels have positive effects on problem-solving skills.
- Nurses' autonomy levels have positive effects on their job satisfaction.
- Problem-solving skills have a mediating role in the effect of autonomy level on job satisfaction.

2. METHODS

2.1. Study Design

This study utilized a descriptive and relational screening design and was conducted in public, private and university hospital of a city center in the west of Turkey between July and September 2021.

2.2. Target Population and the Sample

The target population of the study was 476 nurses who worked in public, private and university hospital for at least one year. A purposeful sampling method was utilized for the sample, and the 300 data collection forms were administered to the nurses who agreed to participate in the study. Twenty-two forms that had missing or inaccurate data were excluded, so the study was completed with 278 nurses. Due to the pandemic process, it was tried to reach all the nurses in the universe.

2.3. Data Collection Tools

Data were collected through the Socio-demographic Form developed by the researchers to determine nurses' individual and professional characteristics, the Problem-Solving Inventory, the Sociotropy-Autonomy Scale, and the Minnesota Job Satisfaction Questionnaire (MJSQ).

2.3.1. The socio-demographic form

The form was composed of 8 questions regarding the participants' individual (age, the city where they live, marital status, education level, etc.) and some professional characteristics (the institution where they work, duration of working, the clinic where they work, etc.). "To what extent do you think the nurses are able to fulfill their professional roles in the clinics they work in?" The answers given to the question are expressed as levels. Participants were asked to mark from 1 to 5. 1=Very low, 5= Very high.

2.3.2. The Problem-Solving Skills Inventory (PSSI)

The Problem-Solving Inventory developed by Heppner and Petersen (1982) is a tool that is utilized to determine the phases of the problem-solving process and evaluate what individuals think about their own problem-solving behaviors and approaches (17). Turkish reliability and validity of the scale were performed by Taylan et al. (1990), and the form is composed of 35 items rated on a 6-point Likert scale. While 1 point indicates strong agreement, 6 points indicate strong disagreement (18). The inventory is composed of three sub-scales, which include Problem-Solving Confidence, Approach-Avoidance, and (Ability to Maintain) Personal Control. The original Cronbach alpha value of the scale is 0.88. This study Cronbach's alpha value was found to be 0.92.

2.3.3. The Sociotropy-Autonomy Scale

The scale was developed by Beck, Nepstein, Harrison, and Emery in 1983 and was adapted to Turkish by Şahin et al. in 1993 (19). The test-retest reliability coefficient of the original form ranged between α .65 and α .88 for the sociotropy feature, and between α .66 and α .75 for the autonomy feature. The reliability alpha coefficient of the Turkish version of the scale was found to be α .70 and α .81 for sociotropy and autonomy, respectively. The scale is responded on a 5-point Likert scale with responses including 0= not at all like me, 1= somewhat like me, 2=quite like me, 3= like me, and 4=very much like me. The scale is composed of two sub-scales called Sociotropy and Autonomy. The top score to be obtained from each sub-scale is 120. The sub-scales can also be utilized separately. This study utilized the autonomy sub-scale. Higher scores indicate higher autonomy levels. The autonomy sub-scale includes personal accomplishment, independence, and enjoying loneliness sub-scales (20-22). Cronbach's alpha value of the autonomy sub-scale was reported 0.81. This study found the Cronbach's alpha value as 0.93.

2.3.4. The Minnesota Job Satisfaction Questionnaire (MJSQ)

The Minnesota Job Satisfaction Questionnaire, developed by Weiss et al. (1967) to measure job satisfaction, was first translated to Turkish as 20 items by Baycan (1985). Each item includes five options defining one's level of satisfaction with his job: not satisfied at all (1), not satisfied (2), not sure (3), satisfied (4), and very much satisfied (5). Scores to be obtained from the scale range between 20 and 100, and the 60 points in the middle indicate neutral satisfaction. While scores close to 20 indicate a low satisfaction level, scores close to 100 indicate higher satisfaction. Cronbach's alpha coefficient of the scale was reported 0.90 (23,24). Cronbach's alfa coefficient was found 0.88 in this study.

2.4. Data Analysis

The analysis of the data was performed using SPSS 26 and AMOS 24 programs. Construct validity of the scales

utilized in the study was analyzed using the second-order Confirmatory Factor Analysis and the research hypotheses were tested using structural equation models (SEM). Since the kurtosis (Mardia's coefficient) value of the multivariable normality assumption in the CFAs performed in the AMOS program did not meet the critical ratio (<20), the Unweighted Least Squares (ULS) method was utilized as the estimation method. As for the SEM analyses, since the multivariable normality assumptions were met, the Maximum Likelihood (ML) method was used as the estimation method (25-27). With 5000-sample utilized in the analyses conducted, factor loads and effects between the variables were analyzed with a 95% confidence interval. The fitness and reliability of the model in the CFA and SEM analyses were analyzed using fit index values. The reliability of the scales was analyzed using Cronbach's alpha internal consistency coefficient. Descriptive findings were demonstrated using numbers, percentages, minimum/maximum values, means, and standard deviation values. Statistical significance was accepted p< .05.

2.5. Ethical Considerations

Before the study was conducted, ethics committee approval was obtained from the Non-invasive Clinical Research Ethics Committee of the Related University (Decision no: 2021/025 and Code: 2021019). Written permission was obtained from the hospital where the study was conducted. The nurses who were invited to participate in the study were informed about the purpose of the study and those who gave verbal consent were included.

3. RESULTS

The distribution of participating nurses' descriptive characteristics is presented in Table 1. The average age of the nurses was found 35.35±7.17. When the educational status of the nurses is examined, 7.2% of them are associate degree, 17.6% are undergraduate, 59.7% and 15.5% are graduate education graduates. Of all the nurses, 74.5% were married and 32.06% have children.

The scale items were included in the CFA models as the observed variables to determine the construct validity of the scales utilized in the study, and related implicit variables were formed. Items were eliminated from the analyses to make model fit index values in the analyses have the desired level, and analyses were reperformed after each item elimination.

Confirmatory Factor Analysis Findings of the Autonomy Scale

Model Fit Values; χ^2/df: 2.128 GFI: .976 AGFI: .971 NFI: .969 RMR: .080

Confirmatory Factor Analysis Findings of the Problem-Solving Inventory

Model Fit Values; χ^2/df: 2.018 GFI: .959 AGFI: .951 NFI: .943 RMR: .078

Confirmatory Factor Analysis Findings of the Minnesota Satisfaction Questionnaire

Model Fit Values; χ^2/df: 1.623 GFI: .976 AGFI: .967 NFI: .963 RMR: .065

These findings showed that the final models related to the scales fit the model fit indexes and were reliable, and the factor loads were statistically significant.

Table 1. *Distribution of the descriptive characteristics of the nurses (n=278)*

Descriptive Characteristics		n	%
Marital Status	Married	207	**74.5**
	Single	71	25.5
Place of living	City center	136	48.9
	District	138	**49.6**
	Town/Village	4	1.4
Education level	High School	20	7.2
	Associate degree	49	17.6
	Undergraduate degree	166	**59.7**
	Postgraduate degree	43	15.5
Working Institution	Public hospital	245	88.1
	Private hospital	7	2.5
	University hospital	26	9.4
Working Clinic	Intensive care	91	32.7
	Emergency Service	48	17.3
	Clinics or Polyclinics	95	34.2
	FHC, City Health Administrative	42	15.1
	Other	2	0.7
Level of nurses' fulfilling their professional roles in the clinics they were assigned	Very low	2	0.7
	Low	40	14.4
	Middle	123	44.2
	High	81	29.1
	Very high	32	11.5
	Mean ± SD		
Levels	3.36±0.89		
Age	35.35 ± 7.17		
Years of working	12.81 ±7.85		

SD: Standard Deviation

Table 2. *Statistical findings and reliability coefficients of the variables*

Variables	Minimum	Maximum	Mean.	SD	Cronbach Alfa
Autonomy	23.00	92.00	66.30	18.38	0.932
Problem – Solving Confidence	10.00	34.00	19.21	6.49	0.852
Approach – Avoidance Style	12.00	46.00	23.08	9.31	0.870
(Ability to Maintain) Personal Control	4.00	15.00	8.76	3.34	0.735
Problem Solving Skills total	26.00	95.00	51.06	17.43	0.921
Internal Satisfaction	16.00	40.00	30.41	4.89	0.815
External Satisfaction	7.00	30.00	21.87	4.81	0.862
Overall Satisfaction	28.00	70.00	52.28	8.69	0.886

SD: Standard Deviation

Statistical findings regarding the variables of the scales and the reliability coefficients are demonstrated in Table 2. These findings show that nurses' autonomy scale total mean score was 66.30±18.38, the Problem-Solving Inventory total mean score was 51.06±17.43, and the Minnesota Satisfaction Questionnaire total mean score was 52.28±8.69. Cronbach's alpha coefficients indicated that the scales and the sub-scales were reliable.

Table 3: *Mediation model analysis findings*

	Standardized Estimation(β)	
	Model 1	Model 2
Overall Satisfaction <— – Autonomy	**0.468***	**0.282***
Problem-solving Skills <— – Autonomy		**0.614***
Overall Satisfaction <— – Problem-solving Skills		**0.297***
	CFI: 1.000 GFI: 1.000 RMR: 0.000 SRMR: 0.000 (Full Model)	X^2/sd: 0.937 CFI: 1.000 GFI: 0.992 AGFI: 0.977 RMSEA: 0.000

**p< .05*

In the first model analyzed, the autonomy score was found to affect the overall satisfaction score (β= .468; p< .05) (Figure 1)

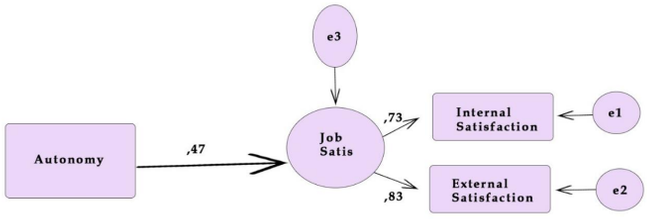

Figure 1. *Structural Equation Model 1*

In the second model analyzed, the autonomy score was found to affect the problem-solving skills score (β= .614; p< .05), problem-solving skills score affected the overall satisfaction score (β= .297; p< .05), and the positive efect of the autonomy score on general satisfaction score was found to decrease (β= .282; p< .05) (Figure 2).

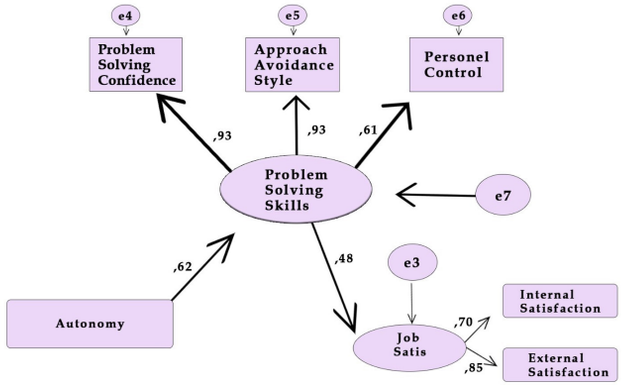

Figure 2. *Structural Equation Model*

These findings indicate the partial mediating role of problem-solving skills in the effect of the autonomy score on job satisfaction.

Modern Mediation analysis findings performed using the Bootstrap method showed that the indirect effect of the autonomy score on overall satisfaction through problem-solving skills was significant (β= .183; %95 GA [0.073, 0.290]).

These findings show that the modern method analyzing the indirect effects at 95% reliability interval, as they did not include 0 (zero) values, also support the mediating role of problem-solving skills on the effect of the autonomy score on job satisfaction.

4. DISCUSSION

This study investigated the effect of nurses' autonomy levels and problem-solving skills on job satisfaction, and the findings obtained are discussed in line with the related literature. The Autonomy Scale total mean score of the nurses was found 66.30±18.38, indicating a medium level of autonomy. This finding could be associated with nurses' responsibilities and authorities indicated by law as well as the assignments varying depending on the institutions where they work. In their study that investigated Iranian nurses' experiences of professional autonomy, Setoodegan et al. (2019) identified autonomy associated with four themes including freedom of speech, independence in the workplace, involvement in professional decision-making processes, and professional accountability, and in each theme reported nurses' dissatisfaction with the nurse autonomy perceptions. Nurses' education, legislation, organizational culture, perceptions of professional autonomy, and desire and capacity for gaining professional autonomy varying from country to country are of importance (28). Participating nurses' Minnesota Satisfaction Questionnaire total mean score was 52.28±8.69, indicating a medium-level satisfaction. An analysis of the studies conducted in Turkey regarding job satisfaction indicates low (29-32) or medium level (33-35) job satisfaction of nurses.

The first structural equation model in the study showed that the autonomy score had positive effects on job satisfaction (β= .468; p< .05). Similar to the present study, a study reported that experiencing a major depression period within the last 12 months was significantly associated with lower autonomy and higher workload (36).

This study found nurses' problem-solving mean score as 51.06±17.43, indicating below-average problem-solving skills. Studies on problem-solving in the field of nursing have generally focused on students. These studies showed that nursing students generally had high levels of problem-solving skills, which had positive effects on their clinical interaction, clinical decision-making, and critical thinking skills (37-39). On the other hand, as they start work-life, nurses' autonomy and problem-solving skills might decrease due to the process and procedures of the institutions where they work.

In the second model, the autonomy score was found to affect the problem-solving skills score (β= .614; p< .05), problem-solving skills score affected the overall satisfaction score (β= .297; p< .05), and the autonomy score was found to have positive effects on the general satisfaction score (β= .282; p< .05). These findings indicated the partial mediating role of the problem-solving skill in the effect of the autonomy score on job satisfaction.

Increasing nurses' job satisfaction is of vital importance as it has the potential to increase nurses' job satisfaction and improve care quality perceptions and adequate nursing labor force. Indirect relationships and predictors of job satisfaction contribute to the understanding of the complicated job satisfaction phenomenon more comprehensively, which might, in turn, could help to develop effective strategies to deal with nursing force deficit and improve the quality of nursing care (40). Job satisfaction has significant effects on nurses' job quality of life and psychological empowerment and organizational commitment (41,42). Aloiso et al. (2021) investigated 28 studies on factors associated with job satisfaction and grouped them under two categories as personal and organizational. While no important organizational factors were determined regarding nurses' job satisfaction, individual factors associated with job satisfaction were found to be age, health condition, determination of one's own destiny/autonomy, psychological empowerment, commitment to the job, job fatigue, and job stress.

5. CONCLUSION

While nurses' autonomy and job satisfaction levels were found to be moderate, their problem-solving skills levels were found to be low. Problem-solving skills were found to have a partial mediating role in the effect of autonomy levels on job satisfaction. It is recommended that multivariate study designs should be conducted to determine the factors enhancing job satisfaction.

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Author Contributions:

Research idea: NB, DSK, OUB, ZG

Design of the study: NB, DSK

Acquisition of data for the study: NB, OUB

Analysis of data for the study: NB

Interpretation of data for the study: NB

Drafting the manuscript: OUB

Revising it critically for important intellectual content: OUB

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Clinical and Experimental Health Sciences

Public Attitudes and Beliefs Towards Childhood Vaccinations: Urban-Rural Differences and the Other Social Determinant of Health

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ABSTRACT

Objective: The study aimed to compare vaccination attitudes and behaviors of individuals living in rural and urban areas by evaluating the social determinants of health.

Methods: This research was a secondary analysis study based on two projects examining vaccination attitudes of individuals, which were conducted separately in urban and rural areas. The researches were conducted in a city center and eight rural areas located in the central Anatolia region of Turkey. In total, 1,164 individuals were studied. Multiple regression analysis (enter model) was used for determinants of public attitude toward vaccination.

Results: In urban areas, the rate of awareness of discussions about vaccination and the rate of consideration that vaccination should be a parental decision were higher than in rural areas. According to the public attitude toward vaccination–HBM Scale, the sub-dimensions of perceived susceptibility, perceived severity, and health motivation scores of participants from rural residents were higher than those from urban residents. Regarding the sub-dimension of perceived barriers, participants from urban areas had a higher score. The social determinants of health, such as lack of health insurance, unemployment or low income, difficulty accessing health facilities, conviction that vaccination is a parental decision, moderate/poor economic perception, especially the profession, are effective in vaccine attitude.

Conclusion: This study showed a difference between vaccination attitudes of individuals living in urban and rural areas.

Keywords: Attitude, childhood, social determinants, rural, urban, vaccine and vaccine hesitancy

1. INTRODUCTION

Infectious diseases may rapidly reach an extent that threatens public health and lead to serious losses (1). Vaccination is considered one of the most effective, inexpensive, easily administrable, and low-risk public health interventions for controlling infectious diseases (2, 3). Effective and safe vaccines are available for certain infectious diseases, and scientists are constantly working on new vaccines (4, 5). An effective vaccination program prevents premature death, hospitalization, and economic losses caused by infectious diseases (5-7). Vaccines protect those who are vaccinated and the entire society by improving community immunity (8). According to the World Health Organization (WHO) data, vaccination presently prevents 2–3 million deaths annually. While 1.5 million more deaths can be prevented by increasing global vaccination coverage, approximately 19.5 million babies are still not vaccinated worldwide (9). Although considerable success has been achieved regarding vaccines in the field of public health, vaccine refusal rates have recently increased, and measles and rubella outbreaks are observed worldwide. There are countries in the European region that have lost measles elimination and are now facing outbreaks of infectious diseases (10). Because the number of vaccine refusal cases has massively increased in recent years, “the anti-vaccination movement” became among the top 10 global health problems that WHO (9) plans to resolve.

Vaccine hesitancy is the problem of accepting the vaccination; in other words, it is a delay in acceptance and rejection of vaccination (11). According to a study conducted in Turkey, 19.7% of parents are hesitant about childhood vaccinations, while the rejection rate is 18.2% (12). 37.7% of children were missing vaccine doses or entire series by the age 24 months

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in America (13). The emergence of vaccine hesitancy appears to be simultaneous with the first application of the smallpox vaccine in the 1790s. However, the 1950s and 1960s are considered the "golden age of vaccine acceptance". This positive progress suffered a deep breakout with the work of Andrew Wakefield, who claimed there is a relationship between measles-rubella and mumps vaccines and autism. Although many studies later rejected this alleged relationship, the negative impact of this study continues (14). Social and political reasons such as lack of information on vaccines and diseases, distrust in vaccines, difficulty in accessing vaccines, fear of side effects, and anti-vaccination news in the media cause vaccine hesitancy (11, 15).

The multifactorial nature of vaccine hesitancy requires the community, health workers, vaccine providers, health systems, and politicians to act to address this problem (16, 17) cooperatively. Understanding and preventing vaccine refusal is an important area of responsibility for health professionals. It may be useful to consider this problem using theories and models associated with health behavior (18-20). Models addressing the health belief system are used more frequently when investigating the causes of vaccine refusal at the community or individual level (21, 22). The most frequently used conceptual framework for explaining health behaviors is The Health Belief Model (HBM) (23, 24). Using this model has explained many health behaviors (25, 26). According to HBM, the probability of a person taking action for disease prevention depends on certain subdimensions. These include awareness about the possibility of being infected with a disease (perceived susceptibility), understanding that the consequences of the disease can be serious (perceived severity), awareness about the need for precautions to be taken before disease onset (perceived benefits), and insufficiency in avoiding risks (perceived barriers) (27). Most conceptual constructs explaining vaccine hesitancy care about the individual motivation that leads to thinking and questioning about vaccination (28). Therefore, it is essential to examine the perceptions of individuals closely. The health belief model has proven to be a successful model for revealing perceptions and attitudes (29) and is preferred by researchers in the evaluation of vaccine hesitancy (30). This model can reveal the opportunity to develop effective strategies in the fight against vaccine hesitancy by identifying the obstacles and motivations of individuals about vaccination.

WHO strongly recommends that social determinants of health be taken into account when assessing health-related situations. It is believed that only in this way will the true nature of the problems be understood (31). Social factors affecting vaccination include race, education level, income level, distance to health institutions, language spoken at home, and the number of children (32-34). Among the social determinants of health, residence is a significant parameter (35). The literature indicates that most sociodemographic factors differ in urban and rural areas (36, 37). This difference is often at the disadvantage of the rural population. Rural areas generally have a lower education level, which leads to lower health literacy rates and is associated with low use of health services. Poverty, agricultural laboring, and difficulty accessing health facilities are less common in urban areas than in rural areas (38, 39).

Different researches have been performed on individuals' attitudes toward vaccination, the significance and benefits of vaccination, and obstacles to vaccination (18, 21, 40)). However, no study covers general population and examines the beliefs, attitudes, and behaviors toward vaccination during childhood based on the social determinants of health, such as place of residence in particular.

2. METHODS

2.1. Design

This study is a secondary analysis based on the research projects that examined vaccination attitudes and behaviors of individuals, which were conducted separately in urban and rural areas. Secondary analysis is the re-analysis of the data collected from previous studies to address a new research question. The new questions of this research are: (a.) Is there a difference between vaccination attitudes of individuals living in urban and rural areas? (b.) What are the social determinants of the public attitude toward vaccination scale–HBM?

2.2. Sample

Researchers conducted these projects between May and June 2018 in a city center and eight rural areas located in the central Anatolia region of Turkey. In the original studies, the study in the rural area was carried out with 392 individuals, and the study in the urban area was carried out with 772 individuals. The secondary analysis was carried out with 1164 people, the sum of these two study groups. All the available data has been used. The sample size of the rural area was determined as a maximum of 384 persons with the rate of delaying or spacing out vaccines of 19% (41), the error rate of 0.04, and a confidence level of 95%. In total, 392 individuals participated in the study. The rate of incomplete vaccination in Turkey's urban areas was 7% (42). Taking this rate into account, the minimum sample size required for the study was found to be 664 with an error margin of 0.03 and a confidence level of 99% 772 individuals, which is a higher value, participated in this study. In the urban area, interviewers collected data from a region where different socioeconomic individuals live together, and the university campus is located. They reached individuals via a Family Health Center (FHC) and municipal social service facilities in three different neighborhoods in this region.

In the rural areas, interviewers collected data through home visits from seven villages and an FHC, to which the villages are connected. Due to the similar population size, approximately 49 individuals from each data collection center were included in the study. Seven interviewers who have received nursing education at the undergraduate level collected data.

Approvals were obtained from the ethics committee for both projects.

2.3. Measures

A questionnaire that included sociodemographic information and awareness about vaccination and the Public Attitude Toward Vaccination Scale –HBM was used as the data collection form.

Sociodemographic information form: This questionnaire included questions regarding demographic (age, marital status, having children/grandchildren aged between 0 and 6 years) and social determinants of health (gender, living place – urban or rural-, education, health insurance, employment status, perception of economic status and difficulty in accessing health facilities)

Awareness about vaccination: The form examined the awareness about childhood vaccination, use of adulthood vaccination, awareness discussions on vaccines (on the arguments of the anti-vaccine movement), and individual opinions on decision-making regarding vaccination.

***The Public Attitude Toward Vaccination Scale –Health Belief Model*:** A scale prepared based on the HBM evaluated the public attitude towards vaccination. This scale comprises 26 items in the following five sub-dimensions: (1) perceived susceptibility (4 items), perceived severity (4 items), perceived benefits (5 items), perceived barriers (8 items), and health motivation (5 items) (43).

In the original validity study of the scale, it was found that these five factors explained 68.9% of the total variance, and in confirmatory factor analysis, acceptable to excellent indices of fit were obtained. The sub-dimensions of the scale have high-reliability coefficients ranging between 0.857 and 0.907. The Cronbach's alpha values of the sub-dimensions for this study ranged between 0.77 and 0.86. Except for the perceived barriers, the increase in the scores of the sub-dimensions shows a positive attitude (43).

2.4. Statistical Analysis

In the comparison of urban and rural areas in terms of (a) sociodemographic characteristics, (b) vaccination behaviors, and (c) scale score, we used independent-samples t-test for continuous variables and chi-square for categorical variables. Multiple regression analysis was performed for determinants of the sub-dimensions. Categorical variables to be analyzed were re-encoded as 1 and 0. Before multiple regression analysis, the assumptions of multiple linear regression were evaluated (linearity, multicollinearity, normality, homoscedasticity, autocorrelation, variance inflation factor, and condition index). Because there was, according to the Phi coefficient, a high correlation between marital status and having children/grandchildren aged between 0 and 6 years, one of the variables was included in the analysis.

3. RESULTS

3.1. Rural and Urban Differences in Characteristics

The mean age of participants from rural and urban residents was similar (p>.05). The rates of being a man, being married, having children between 0 and 6 years of age, illiteracy, and unemployment or having low income was significantly higher in rural residents than in urban residents (p<.001). The perceptions of the economic status of participants from urban and rural residents were similar (p>.05). However, in rural residents, the lack of health insurance and the difficulty in accessing health facilities were higher than in urban residents (p<.05). While 9.2% of participants from urban residents did not have health insurance, 10.5% faced difficulty in accessing health facilities (see Table 1).

Table 1. *Rural and urban differences in some characteristics*

	Rural (n=392)	Urban (n=772)	Test and p-value
Age (mean±sd)	37.0±11.9	36.5±11.3	t=0.643
Gender	n (%)	n (%)	
Man(0)	186 (47.4)	235 (30.4)	X^2=32.57*
Woman (1)	206 (52.6)	537 (69.6)	
Marital Status			
Married	304(77.6)	506(65.5)	X^2=17.71*
Single	88(22.4)	266(34.5)	
Having child/grandchild between 0-6 years of age			
Yes(0)	221 (56.4)	286 (37.0)	X^2=39.51*
No (1)	171 (43.6)	486 (63.0)	
Education			
Illiteracy+only literacy(1)	51(13.0)	15(1.9)	
Primary school(1)	112 (28.6)	137 (17.7)	X^2=157.95*
Middle school – High school(0)	185 (47.2)	288 (37.3)	
University(0)	44(%11.2)	332(%43.0)	
Employment			
Unemployment(1)	179 (45.7)	398 (51.6)	X^2=106.9*
Officer+Retired+self-employment(0)	62 (15.8)	268 (34.7)	
Having low income (1)	151 (38.5)	106 (13.7)	
Perceveid economic status			
Very good+Good(0)	162 (41.3)	348 (45.1)	X^2=5.193
Medium(1)	199 (50.8)	387 (50.1)	
Poor(1)	30 (7.7)	36 (4.7)	
Health Insurance			
Yes(0)	334 (85.2)	701 (90.8)	X^2=8.27**
No(1)	58 (14.8)	71 (9.2)	
Difficulty in accessing health facilities			
Yes(1)	87 (22.2)	81 (10.5)	X^2=28.82*
No(0)	305 (77.8)	691 (89.5)	

**p<0.001 **p<0.05*

3.2. Rural and Urban Differences in Vaccine Attitude

The rate of hearing about childhood vaccines and having vaccination in adulthood was similar in urban and rural residents (p>.05). The rate of being aware of discussions on vaccines and the rate of consideration that vaccination should be a parental decision was higher in urban residents than in rural residents. Further, 87.8% of those rural residents considered that vaccination should be legally mandatory. According to the public attitude toward vaccination–HBM Scale, the sub-dimensions of perceived susceptibility, perceived severity, and health motivation scores of participants from rural residents were higher than those from rural residents. Regarding the sub-dimension of perceived barriers, participants from urban areas had a higher score (p<.05). Regarding the sub-dimension of perceived benefits, participants from urban and rural residents had similar scores (p>.05) (see Table 2).

Table 2. *Rural and urban differences in vaccine attitude*

	Rural	Urban	Test and p-value
Hearing about childhood vaccines	n(%)	n (%)	
Yes (0)	349(89.0)	684(88.6)	X^2=0.04
No (1)	43(11.0)	88(11.4)	
Having vaccination in adulthood			
Yes (0)	149(38.0)	310(40.2)	X^2=0.501
No (1)	243(62.0)	462(59.8)	
Being aware of discussions on vaccines			
Yes (1)	146(37.2)	426(55.2)	X^2=33.46*
No (0)	246(62.8)	346(44.8)	
Consideration about vaccine service			
Considered that vaccination should be legally mandatory (0)	344(87.8)	518(67.1)	X^2=57.73*
Consideration that vaccination should be a parental decision (1)	48(12.2)	254(32.9)	
Public Attitude Toward Vaccination Scale	*mean±sd*	*mean±sd*	
Perceived susceptibility	16.71±2.31	16.28±3.23	t=2.59**
Perceived severity	16.19±2.54	15.48±3.57	t=3.92*
Perceived benefits	19.71±2.69	19.40±3.93	t=1.611
Perceived barriers	18.25±5.06	20.30±6.34	t=-5.96*
Health Motivation	21.08±2.85	19.74±3.83	t=6.699*

**p<0.001 **p<0.05*

3.3. Determinants of Vaccine Attitude Scale

The determinants of the sub-dimensions of the public attitude toward vaccination–HBM Scale were examined by multiple regression analysis. The determinants of the perceived susceptibility sub – dimension were lack of health insurance (β = – 0.06), unemployment or low income (β = – 0.08), the difficulty in accessing health facilities (β = – 0.07), and conviction that vaccination is a parental decision (β = 0.420); these variables decreased the susceptibility score. The determinants of the perceived severity sub-dimension were age (β = 0.09), being a woman (β = – 0.06), unemployed or low income (β = – 0.07), awareness of discussions and news on vaccines (β = – 0.059) and conviction that vaccination is a parental decision (β = – 0.372). While age affected the severity score positively, other variables affected it negatively. The determinants of the perceived benefits sub-dimension were age (β = 0.09), being a woman (β = – 0.07), unemployment or low income (β = – 0.07), not having vaccination in adulthood (β = – 0.08), and conviction that vaccination is a parental decision (β = – 0.372). Living in urban areas (β = 0.124), unemployed or low income (β = 0.144), aware of childhood vaccines (β = 0.09), not having vaccination in adulthood (β = 0.08), and conviction that vaccination is a parental decision (β = 0.324) were the determinants that increased the perceived barriers sub-dimension score.

