Clinical and Experimental Health Sciences

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REVIEW ARTICLE

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A Scale Development Study: Gynecologic Cancer Prevention Information Scale

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ABSTRACT

Objective: The aim of this study is to develop a valid and reliable measurement tool to determine the knowledge level of women about gynecologic cancer prevention.

Methods: This study is of the methodological research type. The number of draft scale items in this study is 50. Women were taken to sample 10 times for each item (500 women) and pre-test was applied to 125 women which was 25% of the sample. The scale was re-applied to the first pre-test group after 3 weeks by test-retest method. The data were collected by using the Personal Information Form and Gynecologic Cancer Prevention Information Scale. The suitability of the data for factor analysis was investigated by the Kaiser-Meyer-Olkin coefficient and by Barlett's test of sphericity. In order to test the construct validity of the scale, exploratory and confirmatory factor analyses were performed.

Results: Content validity index of the draft scale was 94%. Kaiser Meyer Olkin test value was 0.902 and the sample was found to be adequate and appropriate. On the other hand, the Bartlett test was obtained as X^2=9542.07 p<0.001 and it was accepted that the scale fulfilled the requirements for exploratory factor analysis. The scale took its final form and consisted of 35 items and 5 sub-dimensions as a result of the exploratory factor analysis and confirmatory factor analysis. Total percentage of variance explained of 5 factors was 66.53%. That the cronbach alpha coefficients of the scale have high coefficients of 0.82-0.95 and that the test-retest values have coefficients of 0.566-0.881 and the Cronbach's alpha coefficient of the scale was 0.951.

Conclusion: The data obtained from this study reveal that scale is a valid and reliable measurement tool to determine the knowledge level of women about gynecologic cancer prevention.

Keywords: Gynecologic cancer, information, prevention, scale, women

1. INTRODUCTION

Health, as defined by the World Health Organization, is a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity. This understanding is based on gaining behaviors that will protect, sustain and improve the individual's well-being, ensuring that the individual makes the right decisions about his/her own health and is protected against diseases (1-4). As cancer is one of the leading causes of death, it is a critical health issue concerning all the world. Cancer is the second leading cause of death in the world and it is estimated to be responsible for approximately 9.6 million deaths in 2018. According to the World Health Organization 2018 report, approximately one in six people in the world die due to cancer, with 70% of these deaths occurring in low and middle income countries (4). The importance of early diagnosis in the fight against cancer and preventability of approximately one third of cancer dignoses are emphasized (3-5).

Gynecological cancers are the fourth most common type of cancer worldwide and an important public health problem that increases the risk of mortality and morbidity in women. Nowadays, gynecological cancers account for about 15% of all cancers and 10% of all cancer-related deaths. Gynecologic cancer-related deaths are among the top ten cancer-related deaths that are most commonly seen and cause death in women in Turkey (#6 ovarian cancer, #4 endometrial cancer, #9 cervical cancer (4,6,7). The symptoms of gynecological cancers differ according to the organ and the negative effects on women's health are multidimensional. Diagnosis and treatment procedures applied in gynecological cancers negatively affect the quality of life of the woman and her family regarding body image, sexual identity and reproductive ability, as well as problems in other organ cancers (8).

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Care approach focusing on individual needs plays an important role in raising awareness and gaining healthy lifestyle behaviors in gynecologic cancer prevention (7,9-11). Health professionals (nurses, midwives, doctors, etc.) have the responsibility of protecting and improving health, and reaching women who constitute a large group of the society (12-15). Accordingly, the first thing that should be done is to determine the current knowledge levels of the women objectively. Knowing the level of knowledge of women contributes to the realization of educational plans in a realistic way and the structuring of educational contents according to the needs. However, in the literature review, a measurement tool could not be found to provide the current knowledge level of women related to gynecologic cancer prevention. The aim of this study is to develop a valid and reliable measurement tool to determine the knowledge level of women about gynecologic cancer prevention.

2. METHODS

2.1.Research Type and Place

This study is of the methodological research type. The study was carried out in Cumhuriyet University Research and Application Hospital, Obstetrics and Gynecology Clinic of Sivas Numune Hospital, Aydoğan Family Health Center, Çayyurt Family Health Center, Sivas Yenişehir Hanımlar Culture Center and Sivas Quran Course in Sivas province.

2.2. Research Sample

The population of the study consisted of all women who referred to institutions stated above between April 15 Nisan and August 20, 2018. The sample of the study consisted of women who were at 15-60 age group, who had no psychiatric disease and who agreed to participate in the study. The ideal time to increase knowledge about gynecological cancers is the adolescent period. The knowledge gained from the adolescent period and the acquired healthy lifestyle behaviors can contribute to protection from cancer. Advanced age is a risk factor for many gynecological cancers. In the postmenopausal period, it is very important for early diagnosis and treatment to regularly screen women aged 50-60 for gynecological cancers. For this reason, women between the ages of 15-60 constituted the sample of the study. Many suggestions on sample selection are included in the literature in scale development studies. An important factor in determining the sample size is the number of variables. According to Gorsuch (2008), the number of samples (n) should be at least 5 times the number of variables. Cattell (1978) stated the minimum sample number to be 250 (16) Comrey and Lee (2009) presented a graded scale in determining the number of samples in the factor analysis: 100=weak, 200=moderate, 300=good, 500=very good and 1000=excellent (17,18). Kline (2005) proposes to keep the variable (item) ratio to be taken into consideration for sample size by 10:1 (19). The number of draft scale items in this study is 50. Women were taken to sample 10 times for each item (500 women) and pre-test was applied to 125 women which was 25% of the sample. The scale was re-applied to the first pre-test group after 3 weeks by test-retest method.

2.3. Data Collection Tools

The data were collected by using the Personal Information Form and Gynecologic Cancer Prevention Information Scale.

2.3.1.Personal Information Form: The form was created by the researchers. There are 19 questions in the form that question socio-demographic characteristics (age, education, job, social security, income status, family type, marital status), obstetric characteristics (age of marriage, number of children) and genital hygiene practices of women.

2.3.2.Gynecologic Cancer Prevention Information Scale: The scale consisted of 50 items based on a large literature review and clinical observations to determine the information of women on gynecologic cancer prevention (4-15). The content validity was assessed in order to determine whether the items in the draft scale were sufficient in terms of quality and quantity. For the draft scale, five faculty members specialized in Obstetrics and Gynecology Nursing expressed their opinions. The experts were asked to evaluate each item in terms of conformity to the scale, subject compatibility, comprehensibility and sentence contents (1=Suitable, 2=Not suitable, 3=To be corrected). As a result of these evaluations, the items which were not clear and which needed to be corrected were determined, necessary changes related to language and scientific content were made. According to expert opinions, there was no need to exclude an item from the draft scale.

2.4. Analysis of the Data

After the application of the scale material to the sample group, the data was analyzed by using computer programs such as Statistica Academic 13.3 and SPSS 22.0, and tested whether the scale was a valid and reliable tool.

2.4.1.Validity Analysis: The content validity of the scale was analyzed with the content validity index, and the agreement between the scores was examined. The suitability of the data for factor analysis was investigated by the Kaiser-Meyer-Olkin (KMO) coefficient and by Barlett's test of sphericity. In order to test the construct validity of the scale, exploratory and confirmatory factor analyses were performed.

2.4.2.Reliability Analysis: In the evaluation of the reliability of the scale, item analysis was performed, cronbach α reliability coefficient was calculated and test-retest technique was used.

2.5. Ethical Aspect

Prior to the validity and reliability study of the scale, consent was taken from the ethics committee of the university where the authors were attached (Ethics No=2018-03/19). The

women who would participate in the study were informed about the study and consent were taken.

3. RESULTS

The mean age of the women in the study was 33.89±9.33. 28.2% of women were primary school graduates, 96.6% of them were married and their first marriage age was 21.17±4.24. 81.9% of the women were housewives, 38.8% had 2 children, 76% had nuclear families and 83.5% had moderate level income. The height of the women was 161.74±6.08, their weight was 70.02±12.07, and their body mass index was 26.35±4.95. 88.9% of women did not smoke, 52.2% of them washed their vagina. 72.4% of women washed their vagina for hygiene and 36% washed not to conceive after sexual intercourse. Of 95% of the women without a history of sexually transmitted disease, 76.4% of them did not have regular pap-smear tests, 60.7% did not have breast examination, and 75.2% did not have regular reproductive organ examination. Only 19.8% of the women got information about reproductive health, 15.1% of them about gynecological cancers, and doctors were among their first sources of information.

3.1.Validity Findings

3.1.1.Content Validity Findings

The draft validity index of the draft scale, which consisted of materials developed according to expert opinions, was calculated. Content validity index (CVI) of the draft scale was 94%.

3.1.2.Item Analysis Findings

Item analysis was applied to the scale. In item analysis, standard error, item total correlation and item residual correlation values of each item in draft scale were obtained. According to item analysis findings, item total correlations were statistically significant for all items ($p<0.05$). Similarly, the item residual correlations of all items were found to be statistically significant ($p<0.05$). It was found that all items in the draft scale met the necessary conditions as long as item total and item residual correlation values were taken into consideration in scale development. The results are shown in Table 1.

Table 1. *Item Analysis of Draft Scale*

	Mean	SEM	Total Items	Residual Items		Mean	SEM	Total Items	Residual Items
Item 1	0,87	0,01	0,383	0,356	**Item 26**	0,45	0,02	0,415	0,376
Item 2	0,86	0,02	0,429	0,402	**Item 27**	0,31	0,02	0,294	0,253
Item 3	0,57	0,02	0,454	0,416	**Item 28**	0,31	0,02	0,314	0,274
Item 4	0,48	0,02	0,471	0,434	**Item 29**	0,54	0,02	0,528	0,492
Item 5	0,41	0,02	0,512	0,477	**Item 30**	0,45	0,02	0,613	0,583
Item 6	0,55	0,02	0,547	0,513	**Item 31**	0,54	0,02	0,660	0,632
Item 7	0,44	0,02	0,147	0,101	**Item 32**	0,28	0,02	0,303	0,264
Item 8	0,37	0,02	0,407	0,368	**Item 33**	0,40	0,02	0,565	0,533
Item 9	0,42	0,02	0,529	0,494	**Item 34**	0,46	0,02	0,478	0,441
Item 10	0,33	0,02	0,454	0,418	**Item 35**	0,26	0,02	0,391	0,355
Item 11	0,24	0,02	0,406	0,372	**Item 36**	0,48	0,02	0,275	0,231
Item 12	0,22	0,02	0,354	0,319	**Item 37**	0,39	0,02	0,349	0,308
Item 13	0,25	0,02	0,387	0,352	**Item 38**	0,65	0,02	0,593	0,563
Item 14	0,31	0,02	0,463	0,428	**Item 39**	0,63	0,02	0,624	0,595
Item 15	0,30	0,02	0,248	0,207	**Item 40**	0,71	0,02	0,602	0,574
Item 16	0,24	0,02	0,374	0,339	**Item 41**	0,67	0,02	0,574	0,541
Item 17	0,28	0,02	0,165	0,123	**Item 42**	0,42	0,02	0,366	0,325
Item 18	0,28	0,02	0,310	0,271	**Item 43**	0,52	0,02	0,553	0,519
Item 19	0,58	0,02	0,530	0,496	**Item 44**	0,33	0,02	0,443	0,407
Item 20	0,37	0,02	0,581	0,549	**Item 45**	0,30	0,02	0,379	0,341
Item 21	0,43	0,02	0,631	0,602	**Item 46**	0,28	0,02	0,381	0,344
Item 22	0,56	0,02	0,539	0,505	**Item 47**	0,49	0,02	0,597	0,565
Item 23	0,36	0,02	0,435	0,398	**Item 48**	0,62	0,02	0,637	0,609
Item 24	0,68	0,02	0,408	0,370	**Item 49**	0,65	0,03	0,498	0,449
Item 25	0,45	0,02	0,274	0,230	**Item 50**	0,58	0,02	0,654	0,626

SEM: Standart error of mean

3.1.3.Exploratory Factor Analysis

The suitability of the scale for factor analysis was tested by Kaiser Meyer Olkin (KMO) test before construct validity of the scale. The KMO value was 0.902 and the sample was found to be adequate and appropriate. On the other hand, the Bartlett test was obtained as X^2=9542.07 p<0.001 and it was accepted that the scale fulfilled the requirements for exploratory factor analysis. The principal components analysis method was used in the application of exploratory factor analysis. Varimax perpendicular rotation method was applied to ensure the significance between the factors. Items with a factor load >0.40 were included in the scale. The eigenvalue to be >1 and determination of the break point in scree plot were taken as a basis in determining the number of factors. In the first stage, 11 factors with eigenvalues higher than 1 were determined. The factor analysis was repeated by subtracting 2 items (item 26 and 36) with factor loads less than 0.40 and 10 items (items 5,6,7,19,22,23,29,33,37,45) with factor loads difference less than 0.10 and included in both factors. As a result of the repeated factor analysis, 5 factors were formed. The eigenvalues of the 5 factors were greater than 1 and breaking in the scree plot occurred after the 5th factor. The eigenvalues of factors and the 5th factor are shown in figure 1. According to the factor loadings, all items were above 0.40 and since there was no item in both factors, 38 items were obtained (Table 2, Figure 1).

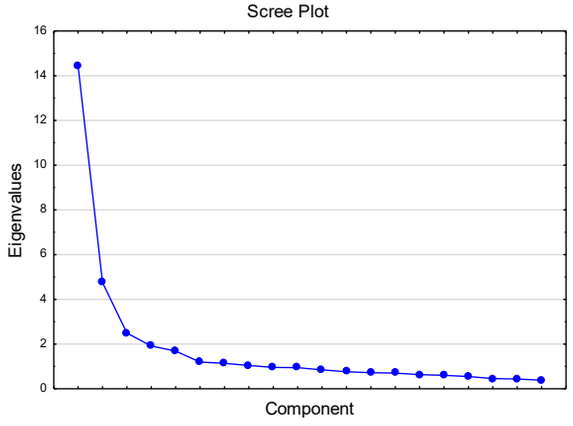

Figure 1. *Eigenvalues of components*

Table 2. *Eigenvalues of factors and total percentage of variance explained*

	Eigenvalues	Total variance	Cumulative eigenvalue	Cumulative variance
Factor 1	14,43715	37,99251	14,43715	37,99251
Factor 2	4,75365	12,50961	19,19081	50,50213
Factor 3	2,49062	6,55427	21,68143	57,05640
Factor 4	1,91392	5,03663	23,59535	62,09303
Factor 5	1,68781	4,44161	25,28317	66,53464

The eigenvalues of the factors and the percentage of variance explained are shown in Table 2. According to the table, total percentage of variance explained of 5 factors was 66.53%. Names of the factors were formed according to the contents of the items in factors. Factor 1 is "prevention from female reproductive system (FRS) cancers" sub-dimension, factor 2 is "female reproductive cancers symptoms" sub-dimension, factor 3 is "observations on female reproductive system related diagnosis" sub-dimension, factor 4 is "early diagnosis of female reproductive cancers and physiological factors" sub-dimension and factor 5 is "birth-related risks of female reproductive system" sub-dimension. Factor loads of items are explained in Table 3.

3.1.4.Confirmatory Factor Analysis

As a result of the exploratory factor analysis, the validity of the draft consisting of 38 items and five sub-dimensions, and the factors were examined by confirmatory factor analysis (CFA). In the model, there are total 38 items and 5 sub-dimensions; 12 items for the prevention from FRS cancers sub-dimension, 13 items for FRS cancers symptoms sub-dimension, 6 items for observations on FRS related diagnosis sub-dimension, 4 items for early diagnosis of FRS and physiological factors sub-dimension and 3 items for birth-related risks of FRS. The draft scale was tested with confirmatory factor analysis (CFA). According to CFA, items 8, 14 and 16 of FRS cancer symptoms were excluded due to not adjusting to the model. Goodness of Fit Index (GFI), Chi-Square (X^2), Adjusted Goodness of Fit Index (AGFI) and Root Mean Square Error of Approximation (RMSEA) were examined with confirmatory factor analysis and conformity index values. According to these values, the remaining 35 items were found to be in good agreement with the exploratory factor analysis findings.

Table 3. *Factor structure of the scale according to exploratory factor analysis*

Factor 1		Factor 2		Factor 3		Factor 4		Factor 5	
Item	Factor load	Item	Factor load	Item	Factor load	Item	Factor load	Item	Factor load
34	0.541	**8**	0.564	**24**	0.546	**1**	0.724	**15**	0.713
38	0.803	**9**	0.588	**25**	0.661	**2**	0.774	**17**	0.687
39	0.799	**10**	0.806	**27**	0.870	**3**	0.737	**18**	0.773
40	0.836	**11**	0.829	**28**	0.854	**4**	0.605		
41	0.846	**12**	0.895	**32**	0.468				
42	0.485	**13**	0.827	**46**	0.553				
43	0.673	**14**	0.671						
44	0.457	**16**	0.737						
47	0.690	**20**	0.728						
48	0.861	**21**	0.656						
49	0.871	**30**	0.415						
50	0.793	**31**	0.431						
		35	0.567						

Table 4. *Relationship between the scale and its sub-dimensions*

	General Scale		PFFRC.		FRCS		OFRSRD		BRRFRS	
	r	p	r	p	r	p	r	p	r	p
PFFRC	,880	**p<0,001***	1							
FRCS	,785	**p<0,001***	,534	**p<0.001***	1					
OFRSRD	,561	**p<0,001***	,394	**p<0.001***	,221	**p<0.001***	1			
BRRFRS	,36	**p<0,001***	,202	**p<0.001***	,148	**p<0.001***	,254	**p<0.001***	1	
EDFRSCPF	,60	**p<0,001***	,448	**p<0.001***	,431	**p<0.001***	,181	**p<0.001***	,130	**0.003***

*PFFRC: Prevention From Female Reproductive Cancers; FRCS: Female Reproductive Cancers Symptoms; OFRSRD: Observations on Female Reproductive System Related Diagnosis; EDFRSCPF: Early Diagnosis of Female Reproductive System Cancers and Physiological Factors; BRRFRS: Birth-Related Risks of Female Reproductive System; Pearson Correlation test; a; 0.05; *Relationship is statistically significant*

The scale took its final form as a result of these findings. The scale consisted of 35 items and 5 sub-dimensions. In factoral terms, prevention from FRS cancers sub-dimension consisted of 12 items (34, 38, 39, 40, 41, 42, 43, 47, 48, 49, 50), FRS cancers symptoms sub-dimension consisted of 10 items (9, 10, 11, 12, 13, 20, 21, 30, 31, 35), observations on FRS related diagnosis sub-dimension consisted of 6 items (24, 25, 27, 28, 32, 46), early diagnosis of FRS cancers and physiological factors sub-dimension consisted of 4 items (1,2,3,4) and birth-related risks of FRS sub-dimension consisted of 3 items (15,17,18).

The relationship between the sub-dimensions of the scale was examined by Pearson Correlation Test. According to the findings in Table 4, the relationship between all sub-dimensions and scale was statistically significant.

3.2. Reliability Findings

3.2.1.Cronbach α Reliability Coefficient

The validity and reliability of the scale were tested with the Cronbach alpha coefficient. The Cronbach's alpha coefficient of the scale was 0.951. The cronbach alpha coefficient of the 5 sub-dimensions varied between 0.82-0.95. Reliability coefficients are given in Table 5.

Table 5. *Reliability coefficients of the scale and sub-dimensions*

Factors	Cronbach Alpha Coefficient
Prevention From FRS Cancers	0.938
FRS Cancers Symptoms	0.928
Observations on FRS Related Diagnosis	0.820
Early Diagnosis of FRS Cancers and Physiological Factors	0.830
Birth-Related Risks of FRS	0.863
General Scale	0.951

FRS: Female Reproductive System

3.2.2.Test-Retest Reliability

The test-retest validity was applied to the pre-test group consisting of 125 individuals with 3-week intervals. Test-retest correlations were between 0.566-0.881 and the agreement between test-retest findings was statistically significant. The test-retest results are given in Table 8. That the cronbach alpha coefficients of the scale have high coefficients of 0.82-0.95 and that the test-retest values have coefficients of 0.566-0.881 and it is statistically significant show the scale has a strong reliability (Table 6).

Table 6. *Test-retest reliability coefficients of the scale*

Factors	Test-retest (n:125)	P
Prevention From FRS Cancers	0.806	p<0.001*
FRS Cancers Symptoms	0.781	p<0.001*
Observations on FRS Related Diagnosis	0.566	p<0.001*
Early Diagnosis of FRS Cancers and Physiological Factors	0.670	p<0.001*
Birth-Related Risks of FRS	0.747	p<0.001*
General Scale	0.881	p<0.001*

** The aggrement between the first and second test results is statistically significant; α:0.05*

4. DISCUSSION

Content validity ratio (CVR) is a statistic item that is used in rejection or retention of certain items. The content validity index (CVI) value is obtained by calculating means of the CVR values of items which will retain in the scale. If the CVI is equal to or greater than the CVR, the content validity of the entire scale is considered statistically significant (20,21). If the CVI is greater than 0.80, the item is accepted sufficient in terms of content validity (21). In this study, the CVI value of the scale was found to be 94%. The content validity of the items in the scale was found to be statistically significant.

Factor analysis is a multivariate statistic to obtain few and identifiable meaningful variables from a large number of variables that measure the same structure. Factor analysis is not applicable to each data group (22). In order to apply factor analysis to a data group, the data should be adequate for the factor analysis and the sample should be sufficient. When applying factor analysis, the results of the two tests are examined first. These are Kaiser-Meyer-Olkin (KMO) and Bartlett's Test of Sphericity. If the KMO value is above 0.90 and the Bartlett test is significant, it is accepted that the sample adequacy is "excellent" and the data are suitable for factor analysis (23,24). In this study, the KMO value of the scale was over 0.90 and the Bartlett test was found to be significant. Based on the analyses, it was decided that the

sample was adequate and the data were suitable for factor analysis.

Exploratory factor analysis (EFA) is the most effective method for testing the structural validity of the scale. However, it is not right to apply an exploratory factor analysis on the scales which measure the level of knowledge and scored in two categories as 0-1. Therefore, it is necessary to convert the data scored in two categories as 0-1 to the appropriate correlation matrix for exploratory factor analysis (25). For this process, tetrachoric correlation coefficients of the items were calculated and tetrachoric correlation matrix was obtained. The construct validity of the items transformed into a tetrachoric correlation matrix was tested with EFA. In the literature, it is recommended that the factor eigenvalues be above 1 when determining factors to be included in the scale after EFA. The eigenvalue of a factor informs about the relationship between the factor and the original variables. The higher the eigenvalue, the more the variance explained by the factor (25). The effect of common variance values is significant in factor analysis. Low common variance values affect the results of factor analysis. Common variance values are discussed in 3 groups as low, large and high. The common variance values range from 0.2 to 0.4 in the low group, from 0.2 to 0.8 in the large group and from 0.6 to 0.8 in the high group (18,26-28). In this study, a 5-factor structure with an eigenvalue above 1, revealing 66.53% of the variance after varimax rotation was found.

Factor load should also be taken into account in EFA. Factor load is a coefficient explaining the relationship between the factors and the items. Factor loads of the items explaining the factors are expected to be high. In order to say that an item measures a structure or factor well, this factor load must have a value of 0.30 or above. It can be deduced that an item with a factor load of 0.30-0.59 measures the structure moderately and an item with a factor load higher than 0.60 (positive or negative) measures the structure well (19). In addition, it is recommended that each item be placed under only one factor and that the items have a difference of at least 0.10 between the two factors (22,29). In this scale study, the factor load of items varied between 0.41-0.87. The factor analysis was repeated by subtracting 10 items (5,6,7,19,22,23,29,33,37,45) from the scale with factor loadings below 0.40 and the factor loads difference less than 0.10.

Confirmatory factor analysis (CFA) is a technique used to test theories about latent variables (25). The correlation between the factors determined in exploratory factor analysis and the theoretical factors is investigated by CFA. CFA calculates the common variables among factors, the load on the factors to which the indicators are related and the measurement errors for each indicator (30). In the CFA, it is decided whether the model is compatible with the theory according to the various fit index results (χ2 / sd, GFI, AGFI, CFI, NFI, S-RMR, RMSEA). In the literature, there is no consensus about which of the fit indices will be accepted as standard and about the acceptable range of values. However, the low chi-square value (<2/ sd), depending on the degree of freedom, has a value of <5 indicates that the data fit of the proposed model is sufficient. In this study, the data fit of the model (χ2 / sd=314) was found to be sufficient. In the literature, it is accepted that the data fit of the model is sufficient for CFI, GFI, AGFI> 0.90 and <0.05 for RMSEA (31,32). In this study, for fit indices >0.90 for GFI, CFI, AGFI and <0.05 for RMSEA were accepted as criteria. It has been observed that there is a fit between the model and the observed data in terms of these fit index values and the scale is fit at a good level.

The Cronbach Alpha Coefficient is considered as an indicator of the homogeneity of the measurement tool. Measurement tool is considered to have reliability if the calculated Cronbach Alpha Coefficient is close to 1. If the coefficient is $0.00<a<0.40$, the scale is not reliable, $0.41<a<0.60$ indicates low reliability, $0.61<a<0.80$ indicates moderate reliability and $0.81<a<1.00$ indicates high reliability.[33,34] In this study, the total Cronbach's alpha coefficient of the scale was found to be 0.95, and the scale was found to be of high reliability. The test-retest method is to apply a measuring tool to the same group for the second time under the same conditions and at a certain time interval. The measurement values obtained from these two applications show the reliability coefficient of the scale (35). The important point is the time interval between the two measurements. This time interval varies according to the measured behavior and the target audience but the average 2-4 weeks duration is sufficient (22,29). Correlation coefficients give information about the degree and direction of the relationship between the two variables and take values between – 1 and +1. The fact that the coefficient is +1 indicates a positive and perfect relationship. It should be at least 0.70 for the acceptance of the stability of a scale (29). In this study, it was determined that the scale total and sub-dimension values of the correlation coefficient were between 0.566-0.881 after test-retest application of the scale with 3 weeks intervals, and the agreement between the first and second test results was statistically significant. The item-total score correlation explains the relationship between the scores obtained from the test items and the total score of the test. Positive and high item-total correlation indicates that the items exemplified similar behaviors and the internal consistency of the test was high (22). In this study, item total correlations and item residual correlations were found to be statistically significant ($p<0.05$).

5. CONCLUSION

The data obtained from this study reveal that Gynecologic Cancer Prevention Information Scale is a valid and reliable measurement tool to determine the knowledge level of women about gynecologic cancer prevention. The scale can be used to determine the level of knowledge of the participants before the training programs, to structure the educational contents according to the information needs of

the women and to measure the effectiveness of the training programs.

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Clinical and Experimental Health Sciences

Efficacy of MTAD Solution and Er:YAG Laser in Smear Layer Removal from Extracted Root Canals: A SEM Evaluation

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ABSTRACT

Objective: The aim of this study was to compare the efficacy of MTAD (a mixture of a tetracycline isomer, an acid, and a detergent), Er:YAG laser, 17% EDTA, and 5.25% NaOCl in removing the smear layer from the surface of instrumented root canals.

Methods: Various organic acids, instruments and lasers have been used to remove the smear layer from the surface of instrumented root canals. Twenty-eight extracted maxillary and mandibular permanent incisors were prepared with rotary files. The teeth were randomly allocated to four treatment groups for final irrigation as follows: (1) 17% EDTA (followed by NaOCl), (2) 5.25% NaOCl, (3) Er:YAG laser, and (4) MTAD. All teeth were processed for scanning electron microscopy (SEM) and the removal of the smear layer was examined in the apical, middle and coronal thirds.

Results: At coronal location, NaOCl(2.2±0.4) group had significantly higher scores than MTAD(0.0±0.0), EDTA(0.6±0.4) and Er:YAG laser(0.6±0.4) groups ($p<0.001$, $p=0.039$, and $p=0.039$, respectively). At the middle third, NaOCl(2.6±0.5) scores were significantly higher than MTAD(0.0±0.0) and EDTA(0.8±0.4) groups ($p<0.001$ and $p=0.036$ respectively). At apical MTAD (0.4±0.3) group had significantly better scores ($p<0.001$).

Conclusion: The results of this suggest that MTAD is an effective final irrigator agent, particularly in the apical segment of the root canal, which presents challenges during cleaning.

Keywords: MTAD, Er:YAG laser, smear layer, scanning electron microscope

1. INTRODUCTION

The long-term survival of endodontic treatment is an issue of high priority focus in pediatric dentistry. The success of endodontic treatment depends on a number of factors including a good biomechanical shaping, disinfection, and three-dimensional filling of the root canal system (1-3). The mechanical instrumentation utilized for the purpose of cleaning and shaping the root canal system results in the formation of smear layer covering the walls of dentin that not only contains dentin residues, but also the remnants of odontoblastic processes, pulp remnants, as well as bacteria (1, 4). Therefore, canal treatment should not consist only of removing the pulp and forming the root canal, we must focus on the complete removal of the smear layer. Its removal provides more efficient penetration of canal sealers and adhesion to the dentin walls (1, 2, 4). Structural irregularities of the root canal system do not allow complete removal of the debridement by mechanical instrumentation.

A number of different procedures including chemical, ultrasonic, and laser-assisted methods have been tested for removing the smear layer. At the same time, numerous irrigation agents with specific chelation action, dissolving capacity, and antibacterial properties have been introduced over the years (5). Recently, compounds combining acids, detergents, and antibiotics have been the focus of attention as a means of reducing the surface tension as well as for providing better penetration to dentin tubules (6).

For many years, NaOCl (sodium hypochlorite) has been conventionally used as a canal irrigation agent due to its antibacterial properties (7). Although NaOCl is effective in removing the organic constituents of the smear layer, chelating agents such as citric acid, polyacrylic acid, tannic acid, phosphoric acid or ethylenediamine tetra-acetic acid (EDTA) are required for the removal of inorganic material(8). Originally, 17% EDTA was used to demineralize the root canal dentin in narrowed and sclerosed canals (6). Dental laser Er:YAG (erbium:yttrium-aluminum-garnet) showed that it can remove most of the smear layer in the root canal wall and open dentin tubules (9). The use of these methods has

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been tested and the smear layer has not been able to remove the entire length of the channel (3).

MTAD, which is a tetracycline isomer, is a bio-compatible material consisting of a mixture of acid and detergent. It exerts a dissolving effect on the pulp and dentin similar to that of EDTA. Torabinejad et al.(10) reported that MTAD is able to effectively remove the smear layer, to eliminate micro-organisms resistant to previous agents, and to provide long-lasting anti-microbial activity.

Therefore, this in vitro study was designed to compare the efficacy of MTAD (a mixture of tetracycline isomer, acid and detergent) for the removal of intra-canal smear with that of 5.25% NaOCl, 17% EDTA, and Er:YAG laser using scanning electron microscope (SEM) images.

2. METHODS

Our study is an in-vitro study performed on teeth extracted due to different extraction indications and all stages of our study were conducted in accordance with the World Medical Association Declaration of Helsinki, IKU/ILU criteria. Twenty-eight maxillary and mandibular single rooted permanent teeth having mature root apex and single root canal with similar anatomic characteristics without anatomical variations and root resorption were selected and included in the study. These extracted teeth were stored in %1 thymol solution. Teeth were decoronated from the cemento-enamel junction with a low-speed rotary diamond disk (90 µm; Microdont, Brazil) under coolant water. The root length of teeth was measured using #10 K-file (Mani®, Mani Inc., Japan). The root canal length measurements were performed in such a manner that the tip of the canal equipment could be visible at the apex of the root canal, and 1 mm was subtracted from the reading to obtain the measurement length to be used for study analyses. The shaping of the root canals was performed with crown-down technique and ProTaper Universal (Dentsply Maillefer, Ballaigues, Switzerland) rotary files (S1 through F3). Each file was used for preparing only for four root canals. Following each file use, root canals were irrigated with 3ml 5.25% NaOCl. After a final irrigation with 3 ml of 5.25% NaOCl, rinsing with 3 ml of distilled water was carried out to prevent the formation of NaCl crystals, and the canals were dried with absorbent paper points. After shaping the canals, the apical foramina of all canals were sealed with modeling compound to prevent outflow of the irrigation solution. The teeth were randomly allocated to four treatment groups for the removal of smear layer: (1) 17% EDTA (followed by NaOCl), (2) 5.25% NaOCl, (3) Er:YAG laser (Fotona Medical Lasers, Fidelis PLUS, Er:YAG and Nd:YAG Dental Laser, Slovenia) and (4) MTAD (MTAD Biopure, Tulsa, OK, USA). The final irrigation protocol in each group determined in Table-1.

During Er:YAG procedure, fiber tip of the laser applicator was positioned within the canal as to lie along the length of the canal and parallel to the surface. Procedure involved activation of the laser equipment at apex and small rotation-like movements in apical-coronal direction for 15 seconds while taking care to provide contact between the fiber tip and whole surface of the root canal. The procedure was repeated for 4 times, with 15 second intervals to prevent excessive heat formation.

In order to ensure that no test solution has remained, all study specimens were washed out with 3 ml of distilled water and cotton pellets were used to seal the coronal tip of the root canals for preventing the entry of foreign material into the canal. Two longitudinal grooves were prepared on the buccal and lingual aspects of each root using diamond bur without penetration into the canal. The roots were then split into two halves with chisel. For each root, the half containing the most visible part of the apex was conserved and coded, the other part was discarded. The specimens were kept overnight in a desiccator at 60 ℃. The coded specimens were then mounted on metallic stubs, covered with 0.02-micron thick (226.8 A°) gold and examined on scanning electron microscope (SEM, JEOL JSM-5910, Tokyo, Japan). Serial (SEM) photomicrographs at X 1000 magnification were taken at the coronal, middle and apical thirds of the root canals. The photographs were evaluated for the presence of a smear layer. SEM images obtained were assessed using the scoring system proposed by Takeda et al. and modified by Prado et al.(11). A score between 0 and 3 was assigned to each photomicrograph based on the presence of debris and smear layer as well as the patency of the dentin tubules. Scoring system was as follows: 0, no smear layer and debris at all, with all tubules cleaned and opened; 1, a few areas covered by smear layer and debris, with most tubules cleaned and opened; 2, smear layer and debris covering almost all surface, with few tubules opened; 3, smear layer and debris covering all the surfaces. Scoring was done in a blinded manner by two dentists who were not informed on the nature and purpose of these experiments and the average scores were used for the analyses. The kappa values for inter-examiner agreement were 0.730, 0.949, and 0.801 for coronal, middle and apical measurements, respectively.

2.1. Statistical analysis

For the analysis of data, SPSS (Statistical Package for Social Sciences) version 23 was used.

One-way anova test was used for intergroup comparisons of data and built-in post hoc Bonferroni test was used for pairwise comparisons. For inter-examiner agreement, Kappa values were calculated. A p value smaller than 0.05 was used as an indication of statistical significance.

3. RESULTS

Figure 1 show the representative images of the apical, middle, and coronal thirds of root dentin covered by smear layer various irrigation protocols of 4 groups. Group 1 (17% EDTA + 5.25% NaOCL): In the coronal third of the samples, a few areas covered by smear layer more intense smear layer was observed in the middle triple. In apical one third, smear

layer covering almost all surface, with few tubules opened. Group 2 (5.25% NaOCL): In all parts of the root of the tooth, a dense smear layer was observed in a third of the coronal, middle and apical. Group 3 (Er:YAG laser): In the coronal third of the samples, a few areas covered by smear layer and in the middle and apical third smear layer covering almost all surface, with few tubules opened. Group 4 (MTAD): No smear layer was observed in the coronal and middle third of all samples. The smear layer was not observed in the apical third of the 2 samples, in 5 samples of 7, small amount of smear layer was observed in the apical third part of the root canal.

When the data obtained from SEM images were examined, MTAD group from all groups showed that the best result was obtained in the coronal, middle and apical third Scores differed significantly across groups at all locations (coronal, middle and apical) p<0.001. Table 2 shows comparisons of the scores between study groups. At coronal location, NaOCl group had significantly higher scores than in MTAD, EDTA and Er:YAG laser groups (p<0.001, p=0.039, and p=0.039, respectively). At the middle third, NaOCl scores were significantly higher than MTAD and EDTA groups (p<0.001 and p=0.036 respectively). At apical MTAD (0.4±0.3) group had significantly better scores (p<0.001).

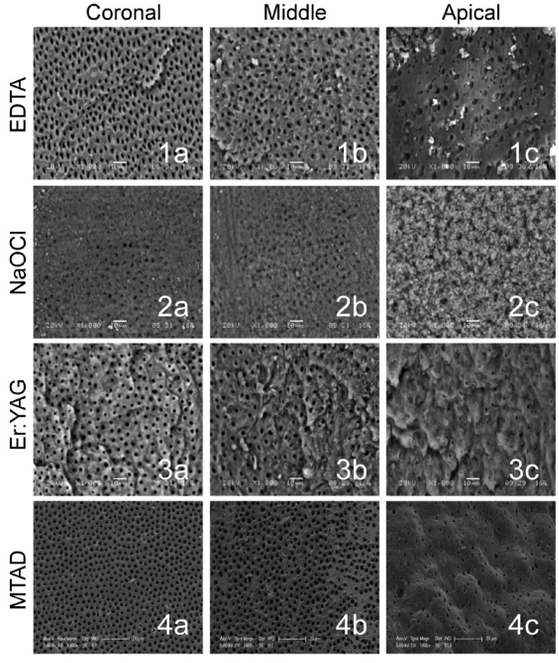

***Figure 1**. Exemplary scanning electron microscope (SEM) images. In the coronal (1a) and middle (1b) thirds of the root canal in Group 1 (17% EDTA), the dentin tubules are clearly observed, while smear remnants together with open dentin tubules can be seen in the apical third (1c). In Group 2 (5.25% NaOCl), the smear layer covering the dentin tubules in all root surface are seen (2a, 2b, 2c). The open dentin tubules of the root canal in the coronal (3a) and middle (3b) thirds are seen in a specimen from Group 3 (Er:YAG laser), while the apical (3c) segment displays smear remnants together with open dentin tubules. In group 4, MTAD (4a,4b) No smear layer was observed in the coronal and middle third. small amount of smear layer was observed in the apical third (4c).*

***Table 1.** Final irrigation protocols of the study groups.*

Group	Final solution for removal of the smear layer	Application procedure
Group 1 (n=7)	17% EDTA	1 min 5ml 17% EDTA plus 3ml 5.25%NaOCl
Group 2 (n=7)	5.25% NaOCl	1 min 5ml
Group 3 (n=7)	Er:YAG laser	Short pulse mode, optical fiber diameter 0.3mm, wavelength 2940nm, output power 1W, pulse energy 100mJ, pulse frequency 10Hz.
Group 4 (n=7)	MTAD	Prepared freshly by mixing the powder Part A and liquid Part B; agitated with a no:15 k-file using 4ml solution for 5min

EDTA, ethylenediamine tetraacetic acid; NaOCl, sodium hypochloride; Er:YAG, erbium yttrium aluminium garnet; MTAD, a mixture of a tetracycline isomer, an acid, and a detergent

***Table 2.** Comparisions of the scores between study groups*

	EDTA (n=7)	NaOCl (n=7)	Er:YAG laser (n=7)	MTAD (n=7)	p*
Coronal	0.6±0.4	2.2±0.4	0.6±0.4	0.0±0.0	<0.001
Middle	0.8±0.4	2.6±0.5	1.0±0.6	0.0±0.0	<0.001
Apical	1.4±0.5	3.0±0.0	1.4±0.5	0.4±0.3	<0.001

*Data presented as mean ± standard deviation. *p for overall difference*

4. DISCUSSIONS

In this study comparing the efficacy of three different irrigation agents and Er:YAG laser in removing the smear layer formed after mechanical shaping of the root canal, MTAD showed superiority over NaOCl in all parts of the root canal, while Er:YAG laser and 17% EDTA were more effective than NaOCl in the coronal and middle third of the root, and NaOCl had the worst performance statistically in all root sections with regard to smear scores. Despite numerous previous comparisons between MTAD and different irrigation agents in terms of their ability to remove the smear layer from the root canal, studies comparing this agent with laser are scarce in number (1, 12-14).

Scanning electron microscopy (SEM) was used in this study to assess the smear layer as an effective means to examine the morphological changes occurring on the surface of the root canal surface. In SEM images, smear layer has an appearance that is akin to remnants of mug (1). The diameter and density of the dentin tubules exhibit variations along the root canal from coronal to apical segments, with dentin tubules showing irregular frequency and angles in the apical segment. The apical third of the root canal represents the most challenging segment for cleaning as the size of the canal is significantly reduced (15). Due to these anatomical variations, the root canal was evaluated in three segments as apical, middle and coronal parts.

An ideal canal irrigation agent should have antibacterial properties, be able to dissolve the organic and inorganic

material without causing erosions in the dentin, remove the smear layer, have no toxic effects on periapical tissues, be bio-compatible, and should be able to dissolve and remove the debridement in the root canal system (8, 15, 16).

NaOCl with concentrations ranging between 0.5 to 5.25% is the most widely used agent for root canal treatments, based on its antimicrobial properties as well dissolving capacity (17). However, its efficacy in the removal of the smear layer is limited. In the study by Andrabi et al. (18) examining the efficacy of a number of different irrigation agents in removing the intra-canal smear layer, 3% NaOCl was no more effective than distilled water and the authors concluded that NaOCl was ineffective for this purpose when used alone (18). Similarly, Gupta et al. found that NaOCl had an effect only on the organic tissue, with no efficacy in the removal of the smear layer (1). In line with these previous observations (3, 12), in this study, NaOCl solution failed to clear the smear layer in the root canal.

The amount of the material used and duration of administration have been reported to influence the smear-removing capacity of different irrigation agents, and these agents have been generally found to be more effective in the coronal and middle thirds of the canal as compared to the apical third, due to wider root canals in the coronal and middle segments than in the apical segment allowing a more extensive contact between the solutions and root surface (15). It has been reported by Torabinejad et al. (10) found that MTAD, when used as a final irrigating agent together with NaOCl, can effectively remove the smear layer without altering the structure of the dentin tubules. MTAD is an acidic solution with a pH of 2.15 that can also eliminate the inorganic substances, (10) and in this study, it was effective in the most challenging apical third of the root canal similar to the reports by Gupta et al., Andrabi et al., Poul et al., and Kumar et al. (1, 3, 4, 13, 18).

A clean root canal surface was achieved by the combination of 17% EDTA and 5.25% NaCl in the study by Yamada et al.(19). Currently, sequential administration of 17% EDTA and 5.25% NaOCl irrigation solutions represents a widely accepted practice for the elimination of organic and inorganic constituents of the smear layer, (1) although this formulation may fail to eradicate the smear layer completely in the apical segment, despite complete removal in the coronal and middle thirds(3). Torabineajad et al.(10) found similar efficacy between 17% EDTA and MTAD in the coronal and middle segments, while MTAD was more effective in the apical part; furthermore, 17% EDTA was associated with erosion of root surface dentin (11). Despite the success of 17% EDTA in the removal of the smear layer, this solution has also been reported to lead to erosions in the peri-tubular and inter-tubular dentin in the coronal and middle segments of the root canal as well as in open dentin tubules (1, 11, 13, 18).

In the study by Kalyoncuoglu and Demiryurek, (9) assessing a number of different irrigation agents and methods for the removal of smear layer (Er:YAG, Nd:YAG, 5.25%NaOCl, 17%EDTA, MTAD) , combination of 17% EDTA and 5.25 NaOCl was the most effective means for smear removal and the authors underscored the fact that this efficacy was associated with allowing a 5 minute treatment period for 17% EDTA solution within the root canal. On the other hand, in a study by Teixeria et al. where SEM was used to compare different durations of treatment with 17% EDTA and 5.25% NaOCl with respect to smear removal in the root canal, treatment durations of 1, 3, or 5 minutes were found to have comparable efficacy (7). In the light of this latter piece of information, a 1-minute treatment duration was chosen for 17% EDTA application in our study in order to minimize the erosive effect. Significant differences between 17% EDTA/5.25% NAOCl combination and other methods were observed in the removal of the smear layer in the apical third of the canal except MTAD in the current study. These were parallel with the report by Charlie et al.(3) but in contrast with the report by Kalyoncuoglu and Demiryurek (9).

Laser has also been used within the root canal for the eradication of organisms and removal of the smear layer. Ashraf et al. (20) reported higher efficacy for 17% EDTA than Er:YAG laser in their SEM study which comparing two irrigation solutions and laser treatment in the apical third. Kalyoncuoglu and Demiryurek, (9) failed to observe a smear removal effect for Er:YAG or Nd:YAG laser methods. Guidotti et al.(5) evaluated Er:YAG laser in combination with NaOCl and EDTA with regard to smear removal capacity and found that combination of laser with EDTA was statistically superior in all root canal surfaces as compared to treatment with laser or EDTA alone. In that same study, laser alone was the least effective approach. In contrast, Er:YAG laser was significantly more effective than NaOCl solution in the coronal third of the canal in our study, despite the absence of a difference from other irrigation agents in the middle and apical thirds. These observed differences may have resulted from a number of factors such as the strength of the laser beam used, the amount of light absorbed by the tissues and duration of exposure, differences in the tips and tip-to target distances, and also from other methodological discrepancies.

5. CONCLUSIONS

Although our findings may be considered preliminary due to limited sample size, SEM results suggest that MTAD, laser and EDTA exhibit comparable efficacy in smear removal in the middle and coronal third of the root canal, while MTAD may distinguish itself from other methods as an effective irrigation agent in the apical third of the root canal. This is notable considering the difficulty apex presents when cleaning the root canal.

Conflict of Interest

There are no conflicts of interest in connection with this paper.

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Clinical and Experimental Health Sciences

Autism Awareness Scale for Security Officers Working in Hospitals: A Study of Validity and Reliability

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ABSTRACT

Objective: The purpose of this study was to evaluate the knowledge and awareness toward autism among security officers working in hospitals and to explore the validity and reliability of the Autism Awareness Scale for Security Officers (AASSO) developed by the researchers.

Methods: This methodological study was conducted at two Government Training and Research Hospitals in Istanbul, Turkey. A total of 135 security officers were included in the study and the data were obtained between February and April 2019. The AASSO is a 4-point Likert type scale and consists of 20 items. The factor structure of the scale was extracted by performing exploratory factor analysis.

Results: Most of the participants were male (n=79; 58.5%) and high school graduates (n=86; 67.7%). Participants considered autism mostly as "a kind of mental retardation" (n=69; 51.9%), followed by "social interaction problem" (n=24; 18.0%). The AASSO reduces autism awareness into 3 factors ('*Coming across', 'Communication' and 'Interaction with the environment'*), with eigenvalues ranging from 9.417 to 1.201 and explained 65.45% of all variance. Cronbach's alpha values for the AASSO was 0.936 and ranged from 0.921 to 0.809 for the subscales.

Conclusion: According to these results AASSO is a valid and reliable scale. The consequence of further studies especially conducted on non-healthcare professionals of hospitals should be establishing autism-friendly hospitals.

Keywords: Autism, awareness, reliability, scale, validity.

1. INTRODUCTION

Autism spectrum disorder (ASD) is a neurodevelopmental condition that has many forms and affects people in varying degrees. Characteristic features include; lack of social interaction and communication, and limited and repetitive behavior patterns in terms of interests and activities (1,2). Although the frequency is unclear, studies have shown that the prevalence of ASD is increasing and has recently found as 1 in 54 (18.5 per 1,000) among children aged 8 years (3). The exact causes of autism are unknown; however, it was concluded that autism is resulting from combination of genetic and environmental risk factors (4).

Autism awareness is important for many reasons both in society and in various professions. Raising awareness about autism will allow recognition of the differences of individuals with autism and ensure the right approach in social areas. Autism is commonly associated with poor emotional control, anxiety and impulsivity (5,6). The challenging behaviors of individuals with autism including aggression to others are frequently observed and not socially acceptable (7,8). Because people with autism cannot hide their minds, can be overly impulsive and cannot internalize social rules they can often remain in a disorder or debate. In addition, a stressful and controversial environment, such as a hospital, can cause anger and crisis in people with autism. In such cases in the hospital, security officers are the first responders. The officers should recognize autism and the moment of crisis and be able to distinguish it from other situations such as the substance and alcohol addiction crisis. They should be able to manage this moment of crisis by calming the individual with autism and his/her family. Furthermore, in most hospitals in Turkey, security officers are the first personnel individuals having autism meet at the hospital environment. The security officer's attitude and behaving as a facilitator for them reaching the hospital's health services may be very important.

Although there are previous studies with community, teachers and healthcare professionals about knowledge and awareness of autism, this subject has not been adequately researched on the security officers (9-12). The aim of this research was to evaluate the knowledge and awareness

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toward autism among security officers working in hospitals and to explore the validity and reliability of the Autism Awareness Scale for Security Officers (AASSO) developed by the researchers.

2. METHODS

2.1. Study Design and Participants

The study was designed as a methodological research. The population of the study consisted of 155 security officers working in two Government Training and Research Hospitals in Anatolian side of Istanbul. All security officers who was volunteer to participate in the research were considered eligible for the study. A total of 135 security officers working in these hospitals were participate (response rate=87.1%).

2.2. Data Collection and Instruments

The data of the study were collected between February and April 2019. An anonymous self-completed survey consisted of 32 questions was used for data collection. Before collected the data, a pilot study was done to test the survey with a smaller sample for determine whether questions are interpreting as intended.

The initial question of the survey was a case question describing a typical situation an individual having autism might come across while waiting at the hospital and the security officer's attitude while handling the situation. The English version of initial case question is presented at the appendix. In addition, five questions about sociodemographic characteristics of the participants, two questions evaluating the general level of knowledge on autism and four questions inquiring any experience of the participants with an individual having autism were included in the first part of the survey.

The AASSO was developed by researchers to measure autism awareness level of security officers working in hospitals. It is a 4-point Likert scale (1=strongly disagree, 2=disagree, 3=agree, 4=strongly agree) and consists of 20 items. The total scores of respondents vary from 20 to 80, and higher scores indicate better level of awareness.

2.3. Ethical Statement

Ethical approval for the study was obtained from the Ethics Committee of Zeynep Kamil Women and Children Diseases Training and Research Hospital (19/12/2018 – 159) and research permissions were taken from the Health Promotion Unit of Istanbul Provincial Health Directorate. Our study was conducted according to the Declaration of Helsinki and written informed consent was obtained from all participants.

2.4. Statistical Analysis

The statistical analysis was carried out by using IBM SPSS Statistics for Windows, version 23 (IBM Corp., Armonk, N.Y., USA). Sample characteristics are presented as frequency tables and mean ± standard deviation values. Exploratory factor analysis was performed on the scale items to determine the construct validity of the AASSO. Factors were extracted by using eigenvalue-one criterion. The Kaiser-Meyer-Olkin measure of sampling adequacy and the Bartlett's Test of Sphericity were calculated to verify the suitability of applying the factor analysis to the data. The internal consistency was measured using Cronbach's alpha, item-total correlations, and the mean inter-item correlation. Correlations between the AASSO and the subscales were examined using the Spearman's rank correlation coefficient.

3. RESULTS

Sociodemographic characteristics of the participants are presented in Table 1. Most of the participants were men (n=79; 58.5%) and high school graduates (n=86; 67.7%). The mean age of participants was 34.43 ± 6.76 years. A high fraction of participants stated that they have ever heard of the word of autism (n=128; 95.5%) and they saw an individual with autism (n=93; 68.9%). In addition, twenty three participants (17.0%) stated that they have a relative with autism.

Table 1. *Characteristics of the Participants*

		n	%
Gender	Female	56	41.5
	Male	79	58.5
Age (years)			34.43 ± 6.76*
Graduate	Elementary school	2	1.6
	Junior high school	23	18.1
	High school	86	67.7
	University	16	12.6
Working experience as a security officer (years)			6.12 ± 3.90*
Working experience in current hospital (years)			4.30 ± 2.97*
Ever heard of autism	Yes	128	95.5
	No	4	3.0
	Do not know	2	1.5
Family member with autism	Yes	23	17.0
	No	111	82.2
	Do not know	1	0.7
Anyone with autism in close social circle	Yes	34	25.2
	No	94	69.6
	Do not know	7	5.2
Ever saw an individual with autism	Yes	93	68.9
	No	38	28.1
	Do not know	4	3.0

** Mean ± standard deviation*

The most common answer of the participants for describing the character in the initial case question was "autism" (n=66; 50.0%), which has been followed by "mental retardation" (n=48; %36.6). An important fraction of the participants answered the onset symptoms of autism show up "between

the ages of 0-1" (n=64; 47.8%). Most participants considered autism as "a kind of mental retardation" (n=69; 51.9%) and "social interaction problem" (n=24; 18.1%) (Table 2).

Table 2. *Participants' Answers to Knowledge Questions About Autism*

		n	%
Case question	Autism	66	50.0
	Mental retardation	48	36.4
	Do not know	8	6.0
	Alcohol or substance abuse	5	3.8
	Conversion	3	2.3
	Other	2	1.5
Definition of autism	A kind of mental retardation	69	51.9
	Social interaction problem	24	18.0
	Strange repetitive behaviors	21	15.8
	Speaking problem	7	5.3
	Other	12	9.0
Onset symptoms of autism show up	Between the ages of 0-1	64	47.8
	Between the ages of 2-5	61	45.5
	Between the ages of 6-12	7	5.2
	Between the ages of 12-18	2	1.5

The Kaiser-Meyer-Olkim (KMO) measure of sampling adequacy was 0.864 and the Bartlett's Test of Sphericity ($\chi 2$=1753.83, p<0.001) was statistically significant, supporting the factorability of the data. According to the principal factor analysis with varimax rotation were extracted 3 factors with eigenvalues greater than 1.0 ranging from 9.417 to 1.201. These 3 factors explained 65.44% of all variance. Factor 1 (*Coming across*) explained that 47.09% of the total variance and contained 8 items regarding the events and observations experienced by the security officers when they come across with an individual having autism. Factor 2 (*Communication*) contributed to the explanation of 12.36% of the total variance and had 7 items about the communication problems, which may be experience between the security officers and an individual with autism. Finally, factor 3 (*Interaction with the environment*) explained 6.00% of the total variance, contained 5 items regarding the interaction of an individual having autism with his/her environment (Table 3).

Table 3. *Factor Structure of the Autism Awareness Scale for Security Officers (AASSO)*

		Factor*			Item-Total Correlation
		1	2	3	
	Factor 1: Coming across with an individual having autism				
1.	They may say stereotypic words/sentences again and again, as they talk themselves.	**0.825**	0.217	0.091	0.701
2.	A child with autism may swing his/her hands like a bird clapping its wings.	**.796**	.275	.010	.685
4.	They may scream and act rapidly.	**.771**	.237	.221	.726
7.	They may play with a toy/thing continuously by shaking or turning.	**.760**	.275	.257	.759
5.	A child with autism may shake himself/herself for a long time.	**.746**	.308	.157	.731
6.	A child with autism may stare blank.	**.728**	.379	.193	.777
3.	A child with autism may turn around himself/herself and on his/her toe.	**.695**	.341	-.018	.645
8.	They may be bored during waiting in line.	**.599**	.429	.295	.749
	Factor 2: Communication with an individual having autism				
11.	They will not response when they are called by their name when they are with other people in public.	.190	**.824**	-.020	.565
10.	They may have trouble by understanding what people say to them.	.292	**.769**	.108	.662
9.	They may have trouble by expressing themselves.	.347	**.738**	.113	.683
12.	They may have trouble when they communicate with people with signs and body language.	.432	**.612**	.241	.723
13.	They have trouble by establishing eye contact with people.	.458	**.600**	.256	.743
14.	They may response to questions by repeating them like as parrot.	.444	**.596**	.174	.699
15.	Their voice may be thin and monotonic like a robot.	.497	**.587**	.121	.712
	Factor 3: Interaction with the environment of an individual having autism				
18.	They do not like to be touched during tantrums.	.033	-.040	**.889**	.305
17.	They may overreact to cry of baby, ambulance sounds, or hums/murmurs and they may experience emotional breakdowns/tantrums.	.028	.013	**.839**	.315
19.	If they are taken to a quiet room during their tantrum, their rage reduces rapidly.	.242	.112	**.724**	.473
16.	They may be uncomfortable in loud places and they do not want to hear voices.	.134	.192	**.678**	.432
20.	A child with autism may do wrong things to reach a specifically desired object without considering the criminal element of his/her behavior.	.199	.329	**.571**	.512

**Principal component analysis with orthogonal varimax rotation. Bartlett's Test of Sphericity was $\chi 2$ = 1753.83, p < 0.001 and Kaiser-Meyer-Olkin was 0.864; Cronbach's alpha = 0.936 (0.890 for Factor 1; 0.921 for Factor 2; 0.809 for Factor 3); Total variance explained 65.45% (47.09% Factor 1; 12.36% Factor 2; 6.00% Factor 3); Eigenvalues for the three factors were 9.417, 2.471, and 1.201, respectively.*

The item-total correlations were ranging between 0.305 and 0.777 and the mean inter-item correlation was 0.423 of the 20-item AASSO. The Cronbach's alpha value for the scale was 0.936 and ranging from 0.921 to 0.809 for the subscales (Table 3). The AASSO subscales were highly or moderately correlated with the total scale score (*Coming across*: r=0.924, *Communication*: r=0.928, *Interaction with the environment*: r=0.478) and correlated with each other (*Coming across* and *Communication*: r=0.795, *Coming across* and *Interaction with the environment*: r=0.330, *Communication* and *Interaction with the environment*: r=0.317) (Table 4).

Table 4. *Correlations between the Autism Awareness Scale for Security Officers (AASSO) and the subscales*

	AASSO	Coming across	Communication	Interaction with the environment
AASSO	-	0.924**	0.928**	0.478**
Coming across	0.924**	-	0.795**	0.330**
Communication	0.928**	0.795**	-	0.317**
Interaction with the environment	0.478**	0.330**	0.317**	-

** Spearman's rank correlation coefficient; **p<0.01*

4. DISCUSSION

In this methodological study, most participants having heard the word autism before and having encountered with an individual having autism before. Although the majority of the respondents correctly answered as 'autism' to the initial case question, there was a similar frequency of those who stated, 'mental retardation'. In addition, most participants stated, 'a kind of mental retardation' as the definition of autism instead of 'social interaction problem'. Autism is characterized by social skills deficits, stereotypical behaviors and difficulties in communication and does not have to be with mental retardation. However, a recent study stated that 33.4% of children with ASD were classified in the range of intellectual disability (intelligence quotient [IQ]≤70) and 24.1% were in the borderline range (IQ=71–85) (3). The level of knowledge about the relationship between autism and mental retardation has been questioned in different ways in the literature. In a community awareness study, only 19% of respondents agreed to the phrase 'autism is similar to mental retardation', whereas, approximately half of respondents thought 'autism is a learning and mental disorder' in another study on primary school teachers (9,10).

In our study, most participants stated that onset symptoms of autism show up "between the ages of 0-1" which has been followed by "between the ages of 2-5" (47.8% and 45.5%; respectively). Previous studies suggest that symptoms and signs including lack of eye contact, no social smiling or failure to respond to name may predict autism between 6-12 months; however, identification of autism in the first year of life may not be possible in the majority of affected children (13,14). The American Academy of Pediatrics recommended to screening at 18 and 24 months of age for ASD (15). Early diagnosis is critical because early interventions can provide the best opportunity to promote healthy development and provide lifelong benefits (16,17).

The AASSO developed by us and used in our study was found to be a reliable scale in terms of Cronbach's alpha value (0.936) (18). According to the factor analysis of the data, the model could explain 65.45% of the variance which indicated within acceptable range (19). Factor 1 emphasizes the events which can be experienced by the security officers when they come across with an individual having autism. In a previous narrative, the importance of nonverbal communication in first contact and the desire of the individuals to be recognized, were emphasized (20). If the security officers establish communication with the individual having autism, they may experience the communication challenges defined by the items loaded to factor 2. The communication problems which are experienced by the individuals with autism are well described and may affect social functioning (21,22). The security officers may experience the reactions of the individual with autism defined by the items loaded to factor 3. The reactions of children having autism may range from tantrums to avoidance, thus the management of these reactions in children with autism is challenging (23). Therefore, this model may separate the items according the components of the social interaction between the security officers and individual having autism. All the correlations between the AASSO and subscales were found to be high or moderate. However, the correlations of factor 3 with factor 1 and factor 2 were low (0.330 and 0.317; respectively). This may be the result of while the items loaded on factor 1 and factor 2 are related to interpersonal interactions, items loaded to factor 3 are related to recognize interaction between individual having autism and his/her environment. In our study the mean inter-item correlation was 0.423, which falls in the range of 0.15-0.50 and considered as an acceptable level of consistency (24). Furthermore, item-total correlations of the items were between 0.305 to 0.777, exceeding the benchmark value of 0.30 (25).

The most important novelty of our study is the development of internally valid scale which can be separated into 3 factors, which explain 65.45% of total variance and are loaded with the items explaining various facets of the social interaction between the security officers and individuals with autism. This study is a guidance on issues to be considered in the training of security officers in terms of autism awareness.

The participants' marking autism among other choices as the answers of the initial case question could be interpreted as a bias, and this could not be controlled. This can be a limitation of our study. Conversely, using multiple choice answers for case questions can occasionally be considered being superior to open ended question (26). Thus, in our study we think we used the optimal methods like multiple-choice questions and Likert type of questions to measure the knowledge and awareness of participants about autism. Furthermore, we avoided using open-ended questions thinking the shortage of time of the participants to complete the survey.

5. CONCLUSION

Our results show that the AASSO is a valid and reliable measurement instrument. It inquires autism awareness in this occupational group by reducing it to three main dimensions (*Coming across, Communication* and *Interaction with the environment*) and gives guidance for detection and improvement of weak points in social interaction of security officers with individuals having autism. Not only security officers at hospitals but also other non-healthcare professionals working at hospitals should be inquired for their autism awareness by conducting further researches. The optimum consequence of these further studies should be establishing autism-friendly hospitals.

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APPENDIX

Case Question: A 14-year-old boy was waiting for examination order at emergency with his family since his brother had a fever. The child had little interest in his environment and repeated himself some words. He had a pensive state. During the waiting, he noticed a man trying to get ahead of his mother on the line and started pulling and kicking his arm. In this case, the man started shouting to call security. The child became more irritable with the noise and started to spin around himself. The security officer tried to calm other individuals and prevent the confusion of the environment instead of stopping the child by using force. After the place became calm the security officer talked with the family members in line and directed them to the required department. The child calmed down in a quiet place.

What do you think could be the diagnosis of the 14-year-old boy mentioned above?

( ) Substance or Alcohol addiction

( ) Mental Retardation

( ) Autism

( ) Epilepsy Seizure

( ) Conversion Disorder

( ) I do not know.

( ) Other (please specify ……)

Clinical and Experimental Health Sciences

Comparison of Nutritional Intake and Dietary Behaviors in Overweight/Obese and Non-Obese University Students: A School-Based Study

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ABSTRACT

Objective: This study was conducted to determine of nutritional intake and dietary behaviors in overweight/obese and non-obese university students.

Methods: The sample of this descriptive comparative study consisted of 220 students randomly selected among the university students who were divided into two groups as overweight / obese and non-obese. Data were collected using a daily nutrition log, anthropometric measurements and questionnaire.

Results: Of the 220 students admitted to our study, 110 were overweight/obese and 110 were non-obese. Obesity was correlated with the reason for skipping meals, smoking, amount of physical activity ($p<0.05$). Overweight/obese students have a high scores of Perceived Health Status scale and a low Self-Efficacy to Regulate Eating Habits scale ($p=0.00$). The scores of the Health Promotion Lifestyle Profile Nutrition scale and the Perceived Health Status scale of the non-smoking students were significantly high ($p<0.05$). The Health Promotion Lifestyle Profile Exercise scale was significantly higher in the students with high frequency of physical activity ($p=0.00$). Overweight/obese students had high daily energy, fat, cholesterol, and sodium intake, while all students consumed insufficient amounts of key nutrients like fiber, folate, and calcium ($p=0.00$).

Conclusions: These results emphasize the need to provide students health promotion programs should be implemented at universities to and raise young people's awareness about making healthy choices that will affect their future.

Keywords: Nutrition, obesity, university students

1. INTRODUCTION

Attending university is a time marked by many important changes in a young person's life, such as the first separation from family, a new education environment, and the transition to real life. Difficulties adapting during this period also lead to changes in one's personal life, professional education, and health behaviors. A young person's health attitudes and behaviors affect the individual themselves specifically, as well as their family and the community in general. For these reasons, reaching the younger population, which represents a key section of society that can easily adopt healthy behaviors, gains even more importance (1-4).

University life is also among the most risky periods in terms of acquiring negative health behaviors. Studies indicate that poor dietary habits such as consuming large amounts of fast food and low amounts of fruits and vegetables and skipping breakfast are common among young adults, 80% of the students had irregular eating patterns and half of the students ate two main meals a day. Students who engage in such behaviors are at increased risk of weight gain and development of noncommunicable diseases (NCD) in the future (2, 5-11).

For individuals to be able to acquire healthy dietary habits, it is first necessary to examine their healthy dietary and exercise behaviors and evaluate cognitive factors such as how they perceive their own health and how important they consider health (12).

Although there are studies in the literature on the dietary or lifestyle habits of university students, few have included a detailed analysis of students' daily food and beverage consumption using a scientific and reliable dietary program and offered suggestions to improve nutrition levels. The present study was conducted to determine of nutritional intake and dietary behaviors in overweight/obese and non-obese university students.