Concerning health motivation sub-dimension, living urban area (β = – 0.121), moderate/poor economic perception (β = – 0.06), not having vaccination in adulthood (β = – 0.09), awareness of discussions and news on vaccination (β = – 0.142) and conviction that vaccination is a parental decision (β = 0.279) are important determinants, which decreased the health motivation score. Relevant determinants explained 22% of the susceptibility, 18% of the perception of severity, benefits, and barriers, and 17% of health motivation (Tables 3 and 4).

Table 3. *Determinants of perceived susceptibility and severity*

	Susceptibility		Severity	
Variables	**Beta**	**t**	**Beta**	**t**
Age (scale)	0.05	1.64	0.09	2.93**
Gender (women)	-0.05	-1.71	-0.06	-2.05**
Living place (urban)	0.00	0.10	-0.03	-1.12
Having child/grandchild between 0-6 years of age (no)	0.00	0.18	0.02	0.99
Education(illiteracy+only literacy +primary school)	0.01	0.61	0.02	0.91
Health insurance (no)	-0.06	-2.54**	-0.02	-1.05
Employment (unemployment and having low income)	-0.08	-2.71**	-0.07	-2.21**
Perceived economic status (medium and poor)	-0.01	-0.59	0.02	0.92
Difficulty in accessing health facilities (yes)	-0.07	2.64**	0.01	0.42
Being aware of childhood vaccines (no)	-0.01	-0.17	-0.00	-0.24
Having vaccination in adulthood (no)	-0.03	-1.29	-0.03	-1.35
Hearing about discussions and news on vaccination (yes)	0.02	0.08	-0.05	2.07**
Consideration that vaccination should be a parental decision	-0.42	-15.48*	-0.37	-13.38*
Susceptibility R =0,469 R^2= 0,220 F= 24,89*				
Severity R =0,428 R^2= 0,183 F= 19,83*				

**p<0.001 **p<0.05*

Table 4. *Determinants perceived benefits, barriers, and health motivation*

	Benefits		Barriers		Health Motivation	
Variables	**Beta**	t	**Beta**	**t**	**Beta**	**t**
Age (scale)	0.09	3.08**	0.00	0.18	0.00	0.17
Gender (women)	-0.07	-2.34**	-0.04	-1.55	-0.03	-1.06
Living place (urban)	0.02	0.87	0.12	4.06*	-0.12	-3.93*
Having child/grandchild between 0-6 years of age (no)	0.01	0.26	0.03	1.36	-0.04	-1.68
Education (illiteracy+only literacy +primary school)	-0.00	-0.02	0.01	0.30	0.04	1.46
Health insurance (no)	-0.00	-0.11	-0.00	-0.33	-0.02	-0.80
Employment (unemployment and having low income)	-0.07	-2.27**	0.14	4.47*	-0.01	-0.52
Perceived economic status (medium and poor)	-0.01	-0.45	-0.00	-0.30	-0.06	-2.35**
Difficulty in accessing health facilities (yes)	0.04	1.80	-0.01	-0.40	-0.00	-0.15
Being aware of childhood vaccines (no)	0.00	0.10	0.09	3.48*	-0.02	-0.87
Having vaccination in adulthood (no)	-0.08	-2.97**	0.08	3.15**	-0.09	-3.47*
Hearing about discussions and news on vaccination (yes)	0.01	0.48	-0.01	-0.37	-0.14	4.95*
Consideration that vaccination should be a parental decision	-0.37	-13.43*	0.32	11.67*	-0.27	-9.92*
Benefits:	R =0.430	R^2= 0.185	F= 20.082	p<0.001		
Barriers:	R =0.426	R^2= 0.182	F= 19.648	p<0.001		
Health motivation:	R =0.407	R^2= 0.166	F= 17.565	p<0.001		

**p<0.001*
***p<0.05*

4. DISCUSSION

In this study, the participants' mean age and perceived economic status from urban and rural areas were similar. In addition, in rural areas, the rates of being married, having children/grandchildren aged between 0 and 6 years, being illiterate and unemployed, or having low income, lack of health insurance, and difficulty accessing health facilities were higher than in urban areas. The literature indicates that most sociodemographic factors, especially income and education, differ in urban and rural areas. This difference is often at the disadvantage of the rural population (36, 37).

The rates of awareness about childhood vaccination and receiving adulthood vaccination were similar between urban and rural residents. However, there is a higher rate of awareness about discussions on vaccination and against the legally mandatory vaccination among urban residents. A study from (44) found that two-thirds of the urban population thought vaccines should be mandatory. However, attention is drawn to the inadequacy of this ratio. It is stated that this inadequacy arises due to the evaluation of compulsory vaccination as a violation of individual rights (44). The opinions of urban residents against mandatory vaccination policies have raised concerns that it may negatively affect the vaccination of individuals living in socioeconomically poor regions (36, 40).

According to the public attitude toward vaccination scale – HBM, the susceptibility, severity, and health motivation scores of participants from rural areas were higher than those living in urban areas. In the sub-dimension of barriers, on the other hand, participants from urban areas had a higher mean score. Urban residents have more negative ideas about vaccination. Focusing more on the negative consequences of vaccines, such as side effects, prevents individuals from getting vaccinated (45). The increasing concerns of parents on the safety of vaccines lead to vaccine hesitancy and, subsequently vaccine rejection (46).

The examination of determinants of the public attitude toward vaccination scale –HBM revealed that sociodemographic and vaccination characteristics affect the sub-dimensions in different aspects. Increasing age increases severity and benefit perception scores. Similarly, it was seen that vaccination rates increased with an increase in age (38, 47). This may be associated with the diseases and social problems experienced by people in advanced age. On the other hand, it was determined that severity and benefits scores of women were lower. A study examining the characteristics of vaccination attitudes found that being a woman was a negative determinant of vaccination attitudes (48). However, there are conflicting findings in this regard. The majority of first-time pregnant women had positive beliefs and perceptions about childhood immunizations (22).

Profession is an important variable that affects all sub-dimensions of the scale, except for health motivation. It was found that being unemployed and having a low or irregular income decreased susceptibility, severity, and benefits scores but increased the barriers score. Profession provides information about an individual's economic status, and there is a close association between economic status and health level and indicators (49). Therefore, the negative attitude of the disadvantaged group with a high prevalence of diseases and poor health conditions may worsen possible problems.

Participants from urban areas were found to have high barriers score for vaccination and a low health motivation score. In rural areas, individuals consider that their children might have health problems if not vaccinated, increasing their health motivation. On the other hand, participants from urban areas use social media more actively to obtain information about vaccines (50, 51). However, much negative information from unscientific sources, such as harms of vaccines or diseases related to vaccines, is shared on social media, which affects vaccination attitude. In addition, the fact that infectious diseases have been more controlled in urban areas may have resulted in more attention being drawn toward the side effects of vaccines. Considering that people living in urban areas are more aware of the discussions on vaccines (Table 2), this interpretation is supported.

Absence of health insurance and difficulty in accessing health facilities decreased sensitivity score. The primary factors leading to difficulty accessing health facilities are lack of health insurance and not having a fixed-income job. Although these factors are intertwined concepts, they adversely affect individual sensitivities toward vaccination practices (38).

Moderate/poor economic perception was found to be a determinant of health motivation. In a similar study, it was found that the health responsibilities of individuals with low economic perceptions decreased, and they did not provide their children with sufficient recommended vaccinations (52). Individuals with this perception want to benefit less from health services when they have any health problems and cause a negative picture of taking motivation for health. Income is considered a prerequisite for healthy lifestyle behaviors (53). As in many health behaviors, it can be assumed that individuals will take more individual motivation if economic inequalities are eliminated in vaccination.

Awareness of childhood vaccination is a determinant that increases the barriers score. This finding indicates that if individuals have vaccine awareness, their attitudes toward disease and vaccination may change. As an indication of the fact that their attitudes toward vaccine for preventable illnesses have changed, there are parental attitudes that underestimate infectious diseases, do not consider them seriously, and do not care about their negative consequences (16, 17, 19). Negative social media and incomplete and inaccurate information provided by incompetent people may be the reason why awareness may be inhibitory. Health policies and healthcare workers have an important role in transforming vaccine awareness into desired health behaviors of individuals exhibiting vaccine refusal or vaccine hesitancy.

Not having vaccination in adulthood is a determinant that negatively decreases the benefit and health motivation sub-dimensions while increasing the barriers sub-dimension score. It has been found in the literature that there are negative attitudes toward vaccination in adulthood (54, 55). The consideration that is receiving vaccination services is a parental decision decreased susceptibility, severity, benefit, and health motivation scores but increased barriers score, and it was found to be a determinant in all subdimensions. For families who consider that childhood vaccination decision belongs to parents, negative thoughts about vaccines can be more noticeable and worrying (51, 56).

Awareness about discussions and news on vaccines is a determinant that decreased the severity and health motivation subdimension scores. This reveals that all individuals, particularly families, should follow the information shared on vaccines on scientific platforms. While explaining the beneficial effects of vaccination, its contribution to community development should be emphasized and this awareness should be raised among individuals. All scientific developments related to vaccines and the contributions of vaccination to public health must be shared with the public through media and social media (57).

4.1. Limitations and future research

This study has some limitations. First, since data obtained from the study population are based on personal feedback and this has been voluntary research, no data could be gathered on the vaccine attitude of the non-participant group. Second, in the study, the impact of social determinants on vaccine attitude has been examined. However, individual characteristics (personality traits, decision-making mechanisms, etc..) that shape attitudes have not been examined. In the following studies, it can be suggested that the theories explaining individual characteristics and health belief model be used together.

5. CONCLUSION

This study showed a difference between vaccination attitudes of individuals living in urban and rural areas. Insurance, accessing health facilities, conviction that vaccination is a parental decision, age, being a woman, unemployment, or low income are related factors. In urban areas, the rates of awareness about discussions on vaccination and consideration that vaccination should be a parental decision were higher than in rural areas. Examination of determinants of the community's attitude toward vaccination scale –HBM revealed that social determinants of health affected these attitudes, albeit from different aspects. Profession, especially, is an important determinant.

In eliminating obstacles to vaccination, it is important to determine social norms against vaccination and provide

accurate information about vaccines. Public health nurses need to be aware of these obstacles, guide the public on immunization, and advocate for vaccination using a science-based approach. Taking into account the effect of social determinants of health on attitudes. We can also suggest that it would be an effective way for researchers and practitioners to address vaccination attitudes based on HBM in understanding society and solving the issue.

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Clinical and Experimental Health Sciences

Effect of Thermomechanical Aging on the Surface Roughness and Color Stability of Novel CAD-CAM Materials: An In-Vitro Study

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ABSTRACT

Objective: This in-vitro study aimed to evaluate the surface roughness (R_a) and color stability of novel monolithic CAD-CAM materials after thermomechanical aging.

Methods: Forty specimens were obtained from 4 different materials (a resilient ceramic (RC), ceramic-reinforced PEEK (PE), lithium disilicate glass ceramic (EX), and a resin-based composites (CO) (n=10). Initial R_a and color coordinates were recorded. All specimens were than subjected to thermomechanical aging. R_a and color coordinate measurements were repeated. CIEDE2000 formula was used to calculate the color changes (ΔE_{00}). Kruskal-Wallis and Dunn tests were used to analyze data, while the effect of aging on R_a was analyzed with Wilcoxon test (α=.05).

Results: Regardless of aging, PE showed higher R_a than EX ($p< .001$), whereas the differences between PE and the other materials were nonsignificant ($p\geq .202$). Thermomechanical aging led to higher R_a for all materials tested ($p= .005$). The greatest ΔE_{00} was observed in PE ($p\leq .002$), while the difference among the other materials was nonsignificant ($p> .05$). R_a of ceramic-reinforced PEEK was above clinical threshold, regardless of thermomechanical aging.

Conclusion: Thermomechanical aging caused a color change that was perceptible for all the materials tested, while it was also unacceptable for ceramic-reinforced PEEK.

Keywords: CAD-CAM, dental materials, roughness, color

1. INTRODUCTION

Computer aided design-computer aided manufacturing (CAD-CAM) technologies have facilitated more natural and standardized restorations with a reduced cost and fabrication time (1-3). Parallel to this technology, various monolithic restorative materials have become viable (4). The increasing trend towards monolithic restorations with reduced chipping and easier fabrication has led to material diversity (5). Resilient ceramics, which contain both ceramic and resin have been introduced as an alternative to ceramics. These materials stand out with their favorable Young's moduli, relatively low fracture tendencies, and ease of processing (6). CAD-CAM composite resins offer better mechanical and esthetic properties than those fabricated by conventional methods due to increased polymerization (7,8).

Among the available materials that can be processed by CAD-CAM technologies, polyetheretherketone (PEEK) is a popular high-performance polymer due to its dentin-like elastic modulus, light weight, ease of machinability, and favorable chemical and mechanical properties (9-13). A more recent iteration of this materials is the ceramic-reinforced PEEK, which has improved esthetic and thereby used for the fabrication of frameworks, crowns, abutments, and inlays (3-14).

Mechanical and optical properties of a restorative material are fundamental for its clinical success (1,15-19). Considering the dynamic intraoral environment involving various chemical and mechanical factors that may cause substance loss (20), a restorative material should resist wear as increased surface roughness (R_a) is directly proportional to plaque accumulation, bacterial adhesion, and periodontal problems (21-24). Moreover, previous researches have shown the adverse effects of R_a on the esthetic and color stability of CAD-CAM materials (16,25). The clinical threshold of R_a has been reported as 0.2 µm (26).

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Optical characterizations are an important parameter that affects the longevity, esthetic appearance, and ultimately patient acceptability of a prosthetic material (16). Color stability can be affected by many factors such as water absorption, aging, patient's cleaning habits, R_a, and material content (25). Color alteration may be associated with aging owing to the degradation of the polymer matrix or unreacted monomers, depending on the material type (27).

Previous studies have focused on the R_a and color stability of restorative materials after different aging procedures (1,15,17,27-37), given the constant exposure to the dynamic loads and temperature changes in the oral environment (1,31). However, there is limited information on the effect of thermomechanical aging on the R_a (38) and color stability (34) of PEEK after thermomechanical aging. Thus, the present study aimed to compare the R_a of ceramic-reinforced PEEK with 3 different CAD-CAM materials before and after thermomechanical aging as well as evaluate materials' color change. The null hypotheses were that i) the R_a values would not be affected by the material type and thermomechanical aging, and ii) the color change would not be affected by the material type.

2. METHODS

Table 1 gives detailed information about the materials tested. Forty square-shaped specimens were wet-sliced (Microcut 201; Metkon Instruments Inc.) by using 4 monolithic CAD-CAM materials (BioHPP; Bredent, IPS e.max CAD; Ivoclar Vivadent, Cerasmart; GC Corporation, Brilliant Crios; Coltène) (n=10). All specimens were then wet-ground (#400 and 600) and polished (#1000) with silicone carbide abrasive papers for the final dimensions of 12x12mm and a thickness of 1.2 mm. A digital caliper (Absolute Digimatic; Mitutoyo) was used to control the dimensions of all specimens. EX specimens were then crystallized in a porcelain furnace (Programat P310; Ivoclar Vivadent AG). No additional glazing or polishing was performed (3,39,40).

Table 1. *List of tested materials*

Material	Abbreviation	Chemical Composition	Manufacturer
BioHPP	PE	Polyetheretherketone, ceramic filler (20%)	Bredent
IPS e.max CAD	EX	57%-80% SiO_2, 11%-19% Li_2O,0%-13% K_2O, and other oxides	Ivoclar Vivadent
Cerasmart	RC	(71%) Silica, barium glass, Bis-MEPP, UDMA, DMA	GC Corporation
Brilliant Crios	CO	(70%) Resin matrix cross-linked with methacrylates, amorphous silica, barium glass	Coltène AG

PE: Polyetheretherketone, EX: Glass ceramic, RC: Resilient ceramic, CO: Resin-based composite

A single researcher (A.T.) measured the initial color coordinates (L*, a*, and b* values) of the prepared specimens on a gray background 3 times by using a digital spectrophotometer (VITA EasyShade V; Vita Zahnfabrik) and the mean values were recorded. The spectrophotometer was calibrated before each reading. Standardization was achieved by positioning the probe tip perpendicular to the surfaces during the measurements, and the measurements were not affected by the ambient light (41). After color measurements, a contact profilometer (Marsurf PS1; Mahr) was utilized for the initial R_a values of the specimens. Three measurements were performed on each specimen (stylus tip radius: 5 µm, stylus driving speed: 0.5 mm/s, traversing length: 1.75 mm, and five cut-off length: 0.25 mm) (42) and the mean values were calculated.

Sound maxillary premolar teeth, which were stored in 0.5% chloramine solution at room temperature for a week and afterwards kept in distilled water at 5°C for 14 days (8) were used as enamel antagonists (Biruni University Ethics Committee, Decision No: 2020/37-04). For thermomechanical aging, the specimens were placed in silicone molds in the chamber of the biaxial chewing simulator (MOD CS; MOD Dental) and fixed by position screws. Prior to thermomechanical aging, buccal cusps of the antagonist teeth were adjusted to a spherical shape by using a diamond instrument (801-314-018-C; Coltene/Whaledent AG). Simultaneous mechanical (240.000 cycles, 49 N, 1.2 Hz, and 0.7 mm lateral movement) and thermal aging (5-55°C, 60 s dwell time) were applied in distilled water (3). The specimens were then cleaned in an ultrasonic tester, and measurements were repeated. Color differences (ΔE_{00}) before and after thermomechanical aging were calculated by using the CIEDE2000 color formula and the parametric factors (KL, KC, and KH) were considered as 1 (1,43):

$$\Delta E_{00} = \sqrt{\left(\frac{\Delta L}{K_L S_L}\right)^2 + \left(\frac{\Delta C}{K_C S_C}\right)^2 + \left(\frac{\Delta H}{K_H S_H}\right)^2 + R_T \left(\frac{\Delta C}{K_C S_C}\right)\left(\frac{\Delta H}{K_H S_H}\right)}$$

Based on the results of a previous study (3), the number of samples was determined (effect size f= 0.71, 1-β= 0.95, and α= 0.5) with a priori power analysis. Normality of the data were analyzed by using Shapiro-Wilk tests. Due to the non-normal distribution of the R_a and ΔE_{00} data, Kruskal-Wallis and Dunn's tests were performed. The differences within each material's R_a values before and after thermomechanical aging were further resolved with Wilcoxon tests (SPSS v23; IBM) (α=.05).

3. RESULTS

Descriptive R_a statistics before and after thermomechanical aging are summarized in Table 2. Significant differences were observed among the tested materials before and after thermomechanical aging (p<.001). Regardless of the thermomechanical aging, PE had higher R_a than EX (p<.001),

whereas no significant differences were seen among PE and other materials (p≥.202). EX had lower R_a than RC before (p=.01) and after thermomechanical aging (p=.018). In addition, CO showed similar R_a to the other materials tested before (p≥.056) and after thermomechanical aging (p≥.101). Thermomechanical aging increased the Ra of all materials significantly (p=.005). Material type significantly affected ΔE_{00} values (p<.001), as PE presented the greatest ΔE_{00} (p≤.002). However, the differences among the other materials were nonsignificant (p>.05) (Table 3).

Table 2. *Median (min-max) and mean ±standard deviation values of the surface roughness before and after thermomechanical aging.*

	Before (µm)		After(µm)	
	Median	Mean	Median	Mean
BH	0.33^{bA} (0.12-0.47)	0.32 ±0.11	0.41^{bB} (0.23-0.56)	0.4 ±0.11
EX	0.09^{aA} (0.04-0.16)	0.09 ±0.04	0.12^{aB} (0.08-0.35)	0.14 ±0.08
RC	0.22^{bA} (0.08-0.43)	0.23 ±0.11	0.26^{bB} (0.16-0.48)	0.3 ±0.1
CO	0.18^{abA} (0.1-0.41)	0.2 ±0.1	0.24^{abB} (0.17-0.5)	0.27 ±0.1

**Different superscript letters indicate significant differences (Uppercase letters for rows and lowercase letters for columns, P <.05). PE: Polyetheretherketone, EX: Glass ceramic, RC: Resilient ceramic, CO: Resin-based composite*

Table 3. *Median (min-max) and mean ±standard deviation values of the ΔE_{00}*

	Median	Mean
BH	1.89^{a} (1.16-2.04)	1.78 ±0.27
EX	0.89^{b} (0.62-1.3)	0.91 ± 0.23
RC	0.98^{b} (0.61-1.25)	0.93 ± 0.23
CO	0.97^{b} (0.41-1.2)	0.92 ± 0.24

**Different superscript letters indicate significant differences (P <.05) PE: Polyetheretherketone, EX: Glass ceramic, RC: Resilient ceramic, CO: Resin-based composite, ΔE00 :Color differences*

4. DISCUSSION

R_a values of the tested materials showed differences before and after thermomechanical aging. In addition, thermomechanical aging significantly increased the R_a of all study groups. Therefore, the first null hypothesis was rejected. The differences among the ΔE_{00} values of the materials tested were significant, which led to the rejection of second null hypothesis.

R_a of a restorative material depends on patient and material related factors (4). Even though inherent material properties have direct effects, polishing or glazing may also influence the R_a (44). Therefore, no polishing other than wet-grounding with silicone carbide papers was performed to materials tested prior to the initial R_a measurements (11). Prior to aging procedure, EX showed significantly lower R_a than RC and PE, whereas CO showed similar values to the all-other materials. To authors' knowledge, the number of studies investigating the R_a of PE after sole wet-grounding and without a polishing procedure is limited (12-14). The initial mean R_a value of PE in the present study (0.33 µm) is in line with those previous studies, as the mean R_a of PE ranged from 0.28 to 1.11 µm when polished with silicon carbide abrasive papers (12-14). The effect of various laboratory and chairside polishing systems on the R_a of PE has also been investigated and all mean R_a values have been reported above the clinically acceptable threshold (10). Considering these results, it can be speculated that monolithic use of PE, even after polishing, could be problematic. However, given the limited number of studies, future studies are needed to corroborate this interpretation. CO and RC had similar R_a before and after aging. This can be attributed to the fact that the materials have similar compositions, although their filler shapes and filler sizes are different (3,44).

Thermomechanical aging statistically increased the R_a of all materials tested in the present study. However, EX showed lower R_a values (0.12 µm) than 0.2 µm (26) even after thermomechanical aging, while RC (0.26 µm) and CO (0.24 µm) showed R_a values slightly higher than the clinically acceptable threshold. Given the fact that no additional polishing was performed after thermomechanical aging, polishing RC and CO with chairside kits might decrease the R_a values below 0.2 µm and improve the longevity of restorations fabricated by using these materials. Even though PE showed similar R_a with RC and CO after thermomechanical aging, the roughness value of PE (0.41 µm) was greater than the clinically acceptable threshold. Considering that the number of mechanical cycles applied in our study approximately reflects 1 year of clinical use (45) and the fact that a greater number of cycles would possibly lead to a coarser surface, it may be speculated that monolithic use of PE might result in restorations with lower color stability and higher plaque accumulation than the other materials tested. However, this hypothesis needs further support with studies involving longer aging cycles as well as post-aging polishing of these materials.

Even though a number of studies have investigated the effect of thermomechanical aging on PE (3,10,34-37), to author's knowledge only 1 study has ever researched the effect of thermomechanical aging on the R_a of PEEK (38). PEEK specimens were subjected to thermomechanical aging with a significantly lower number of cycles (60000 cycles) in Benli et al's (38) study compared with the present study. However, a greater R_a difference was reported (0.139 µm before thermomechanical aging and 0.889 µm after thermomechanical aging) and clinically acceptable threshold of 0.2 µm was exceeded only after thermomechanical aging. A higher initial R_a of PE (0.33 µm) was reported in the present study; however, a direct comparison might be misleading considering the differences in the materials tested as Benli et al (38) utilized a conventional PEEK in their study. Considering

the limited number of studies on the R_a of conventional and ceramic-reinforced PEEK after thermomechanical aging, future studies comparing these materials with longer thermomechanical aging cycles are needed.

Mechanical aging increases roughness and surface area of materials that will result in superficial discoloration (21). Previous studies have reported that aging is an important factor affecting the color change of materials (46,47). Among the materials tested, PE showed the greatest ΔE_{00} after thermomechanical aging, which may be related to the high R_a values of the material as a previous study have reported the strong inverse correlation between the R_a and optical properties (16). However, this interpretation needs further support with future studies involving correlation analyzes between the R_a and color stability of the materials tested. Nevertheless, this result corroborates the findings of a previous study (34) where PEEK crowns showed a lower color stability compared with zirconia crowns when subjected to thermomechanical aging. The aging process also can change the molecular structure of high performance polymers, which are hydrophilic in nature, by affecting the water absorption properties (48). Less color change observed in composite and ceramic content groups may be attributed to the lesser change in their molecular structures. Yet, this interpretation needs to be supported with future studies investigating the molecular structure of the tested materials before and after thermomechanical aging.

Paravina et al (43) reported clinically perceptible and acceptable levels of ΔE_{00} as 0.8 and 1.8. Thermomechanical aging caused a color change that perceptible for all the materials tested (ΔE_{00} =1.89 for BH; ΔE_{00} =0.89 for EX; ΔE_{00} =0.98 for C; ΔE_{00} =0.97 for B), whereas only PE showed a color difference that was higher than the acceptability threshold. Considering these results and the fact that PE has a dull appearance, it can be speculated that its indication for esthetic restorations is limited (49). However, this interpretation needs to be corroborated with in vivo studies, which also investigate the clinical performance of PEEK.

This study aimed to compare the inherent R_a of the materials and to evaluate the effect of thermomechanical aging. Therefore, no polishing procedure was performed before the initial R_a measurements. However, the effect of different polishing procedures on the R_a of PEEK has been shown (10). In addition, ceramic-reinforced PEEK was compared with 1 ceramic and 2 resin-based polymers due to chemical similarity. Nevertheless, future studies should compare ceramic-reinforced PEEK with different materials to corroborate the results of our study. The relatively short thermomechanical aging cycle might be considered as another limitation. In addition, thermomechanical aging was performed by using distilled water. However, proteins and enzymes in the saliva may affect the surface properties and the color stability of materials (50). Future studies involving longer aging procedures performed by using saliva might elaborate the understanding of the R_a and color stability of BH, and approximate the findings more to the clinic.

5. CONCLUSION

Based on the findings of this in vitro study, the following conclusions were drawn:

1. Ceramic-reinforced PEEK presented higher Ra than lithium disilicate glass ceramic and the clinical threshold of 0.2 µm regardless of aging.

2. Surface roughness of all materials increased after thermomechanical aging.

3. Ceramic-reinforced PEEK presented the greatest color difference after thermomechanical aging and among the materials tested only the color change of ceramic-reinforced PEEK was greater than the clinically acceptable threshold.

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Acquisition of data for the study: AADT, DH, ÖSK, MBD

Analysis of data for the study: MBD, DH

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Clinical and Experimental Health Sciences

The Effect of Counseling Depending on the Tv Watching Frequency of Children on Their Emotional and Behavioral Problems

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ABSTRACT

Objective: While television continues to exist in many homes, it is thought that the situation of children being affected by the frequency of watching television is an important issue that is overlooked. This study was conducted in order to determine the effect of counselling given to parents and teachers depending on the TV watching frequency of children going to pre-school education institutions on their emotional-behavioural problems.

Methods: The study was conducted as an experimental study with pre-test – post-test control group. Data were collected by using 'Family Introduction Form', and 'Strengths and Difficulties Questionnaire'. Data were analysed using SPSS 20.0.

Results: The study found significant differences between the pre-test and post-test mean scores of the families and teachers in the experimental group and control group for the total score of the Strengths and Difficulties Questionnaire and the mean scores of all subscales, as well as significant difference between the pre-test and post-test mean scores in the experimental group for intra-group analysis ($p < .05$).

Conclusion: The study found that the counselling training given to parents and teachers lead to a significantly positive change in the mean scores for emotional, behavioural, and peer problems as well as attention deficit and hyperactivity disorders and it also has a significant positive impact on the TV watching duration of children.