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2. METHODS

2.1. Study Design and Setting

This comparative descriptive study was conducted at a university in Istanbul, Turkey between January and February 2020. Approval of the Istanbul Medipol University Non-Interventional Research Ethics Committee (10840098-604.01.01-E.5011) and written permission from the institution were obtained before the study. The consent form given to participants by the researchers provided sufficient explanation of the objective and contents of the study, usability of study results, anonymity, protection of personal information, and possible benefits, and stated that there were no potential disadvantages from participating in the study. The participants were instructed to fill out a self-reported questionnaire. After explaining in detail that all data would be stored by the researcher and not shared with any third parties, written informed consent was obtained from the students and all data were secured.

2.2. Participants

The study universe comprised 5451 students studying at a foundation university and enrolled in a formal education program. The sample of this descriptive comparative study consisted of 220 students randomly selected among the students who were divided into two groups as overweight / obese (110 students) and non-obese (110 students), taking into account their body mass index (BMI) [BMI ≥25: overweight / obese; BMI <25: non-obese] who applied to a health screening at university.

2.3. Measures

A data collection tool consisting of 3 parts was used in this study. The first part included questions regarding the students (age, sex, income, tobacco use, daily water consumption, physical activity level) and their body mass index (BMI) calculated from height and weight values obtained by measuring by researchers; the second part included the daily nutrition log, and the third part included assessments of healthy eating behaviors and cognitive factors: Perceived Health Status Scale, Importance of Health Scale, Nutrition and Exercise Scales of the Health-Promoting Lifestyle Profile (HPLP) and Self-Efficacy to Regulate Eating Habits Scale (13-15).

2.4. Data Analysis

Data were analyzed using SPSS (Statistical Package for Social Sciences) for Windows 22.0 software and the basic version of the BeBIS 8.0 program. Normal distribution of data were tested by Shapiro-Wilk. Number, percentage, mean, and standard deviation were used as descriptive statistical methods. Independent Samples Student's t-Test was used for pairwise group comparisons and One-Way ANOVA with Scheffe or Tamhane's tests was used for the comparisons between more than two groups. Relationships between grouped variables were evaluated using chi-square analysis.

BeBIS 8.0 is a program that analyzes the content of food consumed in Turkish society. The foods and measures used in the program were based on the consumption in the Turkish population. All food (and drinks) consumed are recorded in this software and the individual's daily consumption of energy, macro – and micronutrients, fiber, and cholesterol can be determined. The program has been used in numerous studies conducted by dietitians and researchers (16). Before entering data into the software, the age and sex tab specific to each individual was selected. The individual's daily intake was then entered and the ingredients of the food and their amounts were derived using standard recipes. From this, each individual's average daily intake of energy, protein, carbohydrate, fat, fiber, and nutrients was determined. The daily nutrition logs were entered into the BeBIS program by two specialists familiar with the software to prevent data entry errors.

3. RESULTS

The overweight/obese students in the study had a mean BMI of 29.19±2.32 (25.40–34.30) kg/m^2 and mean age of 19.25±1.47 (18–28) years; the non-obese students had a mean BMI of 22.20±2.00 (18.50–24.60) kg/m^2 and mean age of 19.20±1.79 (17–29) years. Of the overweight/obese students, 69% were female and 72.7% were at the middle-income level; of the non-obese students, 71.8% were female and 64.5% were middle-income. Current smokers comprised 34.5% of the overweight/obese group and 22.7% of the non-obese group. The proportion of students who stated they engage in no physical activity was 65.5% in the overweight/obese group and 48.2% in the non-obese group (Table 1).

When asked about skipping meals, 30.9% of the overweight/obese students responded "yes" and another 32.7% responded "sometimes". The reason cited for skipping meals was not being in the habit for 32.7%, not having time for 29.1%, and wanting to lose weight for 27.3%; 63.6% stated that they most often skip breakfast (Table 1).

Among the non-obese students, 34.5% stated "yes" to the question of skipping meals, while 51.8% responded "sometimes". Reason cited for skipping meals was not being in the habit for 30.0%, lack of appetite for 27.3%, and wanting to lose weight for 7.3% of this group; 57.3% stated that they most often skip breakfast (Table 1).

There were no significant differences between overweight/obese and non-obese participants in terms of sex, age, most skipped meal, or daily water intake ($p>0.05$). A significant correlation was observed between smoking and obesity ($X^2=6.060$; $p=0.048$). The proportion of current and former smokers was higher among the overweight/obese students compared to the non-obese students. There was also a significant correlation between obesity and skipping meals ($X^2=16.639$; $p<0.000$). Skipping meals was more common among non-obese students than overweight/obese students.

Furthermore, obesity was correlated with the reason for skipping meals (X^2=33.803; p<0.000). Not being in the habit and lack of time were more commonly cited as reasons for skipping meals by overweight/obese students compared to non-obese students, while wanting to lose weight and lack of appetite were expressed more by non-obese students than overweight/obese students.

Obesity was also found to be significantly correlated with amount of physical activity (X^2=15.152; p=0.004). Higher proportions of overweight/obese students reported doing no physical activity, engaging in at least 20 min of moderate – to vigorous-intensity exercise 1–2 times a week, and engaging in at least 20 min of moderate – to vigorous-intensity exercise 3 times a week compared to non-obese students. In contrast, more non-obese students reported engaging in at least 20 min of moderate – to vigorous-intensity exercise 1 day a week or at least 30 min of moderate – to vigorous-intensity exercise 5 times a week compared to overweight/obese students (Table 1).

Table 1. *Descriptive Characteristics of Overweight/Obese and Non-obese Students*

		Overweight/Obese (n=110)		Non-obese (n=110)		p
		n	%	n	%	
Sex	Female	76	69.1%	79	71.8%	X^2=0.197 p=0.384
	Male	34	30.9%	31	28.2%	
Age (years)	**Mean±SD**	19.25 ± 1.47		19.20 ± 1.79		t = 0.246 sd = 218 p = 0.806
Family Income Level	Low	10	9.1%	7	6.4%	X^2 = 3.835 p = 0.147
	Middle	80	72.7%	71	64.5%	
	High	20	18.2%	32	29.1%	
Body Mass Index (kg/m^2)	**Mean±SD**	29.19 ± 2.32		22.20 ± 2.00		t = 23.856 sd = 218 p = 0.000
Smoking Status	Never smoker	54	49.1%	72	65.5%	X^2=6.060 p = 0.048
	Former smoker	18	16.4%	13	11.8%	
	Current smoker	38	34.5%	25	22.7%	
Daily Water Intake (mL)	**Mean±SD**	1508.18 ± 579.84		1349.09 ± 685.46		t = 1.858 sd = 218 p = 0.064
Meal Skipping	Yes	34	30.9%	37	33.6%	X^2=16.639 p = 0.000
	No	40	36.4%	15	13.6%	
	Sometimes	36	32.7%	58	52.7%	
Reason for Skipping Meals	Not in the habit	23	32.9%	31	32.6%	X^2=33.803 p = 0.000
	Lack of time	22	31.4%	22	23.2%	
	To lose weight	19	27.1%	7	7.4%	
	Lack of appetite	0	0.0%	30	31.6%	
	Other	6	8.6%	5	5.3%	
Most Skipped Meal	Morning	45	64.3%	53	55.8%	X^2=3.237 p = 0.198
	Midday	17	24.3%	35	36.8%	
	Evening	8	11.4%	7	7.4%	
Physical Activity Level	No physical activity	72	65.5%	54	49.1%	X^2=15.152 p=0.004
	At least 20 min moderate – to vigorous-intensity exercise 1 day/ week	19	17.3%	29	26.4%	
	At least 20 min moderate – to vigorous-intensity exercise 1–2 times/week	16	14.5%	11	10.0%	
	At least 20 min moderate – to vigorous-intensity exercise 3 times/week	3	2.7%	11	10.0%	
	At least 30 min moderate – to vigorous-intensity exercise 5 times/week	0	0.0%	5	4.5%	

HPLP: Health-Promoting Lifestyle Profile; p: Significance level; SD:Standard deviation; t: Student t-test;
X^2: Chi-square analysis; %: Percentage

Mean score on the Importance of Health Scale was 8.23±1.87 (1–10) for the overweight/obese students and 7.71±2.21 (1–10) for non-obese students. Mean scores for the HPLP Nutrition and Exercise scales were 13.46±3.96 (6–24) and 9.21±2.88 (5–20) for the overweight/obese students and 13.88±2.87 (6–24) and 9.40±3.40 (5–20) for the non-obese students, respectively. Mean score on the Self-Efficacy to Regulate Eating Habits Scale was 49.05±18.70 (0–100) for overweight/obese students and 52.95±25.5 (30–100) for non-obese students (Table 2).

Table 2. *Comparison of the Nutrition Behaviors of Overweight/Obese and Non-obese Students*

Groups	Overweight/Obese (n=110)		Non-obese (n=110)		t	p
	Mean	SD	Mean	SD		
Perceived Health Status Scale	2.227	0.630	1.936	0.455	3.925	**0.000***
Importance of Health Scale	8.236	1.872	7.718	2.218	1.873	0.063
HPLP Nutrition Scale	13.346	3.960	13.882	2.873	-1.150	0.252
HPLP Exercise Scale	9.218	2.884	9.409	3.409	-0.448	0.654
Self-Efficacy to Regulate Eating Habits Scale	38.473	19.782	59.455	18.266	-8.173	**0.000***

**: p<0.05; HPLP: Health-Promoting Lifestyle Profile; p: Significance level; SD:Standard deviation; t: Student t-test*

Table 3. *Comparison of the Nutrition Behaviors of Students*

		Perceived Health Status Scale		Importance of Health Scale		HPLP Nutrition Scale		HPLP Exercise Scale		Self-Efficacy to Regulate Eating Habits Scale	
Variables	**n**	**Mean±SD**	**Test Value**	**Mean±SD**	**Test Value**	**Mean±SD**	**Test Value**	**Mean±SD**	**Test Value**	**Mean±SD**	**Test Value**
Sex											
Female	155	2.07±0.55	t=-0.417 p=0.678	8.01±204	t=0.317 p=0.752	13.36±3.34	t=-1.155 p=0.268	9.14±3.08	t=-1.223 p=0,224	48.25±21.28	t=0.317 p=0.752
Male	65	2.11±0.62		7.91±2.13		13.95±3.66		9.73±3.33		50.71±22.78	
Smoker status											
Never smoker	126	2.00±0.57	F=3.465 **p*=0.033**	8.10±1.92	F=0.419 p=0.658	14.13±3.50	F=5.369 **p*=0.005**	9.33±3.20	F=0.214 p=0.808	50.17±19.00	F=0.587 p=0.557
Former smoker	30	2.30±0.47		7.80±2.34		13.32±3.15		9.53±2.34		45.66±26.35	
Current smoker	64	2.11±0.57		7.86±2.23		12.42±3.20		9.10±3.38		48.10±24.55	
Meal Skipping											
Yes	72	2.13±0.47	F=0.792 p=0.454	7.97±2.22	F=1.284 p=0.279	12.83±3.13	F=2.196 p=0.114	8.97±2.99	F=0.441 p=0.644	47.78±19.80	F=3.033 p=0.050
No	54	2.13±0.63		8.32±1.79		13.67±4.23		9.37±3.43		43.7±21.84	
Sometimes	94	2.03±0.59		7.76±2.08		13.95±3.11		9.40±3.02		52.72±22.77	
Physical Activity Level											
No physical activity	125	2.13±0.55	F=2.396 p=0.051	7.69±2.22	F=2.956 p=0.061	13.27±3.25	F=0.998 p=0.410	8.31±.2.83	F=12.439 **p*=0.000**	48.02±21.88	F=2.090 p=0.752
At least 20 min moderate – to vigorous-intensity exercise 1 day/week	46	2.15±0.56		8.22±1.78		13.56±3.87		9.74±2.53		50.00±21.66	
At least 20 min moderate – to vigorous-intensity exercise 1–2 times/ week	27	2.00±0.62		9.07±1.33		13.48±3.56		10.07±2.45		42.96±19.38	
At least 20 min moderate – to vigorous-intensity exercise 3 times/ week	14	1.71±0.47		7.86±1.92		14.64±2.92		12.29±3.07		60.71±23.36	
At least 30 min moderate – to vigorous-intensity exercise 5 times/ week	8	1.80±0.45		7.20±2.77		15.60±4.82		14.20±5.12		62.00±22.80	

** p<0.05; F: One way AN OVA testi; HPLP: Health-Promoting Lifestyle Profile; SD:Standard deviation; ; t: Student t-test*

Mean score for Perceived Health Status was higher in overweight/obese students (x̄=2.227) than non-obese students (x̄=1.936) (p<0.000). Mean Self-Efficacy to Regulate Eating Habits Scale score was higher in of non-obese students (x̄=59.455) compared to overweight/obese students (x̄=38.473) (p<0.000). Importance of Health Scale score and HPLP Nutrition and Exercise Scales scores were not associated with obesity status (p>0.05) (Table 2).

The HPLP Nutrition scale scores of non-smoking students were found significantly higher than smoking students (p<0.01). Perceived Health Status scale scores of the students who participated in the "I used but quit smoking" option (x=2.30±0.47) were found to be significantly higher (p<0.05) than all other groups (Table 3).

The HPLP Exercise scale scores of the students who "exercised at least 30 minutes of moderate to severe intensity 5 times a week" (x=14,20±5,12) were found to be significantly higher (p<0,01) than all other groups (Table 3).

In the overweight/obese group, the mean daily energy intake was 2540.96 kcal, 54.00%±8.33% of which came from carbohydrates, 20.00%±4.18% g from protein, and 26.00%±6.98% g from fat. In the non-obese group, mean daily energy intake was 1357.98 kcal, with 45.20%±11.01% g of this amount from carbohydrates, 16.50%±4.32% g from protein, and 38.12%±8.93% g from fat (Table 4).

Daily energy intake was significantly higher in overweight/obese students (x̄=1940.916 kcal) than non-obese students (x̄=1357.986 kcal) (p<0.000). The overweight/obese students also had significantly higher intake of protein (x̄=72.977 g), fat (x̄=80.238 g), carbohydrates (x̄=227.119 g), fiber (x̄=20.026 g), cholesterol (x̄=365.598 mg), vitamin A (x̄=1435.077 mcg), vitamin E (x̄=13.455 mg), vitamin B1 (x̄=0.880 mg), vitamin B2 (x̄=1.364 mg), vitamin B6 (x̄=1.235 mg), folate (x̄=288.938 mcg), vitamin C (x̄=105.310 mg), potassium (x̄=2484.272 mg), calcium (x̄=829.160 mg), magnesium (x̄=279.664 mg), phosphorus (x̄=1146.681 mg), iron (x̄=11.025 mg), and zinc (x̄=10.050 mg) when compared with non-obese students (p=0.002 for vitamin A, p<0.000 for others).

There were no significant differences between the groups in protein, fat, and carbohydrate percentages or sodium intake (p>0.05) (Table 4).

Table 4. *Daily Nutritional Intake of the Students*

Food groups	Overweight/Obese (n=110)		Non-Obese (n=110)		t	p
	Mean	Standard deviation	Mean	Standard deviation		
Energy (kcal)	2533.15	648.328	1357.986	428.827	7.865	**0.000***
Protein (g)	122.800	29.781	54.548	20.256	5.366	**0.000***
Protein (%)	20.000	4.188	16.500	4.328	-1.757	0.080
Fat (g)	74.800	29.659	57.877	23.155	6.233	**0.000***
Fat (%)	26.000	6.983	38.127	8.939	-1.000	0.318
Carbohydrates (g)	334.160	91.961	151.106	62.433	7.172	**0.000***
Carbohydrates (%)	54.000	8.332	45.209	11.011	1.636	0.103
Fiber (g)	16.820	9.092	14.059	6.122	5.710	**0.000***
Cholesterol (mg)	365.598	199.348	245.525	162.473	4.897	**0.000***
Vitamin A (mcg)	1435.077	2164.200	770.037	480.741	3.146	**0.002***
Vitamin E (mg)	13.455	7.579	8.530	4.419	5.888	**0.000***
Thiamine (mg)	0.880	0.351	0.626	0.237	6.286	**0.000***
Riboflavin (mg)	1.364	0.605	0.960	0.495	5.418	**0.000***
Pyridoxine (mg)	1.235	0.486	0.914	0.377	5.476	**0.000***
Folate (mcg)	288.938	118.610	212.345	95.938	5.266	**0.000***
Vitamin C (mg)	105.310	65.186	74.191	48.094	4.029	**0.000***
Sodium (mg)	4555.310	6984.760	3423.699	5000.842	1.382	0.169
Potassium (mg)	2484.272	940.196	1794.873	590.162	6.514	**0.000***
Calcium (mg)	829.160	313.191	601.419	259.248	5.875	**0.000***
Magnesium (mg)	279.664	105.486	204.016	73.590	6.169	**0.000***
Phosphorus (mg)	1146.681	462.009	836.188	313.958	5.830	**0.000***
Iron (mg)	11.025	5.108	7.695	2.754	6.018	**0.000***
Zinc (mg)	10.050	4.040	7.958	3.138	4.289	**0.000***

**: p<0.05; t: Student t-test*

4. DISCUSSION

In this study comparing nutrient intake and dietary behavior of overweight/obese and non-obese university students, we aimed to determine the amount of macro – and micronutrients consumed by obese students. We found that the energy intake of overweight/obese students exceeded the amount consumed by non-obese students and the recommendations stated in the Dietary Guidelines for Turkey (DGT). In the literature, the energy intake of obese individuals was reported as 2229 kcal/day in a study conducted in Brazil, 3056 kcal/day in a similar study conducted in Portugal, and 1745 kcal/day in a study conducted in Chile. At 2535 kcal/day, the energy intake of the obese individuals in our study group was lower than that of obese individuals in Portugal and higher than that of Brazilian and Chilean individuals (17).

The recommended ratios of daily energy intake from carbohydrates, protein, and fats are 50%–60%, 10%–15%, and 25%–30%, respectively. In both groups in our study, the proportion of daily energy intake from carbohydrates was too low, whereas energy intake from fat was too high. Numerous studies have yielded results similar to our findings (6, 18-20). These students' high fat intake may be related to their consumption of convenience foods such as fast food and chips. Due to reports that consuming a diet high in fat is associated with heart disease, it is not recommended to have more than 30% of daily energy intake from fats (21).

In the present study, cholesterol intake was higher in overweight/obese students compared to non-obese students and DGT recommendations. High cholesterol levels were shown to be strongly associated with cardiovascular disease and are a risk factor for arteriosclerotic disease (22). Consistent with our study, Llanaj et al. also reported high cholesterol intake among overweight/obese university students (6).

Daily intake of vitamins B2, C, and E, iron, and zinc levels of the non-obese students in our study was lower than DGT recommendations. Riboflavin (vitamin B2) deficiency leads to hair loss and lesions of the skin and oral/tongue mucosa. Vitamin E deficiency causes muscle weakness and lethargy. Vitamin C deficiency may lead to a weakened immune system, fatigue, irritability, and muscle pain, and vitamin C is necessary for brain and nervous system function. Zinc deficiency causes immune dysfunction, hair loss, dermatitis, changes in skin structure, and delayed wound healing (20). Iron deficiency may lead to problems such as anemia, anorexia, fatigue, disruptions in epithelial tissue, and pallor (21). Based on these data, students can be advised to consume lean red meat, white meat, fish, and eggs, as well as legumes, nuts, and greens as sources of iron and zinc. Moreover, increasing vitamin C consumption alone can enhance absorption of folic acid, vitamin E, calcium, and iron, which were also found to be insufficient in our students (19). The best source of many vitamins is fresh fruits and vegetables, and an adequate and balanced diet can provide all necessary vitamins (21).

When we evaluated daily nutrient intake, we observed that students in both groups had insufficient intake of fiber, vitamin B1, vitamin B6, folate, potassium, calcium, and magnesium. Researchers also reported in their studies on university students that all students had low fiber, vitamin B1, folate, calcium, and magnesium intake (6, 19, 20, 23). Dietary fiber plays a role in the prevention of conditions such as cardiovascular disease, cancer, and diabetes. Students should increase their consumption of legumes, which have high fiber content, as well as whole wheat products, fresh vegetables, and fruit (22). Nausea, muscle atrophy, and digestive system disorders are observed in vitamin B1 deficiency, while cracked skin, nerve inflammation, and migraines may be observed in vitamin B6 deficiency. Insufficient magnesium intake affects neuromuscular, cardiovascular, and renal systems and is associated with diabetes and hypertension. Low intake results in anorexia, nausea, tachycardia, and muscle weakness (22). Intake of calcium, an important mineral for bone health, was found to be insufficient in the present study and numerous others. Insufficient calcium intake in adolescence and young adulthood is an important risk factor for osteoporosis later in life (20).

In both groups of our study, students' intake of vitamin A, sodium, and phosphorus was higher than DGT recommendations. Garipagaoglu et al. also reported that all students in their study had higher than recommended intake of sodium and phosphorus. The high phosphorus intake may result from excessive consumption by students living in student housing/dormitories (20). Excess sodium can increase the risk of heart diseases, hypertension and stroke, and may lead to kidney damage (22). Llanaj et al. also observed high sodium intake among the overweight/obese students in their study, similar to our findings (6).

In this study, a significant correlation was observed between smoking and obesity. The proportion of current and former smokers was higher among the overweight/obese students compared to the non-obese students. Similarly, in a cross-sectional study examining the relationship between body mass indexes and eating habits of university students, a significant relationship was found between students' smoking status and the risk of obesity / overweight (23). According to previous studies, smoking behavior is associated with an increase in fast-food, reduced intake of healthy food components, and adoption of a sedentary lifestyle, all contributing to weight gain (23).

Furthermore, obesity was correlated with the reason for skipping meals. Not being in the habit and lack of time were more commonly cited as reasons for skipping meals by overweight/obese students compared to non-obese students, while wanting to lose weight and lack of appetite were expressed more by non-obese students than overweight/obese students. Frequent consumption of fast-food has been associated with increased BMI and body fatness in children and adults. Eating regular meals may prevent snacking of energy-rich unhealthy foods between meals.

Obesity was also found to be significantly correlated with physical activity level. Higher proportions of overweight/obese students reported doing no physical activity, engaging in at least 20 min of moderate – to vigorous-intensity exercise 1–2 times a week, and engaging in at least 20 min of moderate – to vigorous-intensity exercise 3 times a week compared to non-obese students. In contrast, more non-obese students reported engaging in at least 20 min of moderate – to vigorous-intensity exercise 1 day a week or at least 30 min of moderate – to vigorous-intensity exercise 5 times a week compared to overweight/obese students. Similarly, in a study by Konal Korkmaz et al., physical activity levels of overweight and obese students were found to be lower than those of normal weight students (24). Some studies show that physical activity level decreases throughout the period students graduate from high school and move on to college (25).

The overweight/obese students in our study had higher Perceived Health Status scale scores than the non-obese students. However, this suggests that overweight/obese students might show a tendency to shift towards higher levels of obesity because they may not be aware of the effects of excess weight on health; therefore, this may be the ideal timing to warn these students and involve them in a health promotion program. It is believed that individuals with a realistic perception of their health status will pay attention to healthy eating behaviors and will assume the necessary responsibility in preventing obesity**.**

The non-obese students in our study had higher Self Efficacy to Regulate Eating Habits Scale scores compared to the overweight/obese students. In the literature, a stronger sense of self-efficacy has been associated with better health, greater achievement, and better social integration. Turning the negative experiences of overweight/obese students into positive ones requires helping the individual to determine realistic goals and set targets, followed by the individual monitoring changes in the behaviors identified together. This will increase their self-efficacy in regulating healthy eating behavior (14).

Importance of Health Scale score and HPLP Nutrition and Exercise scales scores were not associated with obesity status. Contrary to the findings of the research, in the studies of Tedik and Hacıalioğlu, Konal Korkmaz and their friends, a relationship was found between the obesity status of the students and the physical activity and nutrition scale scores of the students, and the scores of the obese students were lower than the other students (24, 26).

HPLP Nutrition Scale scores of non-smoking students were found to be higher than other students. Similarly, in the studies of Özkan and Yılmaz, Cihangiroğlu and Deveci, the Nutrition Scale score of non-smoking students was higher than other students (27, 28).

The Perceived Health Status scale score of non-smoking students was found to be higher than other students. In the study conducted by Taşpınar et al., Parallel to the findings of the research, individuals who smoke were perceived their health more negatively and their fatigue levels were found higher than non-smokers (29). It can be said that as the importance given to health increases, individuals develop positive behaviors to increase their well-being potential, and they adopt and apply health-enhancing behaviors more for a healthy life (30).

HPLP Exercise Scale scores of the students who exercised at least 30 minutes of moderate-intensity intensity 5 times a week were found to be significantly higher than all other groups. Similarly, in the study conducted by Alkan et al. with university students, the HPLP Exercise Scale scores of the students with high physical activity level were found to be higher than the other groups (31). In the study of Yalcinkaya et al., The group who exercised 3-4 days or more a week got higher scores in all sub-dimensions of physical activity and overall total (32).

Strengths and Limitations

Strengths of this study are that it focused on the evaluation and comparison of overweight/obese and non-obese students in terms of healthy eating habits, evaluated daily nutritional intake using the BeBIS 8.0 program, and utilized both anthropometric measurements and qualitative data collection tools.

The study was performed at a single university with 220 students selected by nonprobability sampling. Therefore, the inability to generalize our results to all students is the limitation of this study.

5. CONCLUSION

This study comparing nutrient intake and dietary behavior of overweight/obese and non-obese university students showed that overweight/obese students had high fat and cholesterol consumption. Both groups had higher than recommended vitamin A, sodium, and phosphorus intake, while fiber, vitamin B1, vitamin B6, folate, potassium, calcium, and magnesium intake was insufficient. Vitamin B2, C, and E, iron, and zinc levels were also low among non-obese students. Non-obese students had higher nutrition self-efficacy scores compared to overweight/obese students. Health promotion programs should be provided about energy requirements, essential nutrients, the food groups that provide these nutrients and their recommended amounts, balanced menu selection, nutritional problems and practical solutions, portion control, and food label literacy in order to promote adequate and balanced nutrition among university students.

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Clinical and Experimental Health Sciences

Proteasomal System Related Stress Response in Different Cancer Cell Lines

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ABSTRACT

Objective: Proteasomal system is the primary protein degradation mechanism and important for cellular homeostasis. On the other hand, increased proteasome activity protects cancer cells from cell death. The objective of this preliminary study was to determine the response of the proteasomal system to oxidative stress in human cancer cell lines including K562 chronic myelogenous leukemia, U251 glioblastoma, DU145 prostate cancer, HepG2C3A hepatoma, and MCF7 breast cancer.

Methods: Cells were exposed to hydrogen peroxide (H2O2) as a stressor. 20S and 26S proteasome activities and K48-linked protein ubiquitination levels were determined immediately and 3 hours after exposure.

Results: As an immediate response, 20S proteasome activities decreased in only K562 and U251 cells and 20S+26S proteasome activities decreased only in K562 cells. Following 3h of incubation, all cells showed a significant decrease in both 20S and 20S+26S proteasome activities. K48-linked protein ubiquitination levels increased immediately in K562 and DU145 cells. After 3h of incubation, ubiquitination levels increased in all cell lines except MCF7 cells.

Conclusion: The difference in the response of the proteasomal system to stress could be the reason for differential adaptation to oxidative stress in different cancer types.

Keywords: Proteasome activity, 20S proteasome, 26S proteasome, oxidative stress, cancer

1. INTRODUCTION

All cells need energy to maintain normal cell function and primarily cells generate free energy in the form of adenosine-5'-triphosphate (ATP) via oxidative phosphorylation and glycolysis (1). According to oxygen availability, cellular dynamics of energy shifts and in the normoxic conditions, the main energy source is oxidative phosphorylation while under hypoxic conditions, glycolysis serves as the primary pathway to produce energy to compensate weakened oxidative phosphorylation (2). In contrast to normal cells, most cancer cells rely on glycolysis for energy production and this phenomenon is known as the Warburg Effect (3). Generation of reactive oxygen species (ROS) is a consequence of aerobic metabolism and ROS function as signaling molecules to regulate a wide variety of physiologic conditions at low concentrations (4). The imbalance between generation and scavenging of ROS results disturbances in cellular signaling pathways and causes oxidative stress (5). The Warburg effect plays a role in the upregulation of redox homeostasis and causes alterations in mitochondrial redox potential in cancer cells (3). Many cancer cells have increased ROS levels, however it is not clear if mutations in oncogenes cause an increment in ROS production or affect the levels of antioxidant proteins (6). ROS can cause oxidative modifications of the proteins and these modifications can affect their function and/or activity (7,8). Degradation of oxidized proteins is important for maintaining cellular homeostasis and the proteasomal system plays a very important role in the degradation pathway (7,9).

Proteasome is a multicatalytic complex, which is responsible for turnover of most of the cellular short-lived, unnecessary, oxidized and damaged proteins. The proteolytic part of the proteasome is the 20S core particle. 20S proteasome is barrel-like shaped and consists of two stacked heptameric outer α rings and two inner β rings which has catalytically active sites: β1 (caspase-like), β2 (trypsin-like) and β5 (chymotrypsin-like) (10). Several regulatory particles can bind to 20S proteasome and 19S regulatory particle is among them. When 20S proteasome binds one or two 19S subunits, forms 26S and 30S proteasome, respectively (Hereafter

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referred as 26S proteasome), (11). Substrate degradation by 26S proteasome is ATP and ubiquitin-dependent, and polyubiquitin conjugation to a target protein via lysine 48 (K48) is characterized as an essential step for 26S proteasome degradation (12,13). 19S regulatory particle has functions in substrate recognition, deubiquitination, unfolding and it interacts with 20S catalytic core to promote opening α ring and transfer of substrates to catalytically active sites (14,15). While 20S proteasome does not bind 19S particle, it preferably degrades oxidized proteins and this degradation is ATP and ubiquitination independent (16,17). This selective removal of the oxidized proteins by the 20S proteasome plays a crucial role in antioxidant defense against oxidative stress (18). Under oxidative stress conditions, 26S proteasome dissociates into free 19S regulatory particle and 20S core particle, thus free 20S proteasomes can rapidly degrade oxidatively damaged proteins to maintain cellular function (7). Besides, this dissociation is reversible and after a certain period of time 19S regulatory particle and 20S core particle can associate and regenerate 26S proteasome (19).

Cancer cells have increased proteasomal activity due to altered protein homeostasis (20,21). Adaptation to oxidative stress can protect cancer cells from apoptotic and/or necrotic cell death (22,23). Some studies demonstrated that, cancer cells have significant upregulation in proteasomal pathways compared to normal cells and therefore cancer cells are more prone to proteasome inhibition than normal cells (24,25). This situation also brings the question of proteasomal response to chemotherapy and radiotherapy-induced stress and the role of the proteasomal system in cancer therapy resistance.

Despite extensive research on proteasomal regulation in oxidative stress conditions, there is not enough information about proteasome activation in different cancer cell lines. To address that, we aimed to investigate differential 20S and 26S proteasome activities and K48-linked protein ubiquitination as early (immediate activity measurement) and late (activity measurement following 3h of incubation) response after oxidative stress in different cancer cell lines.

2. METHODS

2.1. Materials and chemicals

Human chronic myelogenous leukemia K562 (CCL-243), human glioblastoma U251, human prostate cancer DU145 (HTB-81), human hepatoma HepG2C3A (CRL-10741), human breast cancer MCF-7(HTB-22) cells were purchased from American Type Culture Collection (ATCC) (Rockville, MD, USA). Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), penicillin-streptomycin solution were purchased from Gibco (Grand Island, NY, USA). Suc-Leu-Leu-Val-Tyr-MCA substrate and 2-Deoxy-D-glucose were from Glentham Life Sciences (Corsham, UK). Hydrogen peroxide (H_2O_2), saccharose, HEPES, magnesium chloride ($MgCl_2$), dithiothreitol, ethylenediaminetetraacetic acid (EDTA), adenosine 5'-triphosphate disodium salt hydrate (ATP), hexokinase were purchased from the Sigma Chemical Company (St Louis, MO, USA). BCA protein assay kit was from Thermo Scientific (Fremont, CA, USA).

2.2. Cell Culture

K562, U251, DU145, HepG2C3A and MCF7 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin-streptomycin at 37°C, 5% CO_2. The cells were routinely passaged in the condition of ~80% confluency.

2.3. H_2O_2-treatment and recovery procedure

Cells were divided into 3 treatment groups as PBS (Control), H_2O_2 (0h time point) and Rec-H_2O_2 (3h time point). For the treatment procedure, $2x10^6$ cells were seeded in four different 100 mm dishes and left overnight. Then, the medium was aspirated and the cells were treated with freshly prepared 1 mM H_2O_2 in PBS with Ca^{++} and Mg^{++} for 30 min. The control group received just PBS with Ca^{++} and Mg^{++} for 30 min. At the end of the exposure time, the cells were collected with trypsinization and washed with PBS (0h time point). To study the late response, the cells were incubated with DMEM supplemented with 10% FBS, 100 U/mL penicillin-streptomycin at 37°C, 5% CO_2 for 3 hours (h) directly after H_2O_2 treatment (3h time point). Then, the cells were collected with trypsinization and washed two-times with PBS.