Keywords: Family, preschool child, teacher, television

1. INTRODUCTION

Human life is mostly shaped by the influence of childhood. The pre-school period is the fastest and most critical years of a child's development. The experiences that can be gained during this period become largely determinant in the child's life (1).

To grow up and develop in a healthy way, children need qualified cognitive stimuli, rich language interactions, and an environment where positive social and emotional experiences are offered to the child, and the child's independence is supported. This is only possible with a qualified pre-school education and a healthy family atmosphere (2-4).

Family atmosphere is one of the important factors affecting the development, success and social adaptation of children. In a caring and sensitive family, the child's emotions are perceived correctly, their ideas and wishes are taken into account, and their needs can be addressed timely and properly (1,4). In this context, a family who occupies an important place in their child's life should be careful about how many hours their children spend watching TV and what kind of shows they watch. Because the effects of television vary according to the children's TV watching duration, the contents of the shows they watch and their parents' supervision (1).

Teacher characteristics are also among the main factors affecting the quality of education and the development of the child. Children can discover only in environments where they see value and feel safe and take advantage of the learning opportunities offered in these environments only. The most important component of this supportive environment for children is the secure and consistent relationship established between the child and the teacher (4,5).

Television plays a big role in all these relationships and experiences that affect the development of children because television maintains its existence effectively in most households, regardless of its economic level. As soon as a baby is born, he/she communicates with his parents, as well as the television, which was made, so to speak, a family member in households (1,6). Thanks to its visual and auditory features, television has the ability to attract the child's attention. Since

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it does not require literacy skills, it is a communication tool that can be used by preschool children (7).

Preschool children, who have a great learning potential, need direct experience, social relations with their peers, and mobility for healthy body development during learning (2). In this respect, training to be given to parents and teachers to adjust the TV watching duration will make a great contribution to the emotional and behavioural development of children.

It is thought that nurses, who interact with many parents with different roles, should know the effects of television on child development and provide training and counselling to the family in this context to reduce the negative effect of television on children (8,9) since the effects of television vary according to the children's TV watching duration, the contents of the shows they watch and their parents' supervision (1). In conclusion, the relationship between preschool children and television is an important issue that needs attention. However, it has been observed that the researches on the subject in the international and national literature are very limited and interventional researches are not sufficient.

Therefore, considering that it will be important to determine the effect of the TV watching frequency of pre-school children on their emotional-behavioural problems, a study was conducted to determine the effect of structured counselling given to parents and teachers depending on the television watching frequency of children going to pre-school education institution on their emotional-behavioural problems.

2. METHODS

The study is an experimental study with pre-test and post-test control group.

Two kindergartens were selected to eliminate the possibility of the control group being affected from the structured counselling program to be applied to the families and teachers in the experimental group. The data of the study were collected during the period between January and April 2015, which is the second semester of the academic year, so that students and teachers could get to know each other and the results of the study would not be affected by the fresh starting of the students to school.

2.1. Hypotheses of the Study

Ho: Counselling given to parents and teachers depending on the TV watching frequency will not have an effect on the average score for emotional behavioural problems of children.

H1: Counselling given to parents and teachers depending on the TV watching frequency will have an effect on the average score for emotional behavioural problems of children.

2.2. Sample

Population of the study consists of children going to independent kindergartens (N:13) affiliated to the Ministry of National Education in the city centre of Erzurum, as well as their parents and teachers.

The sample of the study consists of the children going to the independent Kindergarten A (experimental group) and Kindergarten B (control group) affiliated to the Ministry of National Education in the city centre of Erzurum, who were selected from this population by simple random method, as well as their parents and teachers.

Priori power analysis program was used to determine the sample size of the study. During the power analysis, it was determined that minimum 18 children-families can be taken in each group. Therefore, it was determined that the sample taken in the study could reach 99% power at 0.05 significance level and 95% confidence interval. There are 280 students and 12 teachers in the Kindergarten A, while the Kindergarten B has 110 students and 5 teachers. The sample of the study consists of 70 students and 12 teachers from the Kindergarten A and 70 students and 5 teachers from the Kindergarten B, who meet the inclusion criteria and agreed to participate in the study.

The sampling included children watching TV for more than 2 hours a day, their first-degree relatives who could follow them closely, and teachers willing to follow the children's emotional and behavioural changes during the study. Furthermore, the selected participants did not have any mental or physical illnesses that could affect the course and result of the study.

2.3. Data Collection Tools

The following data collection tools were used in the study.

Family introduction form: This form prepared by the researcher by reviewing the literature, consists of 13 questions investigating the introductory characteristics of children and their families, the frequency of watching TV by children, and the attitudes of families towards television (1,3,7,9).

Strengths and Difficulties Questionnaire (SDQ): It was developed by the British psychiatrist Goodman in 1997 (10,11). The SDQ was made valid and reliable in Turkish by Güvenir et al. (12). SDQ was grouped under 25 questions, some of which investigate positive behavioural characteristics, and other investigate negative behavioural characteristics, and five subtitles each containing five questions: Attention Deficit and Hyperactivity, Behavioural Problems, Emotional Problems, Peer Problems, and Social Behaviors.

Television watching follow-up form: This form, prepared by the researcher, by reviewing the literature, consists of questions aiming to monitor the child's weekly television watching frequency; 'How many hours did your child watch television this week? How do you evaluate your child's television watching frequency this week? (Less or more compared to the last week) (1,3,7,9).

TV watching schedule: It is a schedule prepared by the researcher by scanning the literature in a way that can attract the attention of the child visually in order to record the approximate number of hours the child watches in that week in order to follow the weekly TV-watching frequency of the children.The schedule is prepared as a refrigerator magnet so that it will be kept in view (1,3,7,9).

Student award plaque: It was prepared by the researcher after a literature review for teachers to follow and reward children's weekly television watching frequencies (1,3,7,9).

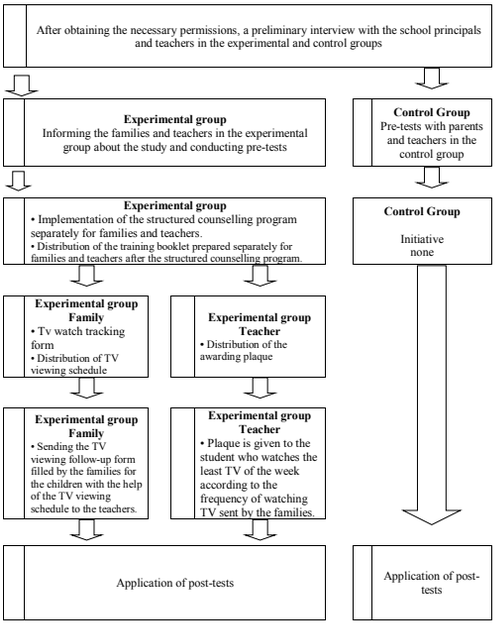


Figure 1. *Research Plan*

2.4. Collection of Data

Prior to the study, the principals and teachers of Kindergarten A and Kindergarten B where the study would be conducted were visited to give information about the study. It was underlined that, before and after the counselling program, the teachers should observe emotional and behavioural changes by closely following up the children.

The families and teachers in the experimental group included in the research were informed about the purpose, importance and duration of the study. Then, they were told that a structured consultancy program on 'The Role of Television in the Social, Emotional and Behavioural Development of Children' will be held, and information, content and application of program, and pre-tests were applied.

After the structured consultancy program planned for the experimental group, the families were provided with a TV watching schedule prepared by the researcher as a refrigerator magnet that can visually draw the children's attention, so that they can note their children's TV watching durations in order to determine their weekly television watching frequencies every week.

Then, the families were asked to note their children's TV watching frequencies in the distributed TV watching follow-up form and send it to teachers with the students weekly. In the light of these forms, the teachers were asked to award the student who watched TV least in the week with a student awarding plaque prepared by the researcher.

Four weeks after the training, post-tests (strengths and difficulties questionnaire) were applied to the families and teachers in the experimental group.

The structured counselling program was given to the families and teachers in the control group after the post-tests.

Family counselling program and materials: Structured consultancy program was held in three sessions, namely 'The Role of Television as a Mass Medium in Our Lives', 'The Effects of Television on Children', 'What Should Parents Do or Shouldn't Do?'. The techniques used in each session were determined according to the purpose of the session, and each session took place over a period of 40 minutes.

At the end of the program, training booklets for families were distributed by the researcher in line with the literature.

Teacher counselling program: Structured consultancy program was held in three sessions, namely 'The Role of Television as a Mass Medium in Our Lives', 'The Effects of Television on Children', 'What Should Teachers Do?'. The techniques used in each session were determined according to the purpose of the session, and each session took place over a period of 30 minutes.

At the end of the training, training booklets were distributed to the teachers.

Before starting the study, approval was obtained from the Ethics Committee of the Atatürk University Institute of Health Sciences (20369917-72.00-3901). Necessary permissions were obtained from the Provincial Directorate of National Education in order to start the study at the Kindergartens A and B.

3. RESULTS

The Table 1 shows the distribution of the descriptive characteristics of the families and children in the experimental and control groups included in the study. In the study, it was determined that 50.0% of the mothers in the experimental group were 32-37 years old, 37.1% were university graduates, 50.0% of the fathers were 32-37 years old and university graduates and 60.0% had 2 children.

According to the Table 1, the study found that 42.9% of the children in the experimental group watch TV series, 44.3% shared their opinions about the show they watched, 70.0% watch TV in their spare time, and 61.4% spent most of their time watching TV. 47.1% of the families in the experimental group also stated that their children were calm.

It was found that 71.4% of the children in the experimental group watched TV for 3-4 hours a day on weekdays, 51.4% watched TV for more than 5 hours per day on weekends, and 41.4% prefers to watch TV between 06:00 and 09:00 PM.

Table 1. *The distribution of the descriptive characteristics of the families and children in the experimental and control groups*

Descriptive Characteristics		Experimental Group (n=70)		Control Group (n=70)		Test and p values
		S	%	S	%	
Mother age	20-25	5	7.1	10	14.3	χ^2=4.011 p=0.260
	26-31	20	28.6	26	37.1	
	32-37	35	50.0	27	38.6	
	38-43	10	14.3	7	10.0	
Father age	26-31	7	10.0	15	21.4	χ^2=3.742 p=0.291
	32-37	35	50.0	31	44.3	
	38-43	23	32.9	21	30.0	
	44 – >	5	7.1	3	4.3	
Mother education level	Primary school	7	10.0	17	24.3	χ^2=2.409 p=0.661
	Middle School	8	11.4	12	17.1	
	High school	19	27.1	28	40.0	
	Junior college	10	14.3	2	2.9	
	College	26	37.1	11	15.7	
Father education level	Middle School	7	10.0	8	11.4	χ^2=2.536 p=0.496
	High school	19	27.1	26	37.1	
	Junior college	9	12.9	10	14.3	
	College	35	50.0	26	37.1	
Children in the family number	Only chıld	11	15.7	10	14.3	χ^2=2.090 p=0.554
	2 chıldren	42	60.0	36	51.4	
	3 chıldren	12	17.1	19	27.1	
	4 and >	5	7.1	5	7.1	
Child's weekday daily tv viewing time	1-2 hours	9	12.9	10	14.3	χ^2=1.029 p=0.598
	3-4 hours	50	71.4	53	75.7	
	5 hours and >	11	15.7	7	10.0	
Child's weekend daily tv viewing time	1-2 hours	3	4.3	9	12.9	χ^2=3.545 p=0.170
	3-4 hours	31	44.3	31	44.3	
	5 hours and>	36	51.4	30	42.9	
Child's TV viewing hours	06:00-10:00	5	7.1	7	10.0	χ^2=4.153 p=0.245
	10:01-12:00	18	25.7	25	35.7	
	12:01-18:00	18	25.7	20	28.6	
	18:01-21:00	29	41.4	18	25.7	
The programs that the child most preferred to watch	Children's programs	27	38.6	30	42.8	χ^2=5.035 p=0.169
	TV series	30	42.9	29	41.4	
	The News	13	18.6	11	15.7	
The situation of child to share their opinions about the TV programs they watch	Shares	31	44.3	32	45.7	χ^2=1.812 p=0.404
	Don't shares	17	24.3	11	15.7	
	Sometimes shares	22	31.4	27	38.6	
The child What made it in spare time	Watches TV	49	70.0	47	67.1	χ^2=0.418 p=0.937
	Plays with friends	12	17.1	14	20.0	
	Painting	5	7.1	4	5.7	
	Spending time with her/his parents	4	5.7	5	7.1	
Where the child spends the most time	Across TV	43	61.4	40	57.1	χ^2=3.201 p=0.362
	With friends	8	11.4	7	10.0	
	With parents	19	27.1	23	32.9	
Your child how would you describe	Calm	33	47.1	34	48.6	χ^2=1.187 p=0.756
	Angry	10	14.3	13	18.6	
	Agressive	7	10.0	8	11.4	
	Withdrawn	20	28.6	15	21.4	

The Table 2 below gives the comparison of the SDQ intragroup pre-test and post-test mean scores of the families and teachers in the experimental and control groups. When the table was analysed, the difference between the pre-test mean scores for Strengths and Difficulties Questionnaire subscale and total scores of the families and teachers in the experimental group and the parents and teachers in the control group was found to be statistically insignificant (p> .05).

According to the table, the difference between the post-test mean scores for Strengths and Difficulties Questionnaire subscale and total scores of the families and teachers in the experimental group and the parents and teachers in the control group was found to be statistically significant (p< .05).

The Table 3 shows the comparison of the SDQ intragroup pre-test and post-test mean scores of the families and teachers in the experimental group and control group. When the table was analysed, the difference between the Strengths and Difficulties Survey intragroup pre-test and post-test subscale and total scores of the families and teachers in the experimental group was found to be statistically significant (p< .05).

Table 2. *The comparison of the SDQ* intragroup pre-test and post-test mean scores of the families and teachers in the experimental and control groups*

Strengths and Difficulties Questionnaire Subscales and Total Scores			Experimental Group Pretest (n=70)		Control Group Pretest (n=70)		Test and p values		Experimental Group Posttest (n=70)		Control Group Posttest (n=70		Test and p values	
			$\bar{x}$	Sd	$\bar{x}$	Sd	t	p	$\bar{x}$	Sd	$\bar{x}$	Sd	t	p
SUBSCALES	Social Behaviors	Family	10.60	3.74	10.10	3.21	0.848	0.398	16.96	2.23	10.34	3.13	**14.375**	**.000**
		Teacher	4.14	1.63	4.67	1.82	-1.805	0.073	17.53	2.51	6.69	1.98	**28.310**	**.000**
	Attention Deficit and Hyperactivity	Family	10.83	2.47	11.36	2.69	-1.208	0.229	4.71	1.42	11.63	2.62	**-19.383**	**.000**
		Teacher	10.46	1.82	10.81	1.97	-1.112	0.268	9.57	2.55	10.83	1.88	**– 3.319**	**.000**
	Emotional Problems	Family	9.49	6.54	10.61	4.91	-1.154	0.251	3.84	2.50	10.61	4.91	**-10.275**	**.000**
		Teacher	12.77	6.03	12.06	6.08	0.697	0.487	2.33	2.48	11.90	5.98	**-12.353**	**.000**
	Behavioral Problems	Family	7.66	3.60	8.33	3.20	-1.165	0.246	3.96	2.16	8.40	3.18	**– 9.651**	**.000**
		Teacher	12.16	4.11	11.03	4.01	1.644	0.102	3.41	1.34	10.71	4.11	**-14.108**	**.000**
	Peer Problems	Family	10.89	1.82	11.36	1.87	-1.509	0.133	5.77	1.35	11.50	1.83	**-21.046**	**.000**
		Teacher	12.63	2.75	13.01	2.43	-0.878	0.381	6.39	2.37	12.91	2.41	**-16.142**	**.000**
TOTAL SCORES		Family	38.86	9.73	41.66	8.72	-1.792	0.075	18.29	4.12	42.14	8.49	**-21.134**	**.000**
		Teacher	48.01	9.03	46.91	8.74	0.732	0.465	21.70	4.54	46.36	8.53	**-21.329**	**.000**

**SDQ: Strengths and Difficulties Questionnaire*

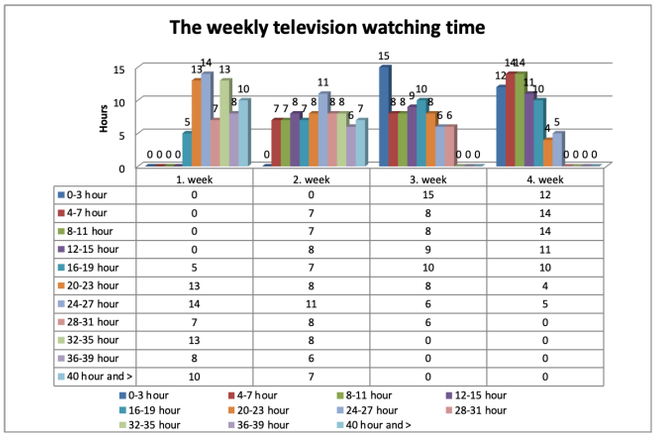
Table 3. *The comparison of the SDQ* intragroup pre-test and post-test mean scores of the families and teachers in the experimental group and control group*

Strengths and Difficulties Questionnaire Subscales and Total Scores			Experimental Group Pretest (n=70)		Experimental Group Posttest (n=70)		Test and p values		Control Group Pretest (n=70)		Control Group Posttest (n=70)		Test and p values	
			$\bar{x}$	Sd	$\bar{x}$	Sd	t	p	$\bar{x}$	Sd	$\bar{x}$	Sd	t	p
SUBSCALES	Social Behaviors	Family	10.60	3.74	16.96	2.23	**– 11.667**	**.000**	10.10	3.21	10.07	2.10	**0.723**	**0.041**
		Teacher	4.14	1.63	17.53	2.51	**– 44.320**	**.000**	4.67	1.82	4.60	1.39	1.676	0.187
	Attention Deficit and Hyperactivity	Family	10.83	2.47	4.71	1.42	**17.078**	**.000**	11.36	2.69	11.63	2.62	– 2.727	0.008
		Teacher	10.46	1.82	9.57	2.55	**2.502 .**	**000**	10.81	1.97	10.83	1.88	– 0.178	0.859
	Emotional Problems	Family	9.49	6.54	3.84	2.50	**6.353**	**.000**	10.61	4.91	10.71	4.11	**– 13.513**	**0.000**
		Teacher	12.77	6.03	2.33	2.48	**14.929**	**.000**	12.06	6.08	11.90	5.98	1.953	0.055
	Behavioral Problems	Family	7.66	3.60	3.96	2.16	**7.007**	**.000**	8.33	3.20	8.40	3.18	– 1.689	0.096
		Teacher	12.16	4.11	3.41	1.34	**14.809**	**.000**	11.03	4.01	10.71	4.11	1.425	0.159
	Peer Problems	Family	10.89	1.82	5.77	1.35	**17.616**	**.000**	11.36	1.87	11.50	1.83	**– 3.054**	**0.003**
		Teacher	12.63	2.75	6.39	2.37	**13.500**	**.000**	13.01	2.43	12.91	2.41	– 1.000	0.321
TOTAL SCORES		Family	38.86	9.74	18.29	4.12	**14.939**	**.000**	41.66	8.72	42.14	8.49	**– 3.885**	**0.000**
		Teacher	48.01	9.03	21.70	4.54	**20.299**	**.000**	46.91	8.74	46.36	8.53	1.667	0.100

**SDQ: Strengths and Difficulties Questionnaire*

According to the Table 3, it was determined that the difference between the intragroup pre-test and post-test Strengths and Difficulties Questionnaire subscale and total scores of the families in the control group is statistically significant (p< .05) and there is a negative change; and the difference between the intragroup pre-test and post-test Strengths and Difficulties Questionnaire subscale and total scores of the teachers is not statistically significant (p> .05).

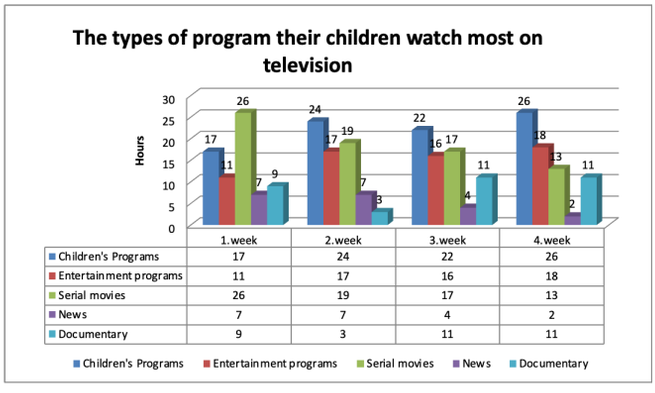
When the Figure 1 was analysed, the difference between the 4-week observations made by the families in the experimental group included in the study on how many hours a day their children watched TV on weekdays was found statistically significant (p< .05).



	1. week	2. week	3. week	4. week
0-3 hour	0	0	15	12
4-7 hour	0	7	8	14
8-11 hour	0	7	8	14
12-15 hour	0	8	9	11
16-19 hour	5	7	10	10
20-23 hour	13	8	8	4
24-27 hour	14	11	6	5
28-31 hour	7	8	6	0
32-35 hour	13	8	0	0
36-39 hour	8	6	0	0
40 hour and >	10	7	0	0

Figure 1. *The observations of the children of the families in the experimental group participating in the study on the weekly television watching time*

According to the Figure, it is seen that while the TV watching time of the children was 40 hours or more in the first weeks, and there was no children watching TV for less than 16 hours; as training progresses, the weekly TV watching time of children gradually decreased, and in the last week, the TV watching time was not more than 27 hours.



	1.week	2.week	3.week	4.week
Children's Programs	17	24	22	26
Entertainment programs	11	17	16	18
Serial movies	26	19	17	13
News	7	7	4	2
Documentary	9	3	11	11

Figure 2. *The observations of the families in the experimental group included in the study, regarding the types of program their children watch most on television during a week*

The Figure 2 gives the observations of the families in the experimental group included in the study, regarding the types of show their children watch most on television during a week. When the observations of the families in the experimental group regarding the types of show their children watch most on television during a week analysed, it was found that 37.1% of children watch TV series in the first week and 37.1% of children watch children's shows in the fourth week, and the difference between them was not found statistically significant (p> .05).

4. DISCUSSION

The findings of the study, which was conducted to determine the effect of structured counselling given to families and teachers depending on the television watching frequency of children going to pre-school education institutions, on their emotional-behavioural problems, were discussed with the relevant literature.

It was determined that 71.4% of the children in the experimental group and 75.7% of the children in the control group watch TV for 3-4 hours on every weekday. It was found that 51.4% of the children in the experimental group watch TV over 5 hours on every weekend day. When the literature was reviewed, it was emphasized that the ideal television watching time of children should be between 5 and 7 hours a week on average, and that the watching time should be less than 2 hours a day and under parental supervision (1,7,13-16). A child's relationship with his friends, and participation in activities such as games, painting, etc. is important in the social development of the child. Therefore, it is a major need of any child to be able to play social games, which are appropriate for his/her age, with his playmates under adult supervision. Important personality traits such as livelihood, compromise, cooperation, leadership, beneficial competition, and altruism can only be learned in this environment (2,17,18). In this context, the children included in the study constitute the risky group in terms of TV watching duration and preferring to watch TV in their spare time.

The study found that 42.9% of the children in the experimental group and 41.4% of the children in the control group watch TV series instead of children's shows suitable for their age, although the shows they watch are chosen by their mothers and their watching is supervised by their parents. The children should not watch all shows, but the shows that are appropriate for them and in certain periods so that they can participate in other activities as necessary for their development. It should be noted that development and learning in the pre-school period is very fast, and the learning and experiences gained in these years will leave permanent traces that are difficult to correct on their future lives and learning (1). When the literature was reviewed in this context, it was found that aggressive behaviours in children are seen especially in children who watch programs that contain aggression rather than age-appropriate children's programs (2,19). Within this framework, the study results once again reveal the necessity of the study.

Zimmermann et al. (20) points out that preschool children watch television for an average of 3.6 hours a day and have 27% risk in terms of behavioural problems, and excessive stimulation on television affects the child's brain development negatively, and there is a significant relationship between watching television and behavioural problems.

In the study, a statistically significant relationship was found between the post-tests in terms of the total score and subscale scores of the strengths and difficulties questionnaire filled by the families and teachers in the experimental group and the control group for children ($p< .05$). It can be said that there is a difference in the results in favour of the experimental group, in other words, the training given and the charts and plaques distributed to the families and teachers in the experimental group positively affect the TV watching frequency.

According to the Table 2, when the results after the training given to the families and teachers in the experimental and control groups are examined, it was seen that there was a positive change in terms of TV watching frequency and an increase in the social behaviour score after training in the experimental group compared to the control group. Considering the directly proportional relationship between the increase in social behaviour score and the increase in social behaviour tendencies in children, it can be said that there is a positive change in social behaviours of the children in the experimental group after the training. In the study, a decrease was seen in the scores of attention deficit and hyperactivity scale, emotional problems scale, behavioural problems scale and peer problems scale after training in the experimental group compared to the control group. Considering the directly proportional relationship between the decrease in those scores and the decrease in the said problems of children, it can be said that it has a positive effective on attention deficit and hyperactivity, emotional problems, behavioural problems, and peer problems**.** In this context, the hypothesis H1 of the study is supported by saying that the counselling given to parents and teachers depending on the TV watching frequency has an effect on the average scores of children's emotional behavioural problems. In this context, when the relevant literature was reviewed (1,21,22), it was seen that the family training programs used for prevention and treatment of emotional and behavioural problems in children have a significant effect, and the emotional behavioural problems decrease in the children of the families trained compared to the children of the families who are not trained.

In line with these results; It is thought that as children's TV watching time increases, their time to communicate with their peers decreases, and peer problems come to light. In the study, in the education within the scope of the study, in which the family and the teacher were discussed together, it was emphasized that the child's TV watching times were followed up weekly, as well as directing the child to activities that could replace TV; It is thought that directing the child to play and activities has a positive effect on peer problems. Just as we adults have a job, children's job is to play. Children express their feelings through play, put their behaviour into play, in fact, play is a kind of place for the child to talk. The decrease in emotional-behavioural problems in children who are directed to play by being removed from the TV when they are still is among the expected results. In addition, considering that in the case of attention deficit hyperactivity, the child is actually unable to focus on one thing because it directs attention to more than one thing, it can be claimed that more than one stimulus such as TV (sound, image transitions, advertisements, etc.) reinforces the attention deficit hyperactivity state. It is thought that removing the child from the TV and directing him to the game or to the activities in which he takes an active role provides support in focusing his attention on a single thing, and in this case, it has a positive effect in the case of attention deficit and hyperactivity, as in the result of the study.

In this direction, the results of the study were discussed with the provincial national education, in-service trainings were carried out, and the continuity of the study results was continued and very positive feedback was received from the relevant institutions.

The study found that the difference between the strengths and difficulties questionnaire pre-test and post-test scores of the families and teachers in the experimental group was statistically significant ($p< .05$). It can be said that the training given to families and teachers and the follow-up of weekly TV watching with the help of a schedule have an effect on the TV watching duration as well as raising awareness about the child's own situation. Besides, it is thought that the results of the study were also affected by the plaques given weekly to the students who watched TV least according to the weekly TV watching frequency results sent to teachers by families through children. It can be emphasized that the results were also affected by the fact that this weekly follow-up was carried out with the cooperation of parent-teacher-child.

According to the Table 3; it can be said that social behaviour scores are below normal values in pre-tests. It is observed that, as the TV watching frequency of the children decreases with the training given to the families and teachers and weekly follow-up of TV watching, there is a positive change in the children's social behaviour scores. Likewise, it should also be noted that when the TV watching frequency decreases, there is a positive change in the means scores for children's emotional, behavioural, and peer problems. Another remarkable result of the study is that in the pre-test results of the families about the children, while their mean scores for emotional behavioural problems are less, the teachers' mean scores for emotional behavioural problems in children were higher. However, it was determined that there was a serious decrease in emotional and behavioural problems in both groups after the training, the TV watching schedule and the plaques given. When the relevant literature was reviewed (1,23,24), it was found that when the families set rules about watching television after the training given to the families, the social behaviour tendencies of the children increased,

their emotional behavioural problems decreased, and the children developed alternative perspectives and they turned to games. Furthermore, the relationship between the child and the teacher, who represents the parents in the school, encourages the children to exhibit positive behaviours (1,25). These results support the hypothesis H1 suggesting that the counselling given to the parents and teachers depending on the TV watching frequency has an effect on the mean scores of the emotional behavioural problems of the children, revealing the effectiveness of the teachers' following up the children regarding the TV watching frequency and awarding the students who watched TV least a week.

When the observations of the families in the experimental group regarding the weekly TV watching time of the children were examined; the difference between them was found to be statistically significant ($p < .05$). When the results were analysed; It was seen that there were no student watching TV for less than 16 hours in the first week. This situation shows that children are in the risky group in terms of TV watching duration. Accordingly, when weekly follow-ups are made after the training given to parents and teachers, it was determined that the weekly TV watching duration of the children decreased. This decrease in the duration of watching TV for children shows the effectiveness of the study as well as its excitability**.**

When the observations of the families in the experimental group regarding the frequency of the shows that children weekly watch most on television were examined; it was found that most of the children watch TV series. In the study by Güngör and Ersoy, parents stated that the most favourite programs of children were domestic movies and TV series, while children's shows ranked third**.**

During the family and teacher training, it was also emphasized that the shows watched by the child must be appropriate for his/her age since the child may not realize that the shows (news, TV series, documentaries, etc.) he/she watches with his/her family are informative. Furthermore, since the child does not have the life experience of an adult and has not yet completed his cognitive and sensory development, he/she is likely to be affected by violent shows or shows which may cause fear or stress (26). Studies suggest that children are affected by the movies they watch and imitate scenes of violence (12). The increase in the rate of watching programs suitable for the age of children (children's shows) as a result of the study shows once again that the structured counselling program provided to families and teachers has achieved its purpose.

5. CONCLUSION

In this study, which was conducted to determine the effect of TV watching frequency of children going to pre-school education institution on their emotional-behavioural problems, it has been found that, after the counselling training given to parents and teachers, there was a significant positive change in the mean scores for children's emotional and behavioural problems, and a significant positive change was achieved in the children's attention deficit and hyperactivity disorders, the mean scores for peer problems and the TV watching durations.

Based on these findings, we recommend to:

- Follow up the children's TV watching frequencies in all institutions by nurses using their role of educator and researcher in pre-school education institutions, and
- Give training to the families and teachers of preschool children on 'the role of television in development of children' and repeat the study with a different sample group.
- The results of the study can be listed among the suggestions to ensure the continuity of the nurses during their educational roles, which is one of the important duties, especially in the in-service trainings in schools.

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Clinical and Experimental Health Sciences

Determination of the Plasma Levels of Growth Arrest Specific 6 in Colon Cancer Patients

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ABSTRACT

Objective: Growth arrest-specific 6 (GAS 6) has a role in cell proliferation and was detected in different cancer types. The aim of this study was to evaluate the plasma GAS 6 levels in colon cancer patients.

Methods: This was a prospective study and conducted in a research and training hospital in Turkey. Thirty-three healthy volunteers were enrolled in the control group, thirty-three colon cancer patients who were diagnosed with colon cancer for the first time. Sociodemographic characteristics of participants were recorded. Blood samples of the control group were taken once a time. Patients' blood samples were taken before and one month after surgery.

Results: There were no statistically significant differences between the sociodemographic characteristics of the two groups. The mean plasma GAS 6 levels in control were significantly higher than that of colon cancer patients ($p<.0001$). There is a statistically significant increase in GAS 6 values after surgery ($p<.0001$).

Conclusion: It was observed that plasma GAS 6 levels of the patients were lower than the control group and were elevated after surgery. We think that our study will contribute to the literature in addition to studies showing that GAS 6 levels may be associated with survival and prognosis in different cancer types.

Keywords: Colon cancer, growth arrest-specific 6, patient, plasma levels

1. INTRODUCTION

Growth arrest-specific 6 (GAS 6) is a ligand of the tyrosine kinase receptors (TKRs) (1-5). GAS 6 is a negative regulator of coagulation (1,6). It was shown that GAS 6 promotes foam cell formation in the atherosclerotic process via inducing scavenger receptor expression in vascular smooth muscle cells (1,4).

GAS 6 a vitamin K-dependent protein that was first identified in growth arrested cells (1). TKRs are important parts of intracellular signaling pathways involved in vital functions of the cell (5,6). GAS 6 has an important role in the phagocytosis of apoptotic cells in vivo (2). GAS 6 is also thought to be effective in cell proliferation because of its strong mitogenic properties for protein S smooth muscle cells and their similarity (2). GAS 6 has a role in maintaining growth and cell viability. It ensures the differentiation of cells, maintains their phagocytosis task and prevents apoptosis (1).

GAS 6 is ligand of Tyro3, Axl and Mer receptors (TAM) and acts as agonist (1). The binding affinity of GAS6 to these three receptors is Axl ≥ Tyro3 ≥ Mer, respectively (3). It is understood that GAS 6 has growth factor-like functions due to its role in intercellular adhesion and stimulating cell migration as a result of interaction with the Axl receptor (1). Axl is the most studied and has been shown to be elevated in many cancers (7). GAS 6 also has an important role in cell proliferation and phagocytosis of apoptotic cells (1-3). In vitro studies have shown that GAS 6 plays a role in cancer development and progression in various cancer cells (8). However controversial results have been reported regarding the prognostic value of the GAS 6 in different cancers (9-14). In a recent study, it was found that the GAS 6 protein has an inhibitory effect on intestinal tumorigenesis (14). According to a review, there are a few studies with GAS 6 and colorectal cancer (15). In this study, we aimed to evaluate to determine plasma GAS 6 levels in patient with colon cancer which is the third most common cancer type around the world.

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2. METHODS

This study was approved by Ethics committee of Marmara University Institute of Health Sciences Health Clinical / Human Research Ethics Committee (01.03.2013-17). This study supported by the Marmara University Scientific Research Projects Commission (project no: SAG – C – DRP – 100.713.0306). Thirty-three healthy volunteers were enrolled to the control group and thirty-three patients who first diagnosed with colon cancer were enrolled to the colon cancer patients' group. Sociodemographic characteristics of two groups were collected and recorded. Additionally, carbohydrate antigen 19-9 (CA19-9) and carcinoembryonic antigen (CEA) levels were recorded for colon cancer patients.

2.1. Determination of Plasma GAS 6 Levels:

Blood samples of control group were taken once a time. Colon cancer patients' blood samples were taken before and one month after surgery. Blood samples were collected from the patients for one time and kept in an Ethylenediaminetetraacetic Acid (EDTA) tubes, then centrifuged at 3500 × *g* for 15 minutes and were kept at −80 °C until the day of analysis. All blood samples were taken in the morning. We have validated Enzyme-Linked Immunosorbent Assay (ELISA) assay for the determination of GAS 6 in plasma with kits provided by R&D Systems (Minneapolis, MN, USA) as previously described in detail (16).

2.2. Statistical Analysis

Statistical Package for Social Sciences (IBM, SPSS Statistics for Windows, version 15.0. Chicago, SPSS Inc.) was used for data analysis. Data were presented as percent and mean ± standard error. Statistical significance was expressed as a *p* value <.05. Distribution of the data was assessed with Kolmogorov-Smirnov test, Shapiro-Wilk, skewness, kurtosis, and histogram. It was found that data were not normally distributed.

3. RESULTS

A total number of 33 colon cancer patients and 33 healthy volunteers were included in this study and socio-demographics features of the groups were as presented in Table 1. There were no significant differences between the groups (p>.05).

Plasma GAS 6 levels of all participants were given in Table 2. The median of the plasma GAS 6 level of control group was found to be as 10.46 ng/mL (7.12-17.13) (n=33) while it was found to be as 7.27 (3.97-10.31) ng/mL (n=33) in colon cancer patients and this difference was significant (p<.01).

Table 1. *Characteristics of the control group and colon cancer patients*

		control group (n=33)		Colon cancer patients (n=33)		p*
		n	%	n	%	
Gender	**Female**	16	48.5	13	39.4	p=.61
	Male	17	51.5%)	20	60.6	
Age (median IQR)		60 (52-69)		63 (54-72)		p=.57
Smoking status	**Yes**	4	12.1	9	27.3	p=.78
	No	29	87.9	24	72.7	
Family history of cancer	**Yes**	6	18.2	8	24.2	p=.47
	No	27	81.8	25	75.8	
Comorbid disease	**Yes**	21	63.6	22	66.7	p=.48
	No	12	36.4	11	33.3	

p: between two groups; IQR: interquartile range (25th percentile-75th percentile); Mann-Whitney U test analysis and Spearman's correlation tests were performed*

Table 2. *Plasma GAS 6 levels of control group and colon cancer patients*

	Control group (median IQR)	Colon cancer patients *(Before the surgery)* (median IQR)	p
Plasma GAS 6 levels (ng/mL)	10.46 (7.12-17.13) (n=33)	7.27 (3.97-10.31) (n=33)	p=.002

n: number of patients; IQR: interquartile range (25th percentile-75th percentile), GAS 6: growth arrest-specific 6; Mann-Whitney U test analysis was performed

The median level of CEA in colon cancer patients were found to be 31.20 (2.87-12.47) (n=22) before surgery and 1.69 (1.27-4.20) (n=22) after surgery (p<.05). Similarly, the median value of CA 19-19 was found to be 9.30 (5.28-24.00) (n=20) before surgery and 10.50 (6.35-19.95) (n=21) after surgery (p>.05) (Table 3).

Table 3. *Plasma levels of GAS 6, CEA and CA in colon cancer patients*

	Plasma GAS 6 levels (ng/mL)	Plasma CEA levels (normal range: 0-3 ng/mL)	Plasma CA 19-9 levels (normal range: 0-35 U/mL)
Before the surgery **(median, IQR)**	7.27 (3.97-10.31) (n=22)	31.20 (2.87-12.47) (n=22)	9.30 (5.28-24.00) (n=20)
One month after the surgery **(median, IQR)**	8.27 (4.75-14.63) (n=22)	1.69 (1.27-4.20) (n=22)	10.50 (6.35-19.95) (n=21)
p	p=.00002	p=.01	p=.51

n: number of patients; IQR: interquartile range (25th percentile-75th percentile), GAS6: growth arrest-specific 6; CEA: carcinoembryonic antigen; CA 19-9: carbohydrate antigen 19-9; Wilcoxon test analysis was performed

There was no significant correlation between the plasma GAS 6 and CEA levels before surgery in colon cancer (Spearman's r = 0.08; p>.05). Similarly, there was no significant correlation

between the plasma GAS 6 and CA 19-9 levels before surgery in the cancer groups (Spearman's r = 0.19; p>.05).

4. DISCUSSION

Tyrosine kinase receptors are the transmembrane proteins, forming extracellular signals to provide adhesion and motility of cells so help the cell living, growth, differentiation (17). In the pathogenesis of many forms of cancer, the increase observed in the secretion or activities of TKR become a current issue and this will help for the investigation of new therapies (18,19). In our study have two results. The first result presents the determination of the plasma GAS 6 levels in colon cancer patients. The second result presents the evaluation of the GAS 6 levels before and after surgery.

In a previous study it has been shown that GAS 6 levels increased in thyroid cancer (10). In another study, GAS 6 over expression is mostly observed in acute myeloid leukemia (AML) and it was reported that GAS 6 expressed by AML blasts could be a marker of poor clinical outcomes (20). Similarly, it was found that high expression of activated Axl was an independent predictor for worse prognosis in patients with osteosarcoma (21). In contrast it was shown that increased activation of tissue GAS 6 levels in the kidney was associated with good prognosis in patients with renal cell carcinoma (22). In another previous study it was shown that higher expression of GAS 6 in breast cancer tissue was associated with improved outcomes (11). Similarly, it was found that a positive correlation was observed between increased GAS6 levels in the tissues of patients with brain tumors and worsening of prognosis (23).

Uribe et al. reported that Axl promotes migration and invasion in colorectal cancer (24). In contrast to this study, Akitake – Kawano et al. showed that higher GAS 6 plasma levels were associated with better survival in patients with colorectal cancer (14). Similarly, we found that the mean plasma GAS 6 levels of patients with colon cancer, were significantly lower than those of the control group (p<.05). In this study, plasma GAS 6 levels increased one month after surgery in patients with colon cancer (p<.05).

Limitations: The number of patients in our study, which was conducted within the scope of the doctoral thesis, is small due to time constraints. In addition, the fact that the Axl levels of the patients could not be evaluated is another limitation.

5. CONCLUSION

In conclusion, considering the results of this study in colon cancer patients, changes in GAS 6 plasma levels were found to be correlated positively with the changes in colorectal cancer markers. In addition, depending on our results and the literature (15,24,25), the increase in GAS 6 plasma levels after surgery suggests that GAS 6 may be used to monitor and evaluate the success of treatment. However further randomized controlled studies are needed.

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Acquisition of data for the study: ST, FVI, FU, OBO, VA, PFY
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Clinical and Experimental Health Sciences

Evaluation of Traditional and Complementary Medicine Applications Used in the COVID-19 Pandemic

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ABSTRACT

Objective: Traditional and complementary medicine practices have been used to protect and treat physical and mental diseases in different cultures and beliefs from past to present. In this study, it was aimed to determine which of the traditional and complementary medicine applications was used and its effectiveness in the pandemic.

Methods: 979 people aged 18 and over (between 18 and 73) participated in the study. In the online survey, province, age, gender, occupation, education status, marital status, presence of children, presence of chronic diseases, COVID-19 diagnosis status, whether there is a COVID-19 vaccine, awareness of traditional and complementary medicine practices and nutritional and food supplements, utilization has been questioned.

Results: It was observed that no nutritional and food supplements used by the participants before and during the coronavirus pandemic did not reduce the frequency of being diagnosed with COVID-19.

Conclusions: In the study, it was observed that no food and nutritional supplement reduced the frequency of receiving COVID-19. However, in order to evaluate the effectiveness of nutritional and food supplements, it was concluded that the sample of the participants should be equal in terms of occupation, age, whether they were infected or not, and that it should be supported by researches by questioning whether the patients diagnosed with COVID-19 had mild symptoms or not.

Keywords: Supplement, pandemic, protect, traditional, complementary medicine.

1. INTRODUCTION

Traditional and complementary medicine (TCM) is the whole of knowledge, skills and practices that can be explained or not, based on beliefs and experiences, fed from different cultures in different geographies for centuries, used to prevent, diagnose, treat and maintain the continuation of people's physical and mental diseases. Today, many individuals in the society often resort to TCM methods in addition to modern medical practices, both for treatment, preventive purposes, and culturally (1).

COVID-19 (COVID-19 – new coronavirus), a new coronavirus disease known to be of zoonotic origin, accompanied by severe acute respiratory problems and rapidly transmitted from person to person, is an important health problem that has become a global pandemic (2). With the failure of antibiotics in this viral infection that is not caused by bacteria, it is reported that the most effective method of fighting it today is not to catch the disease at all. Although vaccination studies and initiation of implementation are hopeful, since it will take time to vaccinate a certain percentage of the population, the importance of strengthening the immune system of individuals with symptomatic approaches and supportive treatments is emphasized (3).

Studies reveal that these practices, which are described as traditional and complementary therapies today, take their place as both an academic and a popular curiosity area, which attracts a lot of attention among the public during the coronavirus global epidemic (4).

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Therefore, in this study, it was aimed to determine which of the traditional and complementary medicine applications is used most by individuals during the pandemic period.

2. METHODS

2.1. Research Design and Participants

The study was conducted as a descriptive online survey between March 01 and April 01, 2021. Persons aged 18 and over who agreed to participate in the study were included in the study, persons under the age of 18, those who were mentally incapable and illiterate were excluded. In line with the inclusion criteria of the study, a total of 979 people aged 18 and over (between 18-73 years old) were included in the study. Necessary explanations were given to the participants aged 18 and over who voluntarily agreed to participate in the study, and their "informed consent" was obtained before the study. All rights of the participants were respected and the principles of voluntariness and confidentiality were taken into consideration.

2.2. Data Collection

The survey we conducted online has 25 questions (1-3) and it questions; personal information consisting of the province where people live, age, gender, profession, education status, marital status, presence of children, presence of chronic diseases, whether they have been diagnosed with COVID-19, whether there is a vaccine for COVID-19, and awareness of traditional and complementary medicine practices, the latest benefit from these applications in 10 years, awareness of nutritional and food supplements and usage status of nutritional and food supplements in the last 1 year.

2.3. Data Analysis

IBM SPSS statistics 22.0 program was used for statistical analysis in the study. While evaluating the study data, in addition to descriptive statistical methods (mean, standard deviation, frequency), Chi-square test was used to evaluate the relationship between variables, Student T test was used to compare data with normal distribution, and Mann-Whitney U test was used for comparison of data that did not show normal distribution. The results were evaluated at the 95% confidence interval and the significance level of $p< .05$.

2.4. Ethical Considerations

After obtaining permission from the Ministry of Health Scientific Research Platform before starting the study, ethics committee approval was obtained from the Ethics Committee of a university with the decision number 22/421 and dated 29.12.2020.

3. RESULTS

The research was carried out between 01 March – 01 April 2021 by reaching a total of 979 people using the online survey method. The distribution of the demographic characteristics of the participants is shown in Table-1, 68.23% (n=668) of the participants were female, 29.8% (n=285) had at least one chronic disease, 24.4% (n=239) were infected with the COVID-19 virus and 42.7% (n=418) were vaccinated against COVID-19 (Table-1).

Table 1. *Distribution of demographic characteristics (N=979)*

	n	%
Gender		
Women	668	68.23
Men	311	31.77
Age range		
Between 18-24	98	10.01
Between 25-34	238	24.31
Between 35-44	387	39.53
Between 45-54	206	21.05
Aged 55 and over	41	5.10
Place of residence		
İstanbul	691	70.6
Ankara	39	4
İzmir	19	1.9
Other cities	230	23.5
Occupation		
Nurse/Health Officer/Midwife	284	29.01
Support workers who are health workers	121	12.36
Teacher, Lawyer, Police officer	95	9.7
Health Academicians, Licensees and Technicians	89	9.09
Housewife	63	6.44
Banker/Accountant/Economist	40	4.09
Freelancer	32	3.27
Doctor	31	3.17
Others	224	22.88
Marital status		
Married	647	66.1
Single	332	33.9
Status of Having a Child		
None	384	39.2
1 Child	214	21.9
2 Children	287	29.3
3 Children and over	94	9.6
Presence of Chronic Diseases		
None	694	70.2
Presence of a Chronic Disease	140	14.3
Presence of Multiple Chronic Diseases	145	15.5
COVID-19 Diagnosis Status		
Yes	239	24.4
No	740	75.6
Availability of COVID-19 Vaccine		
Yes	418	42.7
No	561	57.3

Participants marked more than one option for the questions about benefiting from traditional and complementary medicine approaches, using nutritional and food supplements before and during the coronavirus pandemic in the last 10 years and it was determined that phytotherapy method was the most used (15%) among the TCM methods, and vitamin D (49.9%) was the most used nutritional and food supplements before the coronavirus pandemic, and vitamin C (48.1%) was the most used during the coronavirus pandemic (Table-2).

When the relationship between the use of food and food supplements before the coronavirus pandemic and the status of being diagnosed with COVID-19 was examined, it was seen that no food and food supplement decreased the frequency of being diagnosed with COVID-19. It was determined that individuals using Black Elderberry extract, propolis and vitamin C were diagnosed with COVID-19 at a higher rate ($p<0.05$) (Table-3). In addition, when the relationship between the use of food and food supplements and the status of being diagnosed with COVID-19 during the coronavirus pandemic period was examined, it was determined that no nutritional and food supplement decreased the frequency of being diagnosed with COVID-19. It was observed that individuals using black cumin oil, propolis, vitamin C, ginger and mixed herbal teas were diagnosed with COVID-19 at a higher rate ($p<0.05$) (Table-3).

Table 2. *Utilization of traditional and complementary medicine methods and use of nutritional and food supplements**

Questions	Which and/or which of the traditional and complementary medicine approaches have you benefited from in the last 10 years?			Which and/or which of the following food and nutritional supplements did you use before the coronavirus pandemic?		Which and/or which of the following food and nutritional supplements have you used during the coronavirus pandemic?	
Approache	*n*	*%*	Nutritional and food supplements	*n*	*%*	*n*	*%*
Acupuncture	*85*	*8.7*	***Beta Glucan***	*65*	*6.6*	*91*	*9.3*
Leech Therapy	*41*	*4.2*	***Vitamin D***	*489*	*49.9*	*164*	*16.8*
Cup Therapy	*120*	*12.3*	***Vitamin C***	*428*	*43.7*	*471*	*48.1*
Phytotherapy	*147*	*15*	***Zinc***	*191*	*19.5*	*218*	*20.3*
Ozone Therapy	*56*	*5.7*	***Omega 3***	*243*	*24.8*	*184*	*18.8*
Hypnosis	*14*	*1.4*	***Collagen***	*78*	*8*	*84*	*8.6*
Mesotherapy	*23*	*2.3*	***Elderberry extract***	*72*	*7.4*	*109*	*11.1*
Reflexology	*22*	*2.2*	***Propolis***	*149*	*15.2*	*162*	*16.5*
Homeopathy	*12*	*1.2*	***probiotic***	*208*	*21.2*	*123*	*12.6*
Apitherapy	*3*	*0.3*	***Black cumin oil***	*141*	*14.4*	*107*	*10.9*
Chiropractic	*14*	*1.4*	***Ginger***	*334*	*34.1*	*232*	*23.7*
Osteopathy	*2*	*0.2*	***Mixed herbal teas***	*359*	*36.7*	*182*	*18.6*
Prolotherapy	4	0.4	**Combined vitamins (B, C, E vit etc.)**	243	24.8	241	24.6

**Multiple options ticked*

Table 3. *The relationship between the use of nutritional and food supplements before and during the coronavirus pandemic and the status of being diagnosed with COVID-19 (N=979)*

Nutritional and food supplements	Usage status	Before the coronavirus pandemic			During the coronavirus pandemic		
		People diagnosed with COVID – 19 (n:239)	People who are not diagnosed with COVID – 19 (n:740)	p	People diagnosed with COVID – 19 (n:239)	People who are not diagnosed with COVID – 19 (n:740)	p
Black cumin oil	*Using*	*41*	*100*	*0.163*	*39*	*68*	***0.002****
	Not using	*198*	*640*		*200*	*672*	
Omega-3	*Using*	*64*	*179*	*0.420*	*51*	*133*	*0.247*
	Not using	*175*	*561*		*188*	*607*	
Propolis	*Using*	*47*	*103*	***0.032****	*55*	*107*	***0.002****
	Not using	*192*	*637*		*184*	*633*	
Probiotic	*Using*	*58*	*150*	*0.189*	*29*	*94*	*0.818*
	Not using	*181*	*590*		*210*	*646*	
Collagen	*Using*	*21*	*57*	*0.591*	*18*	*66*	*0.505*
	Not using	*218*	*683*		*221*	*674*	
Combined vitamins	*Using*	*68*	*175*	*0.135*	*60*	*181*	*0.840*
	Not using	*171*	*565*		*179*	*559*	
Black elderberry extract	*Using*	*30*	*42*	***0.000****	*32*	*77*	*0.202*
	Not using	*209*	*698*		*207*	*663*	
Beta glucan	*Using*	*21*	*44*	*0.125*	*22*	*69*	*0.956*
	Not using	*218*	*696*		*217*	*671*	
Vitamin D	*Using*	*132*	*357*	*0.060*	*130*	*352*	*0.070*
	Not using	*107*	*383*		*109*	*388*	
Vitamin C	*Using*	*125*	*303*	***0.002****	*130*	*341*	***0.025****
	Not using	*114*	*437*		*109*	*399*	
Ginger	*Using*	*82*	*252*	*0.942*	*76*	*156*	***0.001****
	Not using	*157*	*488*		*163*	*584*	
Zinc	*Using*	*57*	*134*	*0.051*	*55*	*163*	*0.750*
	Not using	*182*	*606*		*184*	*577*	
Mixed Herbal Teas	*Using*	*80*	*279*	*0.238*	*63*	*119*	***0.000****
	Not using	*159*	*461*		*176*	*621*	

*Mann-Whitney U; *p< .05; **p< .001*

4. DISCUSSION

Although the effect of traditional and complementary medicine practices on modern medicine has not been proven yet, the rate of use by individuals is quite high today. With modern life, the belief that natural products are better and more reliable on individuals instead of pharmacological agents is shown as one of the main reasons for this rise in use (5).

While the use of any of the TCM methods in healthy adult individuals is 40% in the United States (USA), this rate is around 80% in Turkey (6). While the use of TCM in sick individuals with any chronic disease varies between 4 and 79% in various countries of the world; In studies conducted in Turkey on this subject, it has been reported that TCM use is between 12-76% (7). In the study of Güven et al. (8) in 2013, hypertension patients used TCM methods to lower high blood pressure; In the study of Kaynak and Polat (9) in 2017, in order to regulate the blood sugar level of diabetes patients; In the study of Saghatchian et al. (10) in 2014, it was reported that patients with breast cancer resorted to TCM methods to prevent or reduce the side effects of treatment. In this study, it was observed that the majority of individuals (70.2%) did not have any chronic disease, and individuals with one chronic disease (14.3%) and more than one chronic disease (15.5%) were identified. It has been determined that there is no difference in the use of TCM between those with and without chronic disease due to the low number of patients with chronic diseases. When we look at the literature, we see that the elderly population is mostly diagnosed with chronic diseases. The reason for the high rate of those without chronic diseases in the present study was that the age population of the participants was under

55 (94.6%), and we think that this may affect the use of TCM methods.

In the study of Paltiel et al. in Israel in 2001, the types of TCM used in terms of frequency were homeopathy, relaxation therapy and reflexology, respectively; In the USA, it has been reported that there are spiritual treatments (35%), vitamins and herbs (41%), body/mind therapies (10%) (11). In the study conducted by Frass et al. in 2012, it was stated that there was a significant increase in the use of TCM applications in all countries between 1990 and 2006, and the most commonly used TCM methods were herbal treatments, chiropractic applications, massage and homeopathy (12). In the study of Lafci and Kasikci with healthcare professionals, 25% of the individuals used at least one TCM method, along with herbal treatment and massage being the most common TCM methods; It has been reported that the methods with the most information are acupuncture, herbal therapy, dietary support and massage, respectively (13). In this study, it was determined that phytotherapy was used the most (15%) among the TCM methods, vitamin D (49.9%) was the most used nutritional and food supplement before the coronavirus pandemic, and vitamin C (48.1%) was the most used during the coronavirus pandemic. When we look at the differences in the use of TCM methods in the countries and in the present study, the geographical location of the countries, the economic and socio-cultural levels of the countries may be effective in terms of the ease of accessing the TCM methods, the abundance of studies on vitamin D and vitamin C in the literature in recent years, the fact that nutritional and food supplements and medicinal plants are mentioned frequently in social media and news programs, the rate of health workers in this research being high (53.63%) can be said. In the past, herbal medicines have played an important role in the control of infectious diseases. Clinical evidence from a number of studies of herbal medicine in the treatment of SARS coronavirus (SARS-CoV) has shown important results and supported the idea that herbal medicine is effective and beneficial in the treatment/prevention of epidemics (14). In a Cochrane systematic review by Liu et al., it was reported that herbal treatments combined with Western medicine can reduce symptoms and improve quality of life in SARS-CoV patients (15). In the meta-analysis by Luo et al., it was also reported that herbal treatments reduced the rate of H1N1 influenza infection (16). According to the results of Kalayci et al.'s studies; It has been emphasized that the process has been successfully overcome in COVID-19 disease in China by evaluating the mechanisms that affect the immune system in general, phytotherapy and acupuncture, which are TCM methods, together with modern western medicine applications (17). Similarly, in the study of Ucar et al., it is reported that "it would be beneficial to use phytotherapy as an integrative treatment method, alongside conventional treatment" in the treatment of COVID-19 (18). In the review made by Liu et al., it was stated that herbal medicines used in the COVID-19 pandemic in China were effective in the mild course of the disease, but were not effective due to insufficient clinical studies of some drugs (19). In the study conducted by Ma et al., herbal medicines used in Chinese medicine were examined in the laboratory environment, which component was good for which symptom, and determinations were made. The result of this study suggests us to choose the appropriate drug for our symptom and use it (20). In this case, it is necessary to question the clinical study of the drug well before starting to use herbal medicines. In this study, it was determined that none of the nutritional and food supplements reduced the frequency of COVID-19 diagnosis before the pandemic, and individuals using black elderberry extract, propolis and vitamin C were diagnosed with COVID-19 at a higher rate ($p<0.05$). In the pandemic period, it was observed that again none of the nutritional and food supplements reduced the frequency of being diagnosed with COVID-19, and individuals using black cumin oil, propolis, vitamin C, ginger and mixed herbal teas were diagnosed with COVID-19 at a higher rate ($p<0.05$). In this research, 24.4% (n:239) of the participants were infected with the COVID-19 virus, the risk of catching the COVID-19 virus of the healthcare worker was high and the participation rate was high (53.63%), again due to the high percentage of healthcare workers considering that 42.7% (n:418) were vaccinated against COVID-19, it was not possible to evaluate the effectiveness of nutritional and food supplements on individuals. In addition, in the present study, it was not questioned whether the patients diagnosed with COVID-19 had mild symptoms, whether they were hospitalized or not, and it was not possible to obtain information about whether the nutritional and food supplements had mild symptoms. In order to evaluate the effectiveness of nutritional and food supplements, it is thought that there is a need for studies by questioning whether the study sample is equal or close to equal numbers in terms of occupation, age, whether they are infected or not, and whether the patients diagnosed with COVID-19 have mild symptoms.