2.4. Proteasome activity

The cell pellets were suspended in lysis buffer (0.25 M saccharose, 25 mM Hepes, 10 mM $MgCl_2$, 1 mM EDTA, and 1 mM dithiothreitol; pH7.4) and lysed by 3 times freeze-thaw cycles. The cell lysates were centrifuged at 14,000 x g for 30 min at 4 °C. Supernatants were transferred in clean tubes and kept on ice until further analysis, which has been done as soon as possible in the same day. Freeze-thaw of cell lysates are not recommended for proteasome activity analyses.

Fluorogenic peptide substrate suc-Leu-Leu-Val-Tyr-Methylcoumarin (suc-LLVY-MCA) was used to determine proteasome β5 chymotrypsin-like activity. The cell lysates were incubated with suc-LLVY-MCA in buffer containing 225 mM Tris, 45 mM KCl, 7.5 mM $Mg(CH_3COO)_2$, 7.5 mM $MgCl_2$ and 1 mM DTT, pH 7.8 for 30 min at 37 °C. 20S proteasome activity was measured under ATP depleted conditions with the addition of 5 U hexokinase and 15 nM 2-Deoxy-D-glucose. To measure ATP-dependent proteasome activity 2 mM ATP was added into the reaction mixture. Fluorescence intensity of released MCA was measured in a black 96-well plate at 360 nm excitation and 460 nm emission wavelengths. Free MCA standards were used to calculate the proteasome activity. Protein concentrations were measured with BCA assay and data were normalized to the protein concentrations and minute.

2.5. Western blot

Cells were lysed in Cell Lysis Buffer (Cell Signalling Tech. 9803) and lysates were loaded on 12% SDS-PAGE gels then transferred to nitrocellulose membranes. Ponceau S stain was applied to confirm equal protein loading. Membranes were blocked with blocking buffer, which contains 5% non-fat dry milk in TBST for 1 h. The membranes were incubated with K48-linkage Specific Polyubiquitin Antibody (Cell Signaling Tech. 4289S, 1:1000 dilution in blocking buffer) for overnight at +4 °C. Then, the membranes were washed 3 times with TBST and incubated with HRP conjugated secondary antibody (Calbiochem, D0016365, 1:10000 dilution in blocking buffer) at room temperature for 2 h. Finally, the membranes were screened through ChemiDoc chemiluminescence imaging system (Bio-Rad, USA).

2.6. Statistical analysis

All statistical analyses were performed using the Prism 7 software (Graph-Pad, CA, USA). Data are given as means ±S.D. and significance was indicated as $p<0.05$, $p<0.01$ and $p<0.001$.

3. RESULTS

We measured proteasome activity in ATP depleted (20S) and ATP stimulated (20S and 26S) conditions to elucidate the different responses of proteasome activities to oxidative stress in different cancer cell lines. We tested the activities in two different time points (0h and 3h after treatments) to see the immediate (early) and late response. In general, ATP stimulated 20S+26S proteasome activity was found to be more prone to H_2O_2 induced stress condition than the ATP depleted 20S proteasome activity.

K48-linked ubiquitinated proteins are targets for proteasomal degradation and accumulation of K48-linked ubiquitinated proteins may indicate an increase of damaged proteins and/or changes in proteasomal activity (13,26,27). Degradation of K48-linked ubiquitinated proteins requires functional 26S proteasome. In our data, H_2O_2 induced decrease in 20S+26S proteasome activities correlated with an increase in K48-linked ubiquitinated proteins in K562, U251, DU145 and HepG2C3A cell lines.

When we go into detail with the data, 20S and 20S+26S proteasome activities decreased in K562 cells in 0h time points when compared to control (PBS). Immediate response in ATP stimulated condition was more significant when compared to ATP depleted condition. In 3h time point, both 20S and 20S+26S proteasome activities declined in K562 cell line (Figure 1a). A significant decrease was detected in 20S and 20S+26S proteasome activities in late response when compared to early response ($p < 0.001$). Due to decreased 20S+26S proteasome activity, K48-linked ubiquitinated proteins increased ~2-times in 0h time point and ~4-times in 3h time point following H_2O_2 treatment in K562 cell line (Figure 1b).

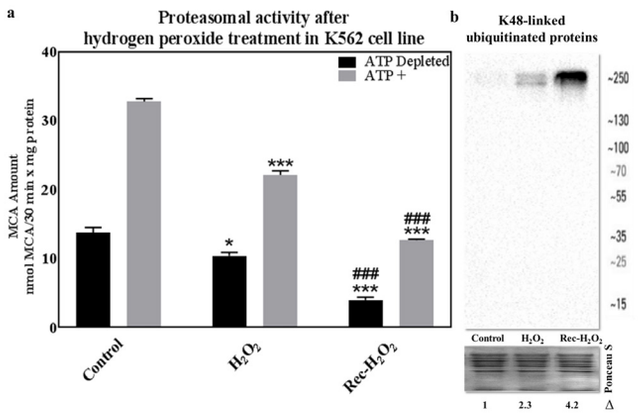

Figure 1. *Proteasomal degradation and K48-linked protein ubiquitination in K562 cells at 0h (H_2O_2) and 3h (Rec – H_2O_2) time points. a. ATP depleted degradation represents the activity of 20S proteasome, whereas ATP+ (ATP stimulated) degradation is indicative of 20S+26S proteasome activities. Values are the mean ± S.D. of three experiments. *$p < 0.05$ vs. control group, ***$p < 0.001$ vs. control group, ###$p < 0.001$ vs. H_2O_2 group. b. Representative immunoblot of K48-linked protein ubiquitination.*

In U251 cells, H_2O_2 treatment decreased 20S proteasome activity ($p < 0.05$ vs control) but did not change 20S+26S proteasome activity in immediate response (0h time point). In 3h time point, both 20S and 20S+26S proteasome activities decreased significantly when compared to the control group (Figure 2a). A significant decrease was detected in 20S+26S proteasome activity in late response when compared to early response ($p < 0.01$). K48-linked ubiquitinated protein levels of U251 cells did not change as an immediate response since 20S+26S proteasome activities did not change. But K48-linked ubiquitinated protein levels increased ~4-times in 3h time point (Figure 2b).

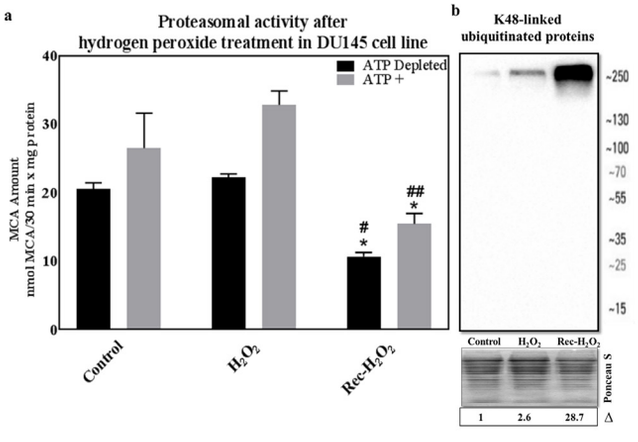

Figure 2. *Proteasomal degradation and K48-linked protein ubiquitination in U251 cells at 0h (H_2O_2) and 3h (Rec – H_2O_2) time points. a. ATP depleted degradation represents the activity of 20S proteasome, whereas ATP+ (ATP stimulated) degradation is indicative of 20S+26S proteasome activities. Values are the mean ± S.D. of three experiments. *$p < 0.05$ vs. control group, **$p < 0.01$ vs. control group. ##$p < 0.01$ vs. H_2O_2 group. b. Representative immunoblot of K48-linked protein ubiquitination.*

Immediate response (0h time point) in DU145, HepG2C3A and MCF7 cell lines were not significant in both 20S and 20S+26S proteasome activities. 20S and 20S+26S proteasome activities significantly decreased in these DU145 ($p < 0.05$ and $p < 0.01$), HepG2C3A ($p < 0.01$ and $p < 0.001$) and MCF7 ($p < 0.001$ and $p < 0.001$) cells in 3h time point showing the late response of these cells (Figure 3a, 4a and 5a) K48-linked ubiquitinated protein levels increased ~2-times in 0h time point in DU145 cell line where K48-linked ubiquitinated protein levels did not change in 0h time point in HepG2C3A cells. In 3h time point, K48-linked ubiquitinated protein levels increased ~28-times in DU145 cells and ~5-times in HepG2C3A cells (Figure 3b, 4b). K48-linked ubiquitinated protein levels in MCF7 cells did not show any significant change in any time point (Figure 5b).

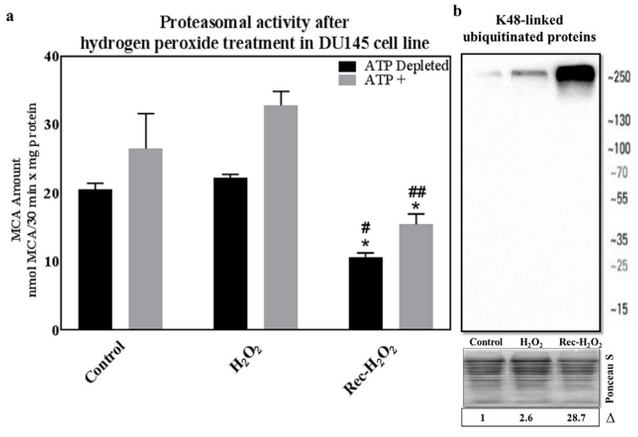

Figure 3. *Proteasomal degradation and K48-linked protein ubiquitination in DU145 cells at 0h (H_2O_2) and 3h (Rec – H_2O_2) time points. a. ATP depleted degradation represents the activity of 20S proteasome, whereas ATP+ (ATP stimulated) degradation is indicative of 20S+26S proteasome activities. Values are the mean ± S.D. of three experiments. *p < 0.05 vs. control group, #p < 0.05 vs. H_2O_2 group, ###p < 0.01 vs. H_2O_2 group. b. Representative immunoblot of K48-linked protein ubiquitination.*

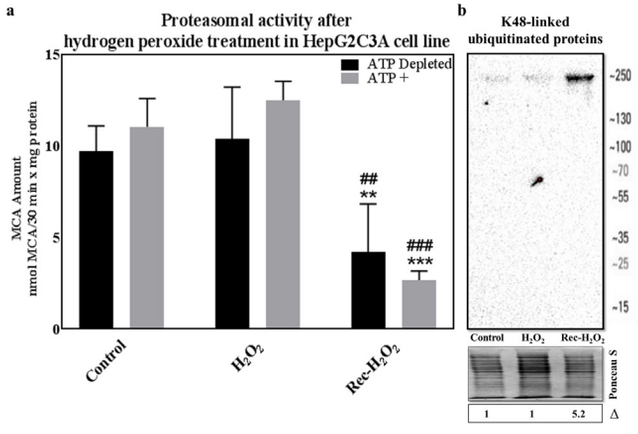

Figure 4. *Proteasomal degradation and K48-linked protein ubiquitination in HepG23C3A cells at 0h (H_2O_2) and 3h (Rec – H_2O_2) time points. a. ATP depleted degradation represents the activity of 20S proteasome, whereas ATP+ (ATP stimulated) degradation is indicative of 20S+26S proteasome activities. Values are the mean ± S.D. of three experiments. **p < 0.01 vs. control group, ***p < 0.001 vs. control group, ##p < 0.01 vs. H_2O_2 group, ###p < 0.001 vs. H_2O_2 group. b. Representative immunoblot of K48-linked protein ubiquitination.*

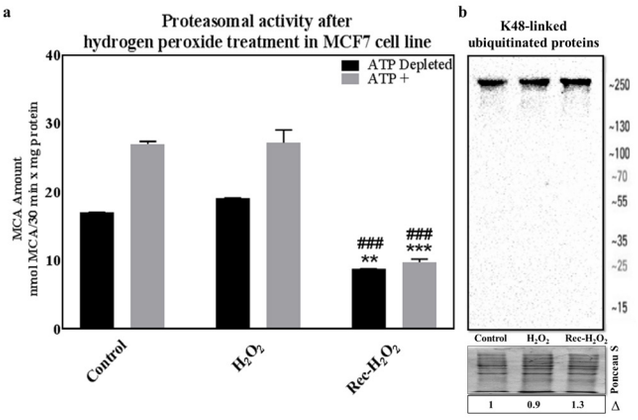

Figure 5. *Proteasomal degradation and K48-linked protein ubiquitination in MCF-7 cells at 0h (H_2O_2) and 3h (Rec – H_2O_2) time points. a. ATP depleted degradation represents the activity of 20S proteasome, whereas ATP+ (ATP stimulated) degradation is indicative of 20S+26S proteasome activities. Values are the mean ± S.D. of three experiments. **p < 0.01 vs. control group, ***p < 0.001 vs. control group, ###p < 0.001 vs. H_2O_2 group. b. Representative immunoblot of K48-linked protein ubiquitination.*

4. DISCUSSION

Under normal conditions, cells maintain a balance between ROS levels and antioxidant systems. However, under increased oxidative stress conditions, balance is disturbed and accumulated ROS cause damage in cellular macromolecules such as lipids, proteins, carbohydrates and nucleic acids (28,29). Such oxidatively damaged macromolecules can disrupt important cellular processes. Among these macromolecules, oxidatively damaged proteins and formed protein aggregates lead disturbances in many molecular pathways and they can inhibit proteasome activity (30). Thus, it is important to remove oxidatively damaged proteins by the proteasomal system and lysosomal pathways to maintain cellular protein homeostasis (31,32). Oxidative stress can modulate the proteasome activity to recycle oxidatively damaged proteins and maintain cellular health. It was suggested that oxidation of proteins increases the hydrophobicity of the proteins and makes them ideal substrates for the proteasome. Therefore oxidized proteins are degraded mainly through the 20S proteasome in an ATP and ubiquitin independent manner (7,15). It was shown that oxidative stress causes reduction of proteasome activity and up-regulation of proteasomal component synthesis and induction of dissociation of 26S proteasome complex into 19S and 20S proteasomes give rise to the 20S proteasomes (33–35). It is known that K48-linked ubiquitination targets the proteins for proteasomal degradation and accumulation of K48-linked ubiquitinated proteins may indicate alterations in 26S proteasome activity and/or increased proteotoxic stress (26,27,36). As mentioned above, oxidized proteins generally do not have to be ubiquitinated in order to be degraded by the proteasomal system (11). For cancer cells adaptation to the oxidative stress and restoring homeostasis can protect cells from apoptotic and/or necrotic cell death (22,23) which is an important point for therapy resistance.

In this study, we investigated proteasomal activity response and K48-linked protein ubiquitination changes of different cancer cell lines in two different time points following oxidative stress to see the immediate and late response. According to our results, immediate and late response following H_2O_2 induced stress presented different changes in 20S and 26S proteasomal activities in different cell lines.

Inhibition of proteasome in cancer treatment has been optimized in mainly hematological cancers since 2003. In our study, we used a chronic myelogenous leukemia cell line K562 as a reference. Following H_2O_2 treatment, ATP depleted 20S proteasome activities decreased only in K562 and U251 cell lines as immediate response when compared to control (0h time point), (Figure 1a and 2a). On the other hand, K562 cells were the only cells that ATP stimulated 20S+26S proteasome activity decreased as an immediate response following H_2O_2 treatment (Figure 1a). The immediate response of 20S+26S proteasome was much more significant when compared to 20S proteasome alone showing the sensitivity of 26S proteasome in K562 cells. This sensitive response of leukemia cells made them suitable for treatment with proteasome inhibitors for many years.

On the other hand, no changes in 20S proteasome and 20S+26S proteasome activities were observed in DU145, HepG2C3A and MCF7 cell lines as immediate response. This shows the resistance of proteasome activities among stress in these cells. It has been observed that oxidative stress-related proteasome activity impairment was compensated with an increased level of PSMA7 subunit of 20S proteasome and proteasome complexes in MCF7 cells (37). The results of a study conducted with a large number of cancer cell lines showed that cells which are especially drug-resistant and highly aggressive need high 26S proteasome activity level for survival (38). In accordance with that, in our study 26S proteasome activities of aggressive cancer cells U251, DU145, HepG2C3A and MCF7 were more resistant to oxidative stress and did not show as much proteasome activity decrease as K562 cells. In our study, none of the cancer cell lines showed increased proteasomal activity after 3 h of incubation when compared with the immediate response. Also, 20S+26S proteasomal activities showed a decline in 3h time point and due to this decline in 26S proteasome activity, K48-linked protein ubiquitination levels increased in all cell lines. In accordance with that, in a recent study protein ubiquitination levels were measured after 0.5 and 16 h of peroxide treatments in WM-451 melanoma cells and, similar to our results, ubiquitination levels showed increase in a time-dependent manner (39). Reports are showing that proteasome activity and proteasomal content could be restored following 6-24 h of oxidative stress (9,34,35,40). According to our results, both 20S and 26S proteasomal activities could not be restored to the basal level after 3 h of recovery and they rather showed decline. These results and recent works indicate that cells cannot restore their proteasomal activity in such a short period of time.

Consistent with our observations, oxidative stress caused reduction in proteasome activity has been reported in different cell types. In ARPE-19 cell line, high levels of H_2O_2 or prolonged exposure to H_2O_2 resulted in greater decrease in the proteasome activity and increase of ubiquitin conjugates. The authors concluded that proteasomal degradation is more susceptible to oxidative stress than the ubiquitin-conjugating enzymes (41). Reinheckel et al. (2000), demonstrated that 20S proteasome is more resistant to oxidative stress than 26S proteasome in K562 cells and after 24 h of recovery time 26S proteasome reconstitution was observed (35). Also in our study 20S proteasome activity of K562 cells was more resistant to oxidative stress. Cancer cells rely on enhanced proteasomal activity for survival (42). Proteasome is a target for oxidative stress and oxidative inactivation of the proteasomal system can be a strategy for cancer therapy.

5. CONCLUSION

Our results present direct evidence that several cancer cell lines show different responses in proteasomal activity and different K48-linked protein ubiquitination levels in response to oxidative stress. Taken together, our data lead the conclusion that proteasome inhibition for cancer therapy should be considered for different cancer types according to their proteasomal response in stress conditions. In addition, the clinical approach for proteasome targeted therapy can be designed according to this truth.

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Clinical and Experimental Health Sciences

The Effect of HbA1c Level on Gender-Specific Long-Term Morbidity and Mortality After Isolated Coronary Bypass in Poorly Controlled Diabetic Patients

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ABSTRACT

Objective: The aim of this study is to evaluate the gender specific effects of the high HbA1c levels in poorly controlled diabetic patients undergoing isolated coronary artery bypass grafting (CABG) procedure on long term morbidity, mortality and re-revascularization (2nd intervention).

Methods: This study was conducted on 532 (346 (65%) males and 186 (35%) females) diabetic patients who underwent CABG procedure at a single center between January 2010 and December 2013.The patients were separated into 4 groups according to gender and preoperative HbA1c level (%). The group1 consisted of females with HBA1c level ≤7(n=46); the group 2 comprised female with HbA1c level >7(n=140); the group 3 comprised men with HbA1c level ≤7(n=117); and the group 4 comprised men with HbA1c level >7(n=229). The groups were analyzed and compared for postoperative complications and mortality.

Results: No statistically significant difference was found among the groups in HbA1c levels and gender with respect to postoperative infection and mortality (p>0.05). The HbA1c level was determined to be statically significant and required the 2nd intervention (p:0.001; 95% CI: 1.249 (1.055,1.478)).

Conclusion: This study suggested that there was no difference in mortality rates after CABG among the groups although the current risk calculator modules stated otherwise. However, the HbA1c levels were associated with a need for a secondary intervention on long term follow up period.

Keywords: Diabetes Mellitus, Coronary Artery Bypass Grafts, Gender

1. INTRODUCTION

The ratio of diabetes mellitus (DM) was 23% among patients undergoing coronary artery bypass graft (CABG)surgery. This percentage is higher in female and currently increasing in both genders [1-3]. Diabetic patients have 2-4 fold higher mortality rate associated with cardiovascular disease as compared with non-diabetic patients, and the morbidity rate is also higher [4].

Hemoglobin A1c (HbA1c) is commonly used as an indicator of long-term average blood glucose levels [5]. HbA1C is an approved criterion for diagnosis of DM by The American Diabetes Association [6]. Higher levels of HbA1c (> 7%) have been found to be related to various complications (stroke, myocardial infarction) and increased risk of all-cause mortality following open heart surgery, but gender specific effects of higher levels of HbA1c are not well sought [7].

In this study, poorly controlled diabetic patients undergoing CABG surgery were retrospectively analyzed. The effect of gender and higher HbA1c levels on the long term morbidity, mortality and the need for repeat revascularization were studied.

2. METHODS

This study was conducted on a total of 532 diabetic cases comprising 346 (65%) men and 186(35%) female who underwent isolated CABG at a single center between January 2010 and December 2013. The mean age of the patients was 60.26±9.32 years (range, 31-84 years).

The Inclusion criteria were as follows: being diagnosed with uncontrolled diabetes (HbA1c > 5,6 %), receiving any kind of treatment for diabetes and undergoing isolated CABG surgery. The patients were separated into 4 groups according to gender and HbA1c levels. The threshold pre-operative HbA1c level of 7% was accepted as a marker of uncontrolled diabetes, according to the current literature [7,8]. The group 1 comprised of female with HBA1c level≤ 7% (n=46); the group 2 comprised of female with HBA1c level >7% (n=140); the group 3 comprised of men with HBA1c level≤ 7% (n=117); and the group 4 comprised of men with HBA1c level > 7% (n=229). Additionally, we evaluated the clinical progress of

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the symptoms of coronary artery disease (CAD) in the long term follow up period. The asymptomatic patients had no recurrent symptoms of coronary artery ischemia at follow-ups. The symptomatic patients were those who presented with symptoms of coronary artery disease and underwent coronary angiography but did not require revascularization. Symptomatic patients (n: 66, 12.4%) who had required re-revascularization were classified as the second intervention patients receiving treatment either with redo CABG or PCI (n: 41, 7.7%). Three of symptomatic patients required redo CABG.

Long-term survival results were searched on hospital database, the national health database and phone-calls with the patients. Also, potential second intervention to the patient in another institution was interrogated during these phone-calls. If the second intervention history is positive, detailed information of this second intervention was obtained from the host-institute by phone-calls too. We excluded the patients with the missing data.

The exclusion criteria were as follows: non-diabetic patients and diabetic patients with low HbA1c level (HbA1c 5,6%) patients with incomplete medical reports, patients using steroids or undergoing chemotherapy, and those receiving a diagnosis of decompensated congestive heart failure, congenital heart disease, cerebrovascular disease within the previous 30 days, dialysis-dependent kidney failure, a clinically active malignancy, endocrinological disorders (hypothyroidism, hyperthyroidism), systemic inflammatory disease, hematological proliferative disease, low hemoglobin levels (Hgb ≤ 10 g/dl), a clinically active infection, or a diagnosis of autoimmune disease.

After the local ethical committee approval, the demographic and clinical characteristics were recorded for each patient. Acute kidney injury (AKI) was evaluated and classified according to the Kidney Disease Improving Global Outcomes (KDIGO) classification by calculating preoperative and postoperative serum creatinine levels [9]. Chronic obstructive pulmonary disease (COPD) was defined as follows: – Patients with obstructive pattern in preoperative spirometry (FEV1/FEVC<0,70). –Patients having active treatment for COPD [10].

DM was accepted as the use of oral anti-diabetic drugs or insulin or a fasting blood glucose level > 126 mg/dl [11]. Before the procedure, peripheral venous blood samples (5-7 cc) were obtained and placed in sterile EDTA tubes to prevent clotting. After 1 hour, hematological parameters were calculated with an automatic blood count device (Abbott CELL-DYN 3700; Abbott Laboratory, Abbott Park, Illinois, USA). All patients had oral antidiabetic-drug or insulin. No patient among all 4 groups received both insulin and oral diabetic drugs at the same time. We evaluated preoperative low density lipoprotein level (LDL > 100 mg/dl and >190 mg/dl) (12).

2.1. Blood Sugar Examinations

Throughout the operation, the blood glucose levels of the patients were evaluated once before the CPB and then at hourly intervals. Crystallized insulin (Humulin R®, Lilly, Indianapolis, USA) was applied intravenously to control blood glucose level. In the intensive care unit (ICU), all patients' blood glucose levels were regulated with an insulin infusion according to the Portland protocol [13]. An average glucose level was calculated for each patient.

2.2. Surgical procedure

All the surgical procedures were performed as on or off pump during CABG surgery. Following the anesthesia induction median sternotomy was performed in all of the patients. Coronary bypass grafts were harvested from the saphenous vein and the left internal mammary artery (pediculate). A non-pulsatile roller pump and membrane oxygenator was used for cardiopulmonary bypass (CPB) in on-pump CABG patients. The surgical procedure was performed at moderate systemic hypothermia (28°C-30°C). CPB was applied at a flow rate of 2.2-2.5 L/min/m^2. Mean arterial pressure between 50-80 mmHg and hematocrit values of 20%-25% were achieved in all patients. Myocardial protection was applied with intermittent antegrade and continuous retrograde hypothermic and hyperkalemic blood cardioplegia. The proximal anastomoses were performed on beating heart under a partial clamp. Additionally; electrocardiogram (ST segments), mean arterial pressure (>70mmHg), cardiac output and activated clotted time (ACT > 250 seconds) monitored continuously during the off-pump CABG procedure.

2.3. Statistical Analyses

For the statistical analysis, NCSS 2007 software was used (Number Cruncher Statistical System, Kaysville, Utah, USA). Descriptive statistical methods such as mean, standard deviation, median, the first quartile, the third quartile, frequency, and percentage, minimum, maximum were used for reporting the data. Independent samples t-test was used to compare normally distributed variables between the 2 groups, Mann-Whitney U-test was used for variables violating normal distribution assumption. One-way ANOVA with Tukey HSD post hoc tests were used to compare normally distributed variables between 3 or more groups. Kruskal-Wallis test with Dunn-Bonferroni post hoc tests were used to compare non-normally distributed variables between 3 or more groups. The Pearson chi-square test, the Fisher-Freeman-Halton exact test, the Fisher's exact test were used to compare qualitative data. Pearson correlation analysis was used to assess the correlation between quantitative variables. The effects of risk factors on postoperative infection and mortality were evaluated via binary backward logistic regression analysis, whereas effects of risk factors on the second intervention were evaluated via multivariate backward logistic regression analysis. The effects of risk factors on length of ICU stay, length of hospital stay and chest tube output were evaluated via linear regression analysis. A p value of <0.05 was accepted as statistically significant.

4. RESULTS

The distributions of the descriptive, the preoperative and postoperative characteristics are shown in Table 1. Among 532 cases included in the study, 467 (87.8%) survived, and 65 died. The CABG procedures were performed with on-pump procedure in 471 patients and on off-pump strategy in 61 patients.

The mean survival time was 72.29±1.00 months. The latest death occurred at 77.1 months, and in that month, the cumulative survival rate was 86.1%, with a standard error of 2.0%. These results are shown according to groups in Figure 1.

Table 1. *Comparison according to Gender& HbA1c levels*

		Total (n=532)	Female & HbA1c≤%7 (n=46)	Female & HbA1c>%7 (n=140)	Male & HbA1c≤%7 (n=117)	Male & HbA1c>%7 (n=229)	P
Gender	Female	186 (35)	46 (100)	140 (100)	0 (0)	0 (0)	-
	Male	346 (65)	0 (0)	0 (0)	117 (100)	229 (100)	
HbA1c (%)		8,74±1,74	6,880±0,29	9,58±1,6	6,98±0,27	9,47±1,5	-
Age (years)		60,26±9,32	61,87±8,55	62,08±9,05	61,15±9,7	58,36±9,13	[a]0,001*
BMI (kg/m^2)		29,3±4,82	31,15±6,11	29,95±5,37	29,19±4,47	28,58±4,19	[a]0,002*
Previous MI		223 (41,9)	14 (30,4)	61 (43,6)	44 (37,6)	104 (45,4)	[b]0,196
COPD		73 (13,7)	3 (6,5)	22 (15,7)	21 (17,9)	27 (11,8)	[b]0,174
Hypertension		300 (56,4)	34 (73,9)	98 (70)	66 (56,4)	102 (44,5)	[b]<0,001*
PVD		26 (4,9)	1 (2,2)	3 (2,1)	6 (5,1)	16 (7)	[b]0,159
EF (%)		54,02±11,15	57,8±9,44	55,5±10,39	54,71±11,12	52,01±11,59	[a]0,001*
LDL > 100; n (%)		335(65,6)	33 (73.3)	87 (63)	84 (73.7)	131 (60.1)	[c]0.053
LDL > 190; n (%)		34(6,6)	3 (6.7)	7 (5.1)	9 (7.9)	15 (6.9)	[c]0.835
Critical stenosis		6 (1,1)	0 (0)	2 (1,4)	0 (0)	4 (1,7)	[c]0,574
LIMA used		484 (91)	41 (89,1)	128 (91,4)	107 (91,5)	208 (90,8)	[b]0,967
Reoperation		11 (2,1)	1 (2,2)	5 (3,6)	2 (1,7)	3 (1,3)	[c]0,463
DM treatment	OAD	403 (75,8)	39 (84,8)	89 (63,6)	101 (86,3)	174 (76)	[b]<0,001*
	Insulin	129 (24,2)	7 (15,2)	51 (36,4)	16 (13,7)	55 (24)	
AKI	0	357 (67,1)	28 (60,9)	83 (59,3)	85 (72,6)	161 (70,3)	[b]0,061
	1	175 (32,9)	18 (39,1)	57 (40,7)	32 (27,4)	68 (29,7)	
Length of ICU stay (hours)		44 (25-69)	47,5 (25-95)	41 (23-49)	46 (24-75)	46 (24-72)	[d]0,010*
Length of hospitalstay (days)		7,5 (6-12)	8 (7-11)	7 (6-10)	7 (7-11)	7 (7-11)	[d]0,251
Followupperiod (months)		50,9 (43,5-68,4)	54,07 (43,65-71,08)	57,27 (42,73-71,5)	54,67 (42,5-69,33)	55,13 (42,87-69,93)	[d]0,867
Intubationduration (hours)		11 (8-16)	11,5 (8-16)	9 (8-12)	10 (8-13)	10 (8-13,5)	[d]0,004*
Number of distal bypass grafts		3 (2-3)	3 (2-3)	3 (2-3)	3 (2-4)	3 (2-3)	[d]0,008*
Chesttubeoutput (ml)		550 (400-700)	450 (350-600)	550 (450-800)	600 (450-800)	550 (400-775)	[d]<0,001*
Perfusionduration (minutes)		82,5 (49-94)	91 (64-115,5)	81 (58-102)	90 (62-112)	88 (61,5-109,5)	[d]0,076
ACC (minutes)		48,5 (30-64)	50 (33,5-70)	48 (30-64)	52 (35-70)	50 (33-68)	[d]0,249
Post-op infection		56 (10,5)	4 (8,7)	20 (14,3)	11 (9,4)	21 (9,2)	[b]0,412
Mortality		65 (12,2)	5 (10,9)	21 (15)	13 (11,1)	26 (11,4)	[b]0,710
2ndIntervention	**Symptomatic (-)**	425 (79,9)	37 (80,4)	106 (75,7)	98 (83,8)	184 (80,3)	[b]0,300
	Symptomatic (+)	66 (12,4)	5 (10,9)	17 (12,1)	15 (12,8)	29 (12,7)	
	2ndIntervention	41 (7,7)	4 (8,7)	17 (12,1)	4 (3,4)	16 (7)	

*BMI: Body Mass Index, MI: Myocardial Infarction, COPD: Chronic Obstructive Pulmonary Disease, PVD: Peripheral Vascular Disease, EF: EjectionFraction, HbA1c: Glycosylated Hemoglobin, LIMA: Left Internal Mammarian Artery, DM: Diabetes Mellitus ICU:Intensive Care Unit, ACC: Aortic Cross Clamp AKI: Acutekidneyinjury LDL: Low Dansity Lipoprotein; [a]One-way ANOVA (reported as mean±sd); [b]Pearsonchi-square test (reported as n (%)); [c]Fisher-Freeman-Haltonexact test (reported as n (%)); [d]Kruskal-Wallis test (reported as median (Q1-Q3)); Q1:First quartile, Q3:Third quartile; *p<0,05*

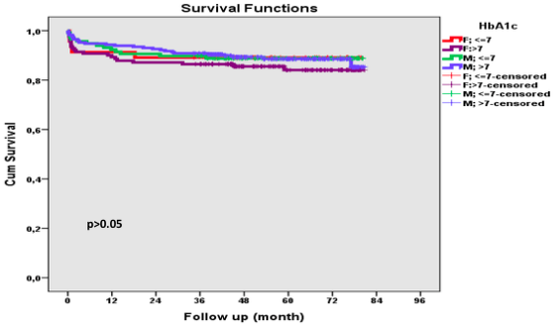

Figure 1. *Survival graphic according to HbA1c levels*

No statistically significant difference was determined among the groups regarding postoperative infection, mortality and the 2nd intervention (p>0.05). The AKI, age, COPD, the length of ICU and the number of distal bypass grafts were found to be statistically significant in mortality (p<0.05). The length of hospital stay was significantly associated with infection (p<0.05). There were no statically significant differences in LDL parameters among the groups (p<0.05).

The postoperative infection, mortality and the 2nd intervention were evaluated according to the characteristics (shown in Table 2).

Table 2. *The patients'scharacteristics determined mortality, infection and the second intervention*

		PostopInfection			Mortality			2ndIntervention			
		No	Yes	P	No	Yes	P	S (-)	S (+)	2ndInt.	p
Age (years)		60,12±9,29	61,45±9,54	[e]0,313	59,5±8,95	65,71±10,15	[e]<0,001*	60,48±9,29	60,17±9,18	58,12±9,82	[d]0,303
BMI (kg/m^2)		29,25±4,8	29,66±5,04	[e]0,553	29,28±4,73	29,38±5,5	[e]0,887	29,28±4,87	29,06±4,06	29,86±5,49	[d]0,697
Previous MI	No	268 (86,7)	41 (13,3)	[b]0,015*	270 (87,4)	39 (12,6)	[b]0,738	250 (80,9)	36 (11,7)	23 (7,4)	[b]0,778
	Yes	208 (93,3)	15 (6,7)		197 (88,3)	26 (11,7)		175 (78,5)	30 (13,5)	18 (8,1)	
COPD	No	415 (90,4)	44 (9,6)	[b]0,076	410 (89,3)	49 (10,7)	[b]0,006*	367 (80)	55 (12)	37 (8,1)	[b]0,597
	Yes	61 (83,6)	12 (16,4)		57 (78,1)	16 (21,9)		58 (79,5)	11 (15,1)	4 (5,5)	
Hypertension	No	208 (89,7)	24 (10,3)	[b]0,905	207 (89,2)	25 (10,8)	[b]0,372	187 (80,6)	30 (12,9)	15 (6,5)	[b]0,625
	Yes	268 (89,3)	32 (10,7)		260 (86,7)	40 (13,3)		238 (79,3)	36 (12)	26 (8,7)	
PVD	No	453 (89,5)	53 (10,5)	[g]0,747	445 (87,9)	61 (12,1)	[g]0,544	403 (79,6)	63 (12,5)	40 (7,9)	[c]0,932
	Yes	23 (88,5)	3 (11,5)		22 (84,6)	4 (15,4)		22 (84,6)	3 (11,5)	1 (3,8)	
EF (%)		53,94±11,28	54,77±10	[e]0,598	54,27±11,27	52,28±10,13	[e]0,178	54,28±11,03	51,44±11,93	55,51±10,68	[d]0,105
Critical stenosis	No	470 (89,4)	56 (10,6)	[g]0,999	462 (87,8)	64 (12,2)	[g]0,544	420 (79,8)	66 (12,5)	40 (7,6)	[c]0,491
	Yes	6 (100)	0 (0)		5 (83,3)	1 (16,7)		5 (83,3)	0 (0)	1 (16,7)	
LIMA used	No	42 (87,5)	6 (12,5)	[b]0,640	41 (85,4)	7 (14,6)	[b]0,600	38 (79,2)	9 (18,8)	1 (2,1)	[b]0,144
	Yes	434 (89,7)	50 (10,3)		426 (88)	58 (12)		387 (80)	57 (11,8)	40 (8,3)	
Reoperation	No	467 (89,6)	54 (10,4)	[g]0,325	458 (87,9)	63 (12,1)	[g]0,632	417 (80)	65 (12,5)	39 (7,5)	[c]0,334
	Yes	9 (81,8)	2 (18,2)		9 (81,8)	2 (18,2)		8 (72,7)	1 (9,1)	2 (18,2)	
DM treatment	OAD	363 (90,1)	40 (9,9)	[b]0,425	357 (88,6)	46 (11,4)	[b]0,317	327 (81,1)	51 (12,7)	25 (6,2)	[b]0,071
	Insulin	113 (87,6)	16 (12,4)		110 (85,3)	19 (14,7)		98 (76)	15 (11,6)	16 (12,4)	
AKI	0	327 (91,6)	30 (8,4)	[b]0,023*	330 (92,4)	27 (7,6)	[b]<0,001*	284 (79,6)	43 (12)	30 (8,4)	[b]0,666
	1	149 (85,1)	26 (14,9)		137 (78,3)	38 (21,7)		141 (80,6)	23 (13,1)	11 (6,3)	
Intubationduration (hours)		10 (8-13)	12 (8-17)	[f]0,035*	10 (8-13)	11 (8-18)	[f]0,103	10 (8-14)	10 (8-12)	9 (8-12)	[d]0,369
Number of distal bypass grafts		3 (2-3)	3 (2-4)	[f]0,329	3 (2-3)	3 (1-3)	[f]0,028*	3 (2-3)	2 (1-3)	3 (2-4)	[d]0,012*
Perfusionduration (minutes)		87 (60-108)	97,5 (78,5-116,5)	[f]0,023*	88 (63-110)	90 (40-109)	[f]0,686	90 (64-110)	68,5 (47-95)	87 (64-108)	[d]0,005*
ACC (minutes)		49 (32-67,5)	56 (43-70)	[f]0,141	50 (35-68)	52 (20-70)	[f]0,607	51 (35-69)	43,5 (20-64)	51 (38-65)	[d]0,044*
Length of ICU stay (hours)		45 (24-71)	47,5 (25-97,5)	[f]0,060	45 (24-70)	51 (23-119)	[f]0,031*	47 (24-72)	44 (24-52)	42 (24-71)	[d]0,606
Length of hospitalstay (days)		7 (6-10)	13 (8-18,5)	[f]<0,001*	7 (7-10)	11 (7-19)	[f]<0,001*	7 (7-11)	8 (7-10)	8 (7-11)	[d]0,360
Chesttubeoutput (ml)		550 (400-800)	550 (425-700)	[f]0,894	550 (400-750)	550 (350-800)	[f]0,610	550 (400-800)	550 (400-700)	600 (450-900)	[d]0,300

*BMI: Body Mass Index, MI: Myocardial Infarction, COPD: Chronic Obstructive Pulmonary Disease, PVD: Peripheral Vascular Disease, EF: Ejection Fraction, HbA1c: Glycosylated Hemoglobin, LIMA: Left Internal Mammarian Artery, DM: Diabetes Mellitus ICU: Intensive Care Unit, ACC: Aortic Cross Clamp AKI: Acutekidneyinjury , S+ : patients who had symptoms of coronary artery disease; [a]One-way ANOVA (reported as mean±sd); [b]Pearsonchi-square test (reported as n (%)); [c]Fisher-Freeman-Haltonexact test (reported as n (%)); [d]Kruskal-Wallis test (reported as median (Q1-Q3)); [e]Independentsamples t test (reported as mean±sd); [f]Mann-Whitney U test (reported as median (Q1-Q3)); [g]Fisher'sexact test (reported as n (%)); Q1:First quartile, Q3:Third quartile; *p<0,05*

There was a statistically significant difference in the need for the second intervention according to number of distal bypass grafts (p<0.05). Also, the patients with the high HbA1c levels required a higher rate of intervention with time (p<0.05). There was no statistically significant difference in the need for re-intervention between off-pump (n: 3, 4.9%) and on-pump (n: 38, 8.1%) procedures (p=0.066).

The risk factors of postoperative infection and mortality were determined with binary logistic regression and for the 2nd intervention with multivariate logistic regression analysis. Gender, HbA1c and variables with p<0.150 in gender comparisons (age, BMI, HT, PVD, EF, DM treatment, AKI, length of ICU stay, Intubation duration, distal bypass grafts, chest tube output and length of hospital stay) were included

as independent variables. In addition to these variables, for each dependent variable, the variables with p<0.150 in the uni-variable analysis regarding that variable were also included in the corresponding model (Table 3).

The model for postoperative infection was found to be statistically significant (χ^2=33.169, p<0.001). Previous MI and length of hospital stay were found to have significant effect on postoperative infection (p=0.022, p<0.001, respectively).

The model for mortality was found to be statistically significant (χ^2=70.242, p<0.001). Age, AKI, length of ICU stay, COPD and number of distal bypass grafts were found to have significant effect on mortality (p<0.001, p<0.001, p<0.001, p=0.043, p=0.005, respectively).

It was found that the model for the 2nd intervention was statistically significant (χ^2=15.862, p=0.003). HbA1c level and number of distal bypass grafts were found to have significant effect on the 2nd intervention (p=0.010, p=0.003, respectively). The one unit changes of HbA1c levels can increase the 2nd intervention risk by 1.249 times.

Table 3. *Logistic regression analyses according to infection, mortality and 2nd intervention*

Dependentvariable		Independentvariables	p	OR (95% CI)
Postopinfection		Constant	<0.001*	0.066
		Previous MI (no)	0.022*	2.116 (1.113, 4.024)
		Length of hospitalstay	<0.001*	1.072 (1.042, 1.103)
Mortality		Constant	<0.001*	0.012
		Age	<0.001*	1.07 (1.036, 1.104)
		AKI (1)	<0.001*	2.905 (1.626, 5.193)
		Length of ICU stay	<0.001*	1.006 (1.003, 1.009)
		COPD (yes)	0.043*	2.074 (1.022, 4.208)
		Number of distal bypass grafts	0.005*	0.667 (0.502, 0.885)
2nd intervention	Symptomatic (+)	Intercept	0.042*	-
		HbA1c	0.347	1.075 (0.924, 1.251)
		Number of distal bypass grafts	0.003*	0.677 (0.523, 0.876)
	2nd intervention	Intercept	<0.001*	-
		HbA1c	0.010*	1.249 (1.055, 1.478)
		Number of distal bypass grafts	0.995	0.999 (0.733, 1.362)

*MI: Myocardial Infarction, COPD: Chronic Obstructive Pulmonary Disease, HbA1c: Glycosylated Hemoglobin, ICU: Intensive Care Unit, AKI: Acute kidney injury; OR: Odds Ratio; CI: Confidence Interval; Sypmtomatic (-) wastaken as the reference category for the 2nd Intervention analysis; *p<0.05*

5. DISCUSSION

In this study, the effect of the HbA1c level was investigated on long-term morbidity, mortality and the need for the second intervention following CABG procedure in poorly controlled diabetic patients with respect to male and female patients. The results of the study showed that there was no difference in mortality rates after coronary bypass among the groups with respect to HbA1c level and gender. However, there was a significantly higher need for the second intervention among patients with high HbA1c level on long term follow up period.

Hyperglycemia has been shown to expand the infarct field in the myocardium, to impair ischemic preconditions and to increase reperfusion injury [14]. Compared to non-diabetic individuals, diabetic patients are known to have a higher morbidity and mortality rate in coronary artery disease (CAD) [15,16]. Diabetes is also an indicator of the development of atherosclerosis and plaque instability. Therefore, and specific to diabetes, the number of vessels affected in CAD is usually more than one, the coronary diameters are small, lesion locations are usually osteal or proximal and completely obstructed, narrowing is more often in the left main artery [6,17], collateral vessel development is impaired [18], and there is greater coronary artery calcification [19]. Similarly, females have coronary arteries of a smaller diameter. Small coronary arteries are more predisposed to occlusion and spasm [20]. The most common cause of death of females worldwide is CAD [21]. However, CAD is seen more often as a male disease than female disease [22]. In the past 10 years, there has been a marked increase in CABG operations in females, and currently, 1 out of every 3 CABG operations is performed on a female patient[23].In this study, females comprised 35% of the study groups. We did not determined difference among the four groups regarding mortality and the 2ndintervention.

In a study by Blankstein et al. who investigated the mortality rates in females, it was reported that female gender was independent risk factors for mortality after CABG. [24]. Ennker et al. stated that there was no effect of gender related to increased risk of CABG operation among females [25]. In our study, gender did not have any effect on mortality rates.

In our study, based on gender, the mortality rates were 13,9% for the female group and 11,2% for the male group, it was not statically different contrary to the widely accepted risk calculators [19,24]. Additionally, the length ICU stay and chest tube output were found to be longer for female patients than for the male patients.

Hemoglobin A1c (HbA1c) is an index of long-term average blood glucose levels and outcome predictors in diabetic patients [5,7]. Higher levels of HbA1c have been associated increased major adverse cardiac events and death (7,26). Halko et al. studied 3089 diabetic and non-diabetic patients in a prospective study and they found that HbA1c levels had significant effect on hospital stay, morbidity and mortality. Mortality was found to be increased in those with high HbA1c, ≥8.6% [27]. Robich et al., found poor long-term survival in increased HbA1c among patients undergoing CABG (ratio of death risk increased by %13 for every unit increase in HbA1 [28]. Nystrom and colleagues found that high levels of HbA1c resulted in increased rates of mortality and repeat revascularization on type 1 diabetic patients who underwent CABG within 4,7 years follow up (29). In this study, there was no difference between the groups according HbA1c levels (HbA1c >7: 12, 7%; HbA1c <7: 11, 0%) for long term mortality. Also one of the findings of this study was that there was a statistically significant higher requirement for the secondary revascularization following coronary bypass in patients who had a high level of HbA1c. Revascularization was provided for these patients following angiography. According to the clinical status of the patients, the revascularization was performed as an angiographic procedure or as a redo CABG.

Some other parameters such as preoperative ventricular function can affect early outcome after CABG (30). We did not observe any effect of EF on adverse outcomes, including mortality.

Previous studies have reported that sternal complications are high in DM patients following CABG operations and that this rate can increase to 10% [31] and the level of HbA1c is associated with sternal wound infection (32). Halkos et al. also reported that mortality and sternal infection rates increased with a high HbA1c level [27]. In the current study, contrary to expectations, no statically difference was observed in infection rates with respect to the HbA1c level and gender among the selected diabetic groups.

Czech et al. examined 2881 DM patients undergoing CABG operation and determined that rates of mechanical ventilation were higher and the length of stay in the ICU was longer in females [19]. In this study, all the patients had DM, and the lengths of stay in the ICU and in the hospital were determined to be statistically significantly longer in females with a high HbA1c level compared to the other groups. Additionally, we found that level of HbA1c were a risk factor for drainage.

Some studies showed that high level of HbA1c and diabetes mellitus was associated with increased postoperative AKI after CABG [33-34]. According to the KDIGO 2012 AKI Guidelines, cardiac surgery with CPB is a 1B risk factor (9). Although there is no consensus on AKI and Hba1c levels in patients with no known renal disease, HbA1c of>7% is defined as a Class 1A risk factor for patients with chronic renal disease [27]. However, some studies noted increased HbA1c level for AKI following CABG is unclear (32). Additionally, the mortality rate of our patient group with AKI was determined to be severely increased in all groups.

6. CONCLUSION

This study showed that higher HbA1c levels have no effect in mortality rates after coronary bypass surgery of poorly controlled diabetic patients although the current risk calculator modules stated otherwise. However, the HbA1c levels have been found to be associated with the need for secondary intervention on long term postoperative period. This group of patients should be treated with aggressive medical therapy for blood glucose level regulation and long-term follow up is required during the postoperative period.

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Clinical and Experimental Health Sciences

Mutual Relationship Between Upper Extremity Function and Core Muscle Endurance in Patients with Multiple Sclerosis

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ABSTRACT

Objective: This study aimed to determine the relationship between upper extremity function and core muscle endurance in patients with multiple sclerosis (PwMS).

Methods: Twenty-two PwMS and 20 healthy controls (HC) were included in this retrospective cross-sectional study. The upper extremity function was assessed by the Nine Hole Peg Test (NHPT). In addition, the core muscle endurance was assessed by the side bridge test, trunk flexor endurance test, trunk extensor test, and prone bridge test.

Results: Upper extremity function and core muscle endurance (except extensor endurance) in PwMS were lower compared to HC ($p<0.05$). In addition, the results of correlation analyses in PwMS showed that both sides' upper extremity functions were related to EDSS, and trunk flexor endurance ($p<0.05$). Moreover, the right-upper extremity function was related to the right side bridge endurance test performance while the left-upper extremity function was related to left side bridge endurance test performance and disease duration ($p<0.05$).

Conclusion: These results demonstrated the mutual relationship between upper extremity function and core muscle endurance in PwMS. Therefore, the core stabilization training may improve both the core muscle endurance and upper extremity function in PwMS.

Keywords: Multiple sclerosis, upper extremity, core muscle, endurance.

1. INTRODUCTION

Motor and sensory dysfunctions occur in patients with multiple sclerosis (PwMS) due to central nervous system damage. The cohort study of the North American Research Committee on Multiple Sclerosis (NARCOMS), containing data from 23931 PwMS, yielded that upper extremity dysfunction was reported by 60% of patients in the first years of the disease, and in 86% of patients in the 30th year of the disease (1). Moreover, over time, not only prevalence but also the severity of dysfunction increase in PwMS. Upper extremity dysfunction causes declines in cognitive functions, quality of life, and abilities in activities of daily living (ADLs) in PwMS (2).

Movements of the upper extremity require proximal stabilization during manipulative and targeted activities. Therefore, the prerequisite for upper extremity functions is to provide trunk stabilization (3). The upper extremity moves through trunk stabilization, power generation, and power transfer which are supported by activation of the core muscles (4). That is, core stabilization is required to generate and transmit forces along the kinetic chain between the upper extremity and core region (5, 6). Abdominal muscles co-contract with lumbar extensor muscles to increase the stability of the spine during and before activity (7, 8). Therefore, core stability created by the activation of core muscles is important and essential for many upper extremity activities (9).

It has been demonstrated that there is a positive relationship between upper extremity functions and postural control in children, older people, and Parkinson's patients (10-12). In addition, impaired upper extremity function was also found to be associated with decreased postural control in PwMS, even mild disabilities (13). However, the relationship between core muscles involved in postural control and upper extremity function is not yet known. Defining this relationship may contribute to the development of rehabilitation approaches aimed at improving upper extremity function in PwMS.

Therefore, the aim of this study is to determine the relationship between upper extremity function and core muscle endurance in PwMS.

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2. METHODS

2.1. Participants

PwMS and healthy controls (HC) were included in this retrospective cross-sectional study which was conducted at Gazi University, Department of Physiotherapy and Rehabilitation from 2018 to January 2020. The study protocol was approved by the Gazi University Ethics Commission (No:518). The patients were recruited by their neurologists who gave the diagnosis according to McDonald criteria (14) and determined the Expanded Disability Status Scale (EDSS) score (15). The McDonald criteria are consist of a combination of clinical tests, magnetic resonance imaging, cerebrospinal fluid, and evoked potentials and regarded as the gold standard for the standard diagnosis of MS (14). The EDSS is the most common clinical scale that measures the disability of MS patients (15). The scores of EDSS range from 0 (normal) to 10 (death due to MS) based on eight functional systems consisting of pyramidal function, cerebellar function, brainstem function, sensory function, bowel/bladder function, mental function, and visual function. For PwMS, the inclusion criteria were (1) the diagnosis of definite MS, (2) age between 18-65 years (3) ambulatory without aids (EDSS score<6) (15), (4) relapse-free for the last 3 months. Exclusion criteria for all participants were (1) history of neurological disease (other than MS for the patient group), (2) presence of orthopedic, vision, or hearing problems, (3) presence of low back pain, (4) having a history of spinal, or abdominal surgery.

2.2. Study Design

The demographic information (age, gender, body mass index) of all participants and disease characteristics (disease duration, the number of relapses, and EDSS) of the PwMS were recorded.

2.3. Measurements

First, the assessor explained and showed each test to the participants, then asked the participants to practice the test once. After one practice trial, the participants performed the actual test once. The participants rested for at least 5 minutes between each test. In all assessments, the actual test duration was recorded in seconds by using a stopwatch.

The upper extremity function was assessed by nine-hole peg test (NHPT). All participants were assessed by using the same materials (chair, table, a wooden board with 9 holes, and 9 pegs). The participants sat on a chair and then they picked up nine pegs one at a time as quickly as possible, put them in nine holes, and then removed them again as quickly as possible one at a time (16, 17).

Core muscle endurance was assessed by the side bridge test, trunk flexor endurance test, trunk extensor test, and prone bridge test (18, 19). Only the trunk extensor test was performed on a bed, other endurance tests were performed on the mat, and these materials were standard in all participants.

2.3.1. Side bridge test: The participants lay on their left / right sides with support from the forearm with elbows below the shoulders and extended their legs straight. Then the participants lifted their hip from the floor. The test was terminated when the side bridge position deteriorated or the hip fell (20).

2.3.2.The trunk flexor test: The participants were in a sit-up position while their trunks were supported at the angle of 60 degrees from the floor. They flexed knees and hips at 90 degrees and crossed arms over the chest. Then, the support of the trunk was removed, and the participants maintained this position as long as possible (20).

2.3.3.Prone bridge test: The participants placed elbows and forearms below their shoulders, straightened the legs. Next, they kept their forearms and toes on the floor, lifted hips, and maintained this straight position as long as possible (18).

2.3.4.The trunk extensor test: The participants lied down on a treatment table, aligning their anterior superior iliac spine with the edge of the table, which allowed the upper body to be planked out over the edge. An assessor fixed the participants' pelvis, hip, and knees to the treatment table. The body and the upper extremities were supported using a chair. Next, the chair was removed, and the participants maintained the horizontal body position as long as possible with their arms crossed position on the chest (20).

2.4. Statistical Analysis

Statistical analysis was performed by using the IBM Statistics SPSS v21.0. (IBM Corp. Armonk. NY. the USA). The variables were determined by the measurement (histograms, Shapiro-Wilk test) and expressed as the median and Interquartile Range (IQR) due to non-normal distribution. Categorical variables were expressed as a percentage. A Mann-Whitney U test was used to compare PwMS and HC. A Spearman correlation coefficient was performed to determine the relationship between upper extremity function, demographic characteristics, and core muscle endurance. The correlation coefficient was classified as negligible (0-0.10), weak (0.10-0.39), moderate (0.40-0.69), strong (0.70-0.89), and very strong (0.90-1.00). The statistical significance level was $p<0.05$ for all tests (21).

3. RESULTS

A total of 22 PwMS and 20 HC were included in this study. The PwMS had mild disabilities [EDSS: median 1.75 (IQR:1.00-3.63)], and all of them were the relapsing-remitting clinical course of MS. Also, all participants used their right hands dominantly.

Table 1 shows the demographic characteristics, upper extremity function, and core muscle endurance in PwMS and HC. There was no difference between demographic

characteristics in PwMS and HC. As expected, PwMS were able to finish the NHPT in a longer time than HC (p<0.05). In addition, all core muscle endurance, except trunk extensor endurance were lower in PwMS compared to HC (p<0.05).

[Table 1 near here].

Table 1. *Demographic characteristics, upper extremity function, and core muscle endurance in PwMS and HC*

	PwMS (n:22)	HC (n:20)	p
Demographic characteristics			
Age (years)	34 (27.50-46.00)	32.50 (23.25-43.75)	0.553
BMI (kg/m2)	23.64 (20.86-25.98)	22.68 (19.84-26.21)	0.580
Gender (f/m) (%)	16/6 (72.70/27.30)	15/5 (75/25)	0.867
Disease duration (years)	6 (3.00-11.25)		
Number of relapses	2.50 (1.75-3.25)		
EDSS (score)	1.75 (1.00-3.63)		
Upper extremity function			
NHPT-right (seconds)	18.01 (15.99-21.34)	15.83 (15.04-16.77)	**0.004***
NHPT-left (seconds)	21.39 (17.73-27.76)	17.42 (15.09-18.06)	**<0.001***
Core muscle endurance			
Side bridge-Right	21.11 (10.66-37.06)	38.95 (30.66-55.73)	**0.001***
Side bridge – Left	14.54 (7.71-26.18)	33.75 (22.73-58.88)	**0.001***
Extansion endurance	61.75 (36.43-76.97)	60.59 (49.63-87.51)	0.650
Flexion endurance	30 (19.93-52.62175)	48.26 (32.40-79.59)	**0.009***
Prone bridge	27.68 (20.55-46.64)	49.15 (39.21-68.44)	**0.001***

*Data are presented as number (%) of patients or median (IQR). *p<0.05. chi-square test or Mann-Whitney U test (between two groups). BMI: Body mass index, EDSS: Expanded Disability Status Scale, NHPT: Nine-Hole Peg Test.*

Table 2 shows the relationship of upper extremity function with demographic characteristics and core muscle endurance in PwMS. The correlation analysis in PwMS revealed that both sides' upper extremity functions were strongly and negatively related to EDSS, and these were moderately and positively related to trunk flexor endurance (p<0.05). Moreover, the right-upper extremity function was moderately and positively related to the right side bridge endurance test performance while the left-upper extremity function was moderately and positively related to left side bridge endurance test performance (p<0.05). Also, the left-upper extremity function was moderately and negatively related to disease duration (p<0.05).

[Table 2 near here].

Table 2. *Relationship of upper extremity function with demographic characteristics and core muscle endurance in PwMS*

	NHPT-Right		NHPT-Left	
	r	p	r	p
Demographic characteristics				
Age	0.111	0.623	0.228	0.309
BMI	-0.048	0.832	-0.084	0.710
Disease duration	0.387	0.075	**0.503**	**0.017***
Number of relapses	0.261	0.240	0.372	0.089
EDSS	**0.714**	**<0.001***	**0.765**	**<0.001***
Core muscle endurance				
Side bridge-Right	**-0.446**	**0.038***	-0.321	0.145
Side bridge – Left	-0.401	0.064	**-0.434**	**0.043***
Extansion endurance	-0.391	0.072	-0.348	0.112
Flexion endurance	**-0.504**	**0.017***	**-0.499**	**0.018***
Prone bridge	-0.135	0.549	-0.047	0.836

**p<0.05, Spearman correlation test. BMI: Body mass index. EDSS: Expanded Disability Status Scale, NHPT: Nine-Hole Peg Test.*

4. DISCUSSION

This study investigated the relationship between upper extremity function and core muscle endurance in PwMS. Even though the PwMS in this study had mild disabilities, upper extremity function and core muscle endurance, excluding extensor endurance, were lower than those of HC. In addition, both sides' upper extremity functions of PwMS were related to EDSS. Similarly, Yozbatiran et al. found a relationship between upper extremity functions and EDSS (2). EDSS is the most widely used scale in clinical practice to assess the severity and progression of MS disease (15). However, EDSS focuses much on walking ability rather than upper extremity functions. Therefore, the National Multiple Sclerosis Society's Advisory Committee on Clinical Trials of New Agents in Multiple Sclerosis developed Multiple Sclerosis Functional Composite (MSFC) to overcome the shortcomings of EDSS (22). In this composite, upper extremity functions are also assessed by NHPT as well as the walking ability and cognitive functions. It was reported in a review on upper extremity measures applied in PwMS that the NHPT was the most frequent measure, utilized in 63% of published studies (23). Also, inter-rater and test-retest reliability of the NHPT are consistently high (range, r = 0.86–0.98) (16).

Bertoni et al. reported that 75% of PwMS had bilaterally dysfunctions in upper extremities evaluated by NHPT, and participation level reduced 35% during home activities even in PwMS with mild disability (24). Similarly, in this study, although the PwMS had mild disabilities (median EDSS:1.75), they finished the NHPT in a longer time than HC. Therefore, the upper extremity functions should be evaluated and improved even in PwMS with mild disabilities.

Moreover, it is known that core muscle endurance is important for balance, functional mobility, coordinated movement, and postural control (25). This study showed that PwMS had lower core muscle endurance, excluding extensor endurance, compared to HC. Similarly, Freund et al. demonstrated that PwMS had lower trunk flexor endurance compared to HC and the lower trunk flexor endurance in PwMS was also associated with lower postural control (26). This relationship is expected since the postural control is provided by core stability, in which the activation of core muscles plays an important role (4). Concerning postural control, Cetisli et al. revealed that lower trunk control assessed by the Trunk Control Test and Trunk Impairment Scale was related to impaired upper extremity function assessed by NHPT in PwMS (13). In line with the result of the study by Cetisli et al., this study presented that both sides' upper extremity functions were positively related to trunk flexor endurance, and also, each upper extremity was positively related to bilateral core muscle endurance in PwMS.

To our knowledge, this study is the first to investigate the relationship between upper extremity function and core muscle endurance in PwMS. The results of this study showed that there is a mutual relationship between upper extremity function and core muscle endurance in PwMS. In elite athletes, a relationship was found between the shoulder internal rotation and external rotation peak torque/body weight and all core endurance tests except extension endurance tests (27). In addition, Lehman et al. showed that the transversus abdominus muscle and multifidus contracted 50 milliseconds before shoulder movements to provide trunk stabilization in healthy people (28). This evidence suggests that core-based exercises may be effective in increasing upper extremity functions.

Saeterbakken et al. demonstrated that a 6-week core stabilization training increased the maximal ball throwing speed in female handball players (29) while Misirlioglu et al. demonstrated that both sides' shoulder strength increased significantly after a 6-week core stabilization home-based exercise program (30). Moreover, Miyake et al. investigated the effect of one session of core stabilization training on upper extremity function in healthy people (31). At the end of only one session of core stabilization training including three core exercises (curl-up, side bridge, bird dog) for about 20 min, the upper extremity function was higher in the core exercise group than the control group.

Although the effects of core stabilization on upper extremity function have not been investigated yet in PwMS, Bulguroglu et al. showed that an 8-week core stabilization based Pilates training increased core endurance in PwMS (32). Furthermore, in the study by Freeman et al., 5 of 8 PwMS reported an improvement in upper extremity function (rating of carrying a drink while walking by visual analog scale) after an 8-week core stabilization training (33). These results promise that a core stabilization training program may improve both the core muscle endurance and upper extremity function. Future studies are needed to arrive at conclusive evidence on this issue.

This study has several limitations. First, all of the PwMS in this study had a mild disability and relapsing-remitting clinical course of MS. Therefore, our results may not reflect the performance of patients who have a severe disability and other types of MS. Second, the sample size was small, so the power of this study is low. Third, the upper extremity function was evaluated with NHPT, which reflects more manual dexterity rather than proximal upper extremity function. Fourth, the cognitive status of the participants was not taken into account in the inclusion criteria of the study. Lastly, although the endurance of the core muscles was evaluated, the results could not be supported by the evidence on muscle thickness and muscle activation using assessment methods such as ultrasound and electromyography.

5. CONCLUSION

This study demonstrated that upper extremity function and core muscle endurance were lower even in PwMS with mild disabilities compared to HC, and mutual relationship between upper extremity function and core muscle endurance in PwMS. Therefore, the rehabilitation program including core stabilization exercises may be beneficial to improve both core muscle endurance and upper extremity function in PwMS. In addition, in future studies, investigating the kinematic relationship between the upper extremity and core muscles may provide a better understanding of motor control in PwMS.

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Clinical and Experimental Health Sciences

Evaluation of the Effectiveness of Erbium Lasers on Removing Calcium Hydroxide

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ABSTRACT

Objective: The purpose of this study was to compare the efficacy of Erbium, chromium-doped yttrium, scandium, gallium and garnet (Er,Cr:YSGG), and Erbium-doped yttrium aluminum garnet laser (Er:YAG) lasers with passive ultrasonic irrigation (PUI) and conventional syringe irrigation (CSI) for the removal of calcium hydroxide [$Ca(OH)_2$] paste from standardized canal irregularities.

Methods: Forty single-rooted human teeth were split longitudinally, forming halves, and cut a standardized groove in canal walls. After filling each groove with $Ca(OH)_2$ paste, the teeth were reassembled and stored for 1 week. $Ca(OH)_2$ was removed with CSI by using 10 milliliters (mL) of 5% sodium hypochlorite (NaClO) and 10 mL of 17% ethylenediaminetetraacetic acid (EDTA), respectively. The teeth were randomly divided into four groups (n=10). CSI group was irrigated with10 mL of 5% NaClO, 10 mL of 17% EDTA, and 10 mL of distilled water with CSI (control group), respectively. In Er:YAG group, 5% NaClO was activated with Er:YAG laser, and in Er,Cr:YSGG group, Er,Cr:YSGG laser equipped with Radial Firing Tip 2 (RFT 2) was used. In the PUI group, PUI was used with 2.5% NaClO. All groups received irrigation with 10 mL of distilled water as final irrigation. The root halves were examined with a stereomicroscope using a 4-grade scoring system.

Results: Residues of $Ca(OH)_2$ in artificial grooves were observed in all groups. In Er,Cr:YSGG group, only three specimens got a score of 1 (less than half of the groove is filled). Er,Cr:YSGG group was found slightly higher yet the difference was found to be not significant with other groups.

Conclusions: Although Er,Cr:YSGG laser equipped with RFT 2 tip seems to have a superior effect on removing $Ca(OH)_2$ from the standardized canal irregularities, there was no statistically significant difference between the applications.

Keywords: Er:YAG laser, Er Cr:YSGG laser, passive ultrasonic irrigation, artificial groove, calcium hydroxide

1. INTRODUCTION

Bacteria and their by-products contribute to pulpal and periapical pathologies (1). The elimination of microbiota at the root canal system can be achieved with the intervention of mechanical instrumentation, copious irrigation, and antibacterial medication. However, complex anatomy makes the entire disinfection of the root canal system impossible (1,2).