5. CONCLUSION

The use of traditional and complementary medicine practices has increased rapidly from past to present. In this study, which aims to determine which traditional and complementary medicine applications are used and its effectiveness in the pandemic, it has been observed that no food and nutritional supplement reduces the frequency of receiving COVID-19. In order to evaluate the effectiveness of nutritional and food supplements, it is recommended that the sample of the participants be equal in terms of occupation, age and whether they are infected and whether they are questioned and supported by research. Those diagnosed with COVID-19 have mild symptoms.

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Design of the study: NK, HBK
Acquisition of data for the study: NK, HBK, MK
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Clinical and Experimental Health Sciences

21st-Century Skills and Lateral Thinking Dispositions of Nursing Students: An Example Global Pandemic

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ABSTRACT

Objective: The aim of this study was to determine the 21st-century skills and lateral thinking dispositions of nursing students during the pandemic process. Speed of technological changes accesses the highest level in the current century. Following the technology and usability rate is going down among the world population. The ability of new technology usage created a new social class in society. This differentiation positively or negatively affects the social and economic status of modern human thinking ability, creativity, and life quality. Especially after the COVID-19 pandemic, the ability of current technology usage made a big difference among people to access information and education.

Methods: This cross-sectional study is descriptive and relational. The research was conducted with 409 students studying at the Faculty of Nursing of a state university in Istanbul, Turkey.

Individual Characteristics Questionnaire, Multidimensional 21st Century Skills Scale, and Lateral Thinking Disposition Scale were used to obtain research data. The data obtained were analysed by number, descriptive statistics, Kolmogorov-Smirnov, Kruskal-Wallis and Mann-Whitney U tests.

Results: The results showed that nursing students were able to improve lateral thinking during pandemic. The male students have a significantly higher advantage of using technology compared with female students ($p<0.05$). We must emphasise that the economy of students also affects their lateral thinking ability ($p<0.05$).

Conclusion: In this century, many changes and transformations are taking place in health care systems in parallel with technological developments. It is necessary that we must educate nurses who can contribute to the changes and transformations and adapt to innovations. To educate new generation innovative nurses we must adapt our education programs together with the academic staff. It is necessary that must educate nurses who can contribute to the changes and transformations and adapt to innovations. To educate new generation innovative nurses we must adapt our education programs together with the academic staff.

Keywords: COVID-19, students, nursing, thinking, problem solving.

1. INTRODUCTION

The COVID – 19 outbreak caused a devastating change in the world. A new type of coronavirus (SARS-CoV-2) began to appear in Wuhan, China, in late December of 2019 and affected the entire world with high transmission efficiency (1). The World Health Organization (WHO) declared that the COVID-19 outbreak to be an international public health emergency and characterized a global pandemic (2). To prevent the spread of COVID-19, the social routine was quickly changed. Closed social environments where people gather, such as shopping centres, theatres, etc., and primarily face-to-face education, are suspended in many countries. The governments decided to switch to online distance learning in the education system, to manage this process and the crisis (3).

These dramatic changes in the education system due to the COVID-19 pandemic create big adaptation and technological problems, such as the orientation of students to the online education system, as well as the lack of technological gadgets and technology use difficulties. The current young generation (generation Z), which is famous for as over knowledge and rapid ability to use the latest technology, can adapt to digital environments faster. Although all these benefits of the new young generation seem a positive evolution, the gap among different groups that can access or lack technology in society increased at the international level. Moreover, it is undeniable that these changes have a significant impact on technology, social structure, and education. Currently, developing

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technology causes differences in individuals' skills, and using these skills by individuals is extremely important for keeping up with the century. It is believed that individuals who cannot adapt to the current changes will have a lower success rate in education and business life (4-6).

Adaptation of current changes is only can be possible to improve critical thinking, learning and innovation etc. A society that wants to get power in the world should raise the number of people in the population that has these abilities (7, 8). It is known that the development of these skills is directly related to the thinking and evaluation processes of individuals. To develop the creativity of students with critical thinking skills, it is aimed to educate creative and problem-solving individuals with multiple perspectives by including innovation education in the curriculum (9, 10). Innovation has continued since the beginning of nursing education to improve the contemporary thinking skills of nurses. The American Nurses Association also emphasises that contemporary education techniques that support innovative thinking, critical thinking, problem-solving and research skills should be included in nursing education (11). It is known that creative thinking and 21st-century skills are one of important milestone in expanding innovation in nursing discipline. Currently, the concept of lateral thinking is frequently encountered, and it is thought to be among the 21st-century requirements, where the importance of creative thinking has increased (12). Lateral thinking is expressed as a method of problem-solving using imagination rather than traditional thinking or logic. It requires the use of information in an encouraging and intriguing way to ensure cognitive structuring. It is also known that individuals with a tendency to think laterally are productive; they explore and search for the possibilities, use inductive reasoning, are creative and can use the knowledge (13). As a result of the global changes and transformations, nursing students are expected to have higher-level skills. Innovative thinking should be prioritised in nursing education, and students' efforts in this direction should be supported throughout the education. Therefore, it is believed that more emphasis should be placed on lateral thinking dispositions and 21st-century skills. The transition to online education, especially due to the global pandemic, has led to differences in nursing education which is a practical field. No studies have been found in the literature on how these differences are met by students. Determining the 21st-century skills and lateral thinking dispositions created by the changing and transforming world on nursing students during the pandemic is very important in planning the education to be given to students. In line with these data, determining nursing students' views on online education, their 21st-century skills and lateral thinking dispositions will contribute to our decision on timely and effective practices and improve the outcomes of education. This study aims at contributing to the literature and being a guide in nursing education.

The aim of this study was to determine the 21st-century skills and lateral thinking dispositions of nursing students during the pandemic process.

2. METHODOLOGY

2.1. Design

This research is a cross-sectional study. The research was conducted online with students of the Faculty of Nursing of a state university in Istanbul, Turkey.

The research was conducted at the Faculty of Nursing of a state university in Istanbul between November and December 2020, with 424 nursing students studying in the national program in the fall semester of the 2020-2021 academic year. This study it was aimed to reach the entire universe without sample selection. The study was completed with 409 nursing students who agreed to participate and completed the questionnaire entirely, and the rate of return was determined as 96.4%. The students who continue their education in the nursing department of the university where the research was conducted and individuals who are not over 25 years old and volunteer to participate in the research were included in the research.

2.2. Data collection tools

The data of this study were obtained using Individual Characteristics Questionnaire, Multidimensional 21st Century Skills Scale, and Lateral Thinking Disposition Scale. Individual Characteristics Questionnaire prepared by the researchers in line with the literature consists of 8 questions containing information such as age, gender, income level, and parental education level (14-17).

2.2.1. Multidimensional 21st-century skills scale

Multidimensional 21st Century Skills Scale was developed by Cevik and Senturk (2019). The scale is used to determine the 21st-century skills of individuals between the ages of 15 and 25. The scale has five subscales: Information and technology literacy skills, critical thinking and problem-solving skills, entrepreneurship and innovation skills, social responsibility and leadership skills, and career awareness. It is a five-point Likert scale consisting of 41 items rated as "strongly agree", "agree", "neutral", "disagree", and "strongly disagree". The scale score was between 41 and 205, and the mean value was 123. The information and technology literacy skills subscale score was the lowest, 15, the highest 75, and the mean value was 45. In the critical thinking and problem-solving skills subscale, the lowest score was 6, the highest score was 30, and the mean value was 18. In the entrepreneurship and innovation skills subscale, the score ranged between 10 and 50, and the mean value was 30. The lowest score in the social responsibility and leadership skills subscale was 4, the highest score was 20, and the mean score was 12. In the career awareness subscale, the lowest score was 6, the highest score was 30, and the mean value was 18. 21st-century skills levels of individuals increased along with the score value. Items numbered 16, 17, 18, 19, 20, 21, 35 are reverse coded. The Cronbach's Alpha coefficient of the scale

is 0.86 (9). The Cronbach's Alpha coefficient of the scale in this study was 0.91.

2.2.2. Lateral thinking disposition scale

The lateral Thinking Disposition Scale was developed by Semerci (2016). The scale is one-dimensional and consists of nine items. It is a five-point Likert scale rated as "strongly disagree" (1), "mainly disagree" (2), "partially agree" (3), "mainly agree" (4), and "strongly agree" (5). The lateral thinking dispositions increased along with the score levels. The minimum score of the scale was 5, the maximum score was 45 and the mean value was 25. The Cronbach's Alpha coefficient of the scale is 0.75 (13). The Cronbach's Alpha coefficient of the scale in this study was 0.90.

2.3. Data collection

The data were obtained by sending a link to the questionnaire created by the researchers via Google Forms. In the first part of the prepared questionnaire, a volunteer consent form containing the purpose and scope of the study was included. In the second part of the questionnaire, questions related to individual characteristics, Multidimensional 21st Century Skills Scale, and Lateral Thinking Dispositions Scale were included. The responses of the individuals who answered the survey questions by approving the volunteer consent form were evaluated. The time spent answering the survey questions was approximately 5-10 minutes.

2.4. Statistical Analysis

Statistical Package for the Social Sciences (SPSS 22.0 for Windows, SPSS Inc., Chicago, IL) program was used for the analysis of the research data. In the analysis of the data, the Kolmogorov-Smirnov test was used to evaluate the conformity of the relevant variables to normal distribution to determine the statistical method to be used. Analysis of the descriptive data included number, percentage, median, minimum, maximum, mean, and standard deviation. The difference between three and more groups that did not show normal distribution was evaluated using the Kruskal-Wallis test, and the difference between the two groups was analysed using the Mann-Whitney U test. For the difference between three and more groups, Mann-Whitney U was used as a post hoc test. Spearman correlation coefficient was used to show the relationship between the two variables. In statistical evaluation, significance was accepted as $p < 0.05$.

2.5. Ethical Considerations

Written permission was obtained from the Scientific Research Ethics Committee of the university where the study was conducted (Date: 2020 / Decision No: 23/1) and from the related institution. All students invited to the study were informed about the research, and their consent were taken. Necessary permissions for the use of the scales were obtained.

Limitations of Study

This study has several limitations. Since this study is a single-centered study and limited to the faculty in which the study was conducted, it cannot be generalized to all nursing students. It is also limited to the answers given to the scales used in the study.

3. RESULTS

The mean age of the nursing students participating in the study was 21.10 ± 1.48 years and 82.5% of them were women. It was determined that 90.2% of the students did not work at any job, and 71.6% of the students had equal income and expenses. Due to the global epidemic, nursing education is carried out remotely in an online environment, and it was found that most of the students (76%) have the necessary technological equipment to participate in online education but have difficulty in accessing the internet at different rates (Table 1).

Table 1. *Distribution of the participants' individual characteristics and views on online education*

Characteristics	N=409	
	n	%
Gender		
Female	341	82.5
Male	68	17.5
Year of University		
First Year	83	20.3
Second Year	104	25.4
Third Year	106	25.9
Final Year	116	28.4
Education Level of the Mother		
Illiterate	36	8.8
Elementary	212	51.8
Secondary	46	11.2
High school	82	20.0
University and higher education	33	8.2
Education Level of the Father		
Illiterate	5	1.2
Elementary	153	37.4
Secondary	72	17.6
High school	123	30.1
University and higher education	56	13.7
Income Level		
Income lower than the expenses	77	18,8
Equal income and expenses	293	71,6
Income higher than the expenses	39	9,6
Difficulty in Using Technology		
Yes	26	6.3
No	224	54.8
Occasionally	159	38.9
Difficulty in Internet Access		
Yes	42	10.3
No	165	40.3
Occasionally	202	49.4
Availability of the Device Required for Online Education	311	76.0
Yes	98	24.0
No		

Students' Multidimensional 21st-century Scale total score ranged between 91 and 205, and the mean score was found to be 163.63 ± 16.64. The total score of Lateral Thinking Disposition Scale ranged between 15 and 45. and the mean score was determined as 34.61 ± 5.30 (Table 2).

Table 2. *Distribution of Multidimensional 21st Century Skills Scale and subscale scores and Lateral Thinking Disposition Scale scores*

Multidimensional 21st Century Skills Scale Subscales	M	SD.	Min	Max
Information and Technology Literacy Skills	62.67	7.33	29	75
Critical Thinking and Problem-Solving Skills	22.46	6.22	6	30
Entrepreneurship and Innovation Skills	37.31	6.44	14	50
Social Responsibility and Leadership Skills	14.72	2.37	7	20
Career Awareness	26.47	3.23	10	30
Multidimensional 21st Century Skills Scale (Total)	163.63	16.64	91	205
Lateral Thinking Disposition Scale (Total)	34.61	5.30	15	45

Statistically significant differences were found between Multidimensional 21st-century Scale subscales of information and technology literacy skills, critical thinking and problem-solving skills, entrepreneurship and innovation skills, social responsibility and leadership skills, and career awareness according to the participants' difficulties in using technology ($p<0.01$). There is a statistically significant difference between "Critical thinking and problem-solving skills" subscale mean scores of the students according to the year of university variable ($p<0.01$). It was determined that the mean value (25.04) of the participants in the first year of university was significantly higher than the mean values (22.54 – 21.57 – 21.36) of the participants in the other years. A statistically significant difference was found between "social responsibility and leadership skills" mean scores according to the year of university variable ($p<0.05$). It was established that the mean value of the participants in the second year of university (14.32) was significantly lower than the mean value of the participants (15.02) in the third year (Table 3).

There is a statistically significant difference between Lateral Thinking Disposition Scale total scores according to the income variable ($p<0.05$). It was determined that the mean value of the participants whose income was less than their expenses (33.35) was significantly lower than the mean value of the participants whose income was equal or higher than their expenses (34.82 – 35.51). A statistically significant difference was found between "entrepreneurship and innovation skills" subscale mean scores according to the gender variable ($p<0.05$). It was observed that the mean value of male participants (38.32) was significantly higher than the mean value of female participants (37.11). For lateral thinking disposition scale total scores, the mean value of male participants (36.01) was found to be significantly higher than the mean value of female participants (34.33) (Table 3).

According to the results of the correlation test among the scales used in the study, there was a moderate positive and statistically significant correlation between the total scores of the Lateral Thinking Disposition Scale and the Multidimensional 21st-century Skills Scale ($p<0.001$; $r=0.631$). It was observed that there was a moderate positive, significant correlation between Lateral Thinking Disposition Scale total scores and "information and technology literacy skills, critical thinking and problem-solving skills, entrepreneurship and innovation skills, social responsibility and leadership skills, and career awareness." subscale scores (Table 4).

Table 4. *Examination of the relationship between Multidimensional 21st Century Skills Scale and Lateral Thinking Disposition Scale*

Multidimensional 21st Century Skills Scale Subscales		Lateral Thinking Disposition Scale (Total)
Information and Technology Literacy Skills	r_s	**.589****
	p	**<0.001**
Critical Thinking and Problem-Solving Skills	r_s	-.060
	p	.228
Entrepreneurship and Innovation Skills	r_s	**.613****
	p	**<0.001**
Social Responsibility and Leadership Skills	r_s	**.464****
	p	**<0.001**
Career Awareness	r_s	**.466****
	p	**<0.001**
Multidimensional 21st Century Skills Scale (Total)	r_s	**.631****
	p	**<0.001**

*rs = Spearman Correlation Coefficient, **p<0.01*

Table 3. *Comparison of individual characteristics and Multidimensional 21st Century Skills Scale and Lateral Thinking Disposition Scale*

	Multidimensional 21st Century Skills Scale (Total)	Information and Technology Literacy Skills Subscale	Critical Thinking and Problem-Solving Skills Subscale	**Entrepreneurship and Innovation Skills Subscale**	**Social Responsibility and Leadership Skills Subscale**	**Career Awareness Subscale**	**Lateral Thinking Disposition Scale (Total)**
	M± SD (min-max) Median Range	**M± SD (min-max) Median Range**	**M± SD (min-max) Median Range**	**M± SD (min-max) Median Range**	**M± SD (min-max)Median Range**	**M± SD (min-max) Median Range**	**M± SD (min-max) Median Range**
Gender							
Female	163.88±15.54 (121-205) 163 84	62.74± 6.83 (48-75) 61 27	22.72±5.99 (6-30) 24 24	37.11± 6.28 (14-50) 37 36	14.72±2.38 (7-20) 15 13	26.60±2.98 (15-30) 27 15	34.33±5.14 (18-45) 34 27
Male	162.34±21.42 (91-205) 160 114	62.29 ± 9.51 (29-75) 62.5 46	21.13±7.18 (6-30) 22 24	38.32± 7.19 (17-50) 40 33	14.74± 2.35 (9-20) 14 11	25.85±4.24 (10-30) 26.5 20	36.01±5.88 (15-45) 36 30
Z	-0.094	-0.613	-1.528	**-2.155**	-0.188	-0.928	**2.679**
p	0.925	0.54	0.127	**0.031***	0.851	0.354	**0.007***
Year of University							
First Year	164.82±15.13 (128-195) 165 67	61.69±6.76 (41-75) 62 34	25.04±3.35 (14-30) 26 16	35.78±6.31 (21-49) 35 28	14.98±2.54 (7-20) 15 13	27.34±2.42 (21-30) 28 9	34.69±4.78 (24-45) 34 21
Second Year	162.55±18.05 (121-205) 158.5 84	62.38±7.08 (48-75) 60.5 27	22.54±6.19 (6-30) 24 24	37.08±7.17 (14-50) 37.5 36	14.32±2.59 (8-20) 14 12	26.24±3.30 (15-30) 26 15	34.07±5.75 (18-45) 33.5 27
Third Year	162.48±17.71 (91-201) 163 110	62.19±8.13 (29-75) 60.5 46	21.57±6.56 (6-30) 24 24	37.71±6.39 (17-50) 38 33	15.02±1.92 (9-20) 15 11	26.00±4.05 (10-30) 26 20	34.29±5.18 (15-45) 34 30
Final Year	164.78±15.39 (133-205) 164 72	64.06±7.08 (48-75) 62 27	21.36±6.99 (6-30) 24 24	38.08±5.73 (27-50) 39 23	14.62±2.39 (7-20) 14 13	26.50±2.71 (18-50) 27 12	35.34±5.34 (23-45) 35 22
X^2	2.493	5.357	14.648	5.805	9.891	6.738	3.719
p	0.477	0.147	**0.002***	0.122	**0.020***	0.081	0.293
Difference	-	-	1-2, 1-3, 1-4	-	2-3	-	-
Income Lower Than the Expenses	159.45±19.33 (91-201) 159 110	61.08±8.69 (29-75) 60 46	21.69±6.12 (6-30) 23 24	36.55±7.13 (17-50) 37 33	14.55±2.51 (7-20) 14 13	25.60±4.67 (10-30) 26 20	33.35±5.54 (15-45) 33 30
Equal Income and Expenses	164.58±16.01 (128-205) 14 77	63.04±7.06 (41-75) 61 34	22.65±6.15 (6-30) 24 24	37.46±6.28 (14-50) 38 36	14.75±2.35 (7-20) 15 13	26.67±2.81 (18-30) 27 12	34.82±5.36 (18-45) 35 27
Income higher Than the expenses	164.67±14.63 (125-193) 162 68	62.95±6.11 (51-75) 62 24	22.54±6.97 (6-30) 24 24	37.67±6.33 (25-50) 36 25	14.79±2.33 (9-20) 14 11	26.72±2.41 (22-30) 27 8	35.51±3.86 (28-45) 35 17
X^2	3.292	3.337	3.241	1.244	0.573	1.281	7.145
p	0.193	0.189	0.198	0.537	0.751	0.527	**0.028***
Difference	-	-	-	-	-	-	1-2 2-3
Difficulty in Using Technology							
Yes	161.92±15.46 (133-194) 162 61	62.73±7.34 (48-75) 61.5 27	19.19±7.62 (6-30) 21.5 24	39.73±4.94 (31-50) 39.5 19	14.54±1.56 (12-17) 14 5	25.73±3.33 (18-30) 24 12	34.15±4.89 (24-44) 36 20
No	166.63±17.63 (91-205) 165 114	63.83±7.63 (29-75) 62 46	23.13±5.88 (6-30) 24 24	37.82±6.41 (17-50) 38 33	14.97±2.59 (7-20) 15 13	26.88±3.26 (10-30) 28 20	35.46±5.36 (15-45) 35 30
Occasionally	159.67±14.48 (117-205) 158 88	61.01±6.59 (41-75) 60 34	22.05±6.27 (6-30) 24 24	36.19±6.54 (14-50) 36 36	14.39±2.13 (7-20) 14 13	26.03±3.11 (11-30) 26 19	33.48±5.09 (18-45) 34 27
X^2	**17.22**	**15.326**	**8.162**	**11.089**	**7.668**	**11.124**	**15.698**
p	**0.001***	**0.001***	**0.017***	**0.004***	**0.022***	**0.004***	**0.001***
Difference	2-1, 2-3	2-3	1-2, 1-3	1-3, 2-3	2-3	2-1, 2-3	2-3

4. DISCUSSION

During the COVID-19 pandemic which has reached serious levels worldwide, face-to-face training was suspended, online education was initiated, and the requirements of the digital age gained more importance. During the global pandemic, theoretical and practical courses continued online with video support in nursing education. In this study, it was determined that the 21st-century skills total and subscale mean scores were recorded high. Similarly, Turner et al. (2016) found that nursing students' 21st-century perception overall mean scores were high (18). When the literature was examined, it was found that 21st-century skills were also at a high level in studies conducted with university students studying in different fields (19-22). The findings which were obtained from our study was supported by the previous research. It is known that today's young people, known as the digital generation or generation z, differ from previous generations because they were born and grew up in a digital age. This difference has a positive impact on 21st-century skills.

Lateral thinking, which addresses creative thinking, has become a necessity in the field of nursing in recent years (11). In this study, nursing students' lateral thinking skills mean scores were found as higher level. Although previous studies were conducted on university student education in different fields, our study unique research on lateral thinking skills of nursing studies in the literature. And as we were found in our study, previous researchers also found that lateral thinking dispositions were high (14-16, 23). The results of previous study also support that nursing students, like other university students, have high lateral thinking dispositions.

It is known that the usage of technology is a part of 21st-century skills (17). In this study, it was determined that the "Multidimensional 21st-century Skills Scale" and the "Lateral Thinking Disposition Scale" mean scores of the students who do not have difficulty in using technology during the global pandemic process were detected significantly higher. It was established that 21st-century skills scores and lateral thinking dispositions increased along with digital competence. This indicates that students who experienced difficulties in the usage of technology should be supported to prevent problems in the educational process.

It is stated that the critical thinking and problem-solving skills of nursing students need to be developed, and critical thinking is extremely important in nursing science and nursing interventions (14). In this study, the mean scores of the "Critical thinking and problem-solving skills" subscale of the "Multidimensional 21st Century Skills Scale" were found to be significantly higher in first-year students. A study conducted by Pehlivan (2005) with students of the Faculty of Education, levels of communication competence subscale of 21st-century skills were evaluated, and it was concluded that the mean scores of the first-year students were significantly higher than the final-year students (24). The reason for the higher level of critical thinking and problem-solving skills and communication competence of first-year students is due to the differences experienced in the education system with the changing and developing technology. Besides, in this study, it was found that the lateral thinking disposition mean scores of the nursing students according to the year of university were similar. In other studies, it was noted that the lateral thinking disposition mean scores of university students according to their class levels were similar compared with each other (15, 23, 25-27). This indicates that there is no difference between university students in terms of lateral thinking dispositions.

Meetings-trainings such as courses, congresses, and seminars that individuals attend have a financial dimension. Therefore, the financial status is a tool for individuals to develop a few competencies. In our study, no significant difference was found between the Multidimensional 21st-century Skills Scale subscales and the total scores according to the income level variable. Zeybek (2019) stated in their studies that the mean scores of students with high family income obtained from different subscales of 21st-century skills were significantly higher than those with low income (22). On the other hand, a significant relationship was found between the family income level of the students participating in our study and their lateral thinking dispositions. When the income levels of the participants and their lateral thinking dispositions were evaluated, it was determined that the mean value of the participants whose income was less than their expenses was significantly lower than the mean value of the participants whose income was equal to or higher than their expenses. Students may need financial support resources to develop their lateral thinking dispositions.

The gender of the individuals affects their feelings and behaviours, and this creates differences in entrepreneurship characteristics (28). In our study, it was established that the Multidimensional 21st-century Skills Scale "entrepreneurship and innovation skills" subscale mean scores of male students were significantly higher than female students. In the study conducted by Ozden et al. (2018), in which 21st-century skills of teacher candidates were determined, it was noted that there was no significant difference between gender and 21st-century skills (20). When the literature was examined, it was also concluded in similar studies that there was no significant difference between 21st-century skills according to gender variable (19, 21). In addition, when the Lateral Thinking Disposition Scale mean scores were evaluated, it was determined that the mean scores of male students were significantly higher than female students. In the studies conducted by Lawrence & Xavier (2013) and Semerci (2017), it was indicated that the lateral thinking disposition mean scores of male individuals were high (15, 29). It is believed that male individuals may be more interested in lateral thinking because lateral thinking skills deal with innovative thinking, and there are technical areas in innovative processes. Meanwhile, it seems that the entrepreneurship characteristic of the male gender has a positive effect.

It is obvious that lateral thinking skills and 21st-century skills of nursing students will contribute to nursing interventions and health care systems. In this study, a moderate positive, significant correlation was found between the Lateral Thinking

Disposition Scale total scores and the Multidimensional 21st-century Skills Scale total score, and information and technology literacy skills, entrepreneurship and innovation skills, social responsibility and leadership skills and career Awareness subscale scores. It is observed that a high level of lateral thinking disposition of nursing students has a positive effect on 21st-century skills. In this respective, it is extremely important to support 21st-century skills and lateral thinking dispositions of students during their educational process.

5. CONCLUSION

In our study, 21st-century skills and lateral thinking dispositions of nursing students were found to be high. Students have the skills to easily adapt to the online education process that is mandatory due to the global pandemic. In this century, many changes and transformations are taking place in health care systems in parallel with the technological developments. Therefore, it is necessary to train nurses who can contribute to the changes and transformations and adapt to innovations. Accordingly, it is recommended that the education programs for the faculty members be improved, and the nursing curriculum includes training aimed at developing 21st-century skills and lateral thinking dispositions of the students.

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Clinical and Experimental Health Sciences

Depression Anxiety Stress Levels of Dentists Redeployed to Filiation due to the COVID-19 Pandemic

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ABSTRACT

Objective: The aim of this study is to investigate the levels of depression, stress, and anxiety of dentists redeployed to filiation during the COVID-19 pandemic and to determine the factors that caused any changes observed.

Methods: An online questionnaire was sent to dentists redeployed to filiation due to the COVID-19 pandemic. The questionnaire consisted of three parts: I) demographic characteristics, II) working conditions in filiation, and III) the DASS-21 scale, which evaluates the Depression, Anxiety, and Stress states of participants.

Results: 206 (164 female, 42 male) voluntary dentists participated in the study. Off all participants, 77.2% had symptoms of depression, 73.8% had symptoms of anxiety, and 59.7% had symptoms of stress. All subscales of the DASS-21 were statistically significantly higher in females than in males, in single dentists than in married dentists, and in those living alone than in those living with family or friends. Among the dentists redeployed to filiation during the COVID-19 pandemic, female gender, living alone, and working at a university were found to be effective factors that constituted the high scores of any of the DASS-21 subscales.

Conclusion: Dentists who were redeployed to filiation during the COVID-19 pandemic stated that they were negatively emotionally affected. Dentists can always be redeployed to the public health method in case of social emergencies. Therefore, dentists need to be trained and motivated for redeployment.

Keywords: COVID-19, Filiation, DASS-21, Dentist, Redeployed

1. INTRODUCTION

In December 2019, a disease caused by a novel *coronavirus* (2019 n-CoV) emerged in Wuhan, China (1). The disease was named coronavirus disease 2019 (COVID-19) by the World Health Organization (WHO). COVID-19 was declared a global pandemic by the WHO on 11 March 2020 (2). The number of cases of infection is increasing because of the high speed of transmission, and in consequence, the workload on healthcare services has been getting heavier day by day, around the world. In many countries, health care professionals, including dentists, are being redeployed to the outside of their workplaces to support COVID-19 health services (3).

Dentists have sufficient knowledge about systemic diseases, medical history, craniofacial anatomy, prescribing drugs, using personal protective equipment (PPE), infection control, and effective communication with patients because of the experience they have gained from their training and routine clinical practices (4). In many countries, according to their national health policies during the COVID-19 pandemic, dentists have been redeployed to assist with different medical services, such as physician assistance, radiological diagnosis, sample collection, online counselling and prescribing, the collection and distribution of medical supplies, medical evacuation and transfer, community volunteering, psychological support, intensive care unit support, renal wards support, accident and emergency services, informative telephonic services, and community nursing (5-9). Since aerosol-generating dentistry procedures inherently carry a high risk of COVID-19 transmission, many dental treatments have been postponed during the pandemic period (10). Dentists whose workloads were reduced during

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the COVID-19 pandemic were redeployed to different areas of the struggle against the virus (4).

In Turkey, dentists have been redeployed to filiation during the COVID-19 pandemic. Filiation is an important public health method that aids to interrupt the transmission chain and prevent the spread of infection (11). Filiation, which includes methods known in the literature as contact tracing, constitutes an important pillar of the COVID-19 struggle in Turkey and has been carried out successfully. Since the detection of the first COVID-19 case in Turkey, the filiation teams that have been established by the Turkish Ministry of Health have been working. These filiation teams first reach the infected by phone and then visiting their houses. Their duties are to follow-up on each case, perform contact tracing, take anamnesis, supply medication, and take samples for COVID-19 polymerase chain reaction (PCR) tests when necessary (12). A filiation team is comprised of one physician or dentist, one medical staff member, and one assistant staff member (13). Dentists have sufficient knowledge and a good command of the intraoral anatomy, and they have a high level of hand-skill practices that provide a great convenience in the taking of samples. Thus, many have been redeployed to filiation teams (14). Thus, the dentists, whose main task is to protect oral health in the clinical environment, have been redeployed in the field to support the health system against the current COVID-19 pandemic.

All healthcare professionals have been playing an active and important role during the COVID-19 pandemic, both inside hospitals and outside of the hospital setting (in a filiation team). Healthcare professionals who provide such services may experience psychological problems in the process (15-18). These problems can be triggered by the increasing number of cases, intensive workload, exposure to infected patients, risk of contamination, and need to trace new information about the disease (19). Although many previous studies have shown that the COVID-19 pandemic has increased depression, anxiety, stress, and insomnia in healthcare professionals, only a few studies have reported negative emotional states in dentists redeployed outside of dental clinics (7,20). The aim of this study is to investigate the levels of depression, stress, and anxiety of dentists redeployed to filiation during the COVID-19 pandemic and to address the factors that may cause these conditions.

2. METHODS

This study was approved by the Gazi University Ethics Committee (Date: 10/07/2020, Research Number: 2020–661), and all stages of the study were conducted in accordance with the Declaration of Helsinki.

Dentists redeployed to filiation during the COVID–19 pandemic in Turkey were included in the study. For this study, a special questionnaire consisting of 40 questions in 3 parts was prepared. The online questionnaire was created on Google Forms (Alphabet, Mountain View, CA, USA) and was delivered to volunteer participants via WhatsApp© (WhatsApp Inc, USA) between 20 December 2020 and 20 January 2021.

In the submitted form, there was a paragraph giving information about the study at the beginning of the questionnaire. The distribution of 40 questions according to three topic titles in the questionnaire was as follows. Part 1: 7 questions about demographic characteristics; Part 2: 12 questions about filiation working conditions; Part 3: the 21 questions of the Depression, Anxiety, Stress Scale–21 (DASS–21).

DASS–21 is a 21–item, shortened form of the 42–item Depression, Anxiety, Stress Scale created by Lovibond SH and Lovibond PF (21). DASS–21 aims to evaluate the relationships between environmental demands, emotional disturbances, and physical disturbances in individuals by means of depression, anxiety, and stress subscales. Each of these subscales consists of seven different items to which the participant is instructed to responded according to the mood of the last week. Responses to each item are graded on a 4-point Likert-type scale (0=Never, 1=Sometimes and occasionally, 2= Quite often, 3=Always). The individual's scores are added up, and higher scores indicate a more violent mood in a negative sense. Scores on the depression subscale are interpreted as: 0–4 is 'normal', 5–6 is 'mild', 7–10 is 'moderate', 11–13 is 'severe', and 14 and above is 'extremely severe'. Score on the anxiety subscale are interpreted as: 0–3 is 'normal', 4–5 is 'mild', 6–7 is 'moderate', 8–9 is 'severe', and 10 and above is 'extremely severe'. Score on the stress subscale are interpreted as: 0–7 is 'normal', 8–9 is 'mild', 10–12 is 'moderate', 13–16 is 'severe', and 17 and above is 'extremely severe'.[21] In 2018, Sarıçam conducted validity and reliability studies on the Turkish version of the DASS–21, and the scale was found to be a valid and reliable instrument in the assessment of depression, anxiety, and stress levels (22).

Statistical Analysis

The sample size was calculated before starting the study via a power analysis. The analysis was conducted using the G*Power software package under a power of 90 %, an assumed effect size of 0.3, and a Type–I error (alpha) of 0.05 (23). The appropriate sample size, given these parameters, was found to be 194 individuals.

IBM SPSS Statistics Version 23.0 (SPSS Inc., Chicago, IL, USA) was used for all other statistical analyses. Regarding the presentation of descriptive statistical information, categorical variables are presented as a number (%), and continuous variables are presented as a mean ± a standard deviation. The normality of the data was assessed using the Kolmogorov–Smirnov test. Two-groups comparisons were conducted via Student's *t*–tests, and comparisons between more than two groups were conducted by means of analyses of variance (ANOVA). The associations between age and subscale scores were explored by using Pearson`s correlation test. Multiple linear regression analysis was employed as a method to identify potential effective factors on depression,

anxiety, and stress. A *p*–value of less than 0.05 is regarded to be indicative of a statistically significant result.

3. RESULTS

Two hundred six (164 female, 42 male) dentists voluntary participated in the study. The original questionnaire form and the distributions of the responses, in terms of numbers and percentages, are shown in Table 1.

Table 1. *Distribution, demographic characteristics, filiation working conditions, and means of DASS-21 subscale scores of the participants, according to their responses on original questionnaire, presented as n (%) with N=206*

Part I: Demographic characteristics		n (%)
Age		34.71±8.12#
Gender	Female	164 (79.6 %)
	Male	42 (20.4 %)
Working institution	Ministry of Health institution	157 (76.2 %)
	University	31 (15.0 %)
	Other	18 (8.7 %)
Work experience	0–5 years	79 (38.3 %)
	6–10 years	37 (18.0 %)
	≥11 years	90 (43.7 %)
Marital status	Single	68 (33.0 %)
	Married	138 (67.0 %)
Having child	Yes	111 (53.9 %)
	No	95 (46.1 %)
Household	Living alone	28 (13.6 %)
	Living with family or friends	178 (86.4 %)
Part II: Filiation working conditions		
How did you get information about the procedures of filiation?*		
I was informed by the Ministry of Health via WhatsApp, internet etc.		93 (45.2 %)
I got information from a physician experienced on filiation		101 (49.1 %)
I did research on my own		55 (26.7 %)
How many cases did you probably reach during filiation?		4008.5±23324.4#
How often do you use the N95 / FFP2 mask during filiation?	Never	2 (1 %)
	Sometimes	22 (10.7 %)
	Usually	43 (20.9 %)
	Always	139 (67.5 %)
How often do you wear the face visor / protective glasses during the filiation?	Never	3 (1.5 %)
	Sometimes	23 (11.2 %)
	Usually	50 (24.3 %)
	Always	130 (63.1 %)
How often do you use the disposable apron / overalls during the filiation?	Never	1 (0.5 %)
	Sometimes	12 (5.8 %)
	Usually	57 (27.7 %)
	Always	136 (66 %)
Did you have COVID-19 infection?		
No		157 (76.2 %)
Yes		49 (23.8 %)
If you had COVID-19 infection, when did you?		
Before starting filiation		9 (4.4 %)
Within 14 days after the start of filiation		5 (2.4 %)
During filiation		34 (16.5 %)
Within 14 days after the end of filiation		1 (0.5 %)
In your opinion, which process is risker during COVID-19 pandemic?		
Filiation		16 (7.8 %)
Dental procedures		190 (92.2 %)
Which process would you prefer to do during COVID-19 pandemic?		
Filiation		164 (79.6 %)
Dental procedures		42 (20.4 %)
Worried about being redeployed to filiation?		
No		42 (20.4 %)
Yes		164 (79.6 %)
If you are worried, what are the reason(s)?*		
Workload excess		102 (21.1 %)
Infecting my relatives with COVID-19 or other disease		153 (32.3 %)
Lack of personal protective equipment		40 (8.6 %)
Lack of adequate security measures		82 (17.2 %)
Negative discrimination in the society		81 (17.1 %)
Other (become distant from dental clinic, adverse weather conditions, working at night, fear of traffic accidents, etc.)		17 (3.5 %)
Have you got psychological support at any period in your life?		
No		168 (81.6 %)
Yes		32 (15.5 %)
I started to get after filiation		6 (2.9 %)
Part III: DASS–21		
Depression		10.17±6.37#
Anxiety		7.94±5.54#
Stress		9.95±6.21#

*# : mean ± standard deviation, *: multiple–choice question, DASS–21: Depression Anxiety Stress Scale*

The distribution of the symptoms observed in the participants and their severity according to the DASS–21 scale is shown in Table 2. According to the three different subscales that formed the DASS–21, it was observed that the participants were mostly at normal levels in terms of stress and worse from normal in terms of depression and anxiety. The rest of the participants showed varying severities of depression, anxiety, and stress symptoms. The distribution of symptoms among all participants was as 77.2 % (N = 159) depression, 73.8 % (N = 152) anxiety, and 59.7 % (N = 123) stress. Extremely severe depression and anxiety symptoms were observed at the highest rates (33.0 % and 40.3 %, respectively), and according to the stress subscale, severe stress symptoms were observed at the highest rate (21.8 %).

The findings of univariate analyses for the subscales of DASS–21 in the overall sample are presented in Table 3. The results revealed a statistically significant differences within each subscale score between genders, marital statuses, and

households. All scores were statistically significantly higher in females than in males, in single dentists than in married dentists, and in dentists living alone than in dentists living with family or friends. Statistically significant differences between depression, and stress subscale scores were found between working institutions, and having children. Working in a university was associated with higher depression and stress scores, whereas having child was associated with lower scores in depression and stress. No statistically significant relationship between age and work experience was found with any subscale score.

A multiple linear regression analysis was performed to determine the independent effects of the significant variables obtained from the univariate analysis in Table 3 on the subscale scores of the DASS–21. It was found, statistically significantly, that female gender and living alone are effective factors on depression, that female gender, living alone, and working at a university are effective factors on anxiety, and that female gender is an effective factor on stress. The results of this analysis are shown in Table 4.

Table 2. *Distribution of the symptoms observed in the participants and their severity according to the DASS–21 scale*

Severity	Symptoms		
	Depression n (%)	Anxiety n (%)	Stress n (%)
Normal	47 (22.8 %)	54 (26.2 %)	83 (40.3 %)
Mild	18 (8.7 %)	31 (15.0 %)	20 (9.7 %)
Moderate	48 (23.3 %)	20 (9.7 %)	25 (12.1 %)
Severe	25 (12.1 %)	18 (8.7 %)	45 (21.8 %)
Extremely severe	68 (33.0 %)	83 (40.3 %)	33 (16.0 %)

Table 3*. Univariate analyses of the factors associated with the subscales of DASS–21, (N = 206)*

	Depression			Anxiety			Stress		
	Mean±Sd	Test statistics	*p-value*	Mean±Sd	Test statistics	*p-value*	Mean±Sd	Test statistics	*p-value*
Age		r=0.117	*0.095*		r=0.06	*0.931*		r=0.066	*0.344*
Gender									
Female	10.84±6.33	t=3.071	*0.002**	8.47±5.35	t=2.768	*0.006**	10.74±5.99	t=3.731	*<0.000**
Male	7.52±5.86			5.86±5.85			6.86±6.14		
Working institution									
Ministry of Health institution	9.83±6.31	F=4.969	*0.008**[1]	7.94±5.53	F=2.933	*0.055*	9.61±6.10	F=5.206	*0.006**[2]
University	13.16±6.00			9.39±5.68			12.97±6.05		
Other	7.89±6.10			5.44±4.68			7.78±5.95		
Work experience									
0-5 years	10.95±6.46	F=1.936	*0.147*	7.67±5.69	F=0.351	*0.704*	10.13±6.48	F=0.108	*0.898*
6-10 years	10.89±6.39			8.59±5.93			10.14±6.43		
≥11 years	9.18±6.21			7.90±5.28			9.72±5.932		
Marital status									
Single	12.60±5.96	t=3.991	*<0.000**	9.25±5.71	t=2.414	*0.017**	11.53±6.15	t=2.595	*0.010**
Married	8.96±6.24			7.29±5.36			9.17±6.11		
Having child									
Yes	8.78±6.25	t=-3.450	*0.001**	7.39±5.31	t=-1.543	*0.124*	9.01±5.87	t=-2.381	*0.018**
No	11.78±6.16			8.58±5.75			11.05±6.43		
Household									
Living alone	14.36±6.22	t=3.869	*<0.000**	10.89±6.31	t=3.098	*0.002**	12.96±6.64	t=2.807	*0.005**
Living with family or friends	9.51±6.15			7.47±5.28			9.48±6.02		

**: p<0.05, Sd: standard deviation, r: correlation coefficient, t: Student's t–tests, F: ANOVA test, [1,2]: Post–hoc analysis indicated that the scores are higher for those who work in a university than those who work in a state institution or other institution.*

Table 4. *Multiple regression analysis on each subscale of DASS-21 in the overall sample*

	B	SE	β	t	95% CI	*p–value*
Depression						
Gender	-3.436	1.042	-0.218	-3.296	-5.492, – 1.380	*0.001**
Working institution	-0.758	0.686	-0.075	-1.104	-2.111, 0.595	*0.271*
Marital status	-1.448	1.245	-0.107	-1.163	-3.903, 1.007	*0.246*
Having child	1.308	1.121	0.103	1.167	-0.902, 3.518	*0.245*
Household	-3.415	1.456	-0.184	-2.345	-6.287, – 0.543	*0.020**
Anxiety						
Gender	-2.768	0.929	-0.202	-2.980	-4.600, – 0.936	*0.003**
Working institution	-1.242	0.611	-0.141	-2.031	-2.447, – 0.036	*0.044**
Marital status	-0.795	1.109	-0.068	-0.716	-2.982, 1.393	*0.475*
Having child	-0.073	0.999	-0.007	-0.073	-2.043, 1.896	*0.942*
Household	-3.509	1.298	-0.217	-2.704	-6.068, – 0.950	*0.007**
Stress						
Gender	-4.018	1.034	-0.261	-3.887	-6.056, – 1.979	*<0.000**
Working institution	-0.551	0.680	-0.056	-0.810	-1.892, 0.791	*0.419*
Marital status	-0.465	1.235	-0.035	-0.377	-2.900, 1.969	*0.707*
Having child	1.152	1.111	0.093	1.037	-1.039, 3.344	*0.301*
Household	-2.742	1.444	-0.152	-1.899	-5.590, 0.105	*0.059*

B: Unstandardized beta coefficient, SE: Standard error, β: Standardized beta coefficient, CI: Confidence Interval

4. DISCUSSION

During the COVID-19 pandemic, dentists have been redeployed to different tasks such as filiation, monitoring vital signs and biochemical indicators, collecting of the nasopharyngeal swab for PCR testing, online consultation, prescribing required medications, triaging, supporting intensive care units, neurosurgery/ear nose physicians, and throat/ophthalmology physicians, and supporting community nursing (5-9,20). In Turkey, contact tracing, isolation, and PCR tests are being successfully carried out with the support of the filiation teams within which dentists are redeployed (12). In the present study, the working conditions and emotional states of dentists who were redeployed to filiation were investigated. The results of the study revealed that depression, anxiety, and stress symptoms, ranging in severity from mild to extremely severe, were observed in the study participants. Female gender, living alone, and working at a university were factors that were found to increase the effect of these negative emotional states. Despite all of the negative emotional states reported by dentists redeployed to filiation, and they predominantly preferred filiation to dental procedures during the pandemic.

In many studies, it has been shown that healthcare professionals who are working at the frontline of the COVID-19 pandemic were adversely psychologically affected (15-18). In previous studies in which the emotional states of working healthcare professionals were questioned, the rates of professionals suffering from depression have been between 50.4 %-77.6 %, from anxiety have been between 51.6 %-60.2 %, and from stress have been between 41.2 %-76.4 %, with 50.4 % suffering from insomnia (15,16,24). Considering the transmission mechanism of COVID-19, aerosols formed as a result of dental procedures pose a significant risk (10). Dentists, their assistant staff, and the patients they serve are therefore at a high risk of transmission and spread of the disease, as they are in close contact with potentially symptomatic/asymptomatic COVID-19 patients who apply to dental clinics for treatment (25). There were several studies that found that the degree of stress experienced by dentists increased significantly during the COVID-19 pandemic (20,25-31). Because of the nature of the procedures conducted in dentistry, more than 90 % of dentists are concerned about the transmission of COVID-19 (25). Studies evaluating the emotional states of dental staff during the COVID-19 pandemic period indicate that stress levels vary between 11.5 % and 95 % (20,25-31). According to our knowledge, few studies examined the emotional states of dentists who were redeployed outside of their dental clinics during the COVID-19 pandemic (7,20). Due to the fact that dentists performed fewer dental procedures in the first period of the COVID-19 pandemic, as reported by a study conducted during this period, the level of occupational burnout was higher in dentists redeployed to filiation (20). In the present study, which was conducted in the late period of the COVID-19 pandemic, most of the participants reported that dental procedures are riskier than filiation and preferred filiation to performing dental procedures. Unlike in the previous study, the reason why dentists now prefer filiation to dental treatments in the current study may be that aerosol-containing treatments started to be applied in the late period, unlike in the early period of the pandemic. Therefore, it can be considered as an expected situation for dentists to accept dental procedures as riskier than filiation during the COVID-19 pandemic. In a study of dentists redeployed to different

medical services of a hospital during the COVID-19 pandemic, it was noted that 52.6 % of the participants felt anxiety and, while 43.9 % felt confident about the new assignments (7). In this study, most of the participants who were redeployed to filiation showed symptoms of varying severity of depression, anxiety, and stress. Consistent with studies in the literature, 77.2 % of all participants had depression, 73.8 % had anxiety, and 59.7 % had stress. The differences can be attributed to the different sample sizes, study methods, questions, the fact that the studies were conducted at different times during the pandemic period, and the dentists' increased knowledge about the disease. Also, it was stated that dentists in different countries will exhibit different levels of subjective overload due to their healthcare systems' instructions, which in turn may affect their psychological distress and burnout (27). It was an expected situation to observe different psychological negativities in healthcare professionals in both those working directly in treatment and those redeployed to filiation of COVID-19 infected or suspected patients. This was due to continue about the extent of the pandemic and how vaccination would yield results.

Although dentists redeployed to filiation teams have several advantages, the personnel should be trained in the collection of the swab samples, infection control measures, and the transport of the samples (32). Also, it is recommended to develop a dental education curriculum on the management of disasters and pandemics (4,5). In a study conducted on redeployed dentists during the COVID-19 pandemic, although 71.9 % of participants received additional training, 42.1 % reported that they felt they did not receive definitive guidance on their redeployment (7). Most of the participants in the present study reported that they were informed about filiation from a physician experienced in filiation and by online services provided by the Ministry of Health. In addition, it may be important to increase the motivation of these personnel with additional information such as scientific articles, or visual or video trainings.

The use of PPE and the training of healthcare professionals in the use of PPE have important roles in the management of the pandemic. The WHO recommends that healthcare professionals who take samples from patients with suspected COVID-19 must use appropriate PPE (e.g. eye protection, N95 / FFP2 mask, long-sleeved gowns, and gloves) and train their staff on this issue (32). In the United Kingdom, it has been reported that 14 % of dental core trainers who were redeployed to areas treating COVID-19 positive or COVID-19 suspect patients did not have sufficient or correct PPE as per current national guidelines (7). In an international study investigating the use PPE by healthcare professionals working in the COVID-19 intensive care unit, it was found that most of the participants used N95 / FFP2 masks, waterproof long-sleeved gowns, and face shields / visors (58 %, 67 %, and 62 %, respectively) (33). In Italy, the usage rates of FFP2 masks, FFP3 masks, face shields, and glasses by healthcare professionals during medical treatments in the COVID-19 pandemic were reported as 50 %, 43 %, 86 %, and 14 %, respectively (34). In this study, the usage rates of N95 / FFP2 masks, face visors / protective glasses, and disposable aprons / overalls in filiation were found to be slightly higher than in previous studies (67.5 %, 63.1 %, and 66 %, respectively). These findings are important, as they show that the need for protective equipment is high during the COVID-19 pandemic. For this reason, the production of such protective equipment needs to be supported by local governments or globally.

Healthcare professionals may increase the risk of COVID-19 infection transmission due to close contact with confirmed or suspected COVID-19 patients. The frequent exposure of healthcare professionals to the virus and their heavy viral loads and lack of protective equipment in some regions not only increases the risk in the relevant regions but also may turn into a global threat (35). The rate of COVID-19 infection among healthcare professionals has been reported as 28 %, 14 %, and 5 % in various studies (36-38). In this study, the rate was 23.8 %, and 16.5 % of participants reported that they were infected during their redeployment to filiation. The differences may depend on the use of PPE, the working conditions, and the number of samples differing across the studies.

Healthcare professionals have been taking an active role in the current COVID-19 pandemic (16). It is known that this situation may cause concern in healthcare professionals, due to the risk of infection, the fear of infecting relatives, and negative discrimination from society (16,19,25). In the present study, most of the participants reported that they were worried because of the redeployment to filiation, and the main cause of worry was the risk of infecting their relatives. Although the dentists now stated that they prefer filiation to dental procedures, working directly in contact with confirmed or suspected COVID-19 may still cause concern for dentists. In addition, a small rate of the participants in this study reported that they started to get psychological support after their redeployment to filiation.

In studies conducted during the COVID-19 pandemic, it has been reported that females, young people, those with a history of psychiatric disorders, and front-line healthcare professionals show high levels of depression, anxiety, stress, and insomnia symptoms (15,16,20,24,39). In addition, single individuals were found to show higher levels of depression symptoms as compared to married individuals, and those living alone at home were found to show higher levels of depression symptoms as compared to those living with family or friends (16,39). In this study, depression, anxiety, and stress levels were higher in females than in males, in singles than in married individuals, and in those living alone than in those living with family or friends. Working at a university was associated with higher scores in the depression, and stress subscales, whereas having children was associated with lower scores on these subscales. A regression analysis showed that female gender, living alone, and working at a university were independent effect factors for negative psychological conditions across almost in all subscales. The findings of the present study were consistent with the findings of previous studies. This may be related to the fact

that the pandemic is global and that healthcare professionals around the world are faced with similar situations.

This study has some limitations. Because of this questionnaire was conducted in a cross-sectional period of the pandemic, we cannot know the long-term impact of the findings. However, the high levels of depression, anxiety, and stress symptoms observed in the participants are important results to monitor in the future. Another limitation is that since participations in the questionnaire study was voluntary, the findings presented here may not reflect the views of all dentists redeployed to filiation.

5. CONCLUSION

According to the findings of the present study, different intensities of depression, anxiety, and stress symptoms, ranging from moderate to extremely severe, were observed in dentists redeployed to filiation during the COVID-19 pandemic. Female gender, living alone, and working at a university were determined to be effective factors on negative emotional states including depression, anxiety, and stress. However, despite all of these negativities, it was an interesting finding that during the COVID-19 pandemic, filiation was seen as less risky and preferred by dentists as compared to the dental procedures they routinely perform in their careers.

Because dentists can be redeployed to public health roles in emergencies, they need to be trained and motivated in this regard.

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Clinical and Experimental Health Sciences

Effects of Melatonin Administration on Vasomotor Activity and Histological Structure of Isolated Thoracic Aorta in Rats Treated with Thyroxine

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ABSTRACT

Objective: The goal of this study was to examine the effect of in vivo melatonin (MEL) administration on isolated thoracic aorta in rats with thyroxine treatment and its duty in aortic response to contractile agents, such as potassium chloride (KCl) and phenylephrine (PE). In addition, immunohistological alterations were also examined.

Methods: Experimental groups were as follows: control group (n= 5), thyroxine group (n= 5), melatonin group (n= 6), and thyroxine + melatonin group (n= 6). L-thyroxine was given by intraperitoneal (i.p) administration at 0.3 mg/kg/day for 14 days. MEL was administered i.p., at 3 mg/kg/day for 14 days. The thoracic aorta was isolated from rats euthanized by cervical dislocation. Then, vascular rings were prepared. Concentration-response curves for KCl and PE applications were recorded in an isolated organ bath. Tissue samples were fixed in 10% formalin for histopathological and immunohistological evaluation.

Results: KCl and PE-induced contractions were reduced significantly in the thoracic aortic rings of the thyroxine-treated rats. MEL administration partially attenuated the reduction in the contraction responses due to thyroxine treatment. Immunohistological findings showed that MEL inhibits the thickening of the vessel wall by probably suppressing collagen formation due to thyroxine treatment in the aortic tissue.

Conclusion: Our results suggest that MEL may attenuate the decrease in vascular resistance caused by thyroxine treatment.

Keywords: Aorta; histology; melatonin; thyroxine; phenylephrine; potassium chloride.

1. INTRODUCTION

Hyperthyroidism is an endocrine disease in which excess thyroid hormone (TH) is synthesized and secreted by the thyroid gland (1). Although TH is effective in almost all tissues and metabolic processes, it shows its effect significantly in the cardiovascular system. Thyroid hormones play a significant role in cardiac structure and function. Excessive thyroid hormone influences cardiovascular functions increasing cardiac output, which causes heart failure and, finally ends with dilated cardiomyopathy. In hyperthyroidism, an increase in cerebrovascular morbidity and mortality rates, especially in the cardiovascular system, as well as disorders in cardiac structures and functions are observed (2).

Melatonin is a hormone that is released at night with a circadian rhythm and is produced especially in the pineal gland (3). Since Lerner et al. described melatonin in 1958, this hormone has been shown to be involved in the regulation of many physiological functions including the cardiovascular system (4). Melatonin has effects on blood pressure and myocardial contractility, and it also increases cardiac antioxidant capacity (5). Melatonin receptors have been discovered in the heart and arteries (4). However, the mechanisms of melatonin actions on the cardiovascular system functions are still not fully understood.

It is obvious that melatonin has various beneficial effects in supportive treatments of cardiopathological conditions including high blood pressure, reperfusion injury, drug toxicity, and hypertrophy of the heart (6-12). These severe conditions and low toxicity of melatonin show that it may be correct to use this indole in clinical trials. In animal studies, findings suggest that melatonin may have much the same protective effects in the human heart, unless it is completely misleading (13). The relationship between thyroid and melatonin hormones has been recently reviewed (14,15).

Although thyroid hormones reduce systemic resistance in small and medium-sized arteries due to smooth muscle

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relaxation, there is limited literature on their effects on the histomorphological structure and mechanical properties of the aorta (16). Thus, the goal of this study was to investigate the in vivo effects of melatonin on the peristaltic activity of the aortic ring in an isolated organ bath and also histological effects in thyroxine-treated rats.

2. METHODS

2.1. Animals

Wistar Albino type male rats (300-350 g, n= 22) used in this study were provided by Ataturk University Experimental Research Center (ATADEM). Rats were housed where 12/12 hours light/dark light cycle, 22±0.5° C room temperature and humidity 50-60% in the environment were provided, and standard pellets and tap water were placed in cages. Experimental groups were as follows: control (n= 5) group, thyroxine (n= 5) group, melatonin group (n= 6), and thyroxine + melatonin group (n= 6) (Table 1). All procedures and protocols used in the study were approved by the Local Ethics Committee of Animal Experiments of the Atatürk University on January 27, 2017, with the document number 75296309-050.01.04-E.170.003.4126.

Table 1. *Experimental groups.*

Group	n	Treatment
I: Control group	5	0.9 % NaCl, i.p., for 14 days
II: Thyroxine	5	L-Thyroxine (0.3 mg/kg/day), i.p., for 14 days
III: Melatonin	6	Melatonin (3 mg/kg/day), i.p., at 21:00 o'clock, for 14 days
IV: Thyroxine + Melatonin	6	L-Thyroxine + melatonin

2.2. Isolated Organ Bath Experiments

2.2.1. Drugs

Phenylephrine (Sigma, CAS number: 61-76-7) was used to induce in vitro isolated rat thoracic aortic smooth muscle contractions at a concentration of 10^{-7} M. KCl (Sigma, CAS no: 7447-40-7) was used to induce isolated thoracic aortic contractions at a concentration of 40 mM. L-Thyroxine (Sigma, CAS number: 51-48-9) was given at 0.3 mg/kg/day intraperitoneally for 14 days as described by Shinohara et al. (17) . Melatonin (Santa Cruz, CAS number: 73-31-4) was also administered by i.p. way to each rat at 3 mg/kg/day at 21:00 o'clock in vivo. All drugs were dissolved in distilled water.