Standard practice prefers calcium hydroxide [$Ca(OH)_2$] because of its antimicrobial properties against the majority of pathogens associated with endodontic lesions (3). Using $Ca(OH)_2$ is an effective supplement to mechanical instrumentation. Also, because the tissue-dissolving ability of $Ca(OH)_2$ allows remaining pulp tissue to be flushed away after the usage of $Ca(OH)_2$ paste (4). Nevertheless, many studies suggest $Ca(OH)_2$ is ineffective at eliminating pathogens in canal irregularities because of its low solubility and vulnerability to the neutralization of dental tissues.

Cleaning $Ca(OH)_2$ paste before root canal filling is vital not only to prevent any undesired interactions between dressing and root canal sealer but also to ensure superior adhesion of the filling material to the root dentin. However, complete removal of $Ca(OH)_2$ paste by using files and irrigating solutions exclusively is unfeasible (5). Research and practice have established various techniques in the field of $Ca(OH)_2$ paste removal from the root canal system (6). The most widely used method is the utilization of the master apical file combined with conventional syringe irrigation (CSI) using NaClO and EDTA (7). Unfortunately, this technique may result in $Ca(OH)_2$ residuals remaining particularly in irregularities of the apical region of the root canal due to the insufficient nature of CSI (8).

Passive ultrasonic irrigation (PUI) has been known to enhance the efficacy of irrigant delivery and augment root canal cleaning (9). Energy expansion resulting from an ultrasonically oscillating tip applied to the irrigant causes

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acoustical streaming and cavitational effects in the root canal (10). PUI performed after root canal preparation and before application of the master apical file allows the instrument to oscillate without constraint. Accordingly, the cutting action of the file is minimized and acoustic streaming and cavitation are more effective (11).

Laser-activated irrigation (LAI) is a promising procedure for root canal irrigation. The cleaning effect of LAI within the root canal results from rapid fluid motion provoked by expansion and implosion of laser-induced vapor bubbles at the fiber tip (12). The Erbium-doped yttrium aluminium garnet laser (Er:YAG) laser with an endodontic fiber tip effectively removes used $Ca(OH)_2$ paste from the root canal without causing structural harm or anatomic changes inside the root canal or to periapical tissues (13). Studies have confirmed that using a plain fiber tip can impel an irrigation solution to the apical foramen without injurious effect on periapical tissues (14). This study aims to evaluate the efficacy of Erbium, chromium-doped yttrium, scandium, gallium and garnet (Er,Cr:YSGG), and Er:YAG lasers with passive ultrasonic irrigation and conventional syringe irrigation for the removal of $Ca(OH)_2$ paste from standardized canal irregularities.

2. METHODS

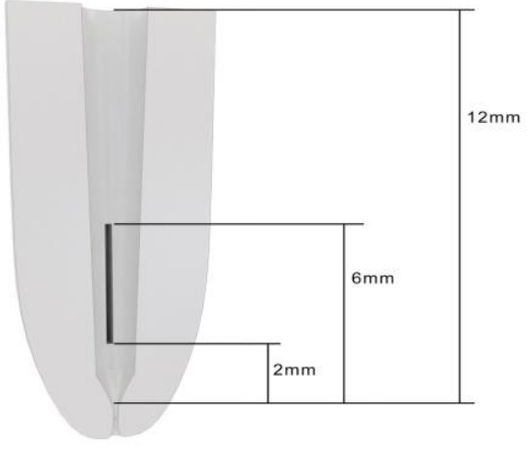
This project was approved by the Ethics Committee of Gaziantep University in Gaziantep, Turkey (Application No: 2014-433). Forty single-rooted, noncarious maxillary human anterior teeth extracted for various reasons were selected. Only Vertucci type I teeth with a single canal were included and teeth with caries, resorptions, cracks, and immature apices were excluded. Buccolingual and mesiodistal radiographs were taken to confirm the existence of single canals without previous root canal treatment, variations in root canal configurations, resorption, or calcifications. Soft tissues and calculus were mechanically removed from root surfaces with the help of a periodontal scaler. The teeth were stored in distilled water at room temperature at the time of experimental procedures.

The crowns of the teeth were removed with a diamond disc (Horico, Berlin, Germany) underwater cooling to attain 13 mm a standard length and to eliminate variations in dimensions of the pulp chamber. The working length was adjusted by subtracting 1 mm from the length at which the tip of a size 10 K-file (Dentsply Maillefer, Ballaigues, Switzerland) was just visible. Revo-S rotary instruments were used (Micro-Mega, Besancon, France) for the root canal shaping process using the following sequence: SC1, SC2, and SU (size 25, 0.06 taper). A brushing motion with the SC1 file and a non-brushing action with the SC2 and SU were used until reaching the desired working length. The root canals were flushed with 1 mL of 5% NaClO solution (Wizard; Rehber Kimya, Istanbul, Turkey) between each file change and applied a final flush using 2 mL of 17 % EDTA and 2 mL of 5 % NaClO, each for 1 minute (min).

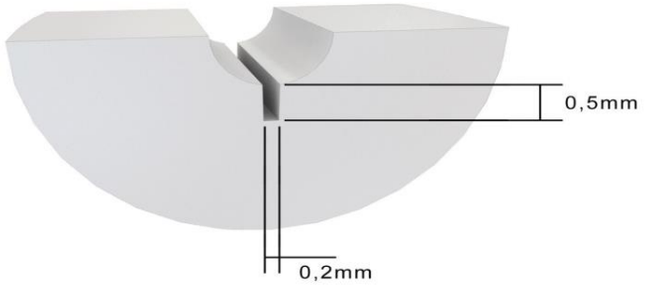
2.1. Preparation of Specimens

The experimental root canal model is based upon the norm designed by Lee et al. and has been implemented in many studies analyzing the $Ca(OH)_2$ paste removal (6,9). All the roots in the study were grooved with a diamond disk longitudinally on the buccal and lingual surfaces along their long axis under water cooling and abstaining any penetration. Then the roots were split into two halves in a buccolingual direction with a hammer and chisel. The roots that could not be reassembled were rejected, retaining a total of forty specimens. To replicate an uninstrumented apical region, a longitudinal groove with nearly 4 mm length, 0.5 mm width, and 0.2 mm depth was cut into the root canal dentin of one half of each tooth at a distance of 2 mm to 6 mm from the apex (Fig 1). A customized ultrasonic tip was used (Dentsply Maillefer, Finland) with an ultrasonic device (EMS miniMaster Piezon; Nyon, Switzerland). After drying the halves with air and the grooves were filled with $Ca(OH)_2$ paste (Best; Bem Dental Kimya İlaç San, İzmir, Turkey) with the help of paper points #30-40 to replicate the occurrence of the retention of $Ca(OH)_2$ paste to canal irregularities. Then root halves were reassembled with orthodontic latex elastics and all the gaps and the apical foramina were sealed with sticky wax to preclude the overflow of the irrigation solution and to achieve a vapor lock effect. After fixing the specimens in the Eppendorf vials (Fırat Plastik Kauçuk San, İstanbul, Turkey) with a silicone material (Optosil; Heraeus Kulzer, Hanau, Germany), the canals were temporarily sealed with cotton and temporary filling material (Cavit; Espe, Seefeld, Germany). The specimens were kept at 37°C with 100% humidity for a week to replicate an interappointment root filling. The specimens were divided into four groups (n=10) at random, each defined by the respective irrigation techniques with which they would be treated (CSI, PUI, Er:YAG and Er,Cr: YSGG).

***Figure 1**. Schematic representations of the specimen preparation*



***a.** On one half of the instrumented root canal, a groove was cut 2 mm to 6 mm from the apex.*



b. *The groove was 0.5 mm depth and 0.2 mm wide.*

For CSI group (n=10), hand irrigation with 10 mL of 5% NaClO, 10 mL of 17% EDTA, and 10 mL of distilled water, alternately, using a 10 mL syringe (Set Medikal, İstanbul, Turkey) with a 27-gauge needle was applied. The needle was inserted 1 mm above the working length and moved back and forth at the apical region for 20 s.

In Er:YAG group (n=10): Er:YAG laser system (Fidelis AT; Fotona, Ljubljana, Slovenia) was used with an R14 handpiece attached with a 300 µm diameter and 14 mm flat fiber tip, (Preciso 300/14; Fotona, Ljubljana, Slovenia). The tip was inserted 5 mm above the working length and immobilized for 5 s at laser activation, repeated 4 times with 5 s intervals. When the irrigating solution in the coronal reservoir ran low, it was refreshed with 5% NaClO. The pulse energy measured at 50 mJ at 20 Hz and 50 µs super short pulse. The water and the air systems were turned off.

In Er,Cr:YSGG group (n=10): Er,Cr:YSGG laser system (Iplus; Biolase, San Clemente, CA, USA) was equipped with a gold handpiece attached a 275 mm diameter and 21 mm length endodontic fiber tip (Radial Firing Tip 2; Biolase, San Clemente, CA, USA) were used at 1.25W at 50 Hz in H mode. The settings were 34 for water and 24 for air. The fiber was held at 1 mm short of working length. This procedure was repeated 3 times by removing the tip in a continuous spiral motion at a rate of 1 s per mm. Researchers paused for 15 s between each exposure.

The specimens in the PUI group (n=10) were activated by placing 0.5 mL of 5% NaClO into the canal and performing passive agitation using an ultrasonic handpiece (Piezon miniMaster; EMS, Nyon, Switzerland). A flat ultrasonic file was placed (size 15 with 0.02 taper) (ESI instrument, EMS) into the canal 1 mm above the working length by avoiding to the root canal walls and allowing it to pulsate. After switching on the ultrasonic device, the intensity was set to the “Endo” mode. The file was activated for 20 s and repeated this procedure 3 times with 5 s intervals.

Following each irrigation procedure, the final irrigation of the root canals with 10 mL of distilled water was performed to avoid undesirable irrigant action.

2.2. Digital Imaging and Scoring

The specimens were dried with paper points. The roots were removed from the tubes to separate the halves. Stereomicroscope (Leica M165C; Leica Mycrosystem Ltd, Heerbrug, Germany) attached to a digital camera was used to obtain the images of the half of each canal with a groove at 20× magnification and transferred the images to a computer.

The amount of $Ca(OH)_2$ paste in the grooves was evaluated according to the classification described by Van der Sluis et al (6). The specimens were scored as follows:

- Score 0: The groove is empty (Fig. 2)
- Score 1: Less than half of the groove is filled with $Ca(OH)_2$ (Fig. 3)
- Score 2: More than half of the groove is filled with $Ca(OH)_2$ (Fig. 4)
- Score 3: The groove is filled with $Ca(OH)_2$ (Fig. 5)

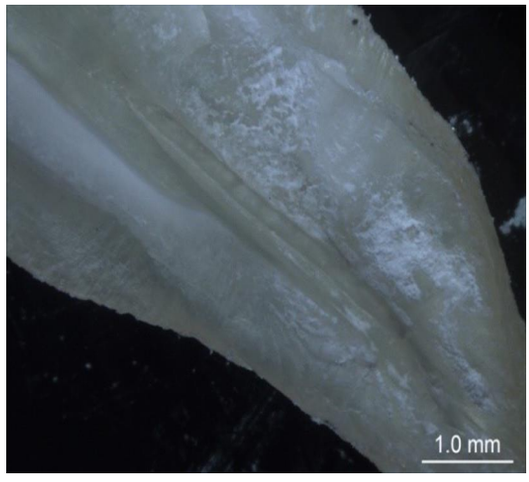


Figure 2. *Score 0: The groove is empty.*

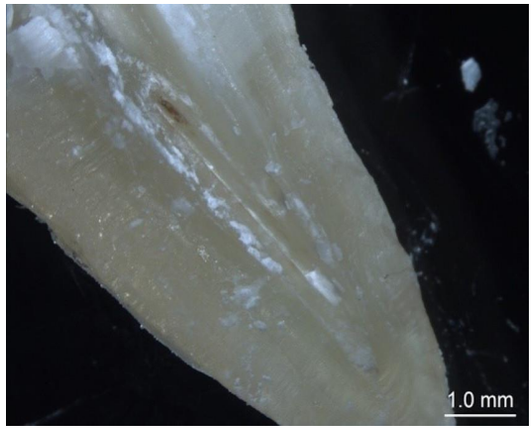


Figure 3. *Score 1: Less than half of the groove is filled with* $Ca(OH)_2$.

Figure 4. *Score 2: More than half of the groove is filled with $Ca(OH)_2$.*

Figure 5. *Score 3: The groove is filled with $Ca(OH)_2$.*

2.3. Statistical Analysis

The data was statistically analyzed by using nonparametric Kruskal Wallis test. The statistical analyses were performed by using software (IBM® SPSS® Statistics 20; IBM SPSS Inc, Chicago, IL, USA).

3. RESULTS

Table 1 shows the numbers of scores after irrigation for each of the four different techniques. An overall significance was not detected among four groups with Kruskal Wallis test (Table 2). The score 0 ratio was the lowest in the CSI group (0%). The highest score 0 ratio was seen in Er, Cr: YSGG (70%), then Er: YAG (60%), lastly in PUI group (40%). The highest Score 3 rate was in the CSI group (30%) . However, the analysis revealed no significant difference between PUI and the laser groups. None of the groups reported complete removal of $Ca(OH)_2$ paste from the grooves. However, In Er, Cr:YSGG group, there were slight $Ca(OH)_2$ remnants as described in Score 1 only in 3 artificial grooves. Thus, the CSI group had the most remaining $Ca(OH)_2$ paste.

Table 1. *The scores of $Ca(OH)_2$ removal*

Groups	Score 0	Score 1	Score 2	Score 3
CSI	0	3	4	3
PUI	4	4	1	1
Er:YAG	6	2	1	1
Er,Cr:YSGG	7	3	0	0

CSI: Conventional syringe irrigation; PUI: Passive ultrasonic irrigation; Er:YAG: Erbium-doped yttrium aluminum garnet laser; Er,Cr:YSGG: Erbium, chromium-doped yttrium, scandium, gallium and garnet

Table 2. *Kruskal Wallis analysis of scores after $Ca(OH)_2$ removal*

	CSI	PUI	Er:YAG	Er,Cr:YSGG
25% Percentile	0.75	1	1	1.25
Median	3	2.5	1.5	2.5
75% Percentile	3.75	4	5	3.75
Mean	2.5	2.5	2.5	2.5
Standard Deviation	1.732	1.732	2.38	1.291

CSI: Conventional syringe irrigation; PUI: Passive ultrasonic irrigation; Er:YAG: Erbium-doped yttrium aluminum garnet laser; Er,Cr:YSGG: Erbium, chromium-doped yttrium, scandium, gallium and garnet

4. DISCUSSION

The Er:YAG laser-activated irrigation uses not only photoacoustic but also photomechanical properties, differentiating it from other activation methods. This technique uses sublative energy levels and short microsecond (µs) pulse rates (50 µs), thus forming impulses that generate expansion and sequential shock waves inducing powerful streaming fluid and facilitating the 3-dimensional movement of irrigation solutions (15). Although the erbium lasers have the potential of thermal side effects such as carbonization and cracks in the root canal walls, using sublative parameters prevents these situations (16).

When water absorbs laser irradiation, the vapor bubble occurs and starts to expand (16). Matsumoto et al. presented evidence that this vapor bubble could increase in size to 1800 µm in 220 µs when a 300 µm laser tip was used, similar to the present study (12). Accordingly, they suggest that it is not essential to place the tip at the end of the canal because these bubbles are effective enough to clean entire of the root canal system, including the apical region. In the present study, this data was verified. By contrast with previous laser applications, flat Er:YAG laser tip inserted 5 mm short of the working length effectively removed $Ca(OH)_2$ that was apically placed. This result can be clarified by the increase in fluid reaction kinetics resulting from laser activation. Therefore, new studies must be designed on the cleanness of the complete root canal system, including anastomoses and isthmuses (17).

According to the results, the Er:Cr:YSGG showed considerable effect for $Ca(OH)_2$ removal from a standard groove at the apical region. There were slight $Ca(OH)_2$ remnants as described in Score 1 only in 3 artificial grooves. The impulsive disposition of laser-produced bubble formations may be the reason for this finding. Bubbles constitute the basis of cavitation. With every pulse, fluid is accelerated, and the acceleration causes inertial forces. By contrast, stabilized flow at CSI or PUI uses viscous stress (18). Besides, the direction of the laser light is toward the root canal walls for RFT in Er,Cr:YSGG, not to the apex of the root canal. As a result, the direct effect of the laser light also may have contributed to the cleanness of the artificial groove.

During the study, a spiral motion wasn't made in the irrigant in Er:YAG group, unlike previous methods that require exposure of the entire root canal wall straight to the laser light. Thus, the possibility of ledge formation appears to be much more with Er:YAG than with Er,Cr:YSGG (19). (16). Also, a distance of 5 mm above the working length was chosen for the place of the laser fiber tip in this study. Because it was demonstrated that there were two times as much dye penetration from the apical foramen by using fiber tip set at 4 mm than at 5 mm beyond the apical foramen.

Deleu et al. documented greater efficacy with the flat Er:YAG laser tip than with photon-initiated photoacoustic streaming concerning the removal of the smear layer (20). Studies have also demonstrated that lesser fiber diameters and higher pulse energies generate greater fluid flow and may increase the occurrence of vapor or a cavity containing bubbles at the irrigant (16). Additionally, bubbles generated in saltwater such as NaClO are prone to be more copious than in fresh water (21). This data may explain the efficacy of water and NaClO in PUI and LAI as irrigants.

All groups in the study produced cleaner artificial grooves compared to CSI. This finding confirms other studies focused on the efficacy of manual dynamic irrigation, PUI, and LAI with erbium lasers (21). Additionally, this study has documented that more artificially placed $Ca(OH)_2$ is removed with PUI than with CSI. Data analysis indicated these results may result from the enhanced velocity and volume of solution flow during PUI.

It was shown that 4×5 s repetitions of LAI with Er:YAG ended up with significantly fewer remnants in the artificial grooves than using PUI for 20 s (21). Contrary to this finding, there were no significant differences among the groups of the present study. This result can be explained with bigger pulse energy and lesser fiber diameter that produce stronger stream and cavitation. To evaluate erbium lasers on the removal of $Ca(OH)_2$ paste from the root canals, further studies must be completed with recently designed tips, different application times, and types of $Ca(OH)_2$ pastes, including anastomoses and isthmuses.

5. CONCLUSION

In this in vitro model, Er,Cr:YSGG maintained the entire cleaning of $Ca(OH)_2$ paste from artificial grooves in most of the straight form root canals. Although there were slight $Ca(OH)_2$ remnants only in 3 roots as described in Score 1, there was no difference between the applications. Besides, using erbium lasers for 20 s is as powerful as PUI for 3×20 s.

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Clinical and Experimental Health Sciences

Men's Choice of Contraception Method

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ABSTRACT

Objective: The aim of this study is to examine the contraception choices of men, aged between 18-60 and living in the center of a province in the Gümüşhane.

Methods: The study is of a descriptive type. As a data collection form, an Individual Information Form consisting of a total of 32 questions was used. This form questions the demographic-physical-biological-social-health histories of the participants and their knowledge, attitudes, behaviors about contraception methods. Averages were given together with standard deviations.

Results: 20.8 % of the participants are aged between 26-33. It was seen that 25.3% of the participants had knowledge about contraception methods and 64.7% did not use any methods. When the variables of the age and the marital status are approached in terms of having sufficient knowledge about contraception and still using contraception methods, there is a statistical significance between the variable of 'is the use of contraception methods harmful to health?' with the status of still using contraception methods. A statistical significance is also found between the variable of being sexually active with the status of having sufficient knowledge about contraception and still using contraception methods ($p<0.05$).

Conclusion: In this study, It has been determined that men who had sufficient information about contraceptions had higher age and higher education levels, were sexually active and married. It has been seen that the age, the marital status, the perception of contraception as harmful to health, the status of being sexually active did not affect receiving any training about contraception.

Keywords: Men, contraception method, choice

1. INTRODUCTION

The aim of family planning services is to prevent unwanted pregnancies and consequent maternal-infant deaths, to present assistance and counseling services for individuals want to have in any number of children at any time. A good family planning service also includes assistance for couples (infertile) that cannot have children. Another aim of family planning services is to increase the health of maternal-infant. The family planning services also develop the skills of family members in deciding and gives the family the freedom to decide freely about having children. Family planning services are an important part of the "Basic Health Services" that should be offered to the community (1).

Increasing the use of modern family planning methods is one of the primary interventions aimed at improving sexual and reproductive health in developing countries. Reducing unwanted pregnancies is important because of the prevention of sexually transmitted infections such as HIV, the reducing of the maternal-neonatal morbidity and mortality. The use of family planning methods is one of the critical developmental aims for maternal-infant survival in low – and middle-income countries (2).

Although the number of women seeking to control fertility in underdeveloped countries has increased significantly, there is no proportional increase in modern contraceptive use (3). It has been stated that the reason is the resistance of men to using family planning methods, the problems in reaching the method and in ensuring the continuity. Except that individual decision making, it has been stated that reproductive behaviors are being influenced by social-institutional norms and policies. For this reason, it is insufficient to focus on only women individualistically (2).

Having knowledge about family planning methods is very important both for the decision whether to use a contraceptive method, to decide on which method to use. Staying on top of fertility control is also a rather important step in reaching these methods and using an appropriate contraceptive method (4).

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In many studies, it has been stated that the use of FP methods is influenced by many factors such as education level of the woman and her spouse, number of children, ideal family size, spouses' decisions, ideal birth interval, men's perspective to FP. It also has been expressed that the participation of men to this process should be ensured in order to achieve the desired success in FP services (5, 6). The number of studies with men related to the family planning process is few. This study was planned to determine the family planning methods used by men, the reasons for using these methods, the factors affecting men.

2. METHODS

2.1. Type of research

This study is of a descriptive type.

2.2. Population of the research and selection of sample

The population of the study has been consisting of males, aged between 18-60, living in the center of Gümüşhane province. According to the 2017 year address-based population registration system, the number of males in this age range is 18303. After the calculations, minimum 377 people were planned to be included in the sample at a 95% confidence interval. However, it was aimed to reach 400 people (approximately 10% of the sample calculated against the probability of possible loss has been reached) in the study considering the possible losses. The sample group was reached according to the improbable sampling method. Participants were divided into 8-year age groups. Thus, 5 groups were formed. Men with cognitive or mental disability and illiterate were excluded from the study. Also, verbal consent was obtained from the participants. Data were collected by face-to-face interviews with participants who voluntarily agreed to participate in the study. Data of the study were collected during a 3-month period.

2.3. Data collection

In the study, an Individual Information Form consisting of a total of 32 questions and developed in accordance with literature by researchers was used. This form, which used as a data collection tool, questions the demographic-physical-biological-social-health histories of the participants and their knowledge, attitudes, behaviors about contraception methods.

The independent variables of the study are the demographic-physical-biological-social data of the participants. The dependent variables are health histories of the participants and their knowledge, attitudes, behaviors about the contraception methods.

2.4. Ethical considerations

The required permission to conduct the study was obtained from the administration unit of the Health Directorate and from the Scientific Research and Publication Board of Gümüşhane University (Number= 95674917-604.01.02-E.831, Approval Date= 08.01.2018).

2.5. Statistical analysis

The statistical package program was used for analysis. Error checks and tables were made through this program. Number and percentage distributions were made in the meta-data; 'chi-suare' and 'fisher's exact test-2-sided' tests were used for data analysis. Averages were given together with standard deviations. $p< 0.05$ was considered significant.

3. RESULTS

20.8% of the participants are aged between 26-33. 53.7% have an education above the high school level. 59.3% are married. 35% do not work. The rate of the participants with a high income is 16%. The average number of children (min: 1; max: 6) is 2.68 (Table 1). The sources of information about sexuality were asked to the participants. 24% of the participants stated that obtained information from friends, 37.5% from the internet, 5.8% from books, 4.8% from newspapers, 2% from magazines, 4.8% from their family, the rest from several sources.

Table 1. *Socio-demographic characteristics of participants (N=400)*

Socio-Demographic Characteristics	n	%
Age		
Between 18-25	80	20.0
Between 26-33	83	20.8
Between 34-41	79	19.8
Between 42-50	78	19.4
Between 50-60	80	20.0
Education Status		
Primary school	34	8.5
Secondary school	49	12.3
High school	102	25.5
Associate degree	66	16.5
College/faculty	149	37.2
Marital Status		
Married	237	59.2
Single	163	40.8
Occupation status		
Educator	31	7.8
Security staff	23	5.8
Medical staff	36	9.0
Craft	76	19.0
Worker	83	20.8
Official	11	2.6
Jobless	140	35.0
Income Status		
Income is less than expense	132	33.0
Income is equal to expense	204	51.0
Income is more than expense	64	16.0
Smoking Status		
Yes	214	53.5
No	186	46.5
The status of being sexually active		
Yes	263	65.8
No	137	34.2
The status of getting a sexually transmitted disease Yes		
No	20	5.0
	380	95.0

The awareness and practices of the men participating in the study related to contraception methods are approached in Table 2.

Table 2. *The awareness and practices of the participants related to contraception methods (N=400)*

Features	n	%
The status of receiving any training about contraception		
Yes	101	25.2
No	299	74.7
The status of having sufficient information about		
contraception	220	55.0
Yes	180	45.0
No		
The heard contraception method		
Condom (Cover, preservative)	217	54.3
Contraceptive pills	40	10.0
Intrauterine device (spiral)	20	5.0
Injection method	7	1.8
Post-tube legation	10	2.5
Vasectomy	7	1.8
Subcutaneous implants	3	0.8
Coitus interruptus	53	13.3
Rhythm method	12	3.0
Spermicidal	1	0.3
Emergency contraceptive methods	6	1.5
Other	17	4.3
All	7	1.8
The status of using any contraception method		
Yes	141	35.3
No	259	64.7
The used contraception method		
Condom (Cover, preservative)	92	64.3
Contraceptive pills	13	9.1
Intrauterine device (spiral)	9	6.3
Post-tube legation/Vasectomy	1	0.7
Subcutaneous implants	1	0.7
Coitus interruptus	17	11.9
Rhythm method	1	0.7
Other	7	6.3
Total	141	100.0
The status of using and quitting any contraception		
method	11	2.8
Yes	389	97.2
No		
Reason for choosing the current method (n=140)		
For finding it safe	74	52.9
Because it is convenient	39	27.9
I only know this method	10	7.1
Easy to use	9	6.4
Other	8	5.7
Reason for not using any contraception method (n=264)		
No need	144	54.5
Lack of regular and frequent sexual intercourse	33	12.5
Don't think your partner will get pregnant	9	3.4
Disbelief contraceptive	12	4.5
Finding contrary to my beliefs	20	7.6
Not having enough information about family planning	31	11.8
Other	15	5.7
The status of experiencing an unwanted pregnancy		
Yes	42	10.5
No	358	89.5
The status of affecting sexuality of family planning		
methods	260	65.0
Yes	140	35.0
No		
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Condom (Cover, preservative)	119	45.2
Contraceptive pills	41	15.6
Intrauterine device (spiral)	30	11.4
Injection method	8	3.0
Post-tube legation	6	2.3
Vasectomy	11	4.2
Subcutaneous implants	5	1.9
Coitus interruptus	33	12.5
Rhythm method	4	1.5
Spermicidal	3	1.1
Emergency contraceptive methods	3	1.1
Total	263	100.0

In the study, it has been seen that 25.3% of the participants had knowledge about contraception methods and 55% of the participants thought that had enough information about contraception methods (Table 2).

In the study, participants were asked what understood from contraception. 20% of the participants stated that contraception was to have a limited number of children, 12% to have children when spouses want, 12.3% to limit the number of member in the family, 51% to have enough number of children to be supported and raised, 1.8% to educate the individuals and families about reproductive health, 0.8% to prevent maternal deaths and save their healths, 0.3% to provide medical help to individuals who want to have children, 1.3% to prevent high-risk and unwanted pregnancies, 0.8% to neuter the individuals.

In this study, 54.3% of the participants said condom/bonnet/sheath/preservative in response to the question of which contraception method heard by. Participants were asked who should be given the family planning method. 89% of participants said that the family planning method should be given to both sexes, 7.5% to just women, 3.5% to just men.

In the answer to the question of where contraception methods can be obtained, pharmacies (38%), community health centers (18%), hospitals (16.5%), centers of maternal and child health (12.3%), private health institutions (8.8%), other places (6.5%) took place respectively. As seen in Table 2, 64.7% of the participants do not use any contraception methods (Table 2).

32.9% of the participants prefer the traditional methods and the rest (67.1%) prefer the modern methods. At the same time, 2.8% of the participants aren't using any method now, although had used any contraception method before.

Table 3. *According to the socio-demographic characteristics of the participants; the statuses of receiving any training about any contraception methods, having sufficient knowledge, using any contraception methods, using and quitting these methods (N=400)**

Socio-Demographic Characteristics	n	The status of receiving training about contraception		The status of having sufficient information about contraception		The status of still using contraception methods		The status of using and quitting contraception method	
		Yes n (%)	No n (%)	Yes n (%)	No n (%)	Yes n (%)	No n (%)	Yes n (%)	No n (%)
Age									
Between 18-25	80	19 (23.8)	61 (76.2)	24 (30.0)	56 (70.0)	13 (16.3)	67 (83.8)	1 (1.3)	79 (98.8)
Between 26-33	83	30 (36.1)	53 (63.9)	52 (62.7)	31 (37.3)	29 (34.9)	54 (65.1)	1 (1.3)	82 (98.8)
Between 34-41	79	20 (25.3)	59 (74.7)	49 (62.0)	30 (38.0)	35 (44.3)	44 (55.7)	3 (3.8)	76 (96.2)
Between 42-50	78	18 (23.1)	60 (76.9)	45 (57.7)	33 (42.3)	39 (50.6)	38 (49.4)	2 (2.6)	76 (97.4)
Between 50-60	80	14 (17.5)	66 (82.5)	50 (62.5)	30 (37.5)	25 (31.3)	55(68.8)	4 (5.0)	76 (95.0)
	Test Value	χ^2=8.05 sd=4 p=0.09		χ^2=25.78 sd=4 **p=0.000**		χ^2=24.02 sd=4 **p=0.000**		χ^2=3.26 sd=4 p=0.515	
Education Status									
Primary school	34	2 (5.9)	32 (94.1)	12 (35.3)	22 (64.7)	10 (30.3)	23 (69.7?	0 (0.0)	34 (100.0)
Secondary school	49	7 (14.3)	42 (85.7)	26 (53.1)	23 (46.9)	15 (30.6)	34 (69.4)	2 (4.1)	47 (95.9)
High school	102	22 (21.6)	80 (78.4)	67 (65.7)	35 (34.3)	48 (47.1)	54 (52.9)	4 (3.9)	98 (96.1)
Associate degree	66	23 (34.8)	43 (65.2)	44 (66.7)	22 (33.3)	22 (33.3)	44 (66.7)	0 (0.0)	66 (100.0)
College/faculty	145	46 (31.7)	99 (68.3)	68 (46.9)	77 (53.1)	45 (31.0)	100 (69.0)	4 (2.8)	141 (97.2)
Master	4	1 (25.0)	3 (75.0)	3 (75.0)	1 (25.0)	1 (25.0)	3 (75.0)	1 (25.0)	3 (75.0)
	Test Value	χ^2=17.05 sd=5 **p=0.004**		χ^2=18.23 sd=5 **p=0.003**		χ^2=8.45 sd=5 p=0.133		χ^2=11.08 sd=5 **p=0.050**	
Marital Status									
Married	237	56 (23.6)	181 (76.4)	151 (63.7)	86 (36.3)	106 (44.9)	130 (55.1)	9 (3.8)	228 (96.2)
Single	163	45 (27.6)	118 (72.4)	69 (42.3)	94 (57.7)	35 (21.5)	128 (78.5)	2 (1.2)	161 (98.8)
	Test Value	χ^2=0.81 sd=1 p=0.368		χ^2=17.84 sd=1 **p=0.000**		χ^2=23.18 sd=1 **p=0.000**		χ^2=2.38 sd=1 p=0.122	
Income Status									
Income is less than expense	132	23 (17.4)	109 (82.6)	65 (49.2)	67 (50.8)	43 (32.8)	88 (67.2)	2 (1.5)	130 (98.5)
Income is equal to expense	204	49 (24.0)	155 (76.0)	115 (56.4)	89 (43.6)	73 (35.8)	131 (64.2)	7 (3.4)	197 (96.6)
Income is more than expense	64	29 (45.3)	35 (54.7)	40 (62.5)	24 (37.5)	25 (39.1)	39 (60.9)	2 (3.1)	62 (96.9)
	Test Value	χ^2=18.09 sd=2 **p=0.000**		χ^2=3.37 sd=2 p=0.185		χ^2=0.76 sd=2 p=0.681		χ^2=1.14 sd=2 p=0.565	
Should contraception be given as education in schools?									
Yes	222	70 (31.5)	152 (68.5)	123 (55.4)	99 (44.6)	76 (34.2)	146 (65.8)	9 (4.1)	213 (95.9)
No	98	15 (15.3)	83 (84.7)	58 (59.2)	40 (40.8)	37 (37.8)	61 (62.2)	2 (2.0)	96 (98.0)
Undecided	80	16 (20.0)	64 (80.0)	39 (48.8)	41 (51.3)	28 (35.4)	51 (64.6)	0 (0.0)	80 (100.0)
	Test Value	χ^2=10.94 sd=2 **p=0.004**		χ^2=1.97 sd=2 p=0.373		χ^2=0.36 sd=2 p=0.831		χ^2=3.35 sd=2 p=0.145	
Is the use of contraception methods harmful to health?									
Yes	79	18 (22.8)	61 (77.2)	53 (67.1)	26 (32.9)	35 (44.3)	44 (55.7)	1 (1.3)	78 (98.7)
No	321	83 (25.9)	238 (74.1)	167 (52.0)	154 (48.0)	106 (33.1)	214 (66.9)	10 (3.1)	311 (96.9)
	Test Value	χ^2=0.31 sd=1 p=0.573		χ^2=5.81 sd=1 **p=0.016**		χ^2=3.46 sd=1 p=0.063		χ^2=0.81 sd=1 p=0.368	
The status of being sexually active									
Yes	263	72 (27.4)	191 (72.6)	158 (60.1)	105 (39.9)	115 (43.9)	147 (56.1)	7 (2.7)	256 (97.3)
No	137	29 (21.2)	108(78.8)	62 (45.3)	75 (54.7)	26 (19.0)	111 (81.0)	256 (97.3)	133 (97.1)
	Test Value	χ^2=1.84 sd=1 p=0.175		χ^2=7.99 sd=1 **p=0.005**		χ^2=24.43 sd=1 **p=0.000**		χ^2=0.02 sd=1 p=0.881	
The status of experiencing an unwanted pregnancy									
Yes	42	8 (19.0)	34 (81.0)	27 (64.3)	15 (35.7)	18 (42.9)	24 (57.1)	3 (7.1)	39 (92.9)
No	358	93 (26.0)	265 (74.0)	193 (53.9)	165 (46.1)	123 (34.5)	234 (65.5)	8 (2.2)	350 (97.8)
	Test Value	χ^2=0.95 sd=1 p=0.328		χ^2=1.63 sd=1 p=0.201		χ^2=1.16 sd=1 p=0.281		χ^2=3.38 sd=1 p=0.066	
Is abortion a contraception method?									
Yes	65	16 (24.6)	49 (75.4)	40 (61.5)	25 (38.5)	25 (38.5)	40 (61.5)	0 (0.0)	65 (100.0)
No	335	85 (25.4)	250 (74.6)	180 (53.7)	155 (46.3)	116 (34.7)	218 (65.3)	11 (3.3)	324 (97.3)
	Test Value	χ^2=0.01 sd=1 p=0.898		χ^2=1.34 sd=1 p=0.24		χ^2=0.33 sd=1 p=0.56		χ^2=2.19 sd=1 p=0.138	

** Rows percentage is taken*

It has seen that when the participants were asked why preferred that methods, finding it safe (52.9%) was placed on the top; when the participants were asked the reason for not using any contraception method, finding unnecessary (54.5%) was placed on the top. In the study, the status of experiencing an unwanted pregnancy was determined as 10.5%. The most common methods used by participants were the use of condoms (64.3%), the method of coitus interruptus (11.9%), the use of contraceptive pills (9.1%). 65% of the participants stated that using contraception affects sexual life. The most common method affecting sexual life is condom with 45.2%. This is followed by the use of contraceptive pills, the method of coitus interruptus the intrauterine device usage (Table 2).

As the FP method which is effective in protecting from sexually transmitted infections (STI), 81% of the participants said condoms, 4.8% contraceptive pills, the rest intrauterine device (IUD), the method of coitus interruptus, tubal ligation.

Finally, the participants were asked how to look at education in schools about contraception methods. It was determined that 55.5% of the participants approved, 24.5% did not approve, the rest (20%) hesitant. On the other hand, 19.8% of the participants stated that the use of contraception methods is harmful to health.

As it is seen in Table 3, cross-tables were made between some socio-demographic characteristics and approaches to contraception of the participants with the statuses of receiving any training about contraception methods, having sufficient information, using any contraception methods, using and quitting contraception methods. Hereunder;

Between the variable of the age with the status of having sufficient information about contraception and the status of still using contraception method,

Between the variable of the education level with the statuses of receiving any training about contraception, having sufficient information about contraception, using and quitting contraception methods,

Between the variable of the marital status with the status of having sufficient information about contraception and the status of still using contraception methods,

Between the variable of the income level with the status of receiving any training about contraception,

Between the variable of 'should the subject of contraception be given as education in schools?' with the status of receiving any training about contraception,

Between the variable of 'is the use of contraception methods harmful to health?' with the status of still using contraception methods,

Between the variable of being sexually active with the status of having sufficient information about contraception and the status of still using contraception methods,

Statistical significance was found between all these data ($p < 0.05$).

4. DISCUSSION

In this study, the family planning methods used by men, the reasons for using these methods, the factors affecting men were determined. 20.8% of men participating from Gümüşhane (a province in the Black Sea Region) are aged between 26-33. 53.7% of men have an education above the high school level.

Participants of the research have various sources of information about sexuality and family planning. These are respectively internet (37.5%), friends (24%), books (5.8%), newspapers (4.8%), family (4.8%) and magazines (2%). In the study in which the information statuses of men in Uganda about family planning was questioned, it was stated that there were various sources of information such as peers, health care personnel, mass communication, partners. The percentages of education received from health care personnel and mass communication campaigns were lower than other sources of information (7).

According to men participated in the study, the contraception is to have a limited number of children, to have children when spouses want, to limit the number of member in the family, to have enough number of children to be supported and raised, to educate individuals and families about reproductive health, to prevent maternal deaths and save healths, to prevent high-risk and unwanted pregnancies.

In a study, the reasons for using a small number of contraceptive methods of young people include cancer, fibroids, reproductive morbidity and fear of infertility for their female partners (8). In another study, it has been shown that the reasons of men for not using the hormonal contraceptives used by their spouses include the excessive bleeding, the menstrual irregularity, the concerns about reducing sexual desire among women (9).

67.1% of the participants prefer modern methods and 32.9% use traditional methods. In this study, the most common contraception method known by participants is the use of a condom (54.3%). The use of contraceptive pills and the method of coitus interruptus follow this. In the study, the large majority of the participants (89%) stated that information about family planning methods should be given to both sexes. In Asian countries such as Pakistan, it is stated that very few men use contraception methods and are generally resistant to the use of modern methods (10). In the researches based on society and population, it was emphasized that it is important to increase the deciding power of women due to their spouses' resistance to contraception (11). For example, according to the Pakistan Demographic Health Survey data (2013), it has been stated that the most commonly used methods were the use of a condom (9%) and the method of coitus interruptus (8.5%) (12). According to 2013 TDHS data, 33% of women and 47% of currently married women used a modern method, while 18% of women and 26% of currently married women used a traditional method. The method of coitus interruptus used by one in four married women (26%) is the most common method of contraception. IUD (17%) is

the most used modern method by married women. The most common method after IUD is the male condom (16%). Health experts emphasized that the use of condoms can be used not only against sexually transmitted infections but also as an alternative method when the use of hormonal contraceptive is not appropriate (13).

It has seen that when men were asked why preferred that methods now, finding it safe (52.9%) was placed on the top; when the participants were asked the reason for not using any contraception method, finding unnecessary (54.5%) was placed on the top. In the study, 65% of the participants stated that using contraception affects sexual life. The status of experiencing an unwanted pregnancy was determined as 10.5%.

It was determined that 55.5% of the participants approved the education in schools about contraception methods. On the other hand, 19.8% of the participants stated that the use of contraception methods was harmful to health. In the meetings, it was stated that family planning services and training provided by health experts were safe at the level of evidence. After appropriate counseling, problems such as failure in the use of contraception methods and unwanted pregnancies are being prevented. The counseling over decreasing fertility associated with the increasing age of women includes the creation of appropriate policies (13).

Women in society have primarily responsible for raising children. Therefore, especially in patriarchal societies, it is important for women to be a decision-maker on the choice of contraception together with men. It has been indicated that in Canada, all methods including abortion should be easily accessible for individuals without taking into account their geographical location. For this; taking the history, screening contraindications, dispensing the method, prescribing, selecting, sexual behaviors of individuals, reproductive health risks, social conditions, belief systems, evaluating of the cultural and political structures, improving the use skills related to the method should be necessary (13).

Sexually transmitted diseases are very important problem today. The use of 'condom', one of the modern methods of family planning, has an important place in the fight against this problem. However, in order to ensure the participation of men in family planning services and works, there are other approaches in addition to popularize the methods that can be used by men. In these approaches, men are expected to be active in all family planning services. In other words, it is emphasized that men should act together with women in deciding the use, the choice, the follow-up of the family planning method and also it is known that social positions of men and women are important in this regard (13).

It is important to include men in family planning practices, to increase modern family planning methods, to plan training for both sexes, to receive necessary training from health experts.

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Clinical and Experimental Health Sciences

Pomegranate Peel Extract Reduces Cisplatin-Induced Toxicity and Oxidative Stress in Primary Neuron Culture

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ABSTRACT

Objective: Cancer is the most common cause of death after cardiovascular diseases. Cisplatin used in most types of cancer produces neurotoxicity. In this study, we aimed to investigate the effects of pomegranate peel extract (1) in different doses, as potent antioxidants, on the prevention of neurotoxicity due to cisplatin, which is frequently used in cancer treatment.

Methods: In our study, newborn rat cortex was used. 2 hours following the application of PPE at 200, 300 and 400 mg/mL, neurotoxicity was established by applying cisplatin in 50 and 100 µM concentrations.

Results: In our study, cisplatin decreased cell viability in increasing doses, while PPE showed the best neuroprotective effect in high doses. Increased total oxidant capacity due to toxicity was significantly improved by PPE4. The antioxidant capacity decreased in the toxicity group showed improvement with the administration of PPE4. At the same time, increased TNF-α mRNA expression after cisplatin administration was significantly reduced with the administration of PPE4. The increased caspase 3 (CAS 3) and caspase 9 (CAS 9) mRNA expression due to cisplatin showed improvement with the administration of PPE4.

Conclusion: These results indicated that PPE could inhibit cisplatin-induced neurotoxicity, and these effects may be related to anti-apoptotic and antioxidants activities.

Keywords: Pomegranate, antioxidant, cisplatin, neurotoxicity, primary neuron

1. INTRODUCTION

Cancer is the second most common cause of death after cardiovascular diseases. The morbidity and mortality rates are increasing day by day. Antineoplastic agents are frequently used as the most effective treatment protocol (2).

Cisplatin is a chemotherapeutic agent used commonly in the treatment of solid tumors in various organs. The clinical use of cisplatin is limited due to its serious side effects on the nervous system (3). In addition, dose-limiting factors such as epilepsy, stroke and neuropathy, changes in consciousness, cerebral violations and ototoxicity are observed. All these side effects are often dose-limiting factors (4). Additionally, oxidative damage, inflammation, mitochondrial dysfunction, DNA damage, and apoptosis are involved in the mechanism of cisplatin neurotoxicity (4). Anticancer drugs, mainly ATPase dependent Na^+/K^+ pumps and Ca^{2+} homeostasis, break down mitochondria. Accumulation of dysfunctional mitochondria causes oxidative stress and peripheral nerve damage (5). Based on this information, studies have shown that cisplatin has a toxic effect by a mechanism caused by oxidative damage.

In shortly, oxidative stress has been recognized as an imbalance between the production of free radicals and antioxidant defense mechanisms that potentially lead to tissue damage. Oxidative stress plays a key role in the development of cerebrovascular and/or neurodegenerative diseases through different molecular pathways (6). Neurons are more sensitive to oxidative damage than other cells. The reasons for this are known to be their high oxygen consumption and low antioxidant enzyme activities etc. (6). From this point of view, it has been of interest to the researchers to search for neuroprotective drugs of natural origin against the neuronal death caused by oxidative stress. The prevention of chemotherapy-induced neurotoxicity associated with the clinical use of cisplatin is thus far an unsolved issue. Neurotoxicity is known to occur as a result of antineoplastic agents managing neuron cells to apoptosis. However, the effects of antineoplastic agents can be permanent in patients after chemotherapy and adversely affect the life of the patient after treatment. Therefore, today both clinical and preclinical studies are being conducted to prevent this neurotoxicity.

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Punica granatum, an important member of the *Punicaceae* family is a fruit with many features known since ancient times. Pomegranate is a perennial herb of the genus *Punica granatum* which is included in the genealogy family (7). Pomegranate peel, seed and fruit have been used for therapeutic purposes for years. Pomegranate peel is rich in 5 phenolic compounds, mainly flavanoids (anthocyanin, catechin and other complex flavanoids) and tannins (punicalin, punicalagin, gallic acid and ellagic acid) (8). Phenolic compounds are natural compounds with antioxidant activity. Proven in-vitro testing is available using four separate testing methods, showing that pomegranate juice and seed extracts have 2-3 times the antioxidant capacity of red wine or green tea (9). In additional in-vivo studies, pomegranate extracts have been shown to clear free radicals, reduce macrophage oxidative stress and lipid peroxidation (10). It has also been clinically indicated to increase plasma antioxidant capacity in older people (11).

Therefore, it was aimed to determine the effects of pomegranate peel extract, which is a natural antioxidant, and an easy to reach product with strong antioxidant activity, on neurotoxicity due to cisplatin.

2. METHODS

2.1. Preparation of Standard and Sample Solutions

Gallic acid, punicalagin A&B, and ellagic acid stock solutions were prepared with methanol at 1.00 mg/mL concentrations and working solutions were obtained by diluting stock solutions with phosphate buffer solution (pH=2.5, 0.025 M) to relevant concentration levels.

Pomegranate bark extract was solubilized with methanol and centrifuged at 10 000 rpm for 5 minutes. The supernatant was diluted with phosphate buffer solution and filtered through HDPE syringe filter with 0.45 µm pore size before injection.

2.2. Chromatographic Conditions

Method development and sample analysis were achieved with 1260 series Agilent HPLC equipped with auto sampler injector, diod array detector (DAD), column thermostat, quaternary pump compartments and Chemstation data acquisition program (Agilent Technologies, Santa Clara, CA, USA). Elution of the compounds were obtained by Kinetex RP_{C18} column (4 µm particle size, 3.9x250 mm I.D., store up at 25 °C), 5.00 µL injection volume. The mobile phase in channel (A) 0.025 M KH_2PO_4 buffer adjusted at pH=2.5 with phosphoric acid and in channel (B) acetonitrile and the gradient elution profile was: 0-10 minutes from 15% to 25% of B, 10-15 mintes from 25% to 60% of B with 0.5 mL/min flowrate. Detection performed at 254 nm for ellagic acid, 262 nm for punicalagin A&B and 272 nm for gallic acid DAD wavelengths.

The method was validated according to the International Council for Harmonization (ICH) guideline. Identification of the peaks were provided by comparison of retention times and DAD spectra of standard compounds and extract samples (Figure 1). Validation parameters and results are summarized in Table 1. Quantification of the pomegranate bark extracts was obtained by injection of three aliquots of extract solution to the system.

Table 1. *Chromatographic Parameters*

Compound	Gallic acid	Punicalagin A	Punicalagin B	Ellagic acid
Wavelength (nm)	272	262	262	254
Retention time (minute)	7.77 ± 0.08	9.56 ± 0.04	10.65 ± 0.05	16.54 ± 0.08
LOD (mg/L)	2.12	1.53	1.45	0.94
LOQ (mg/L)	2.56	1.95	1.85	1.29
Linear Concentration Range (mg/L)	5.00–50.00	40.00–400.00	50.00–500.00	10.00–150.00
Regression Equation (y= mx + b)	0.14x+8.87	0.01x-4.33	0.02x+11.55	0.06-3.97
Regression Coefficient (R^2)	0.9984	0.9996	0.9996	0.9999
Average Recovery	100.54 %	101.47 %	102.18 %	98.92 %
RSD% Peak Areas of Inter-Day Precision (n=3)	2.94	3.12	4.19	0.49
RSD% Peak Areas of Intra-Day Precision (n=9)	3.29	3.85	5.18	1.13

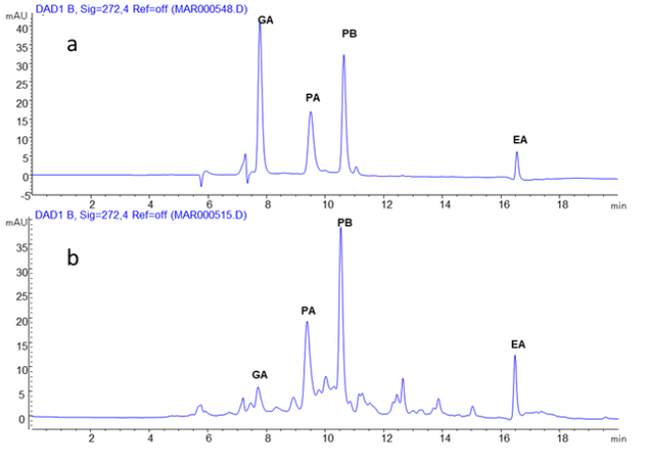

Figure 1. *Chromatograms of (a) standard compounds; (b) pomegranate bark extracts at 272 nm wavelength (GA: Gallic acid, PA: Punicalagin A, PB: Punicalagin B, EA: Ellagic acid)*

2.3. Animal Supply and Ethics Committee

This study was conducted with permission from the Local Ethics Committee of XX University Animal Experiments dated 25.01.2016 numbered 2016–016. A total of 10 newborn

Spraque Dawley rats obtained from the experimental animal laboratory were used.

2.4. Primary Neuron Culture

Primary neuron culture was conducted in the same way as described at our previous study (12). Pomegranate peel extract (1) at 200, 300 and 400 mg/mL concentrations was administered 2 hours before the onset of toxicity. The working order is summarized in Figure 2.

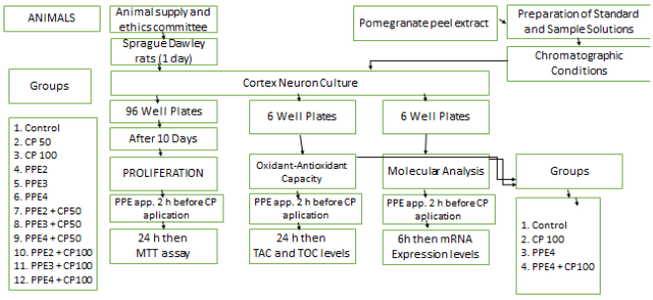

Figure 2. *Description chart of the primary neuron culture experiment*

Experimental groups; 1. Control, 2. 50 µM (C50), 3. 100 µM (C100), 4. PPE2 (200 mg/mL), 5. PPE3 (300 mg/mL), 6. PPE4 (400 mg/mL), 7. PPE2 + C50, 8. PPE3 + C50, 9. PPE4 + C50, 10. PPE2 + C100, 11. PPE3 + C100, 12. PPE4 + C100

2.5. Proliferation Assay

Cell viability was determined by MTT (3-(4,5-dimethylthiazol – 2-yl)-2,5-diphenyltetrazolium bromide) proliferation kit (Cayman Chemical, Ann Arbor, Mi, USA) described as previously (12, 13). 5 wells were used for each group.

2.6. Total Antioxidant Capacity (TAC) and Total Oxidant Capacity (TOC) Analysis

In cisplatin toxicity, commercial kit was used to determine the TAC and TOC levels of neuroprotective endogenous molecule PPE on primary neuron culture cells (Rel Assay Diagnostics, Gaziantep, Turkey). 5 wells were used for each group. The kit application was calibrated with a stable antioxidant, vitamin E analog and called Trolox equivalent (14). A commercial TOC kit was used to determine the TOC levels of neuroprotective PPE on primary neuronal culture cells in cisplatin toxicity (Rel Assay Diagnostics, Gaziantep, Turkey).

2.7. Molecular analysis

For RT PCR analysis, 4 groups (Control, Cisplatin 100 µM, PPE 400 mg/mL, PPE 400 mg/mL Cisplatin 100 µM) were created to work on the most effective doses according to MTT and TAC/TOC levels. 5 wells were used for each group. In our study, the expression levels of mRNA, CAS 3, CAS 9 and TNF-α, were compared between groups. The most toxic dose for cisplatin (C100) and the most effective dose for pomegranate (PPE 400) were preferred when determining real time PCR groups. mRNA extraction and complementary DNA (cDNA) synthesis were performed according to the methods described in our previous studies (15). mRNA extraction was performed from previously homogenized cells (pooled, 20 mg). Total mRNA was purified on the QIACUBE (Qiagen, Hilden, Germany) device according to the manufacturer's instructions using the RNeasy Mini Kit (Qiagen, Hilden, Germany). RNA samples were reverse transcribed to cDNA using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, CA, USA).

Relative mRNA expression analyzes of caspase-3 (CAS 3), caspase-9 (CAS 9) and tumor necrosis factor-alpha (TNF-α) from cDNAs obtained from RNAs of cell was performed on StepOne Plus Real Time PCR instrument (Applied Biosystems) using the Taqman Gene Expression kit (Applied Biosystems). β-actin was used as housekeeping gene. All data were expressed as fold change in expressions compared to the control group using the $2^{-\Delta\Delta Ct}$ method.

2.8. Statistical Analysis

The results of our studies were statistically evaluated by IBM SPSS 20 package program. The groups were checked primarily by Skewness and Kurtosis tests to see if they fit the normal distribution. Kruskal-Wallis test was used for analyses of the non-parametric data as they do not conform to normal distribution. $P<0.05$ was considered significant. Median and range values were used for graphics.

3. RESULTS

3.1. HPLC-DAD Analysis of Contents in PPE

HPLC-DAD system was used for identification and quantification of individual phenolic compounds of PPE. Figure 1 shows standard compounds and extract samples containing sharp, symmetrical and well-resolved peaks that were observed in four compounds. The corresponding result is given in Table 1, which indicates that PPE amounts were calculated as 14.45±0.53 mg/g, 191.56± 0.36 mg/g, 189.48± 0.62 mg/g, 68.02± 0.42 mg/g for gallic acid, punicalagin A, punicalagin B and ellagic acid respectively.

3.2. Proliferation Assay Results

Primary neuron cells were treated with PPE 200, PPE 300, PPE 400 mg/mL, and cisplatin 50 and 100 µM dosing together and/or alone. After application of PPE for 2 h, cisplatin toxicity was determined and MTT test was applied. According to the results of our study, the dose of PPE 300 and PPE 400 mg/mL increased cell viability significantly when compared to the control group ($p<0.01$). It was determined that cell viability decreased with cisplatin application only. PPE3 and PPE4 were able to prevent toxicity against low-dose and high-dose cisplatin-induced toxicity ($p<0.001$) (Figure 2).

3.3. Antioxidant and Oxidant Results

Neurotoxicity with cisplatin: According to the total antioxidant capacity (TAC) and total oxidant capacity (TOC) measurements, in neuron culture medium exposed to PPE 400 mg/mL for 2 h and then cisplatin for 24 h, and there was statistically significant decrease in TAC and statistically significant increase in TOC measurements when compared with the control groups. As shown in Figure 3, cisplatin administration significantly decreased TAC level compared to control. With the application of PPE4, the decreased TAC level increased significantly compared to the control ($p<0.05$). On the contrary, increased TOC level by cisplatin application decreased significantly compared to control with PPE4 administration ($p<0.01$) (Figure 4).

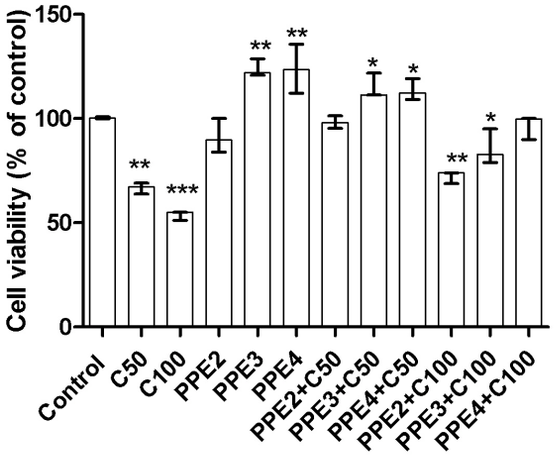

Figure 3. *The effects of PPE against cisplatin toxicity on cell proliferation in neuron cell line in 24 hours. C50: 50 µM Cisplatin; C100: 100 µM Cisplatin; PPE2: Punica granatum peel extract 200 mg/mL; PPE3: Punica granatum peel extract 300 mg/mL; PPE4: Punica granatum peel extract 400 mg/mL; error bars represent standard deviation; *p<0.05; **p<0.01; ***p<0.001 versus control groups.*

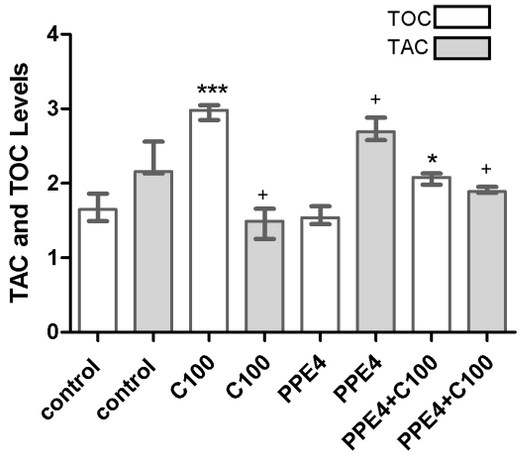

Figure 4. *The effects of PPE against cisplatin toxicity on neuronal oxidative system. TOC: total oxidant capacity (mmol/L); TAC: total antioxidant capacity (mmol/L); C100: 100 µM Cisplatin; PPE4: Punica granatum peel extract 400 mg/mL. The white columns show the TOC, the black columns show the TAC; error bars represent standard deviation; *p<0.05; ***p<0.001 and + p<0.05 versus control groups.*

3.4. Inflammation Findings

We investigated TNF-α mRNA expression level as a marker of inflammation in primary neuron cells. TNF-α expression levels; In our study, cisplatin group had significantly increased TNF-α expression levels compared to the control group. While there was no significant change in PPE4 group, it decreased the level of TNF-α increased by cisplatin in C100+PPE4 group (Figure 4c).

3.5. Apoptosis Findings

CAS 3 expression levels: In our study, CAS 3 expression levels increased significantly in cisplatin group compared to the control group ($p<0.001$). Although there was no significant change in PPE4 group, it decreased the level of CAS 3 increased by cisplatin in C100+PPE4 group (Figure 4a).

CAS 9 expression levels: In our study, CAS 3 expression levels increased significantly in cisplatin group compared to the control group ($p<0.01$). PPE4; CAS 9 level significantly decreased. It also increased the level of CAS 9 with cisplatin to the control level (Figure 4b).

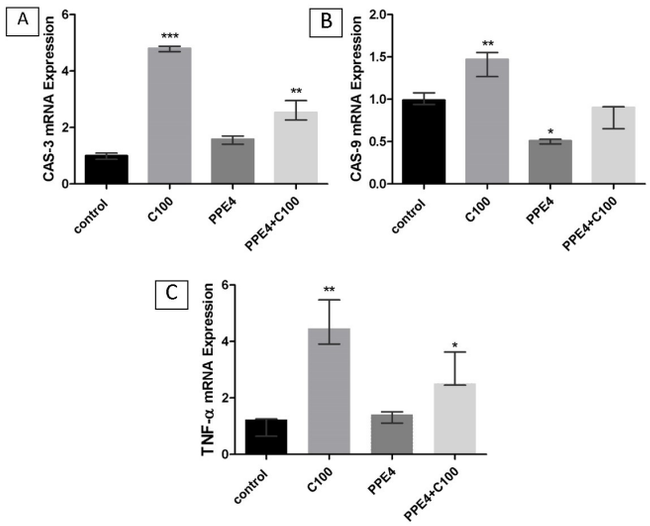

Figure 5. *Effects of PPE against cisplatin toxicity on neuron cells (A), CAS-3 mRNA expression (B), CAS-9 mRNA expression (C), TNF-α mRNA expression levels. The relative expression levels was calculated by the $2^{-\Delta\Delta Ct}$ method. C100: 100 µM Cisplatin; PPE4: Punica granatum peel extract 400 mg/mL; error bars represent standard deviation; *p<0.05; **p<0.01; versus control groups.*

4. DISCUSSION

Some studies have shown the effectiveness of different types of polyphenols using animal models of neurodegeneration to weaken or prevent neuronal death (16, 17). These compounds are antioxidant and anti-inflammatory properties and therapeutic effects due to the pharmacological effects including wide, are considered candidates for clinical trials in neurodegenerative disorders.

Pomegranate possesses a wide range of compounds, including polyphenols, alkaloids, and vitamins with potent free radical scavenging properties (8, 9). Today, *Punica granatum* is one of the most important antioxidant substance (11, 18). Antidiarrheic (19), antifungal (20), anti-ulcer (21), antibacterial (22), antitumoral and anticancer (23) effects have been shown in studies with *Punica granatum*. Oxidative stress has been known as imbalance between the free radicals and antioxidant defense system. Neurons are more sensitive to oxidative stress because of low activity of antioxidant enzymes (24). Some experimental studies support that reactive oxygen species were related with cisplatin cytotoxicity (5, 6).

As a result of stimulation of the expression of antiapoptotic genes in cancer cells, the development of chemoresistance is characterized by cisplatin administration (25). To eliminate this resistance, cisplatin should be administered in high doses, but it causes the most organ / tissue toxicity such as high doses of cisplatin, nephrotoxicity, hepatotoxicity and neurotoxicity

MTT assay was using evaluated cytotoxic effect and/or cell viability. According to the MTT assay, the cytotoxic effects of cisplatin was demonstrated by their strong inhibition on cell viability on neuron cells. One study showed the ability of cisplatin to penetrate into the brain where it inhibits neuronal stem cell proliferation (26). PPE3 and PPE4 significantly reduced toxicity caused by cisplatin ($p<0.001$) and increase the viability. Likewise, it was reported that cisplatin caused significant neurotoxicity via induction of lipid peroxidation and reduction in the potency of the antioxidant defense system (27).

In the present study, we investigated protective effect of PPE against cisplatin-induced oxidative effects by determining TAC and TOC levels. Cisplatin application decreased TAC level and TOC level was increased by Cetin et al. (12). In our current study, TAC level was significantly decreased with C100 application and a significant increase was observed in TOC level. However, in the PPE4 + C100 groups, the TAC level increased significantly compared to the C100 group, and the level TOC decreased significantly. In short, PPE4 reduced the oxidative damage due to its antioxidant effect induced by cisplatin (Figure 3). Changes in TNF-α mRNA expression levels were observed with real time PCR to demonstrate the antioxidant activity of PPE4. Parallel to TAC and TOC ELISA result; compared with the healthy group, the TNF-α mRNA expression level was increased by cisplatin administration, whereas the PPE4 normalized TNF-α mRNA expression levels.

Excessive accumulation of intracellular ROS has been recognized as the strongest trigger of cisplatin-induced initiation of CAS 3 and 9 activities, resulting in increased apoptosis (28). Marullo et al. showed that cells lacking functional mitochondria were more resistant to cisplatin-induced cell death (29). We have reached similar results. The results showed that PPE4 is able to protect primary neuron cells through inhibition of apoptosis, as proved by the attenuation of mRNA expression of proapoptotic gene CAS 3 and CAS 9 activation (Figure 3a).

5. CONCLUSION

Our in vitro experience: PPE4, provided protection against the neurotoxic effects of cisplatin in rat primary neurons. The protective mechanisms may be related to antioxidant activities and botanical phenolic components of PPE. Hence, inclusion of PPE in cisplatin-based chemoradiotherapy can be an effective strategy to counter cisplatin-induced neurotoxicity. Further research is needed to explain the exact mechanism of the potential neuroprotective effects of PPE.

Ethics Committee Approval: This study was conducted with permission from the Local Ethics Committee of Kafkas University Animal Experiments dated 25.01.2016 numbered 2016–016. A total of 10 newborn Spraque Dawley rats obtained from the experimental animal laboratory were used.

Conflict of interest: Part of the Study presented orally at "7th drug chemistry conference: Design, synthesis, production and standardization of drug active substances."

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Clinical and Experimental Health Sciences

Genotoxicity of Plant Mediated Synthesis of Copper Nanoparticles Evaluated Using *In Vitro* Mammalian Cell Micronucleus Test

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ABSTRACT

Objective: Nanotechnology is an emerging technology which has wide applications in many fields. Prime concern of research in nanotechnology is the synthesis of nano-material with the controlled size and shape. Recently, biosynthesis of metallic nano-particles has gained popularity owing to its eco-friendliness. The increasing use of copper nanoparticles (CNPs) in medicine and industry demands an understanding of their potential toxicities.