2.2.2. Preparation of isolated rat aorta

The isolated organ bath is a standard in vitro research method that needs basic pharmacological equipment. Experimental animals were sacrificed by cervical dislocation to prevent possible vascular complications due to anesthesia. The thoracal cavity was carefully opened from the median line. The thoracic aorta was reached by pushing the heart and lungs to the left. The descendent part of the thoracic aorta was removed and put in a Petri dish with Krebs solution in which the tissue was able to maintain its functions under optimum conditions. The surrounding connective tissues of the thoracic aorta were carefully cleaned and vascular rings of 3-4 mm size were prepared. Before the experiment, adjustment control was made by hanging a 2 gram (g) weight on the isometric transducer tip. The thoracic aortic ring was fixed on the retaining hook in a 10 milliliter isolated organ bath in a standard Kreps solution and the other end was attached to the hook connected to the transducer. Kreps solution, with pH 7.4, 37° C and 95% O_2-5% CO_2, was consisted of NaCl 119, KH_2PO_4 1.2, KCl 4.75, $MgSO_4$ 1.5, $CaCl_2$ 2.5, $NaHCO_3$ 25, and glucose 11 as nM. A contraction up to 1g weight tension level was created while the medium was renewed every 15 minutes and incubated for one hour to let the thoracic aortic rings adapt to the bath environment.

At the end of the incubation period, different concentrations of PE (10^{-4}-10^{-9} M) and KCl (20 mM-100 mM) are applied to the isolated organ bath via a micro-pipette where the thoracic aortic smooth muscle rings isolated from the rats were hanged, and the contraction responses to them were recorded. The determined concentrations were applied non-cumulatively to the bath environment, and after each concentration, washing was done three times with an interval of two minutes and the tissue was brought to baseline balance. Another upper concentration was applied to the bath when the tissue appeared to be in basal balance, and the process continued as specified in the test protocol until the last concentration. Concentration-response curves for KCl and PE applications were recorded by reading them on the computer screen. Contractions reported are the means of experiments repeated three times for each concentration.

2.3. Histopathological Examination

2.3.1. Histological examination

Tissue samples were fixed in 10% formalin solution for 2 days. Paraffin blocks were embedded due to tissue follow-up routine. Sections with 4 µm of thickness were captivated from each block. For histopathological examination, the slides were stained with hematoxylin-eosin (HE) and examined with a light microscope (Olympus, Japan). Sections were assessed as absent (-), mild (+), moderate (++), and severe (+++) according to their histopathological findings (18).

2.3.2. Immunohistochemical staining

All sections taken with adhesive (poly-L-Lysin) on slides were passed through the xylol and alcohol series for immunoperoxidase examination. The sections were washed with phosphate buffered saline (PBS), and endogenous peroxidase inactivation was achieved by keeping them in 3% hydrogen peroxide for 10 minutes. In order to determine the antigen in the tissues, they were exposed to antigen retrieval

solution for 2x5 minutes at 500 watts in a microwave oven and then left to cool. Tissues were exposed to primary antibody (COL 1A1, Pro-COL 3A1, Catalog no: sc-293182, sc-166316, Santa Cruz, USA) for 30 min at 37˚C and then followed according to the immunohistochemistry kit procedure (Ab-236466) in order to determine mature and young collagen deposits. The chromogen used was 3-3' Diaminobenzidine (DAB). Hematoxylin was used for floor staining (19).

2.4. Statistical Analyses

Statistical analyses of the experimental data were carried out using IBM SPSS Statistics 20 for Windows. One Way ANOVA with posthoc LSD test was used to find the statistical differences between groups. As the level of statistical significance, P <0.05 value was accepted. Data were presented as mean ± standard deviation (SD).

Five random areas were selected from each image to evaluate the intensity of positive staining using the Imaging Software Program of ZEISS Zen. Mann-Whitney U test was performed to compare positive immunopositive stained areas and immunoreactive cells with controls. As a result of the test, the data were presented as mean ± SD and a P value of <0.05 was considered statistically significant.

3. RESULTS

3.1. Contraction Responses of the Aorta in an Isolated Organ Bath

The contraction responses of rat isolated thoracic aortic endothelial smooth muscle rings of the control group (n= 5) to KCl and PE are shown in Figures 1 and 2. Contractions reported are the means of experiments repeated three times for each concentration.

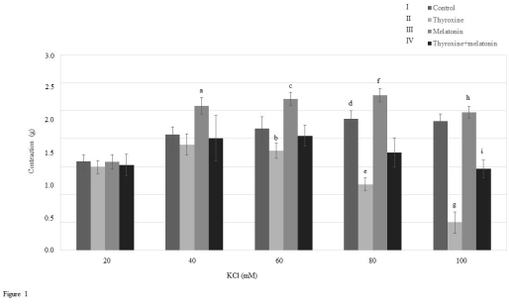

Figure 1

Figure 1. *Isolated thoracic aortic smooth muscle ring contraction responses (mean±SD) induced by potassium chloride (KCl) in control, melatonin, thyroxine treated and thyroxine plus melatonin groups. Contractions are the means of experiments repeated three times for each concentration. Statistical differences between groups are as follows: a: III vs I (p<0.05), II and IV (p<0.01); b: II vs IV (p<0.05); c: III vs I, II and IV (p<0.001); d: I vs II (p<0.001), III (p<0.01) and IV (p<0.001); e: II vs III and IV (p<0.001); f: II vs IV (p<0.001); g: II vs I (p<0.001); h: III vs II (p<0.001); i: IV vs I, II and III (p<0.001)*

As shown in Figure 1, although the contraction response of the isolated aorta to KCl was not affected below 60 mM, it decreased remarkably at 80 and 100 mM concentrations. Melatonin administration increased the aortic contraction compared with the control goup in all concentartions of KCl except 20 mM. At 80 and 100 mM KCl concentrations the decreases in aortic contraction due thyroxine treatment were prevented by melatonin administration.

As seen in Figure 2, contraction responses to PE were higher than all other groups in all concentrations tested. Thyroxine treatment decreased the contraction responses of aorta to PE remarkably in all concentrations of PE compared with control group. These decreases could only be prevented significantly by melatonin administration at 10^{-9} and 10^{-8} M concentrations. Although melatonin administration itself increased the contraction responses of aorta to PE in all concentrations of PE, the increases could have reached the statistical significance at 10^{-9}, 10^{-8} and 10^{-7} M concenrations of PE.

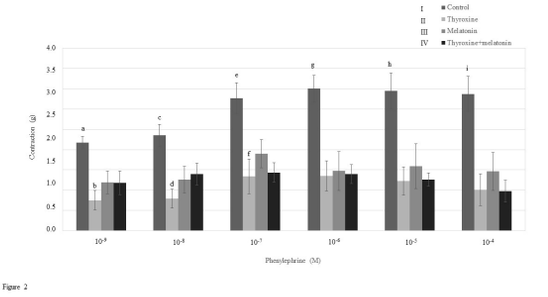

Figure 2

Figure 2. *Isolated thoracic aortic smooth muscle ring contraction responses (mean±SD) induced by phenylephrine (PE) in control, melatonin, thyroxine treated and thyroxine plus melatonin groups. Contractions are the means of experiments repeated three times for each concentration. Statistical differences between groups are as follows:a: I vs II, III and IV (p<0.001); b: II vs III and IV (p<0.05); c: I vs II, III and IV (p<0.001); d: II vs III and IV (p<0.05); e: I vs II, III and IV (p<0.001); f: II vs III (p<0.05); g: I vs II, III and IV (p<0.001); h: I vs II, III and IV (p<0.001); i: I vs II, III and IV (p<0.001).*

3.2. Histopathological Findings

3.2.1. Histopathology

When the aortic tissues were evaluated histopathologically, it was observed that they had a normal histological structure in the control group (Figure 3-A). On the other hand, severe degeneration of endothelial cells in the intima layer, the vessel wall thickening due to severe collagen increase in the media layer, and moderate edema in the adventitia layer were detected in the thyroxine treated group (Figure 3-B). However, the media and intima layers were in normal histological structure in the MEL group (Figure 3-C). Mild endothelial damage in the intima layer, thickening due to mild collagen increase, and mild edema in the adventitia layer were observed in MEL + thyroxine treated group (Figure 3-D).

A statistically significant decrease in aortic wall thickness was found when MEL + thyroxine treated group was compared with the only thyroxine treated group ($p<0.05$, Table 2). Histopathological findings are summarized in Table 3.

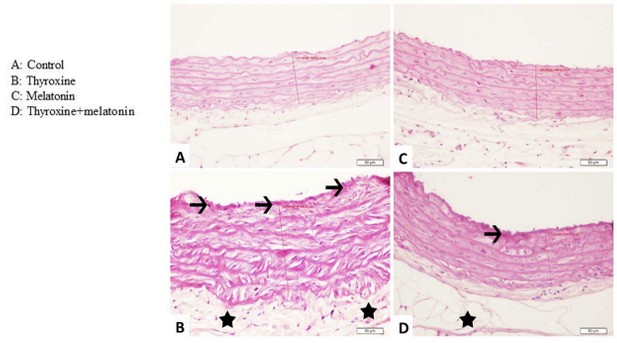

Figure 3. *Control and melatonin groups had normal histological appearance (A, C), thyroxine treated group had degeneration of endothelial cells (arrows), severe thickening of the vessel wall, and adventitial edema (stars) (B), melatonin + thyroxine group had mild thickening of the vessel wall and adventitial edema (star) (D). Aortic tissue, H&E, bar:50µm.*

Table 2. *The thickness of the aortic wall in experimental groups.*

Group	Thickness of the aortic wall (nm)
Control	103 288 ± 1241[a]
Thyroxine	195 425 ± 1062[b]
Melatonin	105 471 ± 1950[a]
Thyroxine + Melatonin	117 642 ± 1204[c]
Different letters (a, b, c) show statistically significant difference (p<0.05).	

Table 3. *Scoring of histopathological findings in aortic tissue.*

	Control	Thyroxine	Melatonin	Thyroxine + Melatonin
Endothelial damage	-	+++	-	+
Wall thickening	-	+++	-	+
Adventitial edema	-	+++	-	++

3.2.2. Immunohistochemical Findings

COL 1 and PRO-COL 3 expressions were evaluated as negative when aortic tissues were examined immunohistochemically in the control group (Figure 4-A and Figure 5-A). On the other hand, severe COL 1 and PRO-COL 3 expressions were detected in the medial layer in the thyroxine treated group (Figure 4-B and Figure 5-B).

COL 1 and PRO-COL 3 expressions were evaluated as negative when aortic tissues were examined immunohistochemically in the MEL group, also (Figure 4-C and Figure 5-C). However, mild COL 1 and PRO-COL 3 expressions were observed in the medial layer of the aorta in MEL+thyroxine treated group (Figure 4-D and Figure 5-D). Immunohistochemical findings are also summarized in Table 4.

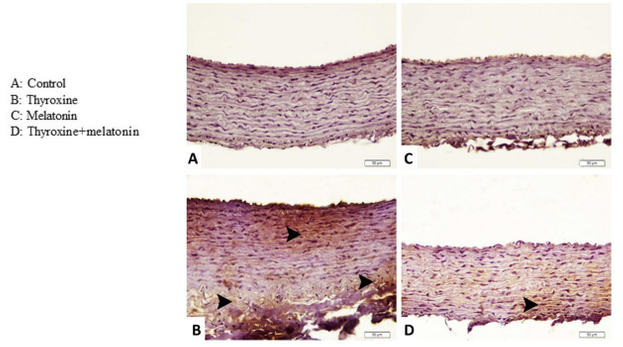

Figure 4. *Control and melatonin groups had negative COL 1 expression (A,C), the thyroxine treated group had severe COL 1 expression (arrow heads) (B), and the melatonin+thyroxine group had mild COL 1 expression (arrow heads) (D). Aortic tissue, immunohistochemistry paraffin protocol (IHC-P), bar: 50µm.*

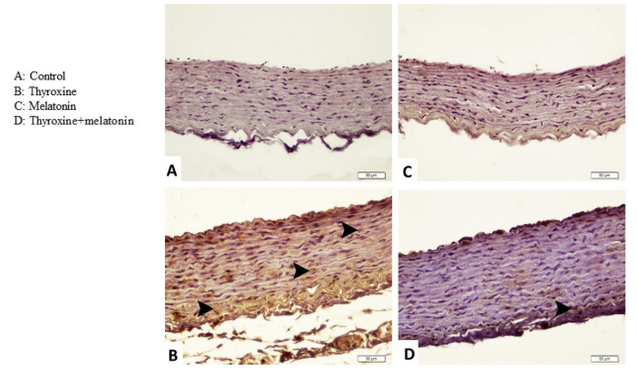

Figure 5. *Control and melatonin groups had negative PRO-COL 3 expression (A,C), the thyroxine treated group had severe PRO-COL 3 expression (arrow heads) (B), and the melatonin+thyroxine group had mild PRO-COL 3 expression (arrow head) (D). Aortic tissue, IHC-P, bar: 50µm.*

Table 4. *Scoring of immunohistochemical findings in aortic tissue.*

	Control	Thyroxine	Melatonin	Thyroxine + Melatonin
COL 1	21.3 ± 2.91[a]	83.1 ± 2.91[b]	20.9 ± 4.65[a]	41.4 ± 3.18[c]
PRO-COL 3	20.4 ± 4.25[a]	80.5 ± 7.16[b]	20.9 ± 5.14[a]	40.4 ± 3.18[c]

Different letters (a,b,c) in rows show statistically significant difference ($p < 0.05$).

4. DISCUSSION

Thyroid hormones have various effects on the cardiovascular system. Although the resistance of the systemic blood vessels and functions of the heart are mainly affected by the thyroid gland, hyperthyroidism decreases the total peripheral resistance and increases the cardiac output (20). However, the underlying mechanisms are still largely uncertain. In literature, although the changes in cardiac and vascular functions due to hyperthyroidism were commonly investigated in vivo (21-26), to the best of our knowledge the

changes in in vitro aortic reactivity in hyperthyroidism were studied in a few studies only (27,28).

In a study where McAllister et al. (28) have given triiodothyronine (Hyper, n=27; 300 µg/kg) to male rats for 6-12 weeks and investigated the effect of hyperthyroidism on vascular contraction and relaxation responses by comparing with the euthyroid control group (euthyroid n=27). They reported that the group with hyperthyroidism had developed hypertrophy in the left ventricle (euthyroid, 2.01±0.04 mg/g/kg; hyperthyroid, 2.70±0.06; p<0.0005), and also had higher oxidative enzyme activity in many skeletal muscle tissues (p<0.0005). When they evaluated the vascular responses of 2-3 mm vascular rings obtained from the abdominal aorta in vitro, and compared with the control group, they observed decreased contraction responses induced by the norepinephrine in the aortic rings with intact endothelium in the hyperthyroidism group (p<0.05), however, they did not find a significant difference when the endothelial layer is removed. They concluded that hyperthyroidism affects vascular contractile and relaxation responses of the blood vessels in male rats. Similar findings have also been reported by other researchers (29-31).

Although the excess amount of thyroid hormones is associated with several effects on the cardiovascular system, the adaptive changes in various arteries are not known well. In a study on acute hyperthyroidism induced vascular changes in isolated rat aorta reported by Honda et al. (27), acute hyperthyroidism was induced with a 3-day L-thyroxine (T_4) (0.5 mg/kg/day) subcutaneous injection to investigate whether muscarinic and adrenergic receptor-mediated responses of the blood vessels change. They stated that T_4 significantly increases tachycardia, cardiac hypertrophy, and weight loss. To see the effects of acute hyperthyroidism, the tension of aortic ring preparations was isometrically measured. As a result, they stated that the contractions induced by norepinephrine (NE) were significantly decreased in the aortic rings of T_4 treated rats compared to the control group, and vascular responses may change in the acute stage of hyperthyroidism and may result from an alteration in the endothelial NO system regardless of the amount of eNOS protein. In recent years, Gachkar et al. in the study of aortic effects of thyroid hormones in male mice, have shown that thyroid hormone affects aortic contractility via genomic and non-genomic mechanisms of action (32). In our study, we examined the effects of in vivo MEL application on contractile agents KCl and PE in isolated thoracic aorta smooth muscle rings in rats with thyroxine treatment, compared to the control group. KCl-induced relaxation responses of the aorta of rats treated with thyroxine at 80 mM and 100 mM concentrations were 49.79% and 78.35% of the control, respectively (Figure 1, p<0.001). When the PE responses were compared with the control group, the decrease in isolated thoracic aortic smooth muscle contractions in the thyroxine treated group was observed at all concentrations, and the highest relaxation response was found to be 70.03% of the control at PE concentration of 10^{-4} M (Figure 2, p<0.001). In rats with thyroxine treatment, the contractions created with KCl and PE showed a vasorelaxation response compared to the control group, and the reduction in vascular resistance in the literature was similar to our results.

Melatonin is involved in physiological processes including the cardiovascular system (33). It has been reported that melatonin causes vascular contraction (34) and contraction in isolated ring segments obtained from rat caudal artery is enhanced by adrenergic nerve stimulation (35). Mahle et al. first obtained pre-contraction induced by 30 mM KCl in the rings obtained from the 28 human coronary arteries, then they found increases in coronary artery rings induced by various concentrations of in vitro melatonin exposure (10^{-13}-10^{-5} M) (36). They also reported that melatonin induces concentration-dependent vasoconstriction in the rings of rat caudal and cerebral arteries.

In a study by Klemm et al., melatonin did not affect the relaxation caused by acetylcholine in the rat aorta; either this response did not possibly change the endothelial nitric oxide (NO) activity or vascular response would not be present to nitric oxide (37). In other studies, when vascular studies with the effect of melatonin in vitro and in vivo are evaluated, it can be seen that the endothelial layer is important in the effect of melatonin, and therefore melatonin may have a suppressive effect on NO (33, 38). Monroe et al. have found that agonist KCl (70 mM) induced contractions in rat aorta were inhibited by melatonin (10^{-5}-10^{-3} M) (33). Melatonin has been found to affect channels other than calcium channels, and in addition, it antagonizes calcium binding of calmodulin, the intracellular Ca^{+2} binding protein (39). In the literature, the fact that there are contradictory results in studies investigating the effects of melatonin on the vascular system suggests that responses to the melatonin used in the bath environment may vary due to different concentrations. In light of all this information, it can be said that the absence of suitable antagonists and agonists of the receptor subtypes of melatonin in the vascular endothelium makes it difficult to investigate the vasoconstrictive effects of melatonin (40).

In literature, there are many studies on the relations between the thyroid gland and pineal gland, which are important organs of the endocrine system. Although there are various results related to the hypothesis, material method and findings in the studies, it is concluded that the thyroid gland affects the pineal gland or vice versa. As a result of the literature review, it has been determined that there are differences between the application times of melatonin and thyroid gland functions. Two studies by Lewinski et al. (15) in rats and a study by Petterborg et al. (41) reveal that melatonin applied in the afternoon is more effective in thyroid inhibition. In the present study, 0.3 mg/kg/day L-thyroxine i.p was applied at 08:00 o'clock and 3 mg/kg/day melatonin i.p at 21:00 o'clock for fourteen days. Considering the results of the literature, it was aimed to increase the bioavailability of the hormone by applying melatonin in the evening.

Studies have shown that melatonin hormone, which has an endocrine effect on thyroid gland functions, has a general inhibitory effect. An increase in the secretion of

the T_4 hormone and hypertrophy in the thyroid gland have been reported in experimental animals with pinealectomy. Melatonin, on the other hand, suppresses T_4 released from the thyroid gland, causing a decrease in plasma T_4 levels. Melatonin also increases TSH (42). Vriend et al. have demonstrated that the administration of melatonin in hamsters with hypothyroidism decreases the high TSH hormone level suggesting that melatonin can play a role in regulating the release of TRH (43). In another study, it is reported that exogenous melatonin administration to blind hamsters reduces T_3 and T_4 levels (44). Studies have also shown high plasma melatonin levels in experimental animals with hyperthyroidism. This result demonstrates that hyperthyroidism can increase plasma levels of melatonin. It is widely agreed upon that there is an important relationship between the thyroid and the pineal gland and that disorders in thyroid function can alter the release of melatonin (45).

In recent studies, it has been shown by biochemical and immunohistochemical methods that MEL affects the cardiovascular system (11,42,46). In this study, severe degeneration of endothelial cells in the intima layer, thickening of the vessel wall due to severe collagen increase in the media layer, and moderate edema in the adventitia layer were observed in the thyroxine treated group. Additionally, mild COL 1 and PRO-COL 3 expressions were observed in the medial layer of the aortic wall in the thyroxine treated group. The aortic wall thickness in thyroxine treated group was significantly decreased by melatonin administration. Immunohistological alterations due to thyroxine treatment were attenuated by in vivo melatonin administration. The histopathological and immunohistological findings suggest that melatonin inhibits the thickening of the vessel wall by suppressing the formation of collagen 3, which is active young collagen, and its conversion to mature collagen, collagen 1, due to thyroxine treatment in the aortic tissue. In a study by Moulakaksis et al., it was stated that rats with thyrotoxicosis show significant changes in the mechanical and microstructural properties of the aortic wall (16). Similarly, there are studies showing that hyperthyroidism causes an increase in aortic stiffness (47). The increase in arterial wall stiffness carries a significant risk in the formation of atherosclerosis together with vascular aging. In SAMP8 mice, Rosei et al. have shown that chronic administration of melatonin reduces the contractile responses to norepinephrine in small arteries of the mesentery (48). In another study, it was demonstrated that in vitro administration of aortic rings of elderly rats with melatonin increases the relaxation response to the acetylcholine exposure (49). All of these studies show the beneficial effect of melatonin on arterial changes parallel to our results in this study.

Although many studies show the cardioprotective effects of melatonin in literature, it was not possible to make one-to-one comparison with our results since we could not find sufficient studies showing the effect of in vivo melatonin on vascular changes caused by thyroxine treatment. It seems that more studies are necessary to determine the mechanism/s of cellular action of melatonin on the vascular smooth muscle in thyroxine treated rats. With the techniques required to identify melatonin receptors, it will be possible to examine the physiological roles of the receptors and how they can act in various clinical situations, such as hyperthyroidism.

There are some limitations of this study. First, the experimental study period was relatively short (only 2 weeks) in our study evaluating the in vivo effect of melatonin on the thoracic aorta of rats treated with thyroxine. Second, studies using the nitric oxide synthesis inhibitor L-NAME for endothelium-independent relaxation could not be performed due to time and equipment limitations. Third, the effect of melatonin administration on the smaller peripheral arterioles of thyroxine-treated rats was beyond the scope of this study. Further studies are needed to investigate the possible role of melatonin in regulating compliance against resistance vessels (including venous capacitance vessels) in different regions and vascular beds of thyroxine-treated rats.

5. CONCLUSION

In summary, KCl and PE-induced contractions were reduced significantly in thoracic aortic rings isolated from thyroxine treated rats. MEL administration partially attenuated the reduction in the contraction responses due to thyroxine treatment. Immunohistological findings suggest that melatonin inhibits the thickening of the vessel wall by probably suppressing collagen formation due to thyroxine treatment in the aortic tissue. Our results suggested that MEL may attenuate the decrease in vascular resistance caused by thyroxine treatment.

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Ethics Committee Approval: *This study was approved by Ethics committee of Atatürk Üniversitesi Animal Studies (Date: 27.01.2017; and number of approval: 6).*

Peer-review: *Externally peer-reviewed.*

Author Contributions:

Research idea: HU, ES
Design of the study: HU, ES, MG
Acquisition of data for the study: HU, ES, SY
Analysis of data for the study: HU, ES, FC, MG
Interpretation of data for the study: HU, MG, FC, SY
Drafting the manuscript: HU, ES
Revising it critically for important intellectual content: HU, MG, FC, SY
Final approval of the version to be published: HU, ES, SY, FC, MG

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Clinical and Experimental Health Sciences

Elevated Matrix Metalloproteinase 9 in Treatment Resistant Bipolar Depression

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ABSTRACT

Objective: Matrix metalloproteinase is a family of proteases with different pathophysiological roles. Matrix metalloproteinase 9 (MMP9) plays an enzymatic role in the restructuring of the extracellular matrix and adhesion molecules. MMP9 is upregulated in pro-inflammatory states and leads to breakdown of tight junctions thereby increasing blood-brain barrier (BBB) permeability. MMP9 may contribute to the pathophysiology of bipolar disorder (BD) via proteolysis of the BBB thus allowing entry of cytokines and neurotoxic agents into CNS. Polymorphisms of the MMP9 gene may pose increased risk for BD and schizophrenia. In this study we sought to determine MMP9 levels in treatment resistant bipolar depressed patients before and after treatment.

Methods: Treatment resistant bipolar depressed patients were treated with escitalopram, in combination with the COX-2 inhibitor, celecoxib. It was hypothesized that combination treatment would reverse resistance and augmented treatment responses. This was a 10-week, randomized, double-blind, two-arm, placebo-controlled study.

Results: MMP9 levels were higher in bipolar depressed patients compared to healthy controls at baseline, however, the difference did not reach significance. Levels decreased after treatment reaching significance in the escitalopram plus placebo group. Female patients had significantly lower MMP9 levels at end of treatment. MMP9 was higher in carriers of the MMP9 SNP, rs3918242, than in noncarriers, but the difference did not reach statistical significance.

Conclusion: MMP9 decreased in bipolar depressed patients with treatment. Age, sex and the rs3918242 polymorphism play a role in MMP9 levels. Future studies should confirm the role of MMP9 in the pathogenesis and pathophysiology of bipolar disorder, as a potential diagnostic biomarker.

Keywords: Bipolar depression, treatment resistance, MMP9, blood-brain barrier, inflammation, celecoxib

1. INTRODUCTION

Psychiatric disorders are highly prevalent around the world and represent a significant health burden (1). Lifetime prevalence of bipolar disorder (BD) is estimated to be 0.5 – 5% for both type I and II, but rates vary significantly across studies (2). BD is the sixth leading cause of disability worldwide (3). BD places a significant burden on affected individuals and their families with serious socioeconomic consequences; it is associated with significant impairment in work, family, and social life, not only during the acute phases of the illness, but also between episodes.

A better understanding of the pathophysiology of BD can lead to better treatment options and quality of life for these patients. There are abundant theories about the pathophysiology of the disorder. Heritability that is calculated to be 56.7% after adjusting for sex and age, plays an important role, with the concordance rate for monozygotic twins being 57% compared to 14% for heterozygotic twins (4). Studies suggest involvement of different brain regions. PET imaging and functional MRI studies have shown elevated activity in amygdala and diminished activity in the hippocampus and prefrontal cortex in BD patients. Structural studies have associated BD with above-average amygdala volume and below-average prefrontal cortex, basal ganglia, hippocampus, and anterior cingulate volume (5). Dysregulation of multiple neurotransmitters, notably serotonin, norepinephrine

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and dopamine, has been suggested as a contributory etiopathological causes of mood disorders (6). There is also evidence that infection with HSV-1 is an independent predictor of cognitive impairment in patients with BD or Schizophrenia (3).

A growing body of evidence supports the role of inflammation and oxidative stress in BD pathophysiology. Studies have shown peripherally measured elevated levels of pro-inflammatory cytokines, such as IL-1b, IL-6 or TNFα, in BD patients in comparison to healthy controls, and these increases occur during both manic and depressive episodes. Cognitive decline as well as abnormalities observed in gray and white matter are considered to be mediated, at least in part, by increased neuro-inflammation. Evidence of increased neuroinflammation through excessive microglial activation has been found in many imaging and postmortem studies (7-9). These pathophysiological changes are in line with numerous studies showing elevated mortality rates due to conditions associated with inflammation and oxidative stress. BD patients die on average 9 years younger than the general population. Moreover, several studies have provided evidence of the role of psychotropic agents, such as lithium, in the modulation of pro-inflammatory and anti-inflammatory pathways (10).

Increasing attention is being given to the role of the blood brain barrier (BBB) disruption in numerous neuropsychiatric disorders. The BBB functions as a diffusion barrier, regulating the transport of micro – and macro-molecules between peripheral blood and the CNS. It is formed primarily by endothelial cells of the capillary wall, astrocyte end-feet ensheathing the capillary, pericytes embedded in the capillary basement membrane, perivascular macrophages and a basal membrane. A tightly sealed monolayer of endothelial cells with tight junctions (TJ) and adherent junctions (AJ) form its barrier. Small lipophilic molecules can freely pass-through the BBB, whereas passage of large and hydrophilic solutes is limited. Specific transporters and receptor mediated endocytosis allow certain complex molecules to pass through it. Thus, a disruption of its integrity can lead to the transport of inflammatory mediators from the periphery to the CNS. Both manic and depressive episodes in BD are associated with increased levels of proinflammatory cytokines in blood, leading to a transient disruption in the permeability of the BBB during each episode.

Matrix metalloproteinase (MMP) is a large family of zinc-dependent, extracellularly acting endopeptidases with several different roles (11). These proteins are involved in the breakdown of extracellular matrix in normal physiological processes, such as embryonic development, reproduction, and tissue remodeling. At the same time, these proteins play a role in disease processes, such as cardiovascular disease, arthritis, cancer and neuropsychiatric disorders (BD, schizophrenia, and multiple sclerosis (12). MMPs are secreted as inactive pro-proteins which are activated when cleaved by extracellular proteinases.

Matrix metalloproteinase 9 (MMP9), also known as gelatinase B, 92 kDa gelatinase, or 92 Da type collagenase, plays an enzymatic role in the restructuring of the extracellular matrix and adhesion molecules (11). Experimental studies have shown that MMP9 plays a role in the plasticity of the central nervous system, as demonstrated by studies with MMP9 null mutant mice that have behavioral impairments in hippocampal dependent memory. (13). Moreover, MMP9 has been suggested in animal models to have a role in the pathogenesis of temporal lobe epilepsy (11).

Of key interest is the upregulation of MMP9 in pro-inflammatory states. In this proinflammatory milieu, MMP9 plays an important role in the breakdown of tight junctions, basal lamina, and extracellular matrix, thus increasing the permeability of the BBB, which can potentially have an impact on CNS milieu (10).

The human MMP9 gene was mapped in the 20q11.2-q13.1 chromosome area. There is a single nucleotide polymorphism (rs3918242), a C to T substitution at – 1562 bp, which results in a functional effect on gene transcription. This polymorphism leads to loss of binding of a nuclear protein to this region and an increase in transcriptional activity in macrophages. Lower transcriptional activity of the gene is associated with C/C genotype, while C/T and T/T genotypes resulted in higher transcriptional activity. Research has linked the T allele with increased severity of coronary arteriosclerosis, increased cardiac mortality and increased risk of more severe progression of some types of cancer and predisposition to BD, schizophrenia, and multiple sclerosis (14). BD patients had significantly higher frequency of the T allele of the polymorphism compared to healthy controls, especially evident in a subgroup of patients with BD type II.1 This could be the genetic link between BD and some somatic illnesses (15). A recent study produced strong evidence that a SNP related to the MMP9 gene, rs3918242, was significantly associated with susceptibility to anxiety disorders (16).

Another study showed increased levels of MMP9 and soluble intercellular adhesion molecule 1(sICAM-1), which is also involved in the restructuring of connective tissues in patients with BD in euthymic state compared to healthy controls. Significantly higher levels of MMP9 and sICAM-1 were associated with a later, progressive stage of the disease compared to earlier stages (17). We hypothesized that MMP9 may contribute to the pathophysiology of BD via proteolysis of the BBB thus exposing the CNS to a pro-inflammatory environment resulting from entry into the brain parenchyma of cytokines and neurotoxic agents. The aim of this study was to investigate the role of MMP9 in treatment resistant bipolar depression (TRBDD) and to establish whether MMP9 levels are increased in such patients. In addition, we sought to determine whether addition of an anti-inflammatory agent would augment the therapeutic response to antidepressant treatment.

2. METHODS

2.1. Study Population

The study was approved by the Institutional Review Board (IRB) of Loyola University Medical Center and was conducted according to the principles of the Declaration of Helsinki (LU # 203368, March 23, 2011). Potential candidates were screened to determine eligibility for the study based on their mental capacity to give informed consent after a study physician extensively explained the procedures of the study and the inclusion/exclusion criteria. Candidates were males and females between the ages of 21 and 65 with TRBDD who met the DSM-IV criteria for bipolar disorder (BD I or II) depressed phase and who met criteria for treatment resistance in the depressive phase of their illness.

Exclusion criteria included any comorbid medical or psychiatric diagnosis or substance abuse/dependence during the preceding 12 months. Subjects with chronic or acute inflammation, liver or kidney disease, arthritis, hypertension, diabetes, anemia or any autoimmune disorders were excluded. Study patients could not be taking stimulants, lithium, anticoagulant agents or any nicotine containing substances. Individuals who were pregnant, lactating, or taking oral contraceptives were excluded.

Treatment resistance was determined by the Maudsley Scale. The scale uses a variety of factors to determine treatment resistant depression, notably, duration and severity of symptoms, number of treatment failures and the use of psychopharmacological augmentation or electroconvulsive therapy. To be considered for the study, patients had to have failed to respond to at least two adequate lifetime trials with antidepressants and/or mood stabilizers or experienced a breakthrough depressive episode despite being maintained on a mood stabilizer and/or atypical antipsychotic agent.

For admission to the study, a minimum score of 18 on the 17-item Hamilton Depression scale (HAM-D17) was required. Moreover, the patients were required to be clinically stable before entering the study on a mood stabilizer and/or antipsychotic medication.

2.2. Study Design

This was a 10-week, randomized, double-blind, two-arm, placebo-controlled study. The study included a screening visit, a 1-week washout phase, a 1-week placebo run-in phase and an 8-week flexible dose phase (NCT0149829). During the screening visit, medical history was obtained using a Past Medical History and a Family History Questionnaire, and a physical exam was performed. Blood and urine samples were collected to obtain CBC with differential, CMP, thyroid function, lipid profile, hCG pregnancy test and toxicology screening. Subjects were evaluated with the MINI International Neuropsychiatric Interview and a variety of scales, HAM-D17, Montgomery-Asberg Depression Rating scale, Hamilton Anxiety Rating Scale, Clinical Global Severity and Improvement, and Columbia Suicide Severity Rating Scale. Once cleared and consented, patients were instructed to stop any antidepressant they were taking for a 2-week washout (4 weeks for Fluoxetine) period. They were then placed on a 1-week blinded placebo run-in phase, to identify potential placebo (PBO) responders, who were excluded from the study and offered conventional care at the same institution.

Patients who continued to meet eligibility criteria at the baseline visit were randomized to receive, on a double-blind basis, either escitalopram (ESC) and celecoxib (CBX) or ESC and PBO. ESC dosing was initiated at 10mg/day and was optimized based on efficacy and tolerability over the first 4 weeks of treatment, with a max dose of 30mg, while CBX was administered fixed at 400mg/day. No dose adjustments were made after the first 4 weeks of treatment. The randomization was done at a fixed assignment ratio 1:1. The study pharmacist generated the randomization code, kept it in sealed envelopes and only broke the seal if a serious adverse event occurred. At every visit the pharmacist prepared and handed to the subjects the study medications. Subjects were required to return the empty vials at every visit to ensure compliance.

A HAM-D17 score of 18 or greater was required for study eligibility. At least a 50% reduction in baseline HAM-D17 score by week 8 of treatment was considered response to treatment as long as the total score was above 7, while remission was defined as a total score less than 7 in HAM-D17 by treatment end. Forty-six patients completed the study; however, MMP9 data for baseline and completion of 8 weeks of treatment was available for only 39 patients. From these 39 patients, 14 were in the placebo group, while 24 were in the Celecoxib group. Healthy control subjects (N=21) were used for comparison of baseline measures, but only 13 of them had MMP9 values.

2.3. Healthy Control Subjects

Potential healthy control (HC) subjects were required to provide written informed consent as approved by the IRB. They then underwent the same screening assessments; routine laboratory tests had to be within normal limits while the main exclusion criterion was any history of psychiatric disorder in themselves or their first-degree relatives. Other exclusion criteria included presence of any medical condition, any inflammatory condition and substance abuse, current or past. HAM-D17 and BDI scores had to be less than 5. A cohort of 21 HC subjects were recruited to match the age range of the study subjects. We obtained MMP9 data for 13 of the 21 HC subjects (Table 1).

2.4. Biochemical Analysis

After an overnight fast, blood draws occurred between 8 and 10 am at baseline, at week 4 and week 8 for patients and once for healthy controls. Peripherally measured MMP9 levels exhibit a diurnal rhythm. The peak is upon awakening and the trough during the nighttime; levels are stable during midday

and into the evening hours. Blood samples were separated into plasma or serum and samples were immediately stored at – 80° C until analyzed. Serum samples were analyzed for cytokines and MMP9 levels by the technique marketed by Randox Technologies using "Evidence Investigator™". Procedures were followed according to the protocols for these assays. Statistical analyses were conducted with GraphPad Prism version 8.0.2 and SPSS version 20. Paired and unpaired t-test was used to compare the mean value of a quantitative parameter between 2 groups, while ANOVA was used to compare 3 or more groups. Pearson correlation was used to investigate the correlation between MMP9 levels and the scores of different scales that were used in the study.

Table 1. *Demographics for TRBDD patients and healthy controls*

		Patients with TRBDD	Healthy controls	p value
Female		61%	54%	0.649[a]
Male		39%	46%	
Age		41.82	42	0.9665[b]
Weight (in Kg)		89.65	73.9	0.0222[b]
BMI		31.85	26	0.0101[b]
Ethnicity	Caucasian	24	10	0.3126[a]
	Non-Caucasian	15	3	
Tobacco users	Yes	13	0	0.0162[a]
	No	26	13	

[a] *Chi-square test*
[b] *t-test*

3. RESULTS

Baseline MMP9 levels in the TRBDD group (n=39) were elevated compared to the HC subjects (n=13), but the difference did not reach statistical significance (P = 0.1668, unpaired t-test), means of 67.81 ng/ml and 43.94 ng/ml, respectively (Figure 1). MMP9 levels in patients with TRBDD declined significantly after 8 weeks of treatment with ESC whether combined with CBX or PBO. Mean week 8 MMP9 level for the entire TRBDD cohort was 50.62 ng/ml compared to 67.81 ng/ml at baseline (p=0.025, paired t-test) and still slightly above the mean of HC subjects (Figure 2).

We compared MMP9 values at baseline and week 8 for the two treatment groups. Surprisingly, there was a statistically significant difference in the ESC + PBO group (p=0.018, paired t-test; Figure 3), but not in the ESC + CBX group (p=0.348, paired t-test; Figure 4). Mean MMP9 value for the ESC + PBO group was 86.41 at baseline and 56.32 at week 8. For the ESC + CBX group the mean MMP9 value was 56.18 at baseline and 47.06 at end of treatment. Both treatment groups showed declines in MMP9 values with treatment. When comparing HC at baseline with the two groups, ESC+PBO and ESC+CBX, there was no statistically significant difference at MMP9 levels. (HC vs ESC + PBO group, p=0.0896; HC vs ESC + CBX, p=0.7744; ESC + PBO group vs ESC + CBX, p=0.1920).

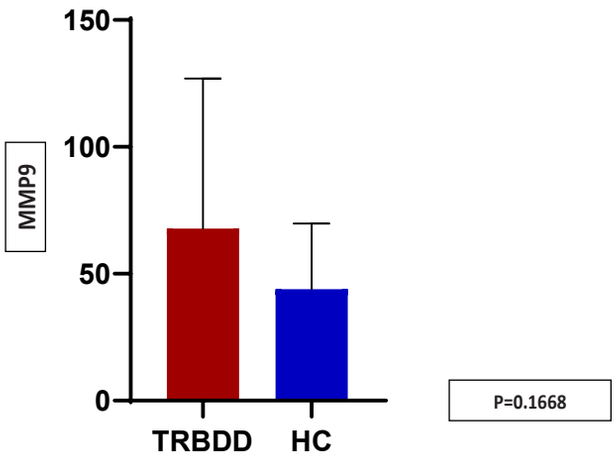

Figure 1. *MMP9 levels at baseline for patients with treatment resistant bipolar depression (Mean=67.81 ng/ml, SD= 59.17) and Healthy controls (Mean=43.94 ng/ml, SD=25.89)*

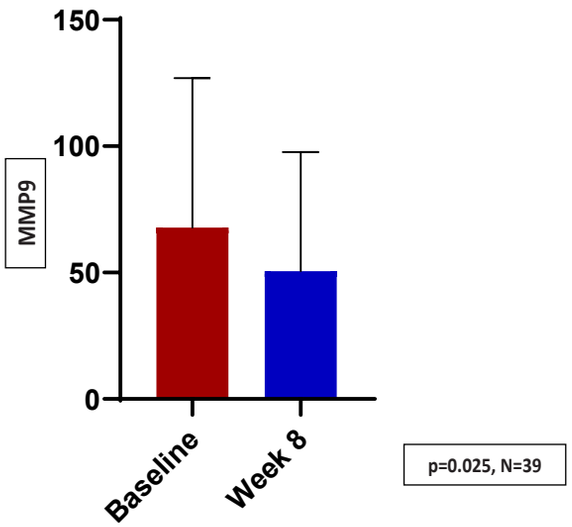

Figure 2. *MMP9 levels for patients with treatment resistant bipolar depression at baseline (Mean MMP9=67.81 ng/ml, SD=59.17) and week 8 (Mean MMP9=50.62 ng/ml, SD=47.04)*

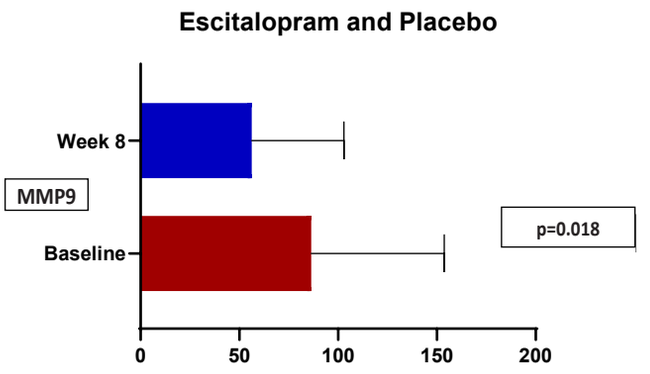

Figure 3. *Placebo group at baseline (Mean=86.41 ng/ml, SD= 67.3) and week 8 (Mean=56.32 ng/ml, SD=46.59)*

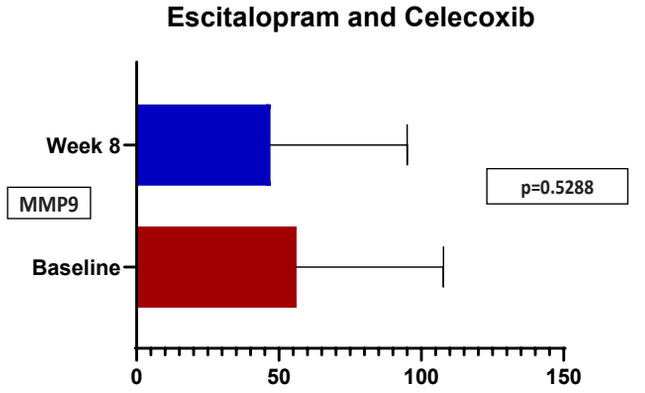

Figure 4. *Celecoxib group at baseline (Mean=56.18 ng/ml, SD=51.58) and week 8 (Mean=47.06 ng/ml, SD=47.96).*

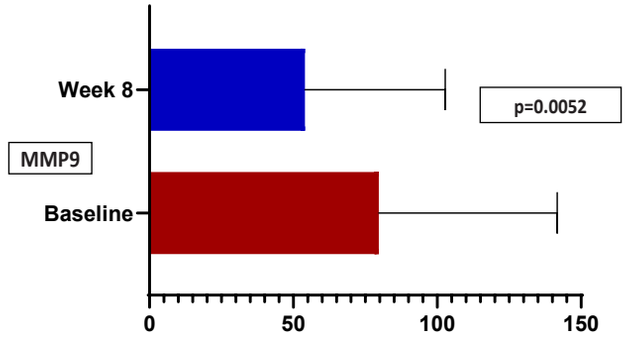

Figure 5. *MMP9 levels for females at the beginning (MMP9=79.71 ng/ml, SD=61.96) and end of treatment (54.03 ng/ml, SD=48.72)*

We then compared MMP9 levels in males (n=15) and females (n=24). There was no statistically significant difference between the two groups at baseline (p=0.113). There was no statistically significant difference when comparing female HC and baseline female BDD patients at baseline or male HC and male BDD patients at baseline. However, females showed a significant change between baseline and week 8 (p= 0.005 vs p=0.793 for men, paired t-test; Figure 5). MMP9 levels declined in the female group from 79.71 to 54.03.

Using the median age of the patient group, we divided them into two age groups, <40 (n=20) and ≥40 (n=19). MMP9 at baseline was statistically significantly higher in the ≥40 patient group (p=0.012). Mean MMP9 values were 91.64 for the ≥40 group, and 45.16 for the <40 group. There was no statistically significant difference in MMP9 levels between baseline and week 8 for the <40 group. MMP9 for the ≥40 group was higher at baseline than week 8 (p=0.075, paired t-test), means of 91.64 and 57.35, respectively (Figures 6 and 7).

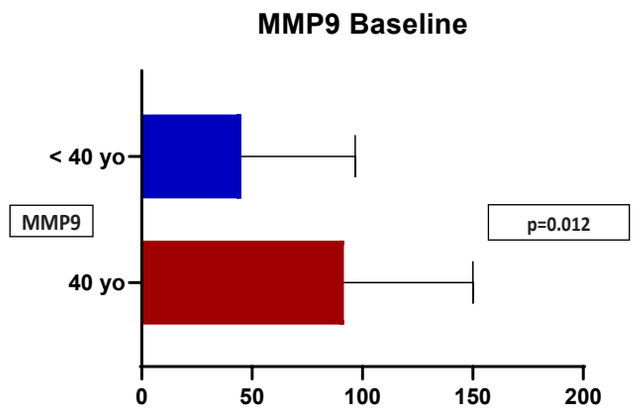

Figure 6. *Baseline levels of MMP9 for the ≥ 40 (Mean=91.64 ng/ml, SD-58.44) and <40 group of patients (Mean=45.16 ng/ml, SD=51.56)*

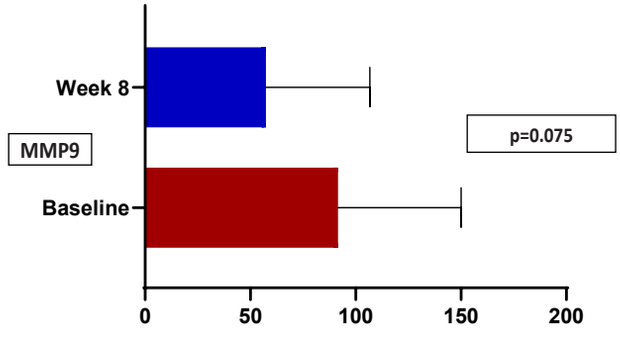

Figure 7. *MMP9 was decreased for patients ≥ 40 years old between baseline (Mean= 91.64 ng/ml, SD=58.44) and week 8 (Mean= 57.35 ng/ml, SD=49.34)*

There was no statistically significant correlation between MMP9 and HAM-D17, HAM-A, MADRS and PSS-14 at baseline, week 4 or week 8. We also assessed the MMP9 SNP rs3918242. Of a total of 39 patients, 30 were non-carriers and 9 were carriers of the SNP rs3918242. In the no-carrier group, 44.1% were males and 55.9% females, whereas in the carrier group 20% were males and 80% females (p=0.169). When adjusting for confounding factors (age, sex, and body mass index), the mean value for MMP9 at baseline was higher for carriers of the MMP9 SNP (80.16) than for the noncarriers (64.09) but the difference did not reach statistical

significance (p=0.486). This is likely due to the small sample size, and we believe that a larger sample size will likely reach statistical significance. Future studies should explore differences in MMP9 levels in carriers and non-carriers of the SNP rs3918242, as it may relate to BBB function. Additionally, in the non-carrier group 38.2% showed no response, while 61.8% responded to treatment. In the carrier group, 30% were non-responders while 70% responded to treatment. That difference was not statistically significant (p=0.634). When we investigated remission, for the non-carrier group 61.8% did not remit while 38.2% were remitters. In the carrier group 40% did not remit while 60% reached remission (p=0.222). We do believe that a greater sample size can reach statistical significance and this difference may have clinical implications.

4. DISCUSSION

In order to more accurately diagnose and more effectively treat BD, we need to better understand its complex pathophysiology. Currently, the diagnosis of BD is established by DSM criteria, that are based on the patient's report and clinical judgement, both of which are subjective. Recent studies have clearly revealed increased levels of pro-inflammatory mediators, such as TNF-a and IL-1b, and decreased levels of anti-inflammatory ones, such as IL-4 and IL-10, in patients with mood disorders. This supports the hypothesis that inflammation plays an important role in the pathophysiology of BD. Biochemical markers can potentially serve as diagnostic markers for mood disorders or even as an adjunctive tool for staging of BD (18).

A potential biomarker that may be used in BD diagnosis, if confirmed in future and larger studies, is matrix metalloproteinase-9 (MMP9), an endopeptidase that plays a role in the plasticity of the central nervous system. Studies have shown an association between MMP9 and unipolar depression (19) as well as symptoms of depression in middle-aged normal population assessed by means of the Center for Epidemiological Studies Depression Questionnaire (CED-S) (20). Increased MMP9 levels are also associated with cardiovascular disease, cancer, multiple sclerosis, schizophrenia and bipolar disorder (11,15).

Our results show a significant association between blood levels of MMP9 in patients with TRBDD compared to HC subjects at baseline. Our findings are in line with the growing body of evidence suggesting a role of neuro-inflammation in the pathogenesis and pathophysiology of BD. Many studies are showing that inflammatory markers are increased in patients with BD in comparison to healthy controls. Therefore, MMP9 can be an adjunctive tool in BD diagnosis In addition, anti-inflammatory treatment could be used to augment antidepressant effectiveness in TRBDD.

Our study showed that women and patients ≥40 years of age rather than men or patients <40 years of age had significantly higher levels of MMP9 at baseline compared to HC subjects. In another study (17), MMP9 levels are higher for BD patients in euthymic state than in HC and patients at a later stage of BD had higher levels of MMP9 levels than patients in earlier stages. More studies with a greater number of patients are needed in order to determine what role MMP9 can play in the diagnosis of BD, its subtypes, the stage of the disorder and treatment resistance. Several polymorphisms of the MMP9 gene have been identified, the MMP9 SNP (rs3918242) seems to have a functional effect on the transcription of the gene. Even though our analysis did not reach statistical significance, the trend shows that MMP9 levels were increased in the carrier group. There was also a trend showing that the group of carriers had greater remission rates. Future studies should focus on the effect of this SNP in the pathogenesis of BD and the response to different types of treatment.

The search for peripheral biomarkers has not, as of yet, resulted in reliable, non-invasive tests that can be used for the diagnosis of psychiatric disorders. The heterogeneity of the pathophysiology and the overlap across different psychiatric disorders make it impossible for a single test to be specific or applicable for any disorder. However, the different biomarkers can be used in addition to the scales and clinical criteria to assist the clinician with the practice of personalized medicine thereby leading to more accurate diagnosis and patient congruent treatment modalities.

There is growing evidence that certain antidepressants and mood stabilizers exert anti-inflammatory and antioxidant effects. In our study, patients were treated with ESC in combination with either CBX or PBO. MMP9 levels were reduced after both treatments. However, the difference was, surprisingly, statistically significant only for the ESC + PBO group. A statistically significant decrease of MMP9 levels was noted for the patients ≥40 years of age and women after treatment. There was a decrease in patients <40 years of age and men as well, but it did not reach statistical significance. Clearly, age and sex are apparent important confounding factors along with the SNP rs3918242 polymorphism; we strongly recommend they should be included in the design of future studies along with further exploration of the MMP9 and potential role of anti-inflammatory treatments.

Limitations: *Relatively small sample size and a short observation period (8 weeks).*

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Clinical and Experimental Health Sciences

Search, Look, and See; Late Recognised Hypereosinophilic Syndrome with Deletion (4) (q12)

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ABSTRACT

The hypereosinophilic syndrome (HES) is a group of rare disorders characterized by persistently high peripheral blood eosinophiles (≥ 1.5x109/L), and related signs or symptoms of organ involvement without secondary causes. Eosinophilia with recurrent genetic abnormalities (PDGFRA/B, FGFR1) comprises a minority of these patients. In this report, we aimed to point out a case with 4q12 deletion whose diagnosis and treatment were delayed for quite a while. The patient was followed for bronchial asthma for a long time and the recognition of hypereosinophilia yielded a suspicion for HES / Chronic eosinophilic leukemia (CEL). During the initial part of his diagnostic evaluation, there was an unawareness of the cryptic deletion which was a target for tyrosine kinases. The symptoms resolved and complete cytogenetic response was achieved with 100 mg imatinib continuing for 57 months.

Keywords: Hypereosinophilic syndrome, del (4) (q12), tyrosine kinase inhibitors

1. INTRODUCTION

The hypereosinophilic syndromes (HES) are a group of rare disorders characterized by a persistently high peripheral blood eosinophilia (≥ 1.5x109/L), signs or symptoms of organ involvement and no secondary causes such as allergies, atopic diseases and asthma, infections (mainly helminthic), autoimmune disorders, exposure to toxins, solid or hematopoietic neoplasias (1).

The incidence of eosinophilias with recurrent genetic abnormalities (PDGFRA/B, FGFR1) comprises a minority of these patients. Larger studies conducted in developing countries indicate that the FIP1L1-PDGFRA fusion occurs in approximately 10–20% of patients with idiopathic HES (2-4).

The severity of eosinophilia has been divided into mild (AEC from the upper limit of normal to 1,500/ mm^3), moderate (AEC 1,500–5,000/mm^3), and severe (AEC > 5,000/mm^3) (5-7).

The most common symptoms of patients have been showed weakness and fatigue, cough, dyspnea, myalgias or angioedema, rash or fever, and rhinitis in two retrospective studies (8). In the laboratory, peripheral eosinophilia in the range of 30–70% and leukocytosis up to 30000/mm3 or higher are common results (9-11). The other hematologic findings; as neutrophilia (in peripheral or bone marrow), basophilia, myeloid immaturity, and both mature and immature eosinophils with varying degrees of dysplasia can also be seen (12,13). All organ systems can be affected with sustained eosinophilia and can be seen insufficiencies of these systems (14).

The screening of the peripheral blood for the FIP1L1-PDGFRA gene fusion is the first step of evaluation of primary eosinophilia. FISH probes that hybridize to the region between the FIP1L1 and PDGFRA genes should be used to detect the presence of the cytogenetically occult 800-kb deletion on 4q12 that results in FIP1L1-PDGFRA. Absence of the FIP1L1-PDGFRA fusion should prompt evaluation for other primary eosinophilia associated with recurrent molecular abnormalities (8). We aimed with our case to point out examining the results of genetic assessment for primary HES. While incomplete or incorrect result evaluations may delay the appropriate treatment approach.

2. CASE PRESENTATION

A 47-year-old male patient admitted to our center for evaluation of leukocytosis with hyper – eosinophilia. He has been treated with inhaled steroids because of chronic cough

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lasting four years. The patient was consulted with hematology for eosinophilia which was noticed during the follow-up. The hypereosinophilia was confirmed by another center with controlling whole blood count and 90% eosinophiles were detected in the peripheral blood smear without blastic cells. The bone marrow smear and flow cytometric analyses revealed 23% eosinophiles without blastic transformation. The secondary causes and organ involvement were excluded by using molecular test of BCR-ABL and JAK2 V617F mutation, karyotype analysis of bone marrow cells, ultrasonographic evaluation of the abdomen, endoscopic review and endoscopic biopsy of the gastrointestinal system, high-resolution chest tomography, and echocardiography.

The fusion of FIP1L1 and PDGFRA was found negative by interphase-FISH and 1 mg/kg metilprednisolon po was initiated. However, there was not any regression in the eosinophilic leukocytosis or clinical symptoms. After the patient's admission to our center, we reviewed all the previous examinations and noticed that a FIP1L1and PDGFRA fusion had been found negative in the interphase FISH analysis with one-month interval, but as a remarkable result, 4q12 deletion had been found 24% and 74% respectively. The screening of the peripheral blood or the bone marrow for the FIP1L1-PDGFRA gene fusion is the first step of the evaluation of primary clonal eosinophilia. FISH probes that hybridize to the region between the FIP1L1 and PDGFRA genes should be used to detect the presence of the cytogenetically occult 800-kb deletion on 4q12 that results in FIP1L1-PDGFRA. This cryptic deletion of 4q12 producing the FIP1L1/PDGFRA fusion gene has been indicated as a distinct CEL subgroup. The results of interphase-FISH had been misinterpreted by the previous center. We have got approval for off-label use of imatinib mesylate from the Ministry of Health and started it immediately. The symptoms resolved and complete cytogenetic response was achieved with 100 mg imatinib continuing for 57 months.

3. DISCUSSION

The identification of FIB1L1/PDGFRA fusion gene in patients with HES/CEL, imatinib mesylate treatment has significantly changed the course of this disease (15). Regarding the reported literature, in one case series, some differences have shown in the clinical features between patients with HES and 4q-/CEL (17). The incidence of hepatomegaly and splenomegaly in the 4q-/CEL group has been found higher than the HES group. In our case, we did not found organomegaly. While this case series has the largest patient number according our knowledge, the authors detected 3 patients whose results did not completely fulfill the WHO criteria for either HES or CEL since they did not show end-organ involvement but del (4) (q12)-FIB1L1/PDGFRA lesion had been found. While except one issue which we did not detect increased blast in our patient's bone marrow examination with flow cytometric analysis, this description was also quite suitable for our patient as we mentioned above and we started imatinib mesylate immediately. We took very impressive results with imatinib treatment in our patient in term of symptoms and hematological parameters even which consist of complete cytogenetic response.

4. CONCLUSION

As targeted therapy with tyrosine kinas' inhibitors could dramatically change the prognosis of FIP1L1-PDGFRA (+) CEL/HES, we should be aware of misinterpreted test results which may retard the accurate diagnosis and treatment.

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