Methods: Genotoxicity of CNPs was assessed using the in vitro micronucleus assay which is standard genotoxicity assay. In this study, CNP was tested in the absence and presence of the metabolic activation (2% v/v S9 mix). Human peripheral blood lymphocyte cultures were exposed to CNPs, at 5 dose-levels between 0.125 to 2μL/mL of culture medium in absence and presence of the metabolic activation system.

Results: Required level of cytotoxicity (55 ± 5% reduction in replicative index, i.e., cytostasis) was observed in absence of the metabolic activation at the test concentration of 2 µL/mL. Therefore, dose levels selected for scoring of binucleated cells containing micronuclei were: 0.5, 1, and 2 µL/mL. From the obtained data of MNBN cells (Binucleated cells with micronuclei) for all three selected test concentration was found in the range of negative and vehicle control.

Conclusion: Our results concluded that, CNPs did not induce statistically significant or biologically relevant increase in number of binucleated cells with micronuclei in absence and presence of the metabolic activation.

Keywords: Metal nanoparticles, micronucleus test, genotoxicity, lymphocytes.

1. INTRODUCTION

The use of nanomaterials (size between 1 nm and 100 nm) in the world has become an important component of consumer products in recent years. This fact has gradually led to an increase in concerns and debates about the impact of nanomaterials on different biological systems (1,2). According to assessments in recent years, engineered metal nanoparticles, which have small size, large specific surface area and surface reactivity, are extensively used in many industries such as electronic, textile, cosmetic, pharmacology, and medicine (1). Because of their unique properties, NPs may be useful in a diverse range of applications, such as the biomedical field, the use of nano vaccines and nano drugs. (3). Hence, nanoparticles increased use enhances the risk of its exposure to humans and other living organisms. These risks may cause cytotoxic, genotoxic, and biochemically adverse effects on the biological systems. Therefore, there is an urgent need to eliminate these adverse effects of nanoparticles on biological systems. Because of the increasing evidence of adverse effect nanoparticles on biological systems, scientists became concerned to eliminate this effect. As a result, studies on the possible adverse effects of nanoparticles, especially genotoxicity, have increased (4). Some tests, including the micronucleus test, comet assay, and gamma H2AX, are commonly used to assess the genotoxicity of many different chemicals (drug, pesticide, NPs, and phthalates) in vivo as well as in vitro studies (5). The in vitro micronucleus test is a standard test for evaluation of genotoxicity. It is simple, useful, and applicable in different cell types. The type performed using cytochalasin B is the most useful micronucleus assay which produces binucleated cells (BNCs) (6).

Genotoxic effects of different types of nanoparticles have been evaluated by several researchers (7-9). Shukla et al. investigated titanium dioxide NPs (TiO_2 NPs) in human epidermal cells which cause reactive oxygen species (ROS) mediated genotoxicity (10).

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In the present study, CNPs was tested in the absence and presence of the metabolic activation (2% v/v S9 mix).Human peripheral blood lymphocyte cultures were exposed to CNPs, at 5 dose-levels (two cultures/dose level) between 0.125 to 2 µL/mL of culture medium in absence and presence of the metabolic activation system.

2. METHODS

2.1. Materials

Mallotus phillipensis were collected from the Botanical Garden of Navsari Agriculture University (N.A.U.), Navsari. Their botanical identification and authentication was done by the authorities of the Herbarium of N.A.U. Copper (II) sulfate pentahydrate salt-$CuSO_4$ $5H_2O$ (Loba Chemie), DMSO (Finar), Methanol (Finar) was purchased. Positive controls such as Vinblastine and Cyclophosphamide, RPMI 1640 medium, β – NADP, D-Glucose-6-phosphate, Cytochalasin-B and Potassium chloride were purchased from Sigma-Aldrich (St. Louis, USA). All other chemicals used in the experiments were also purchased from Qualigens. Giemsa Powder used in the experiment was purchased from Merck. The S9 fraction procured from Dr. G. P. Meshram, Nagpur (MWR/ARI/S9F/01/17. Sep 2017) was used in this assay. Fetal Bovine Serum and 2% Phytohaemagglutinin were procured from Gibco.

2.2. Synthesis of Copper Nanoparticles

Copper ions can be reduced to nanosize by using reducing agent. In this study methanolic extracts of Mallotus phillipensis leaves were used as a reducing agent. For copper nanoparticles synthesis, 10 mL leaf extract of Mallotus phillipensis was added to 100 mL of 1mM aqueous copper sulphate solution in a 250 mL Erlenmeyer flask. The flask was then kept overnight at room temperature. The green colored copper nanoparticles solution thus obtained was purified by repeated centrifugation at 12000 RPM for 15 minutes followed by re-dispersion of the pellet in ethanol.

2.3. Characterization Method

The characterization of the synthesized silver, copper and zinc nanoparticles were carried out using UV-Vis spectrophotometer and Scanning Electron microscope (SEM analysis).

2.4. Test System

This study was performed at the Department of Toxicology, Jai Research Foundation, Valvada – 396 105, Dist. Valsad, Gujarat, India.

The test system used for the *in vitro* mammalian cell micronucleus test was human peripheral blood lymphocytes. The selection criteria for a volunteer were as per JRF standard operating procedure. Blood was drawn from healthy, female volunteer (25 years old), by venous puncture using heparinized syringe (Heparin obtained from Biological E. Limited, Hyderabad). A trained medical laboratory technician collected blood by vein puncture using a 23 G needle attached to a 50 mL disposable syringe.

2.5. Cytotoxicity

CNPs was evaluated for its potential to induce micronuclei at the tested concentrations of 0.125, 0.25, 0.5, 1 and 2 µL CNPs/mL of culture medium in the absence and presence (2% v/v S9) of a metabolic activation.

2.6. Lymphocyte Isolation and Exposure to Test

Whole blood was cultured in RPMI-1640 (Roswell Park Memorial Institute) with L-glutamine and 25 mm HEPES containing antibiotics and antimycotic solution (penicillin: 50 IU/mL; streptomycin: 50 µg/mL and amphotericin B: 0.25 µg/mL) supplemented with 20% heat-inactivated (56 °C; 30 min.) fetal bovine serum. Cultures were prepared separately in a centrifuge tube containing heparin sodium. Each contained 0.5 mL of whole blood in 9.5 mL of complete medium [containing 20% heat-inactivated (56°C; 30 min.) fetal bovine serum and 2% Phytohemagglutinin (PHA-M)] in centrifuge tubes and incubated at 37 ± 1°C and 5% CO_2 in a CO_2 incubator for approximately 48 hours. Same procedure was used for preparation of cultures for all the phases of the experiments.

Peripheral blood lymphocyte cultures are exposed to the test item both in the absence and presence of metabolic activation. The actin polymerization inhibitor, Cytochalasin B, is added to block cytokinesis, resulting in binucleated cells. After exposure to the test item, the cells are grown for a period sufficient to allow chromosome or spindle damage to lead to the formation of micronuclei in interphase cells and for the induction of aneuploidy. At predetermined intervals, cells are treated with Cyto-B, harvested, stained and interphase cells are analyzed microscopically for the presence of micronuclei.

The main study was conducted in two phases with lymphocytes exposed for 4 h (hour) without and with metabolic activation (2% v/v S9) in Phase I and for approx. 24 h exposure without S9 in Phase II. There were two replicates for each treatment concentration including positive, vehicle (DMSO) and negative (untreated control) controls.

Phase I (Absence and presence (2% v/v S9 mix) of metabolic activation and exposure for 4 h): In Phase I, the cultures were exposed to the concentrations of 0.125, 0.25, 0.5, 1 and 2 µL CNPs/mL of culture in the absence and presence (2% v/v S9) of a metabolic activation. For treatment, the first stock solution (stock A) of the test item was prepared by suspending 400 µL of test item in DMSO and made up to 2 mL (200 µL/mL). A volume of 1 mL of stock A was added to 1 mL DMSO to obtain 100 µL/mL (stock B). Further stock solutions of 50 µL/mL (stock C), 25 µL/mL (stock D), and 12.5 µg/mL (stock E) were prepared by serial dilution using 1 mL of relevant stock with 1 mL of DMSO. A concurrent negative control (untreated control) and vehicle control (dimethyl sulfoxide) were also

maintained. Volumes of 180 µL of relevant stock solutions of A-E were added into 17.82 mL of medium to obtain required test concentrations in the absence of metabolic activation.

A volume of 8 mL of this treatment medium was used for treatment of the respective replicate culture tube. Same procedure was followed for treatment in all phases of the main study. Serum free medium with KCl was used in the absence of metabolic activation while medium with S9 mix was used in the presence of metabolic activation. The cultures were exposed for 4 h both in the absence and presence of metabolic activation. At the end of the treatment, cultures were centrifuged at approximately 1500 rpm for 8 minutes and the supernatant was replaced with fresh complete medium containing cytochalasin B (6 µg/mL) and incubated at 37 ± 1 °C and 5% CO_2 in a CO_2 incubator till harvesting.

The cells were harvested and processed for slide preparation approximately after 24 h from the beginning of treatment.

Phase II (Absence of metabolic activation and exposure for approximately 24 h): In Phase II, the cultures were exposed to the concentrations of 0.125, 0.25, 0.5, 1 and 2 µL CNPs/mL of culture (supplemented with 10% FBS), for approximately 24 h without S9 in the absence of metabolic activation with cytochalasin B (6 µg/mL). The cells were harvested and processed for preparation of slides after the exposure period.

As the phase II experiment was performed to confirm the negative results obtained in the absence of metabolic activation in Phase I, slide scoring data were evaluated after evaluating the data of phase I experiment.

Controls: A concurrent negative control (untreated control), vehicle control (dimethyl sulfoxide) and positive controls were maintained, in duplicate, along with each phase of the experiment (except positive control in phase I in absence of S9), both in the absence and presence of metabolic activation, which demonstrate both the activity of the metabolic activation system and the responsiveness of the test system. Cyclophosphamide (30 µg/mL, Phase I) was used as the positive control in the presence of metabolic activation and Vinblastine (0.008 µg/mL) was used as the positive control in the absence of metabolic activation (Phase II).

2.7. Harvesting of Cells

Each culture was harvested and processed separately for the preparation of slides for micronuclei frequency evaluation. Cells were hypotonically treated with cold (2 – 8 ºC) potassium chloride solution (0.075 M KCl) and centrifuged immediately for 8 minutes. Supernatant was removed and replaced with chilled Carnoy's fixative and centrifuged for 8 minutes. Cells were given further 2 changes of chilled Carnoy's fixative washing.

2.8. Slide Preparation

Slides were prepared from each culture tube by pouring approximately 0.5 mL of the fixed cell suspension, drop by drop on two, pre-cleaned, ice-chilled slides. The slides were dried over a slide warmer and labelled with study number, treatment code, absence/presence metabolic activation and slide number. Two slides were prepared per replicate of culture at each test concentration, Out of these two slides, one was used for scoring and the other served as reserve or used for scoring wherever required. The dried slides were stained with 5% Giemsa in phosphate buffer for 30 minutes. The slides were made permanent by mounting a cover slip with DPX mountant. In order to prevent bias in the scoring procedure for micronuclei, the slide numbers were masked with code numbers provided by Department of Biostatistics and Systems Information, JRF. All slides were coded before microscopic scoring and decoded after scoring.

2.9. Scoring of Slides

2.9.1. Replicative Index

All slides, including those of positive, negative control (untreated control) and vehicle control (dimethyl sulfoxide), were independently coded prior to microscopic analysis for replicative index (during main study) and cells with micronuclei (during main study). The slides were examined under a microscope and a minimum number of 500 cells per slide (culture) were counted and numbers of binucleated cells, multi nucleated cells and mono nucleated cells were recorded in different fields to determine the replicative index by using the following formula:

$$RI = \frac{[(\text{No. binucleated cells}) + (2 \times \text{No. multinucleated cells})] \div (\text{Total number of cells})\ T}{[(\text{No. binucleated cells}) + (2 \times \text{No. multinucleated cells})] \div (\text{Total number of cells})\ C} \times 100$$

% Cytostasis = 100 – Replicative index

Key: RI = Replicative index, T = Treated cultures and C = Control cultures

2.9.2. Micronucleus Frequency

Two replicate slides per concentration were used for screening of micronucleated binucleated cells whereas the other two slides were kept in reserve, for scoring whenever required (if binucleated cell frequency is less). The slides were examined for the presence of micronuclei in binucleated cells under a microscope. A minimum of 2000 binucleated cells were screened per concentration to evaluate the incidence of micronuclei. The masked labels were removed and all the slides were decoded after scoring.

2.10. Statistical Analysis

Data on micronuclei containing binucleated cells were subjected to Shapiro-Wilk's test for normality and Bartlett's test to assess homogeneity of variance before conducting Analysis of Variance (ANOVA) and Dunnett's t-test (11). Where the data did not meet suitable homogeneity of

variance, Student's t-test was performed followed by chi-square to determine the level of significance between vehicle control, three selected test concentrations (selected based on the replicative index data) and positive controls.

3. RESULTS

3.1. Synthesis of Copper Nanoparticles

After 24 hours of reaction, the appearance of greenish colour in the reaction vessels suggests the formation of CNPs. The colour tone of the reaction mixture changes from light blue to greenish indicates the reduction of Cu^{++} ions leads to the formation of CNPs (Figure 1). Different colour change observed in the reaction vessel is due to an effect called Localized Surface Plasmon Resonance.

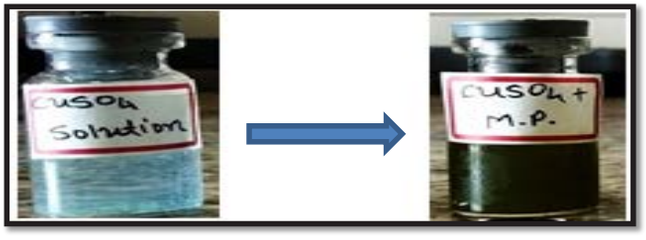

Figure 1. *Biosynthesis of Copper nanoparticles using Mallotus phillipensis*

3.2. Characterization of Copper Nanoparticles

The UV-Vis spectra of CNPs biosynthesized using *Mallotus phillipensis* was shown in Figure 2. The absorption peak of the prepared CNPs was found around 524 nm of UV-Vis range, which is characteristic range of copper. CNPs biosynthesized from *Mallotus phillipensis* have approx. particle size between ~41-50 nm (Figure 2).

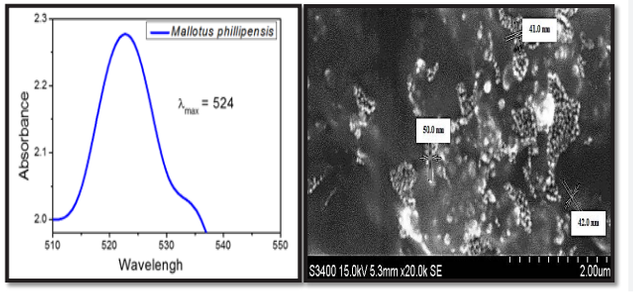

Figure 2. *UV-Vis Spectra and SEM Images of Copper Nanoparticles Prepared Using Mallotus Phillipensis*

3.3. Solubility, Precipitation, pH and Osmolality Tests

CNPs was insoluble in distilled water, while found to form suspension at 2 µL/mL in dimethyl sulfoxide. The solubility limit for final stock concentration of 200 µL CNPs/mL, was achieved in DMSO. Therefore dimethyl sulfoxide was selected as the suitable vehicle in this study.

No significant change in pH (±1 unit) or osmolality (≥ 50 mOsm/kg H2O) were observed in the tested concentrations 0.0625, 0.125, 0.25, 0.5, 1, and 2 µL/mL of culture medium, at 0 h and 4 h. In addition, no precipitation was observed up to the test concentration level 200 µL/mL. Therefore, 2 µL/mL was selected as the highest concentration to be tested in the cytotoxicity test.

3.4. Cytotoxicity

The pH and osmolality at the beginning of the treatment at concentration of 2 µL/mL in absence of the metabolic activation system were 7.33 and 442 mOsm/kg H_2O, respectively (compared to 7.36 and 441 mOsm/kg H_2O in the vehicle control). At the same concentration, but in presence (2% v/v S9 mix) of the metabolic activation system, the pH and osmolality were 7.33 and 445 mOsm/kg H_2O, respectively (compared to 7.35 and 444 mOsm/kg H_2O in the vehicle control). No relevant influence of the test item on pH value or osmolality was observed in absence (Phase I and II) and presence of the metabolic activation (Phase I).

Phase I (Absence and presence of the metabolic activation (2% v/v S9 mix) and exposure for 4 h**):** The cytostasis observed was 1.70, 7.29, 5.02, 8.14 and 12.17% at the tested concentration level of 0.125, 0.25, 0.5, 1, and 2 µL CNPs/mL in absence of the metabolic activation, respectively. The cytostasis observed in presence of the metabolic activation was 7.13, 4.56, 5.91, 9.64, 15.37% at the tested concentrations of 0.125, 0.25, 0.5, 1, and 2 µL CNPs/mL, respectively. The cytostasis observed for positive control in presence of the metabolic activation was 10.92%. The cytostasis of cultures treated with various concentrations of CNPs, with the positive, negative (untreated control) and vehicle (dimethyl sulfoxide) controls in absence and presence of the metabolic activation (Phase I) is provided in Table 1.

Cytostasis, i.e., reduction in replicative index of 12.17% and 15.37% was observed at the tested concentration of2 µL/mL in absence and presence of the metabolic activation system. Therefore, test concentration levels selected for scoring of binucleated cells containing micronuclei were: 0.5, 1, and 2 µL/mL culture medium in absence and presence of the metabolic activation.

Both in absence and presence of the metabolic activation, CNPs did not induce any statistically significant or biologically relevant increase in number of binucleated cells with micronuclei.

Phase II (Absence of metabolic activation and exposure for 24 h**):** The cytostasis observed was 5.77, 8.05, 11.23, 14.12 and 40.03% at the tested concentration levels of 0.125, 0.25, 0.5, 1, and 2 µL of CNPs in absence of the metabolic activation, respectively. The cytostasis observed was 10.57% in the cultures treated with positive control in absence of the metabolic activation. Required level of cytotoxicity (55 ± 5% reduction in replicative index, i.e., cytostasis) was observed in absence of the metabolic activation at the test concentration of 2 µL/mL. Therefore dose levels selected for scoring of binucleated cells containing micronuclei were: 0.5, 1, and 2 µL/mL. The cytostasis of cultures treated with various concentration levels of CNPs, with the positive, negative (untreated control), and

vehicle (dimethyl sulfoxide) controls in absence (Phase II) of the metabolic activation are also presented in Table 1.

Table 1. *Summary of Cytostasis for Main Study*

Concentration of CNPs	Phase I– Cytostasis (Exposure for 4 h)				Phase II– Cytostasis (Exposure for 24 h)	
	Absence of Metabolic Activation		Presence of Metabolic Activation (2% v/v S9 mix)		Absence of Metabolic Activation	
	%RI (Mean)	Cytostasis	%RI (Mean)	Cytostasis	%RI (Mean)	Cytostasis
NC (Untreated control)	100.00	0#	100.00	0#	100.00	0#
VC (DMSO)	100.00	0#	100.00	0#	100.00	0#
T1 (0.125 µL/mL)	98..30	1.70	92.87	7.13	94.23	5.77
T2 (0.25 µL/mL)	92.71	7.29	95.44	4.56	91.95	8.05
T3 (0.5 µL/mL)	94.98	5.02#	94.09	5.91#	88.77	11.23#
T4 (1 µL/mL)	91.86	8.14#	90.36	9.64#	85.88	14.12#
T5 (2 µL/mL)	87.83	12.17#	84.63	15.37#	59.97	40.03#
PC	-	-	89.08	10.92#	89.43	10.57#

NC: negative control; VC: vehicle control; R: replicate; DMSO: dimethyl sulfoxide; T: treatment group, PC: positive control (Cyclophosphamide @ 30 µg/mL in the presence of metabolic activation for Phase I and Vinblastine @ 0.008 µg/mL in the absence of metabolic activation for Phase II); #: concentrations selected for scoring of binucleated cells containing micronuclei; RI: replicative index

Replicative index and micronucleus frequency check (Figure 3) results of negative and vehicle control were comparable and no adverse effect of DMSO was observed on cultures when compared with that of the concurrent negative control (untreated control) during the main study. Individual observation for replicative index of cultures treated with various concentration levels of CNPs, with the positive, negative (untreated control), and vehicle (dimethyl sulfoxide) controls in absence (Phase I and Phase II) and presence (Phase I) of the metabolic activation are presented in Table 2, Table 4 and Table 3, respectively.

In absence of the metabolic activation, CNPs did not induce any statistically significant or biologically relevant increase in the number of binucleated cells with micronuclei.

The number of binucleated cells with micronuclei found in the negative and vehicle control cultures were within the published range and acceptable for addition into laboratory's historical data base (12). The positive controls, vinblastine produced statistically significant increase in the frequency of micronuclei containing binucleated cells in phase II (continuous treatment) in absence of the metabolic activation and cyclophosphamide in Phase I in presence of the metabolic activation (short term exposure). Response of the positive controls demonstrated the efficiency of the test system and suitability of the test procedures and conditions employed in the study.

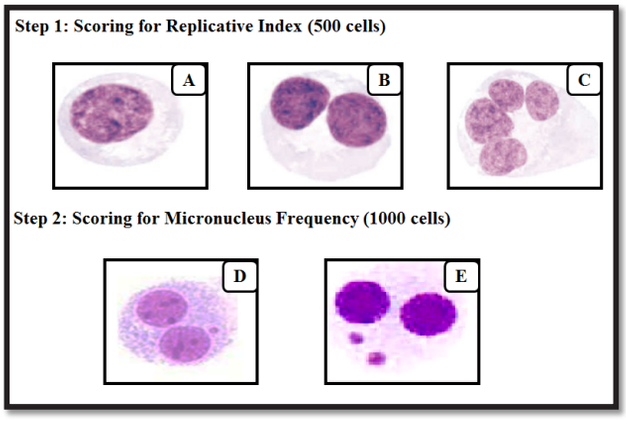

Figure 3. *Photomicrographs of Mononucleated Cell (A), Binucleated Cell (B), Multinucleated Cell (C), Micronuclei Containing Binucleated Cell (D-E)*

Table 2. *Individual Observation for Replicative Index – Phase I (Absence of Metabolic Activation)*

Concentration of CNPs	Replicate	No. of Mononucleated Cells	No. of Binucleated Cells Scored	No. of Multinucleated Cells	Total No. of Cells
NC (Untreated Control)	R1	143	334	56	533
	R2	119	325	78	522
VC (DMSO)	R1	146	301	79	526
	R2	139	328	51	518
T1 (0.125 µL/mL)	R1	152	300	80	532
	R2	141	348	40	529
T2 (0.25 µL/mL)	R1	167	323	40	530
	R2	146	332	50	528
T3 (0.5 µL/mL)	R1	156	329	51	536
	R2	148	327	50	525
T4 (1 µL/mL)	R1	167	306	52	525
	R2	162	310	49	521
T5 (2 µL/mL)	R1	171	305	38	514
	R2	169	316	40	525

R: replicate; T: treatment group; NC: negative control; VC: vehicle control; DMSO: dimethyl sulfoxide

Summaries of percent Micronuclei Containing binucleated cells are provided in Table 5 for phase I and II. Individual observations of Micronuclei Containing binucleated cells and binucleated cells of Phase I and II with individual values are provided in Table 6.

Student's t-test was performed for statistical significance between two groups. The P value is ≤ 0.01 (1% level) is accepted as significant. Summaries of statistical significance of P value are provided in Table 7.

Table 3. *Individual Observation for Replicative Index – Phase I [Presence of Metabolic Activation (2% v/v S9 mix)]*

Concentration of CNPs	Replicate	No. of Mononucleated Cells	No. of Binucleated Cells Scored	No. of Multinucleated Cells	Total No. of Cells
NC (Untreated Control)	R1	112	319	99	530
	R2	141	332	63	536
VC (DMSO)	R1	147	306	93	546
	R2	124	331	76	531
T1 (0.125 µL/mL)	R1	140	309	59	508
	R2	137	316	56	509
T2 (0.25 µL/mL)	R1	134	313	70	517
	R2	139	319	62	520
T3 (0.5 µL/mL)	R1	118	358	58	534
	R2	145	328	49	522
T4 (1 µL/mL)	R1	142	311	52	505
	R2	146	321	50	517
T5 (2 µL/mL)	R1	162	319	35	516
	R2	151	321	38	510
PC	R1	135	362	38	535
	R2	144	351	35	530

PC: positive control (Cyclophosphamide 30 µg/mL in the presence of metabolic activation)

Table 4. *Individual Observation for Replicative Index – Phase II (Absence of Metabolic Activation)*

Concentration of CNPs	Replicate	No. of Mononucleated Cells	No. of Binucleated Cells Scored	No. of Multinucleated Cells	Total No. of Cells
NC (Untreated Control)	R1	134	342	52	528
	R2	140	335	56	531
VC (DMSO)	R1	153	355	23	531
	R2	119	333	76	528
T1 (0.125 µL/mL)	R1	141	325	39	505
	R2	146	327	34	507
T2 (0.25 µL/mL)	R1	160	305	39	504
	R2	157	316	43	516
T3 (0.5 µL/mL)	R1	161	305	35	501
	R2	165	309	32	506
T4 (1 µL/mL)	R1	171	305	32	508
	R2	176	299	30	505
T5 (2 µL/mL)	R1	280	205	21	506
	R2	278	198	32	508
PC	R1	147	327	33	507
	R2	169	305	29	503

R: replicate; T: treatment group; NC: negative control; VC: vehicle control; DMSO: dimethyl sulfoxide; PC: positive control (Vinblastine 0.008 µg/mL in the absence of metabolic activation)

Table 5. *Summary of Mean Binucleated Cells, Micronuclei Containing Binucleated Cells and Percent Micronuclei Containing Binucleated Cells*

Concentration of CNPs	Phase I [Absence of Metabolic Activation]	
	MNBN Cells	% MNBN Cells
	Mean ± SD	Mean ± SD
NC (Untreated Control)	3.00 ± 1.41	0.30 ± 0.14
VC (DMSO)	3.50 ± 0.71	0.35 ± 0.07
T3 (0.5 µL/mL)	2.00 ± 1.41	0.20 ± 0.14
T4 (1 µL/mL)	2.50 ± 0.71	0.25 ± 0.07
T5 (2 µL/mL)	2.00 ± 1.41	0.20 ± 0.14
Concentration of CNPs	**Phase I [Presence of Metabolic Activation (2% v/v S9 mix)]**	
	MNBN Cells	**% MNBN Cells**
	Mean ± SD	**Mean ± SD**
NC (Untreated Control)	2.50 ± 0.71	0.25 ± 0.07
VC (DMSO)	3.00 ± 1.41	0.30 ± 0.14
T3 (0.5 µL/mL)	1.50 ± 0.71	0.15 ± 0.07
T4 (1 µL/mL)	2.00 ± 1.41	0.20 ± 0.14
T5 (2 µL/mL)	2.50 ± 0.71	0.25 ± 0.07
PC (Cyclophosphamide @ 30 µg/mL)	42.00 ± 1.41	4.17 ± 0.17
Concentration of CNPs	**Phase II [Absence of Metabolic Activation]**	
	MNBN Cells	**% MNBN Cells**
	Mean ± SD	**Mean ± SD**
NC (Untreated Control)	3.00 ± 1.41	0.30 ± 0.14
VC (DMSO)	3.50 ± 0.71	0.35 ± 0.07
T3 (0.5 µL/mL)	2.50 ± 0.71	0.25 ± 0.07
T4 (1 µL/mL)	2.00 ± 1.41	0.20 ± 0.14
T5 (2 µL/mL)	2.50 ± 0.71	0.25 ± 0.07
PC (Vinblastine @ 0.008 µg/mL)	44.00 ± 1.41	4.40 ± 0.15

NC: negative control; VC: vehicle control; DMSO: dimethyl sulfoxide; BN cells: binucleated cells; MNBN: micronucleated binucleated cells; SD: standard deviation; PC: positive control; : significantly higher than the control at 1% level (p≤0.01) and : significantly higher than the control at 5% level (p≤0.05)

Table 6. *Individual Observation of Slides for Frequency of Micronuclei Containing Binucleated Cells*

Concentration of CNPs	Phase I								Phase II			
	R	Absence of Metabolic Activation			R	Presence of Metabolic Activation (2% v/v S9 mix)			R	Absence of Metabolic Activation		
		Total Number of BN Cells Scored	Number of MNBN	Percent MNBN		Total Number of BN Cells Scored	Number of MNBN	Percent MNBN		Total Number of BN Cells Scored	Number of MNBN	Percent MNBN
NC (Untreated Control)	1	1004	4	0.400	1	1002	2	0.200	1	1004	2	0.200
	2	1015	2	0.200	2	1003	3	0.300	2	1004	4	0.400
VC (DMSO)	1	1002	4	0.400	1	1004	4	0.400	1	1004	3	0.300
	2	1003	3	0.300	2	1008	2	0.200	2	1005	4	0.400
T3 (0.5 µL/mL)	1	1003	3	0.300	1	1004	2	0.200	1	1004	2	0.200
	2	1004	1	0.100	2	1003	1	0.100	2	1002	3	0.300
T4 (1 µL/mL)	1	1003	2	0.200	1	1004	3	0.300	1	1001	3	0.300
	2	1005	3	0.300	2	1004	1	0.100	2	1002	1	0.100
T5 (2 µL/mL)	1	1005	1	0.100	1	1014	3	0.300	1	1008	2	0.200
	2	1004	3	0.300	2	1006	2	0.200	2	1003	3	0.300
PC	1	NA			1	1003	43	4.290	1	1001	45	4.500
	2				2	1013	41	4.050	2	1003	43	4.290

R: replicate; T: treatment group; NC: negative control; VC: vehicle control; DMSO: dimethyl sulfoxide; BN cells: binucleated cells; MNBN: micronucleated binucleated cells; PC: positive control (Cyclophosphamide 30 µg/mL in the absence and Vinblastine 0.008 µg/mL in the absence of metabolic activation); NA: not applicable.

Table 7. *Statistical Analysis*

Concentration of copper nanoparticle	Phase I [Absence of Metabolic Activation]			
	MNBN Cells	% MNBN Cells	MNBN Cells	% MNBN Cells
	P Values	P Values	Statistical Significance	Statistical Significance
VC vs T3 (0.5 µL/mL)	0.4790	0.4790	Non-Significant	Non-Significant
VC vs T4 (1 µL/mL)	0.7200	0.7200	Non-Significant	Non-Significant
VC vs T5 (2 µL/mL)	0.4790	0.4790	Non-Significant	Non-Significant
Concentration of copper nanoparticle	**Phase I [Presence of Metabolic Activation]**			
	MNBN Cells	% MNBN Cells	MNBN Cells	% MNBN Cells
	P Values	P Values	Statistical Significance	Statistical Significance
VC vs T3 (0.5 µL/mL)	0.4790	0.4790	Non-Significant	Non-Significant
VC vs T4 (1 µL/mL)	0.7200	0.7200	Non-Significant	Non-Significant
VC vs T5 (2 µL/mL)	0.9405	0.9405	Non-Significant	Non-Significant
VC vs PC	0.0013	0.0016	Significant***	Significant***
Concentration of copper nanoparticle	**Phase II [Absence of Metabolic Activation]**			
	MNBN Cells	% MNBN Cells	MNBN Cells	% MNBN Cells
	P Values	P Values	Statistical Significance	Statistical Significance
VC vs T3 (0.5 µL/mL)	0.6209	0.6209	Non-Significant	Non-Significant
VC vs T4 (1 µL/mL)	0.3662	0.3662	Non-Significant	Non-Significant
VC vs T5 (2 µL/mL)	0.6209	0.6209	Non-Significant	Non-Significant
VC vs PC	0.0008	0.0008	Significant***	Significant***

T: treatment group; VC: vehicle control, PC: positive control, MNBN: micronucleated binucleated cells, P value is ≤ 0.01 (1% level) is accepted as higher (***) significant

4. DISCUSSION

The fast development of nano-products in recent years has made significant concerns about their safety. Nanotoxicity as a new branch of toxicity can be described as the study of the adverse effects of nanomaterials on living organisms and ecosystems. Genotoxicity is a vital aspect of studying the damage to genetic information within a cell.

In this study, genotoxic effect of biosynthesized CNPs was evaluated by using in vitro mammalian micronucleus test. Results of the study indicates that biosynthesized CNPs did not induce any toxic potential to form micronuclei in cultured human peripheral blood lymphocytes up to using 0.125 µL/mL – 2 µL/mL concentration of CNPs.

The genotoxicity of nanoparticles may be affected by the nanoparticle properties, including the composition, size, shape, surface properties, physicochemical specifications (pH, temperature, etc.), solubility and other factors such as the nanoparticle concentration, exposure time, cell type used and treatment regime.

5. CONCLUSION

From results of this study, it is concluded that CNPs did not show any potential to induce micronuclei or clastogenic or aneugenic potential in cultured human peripheral blood lymphocytes, both in absence and presence (2% v/v S9 mix) of the metabolic activation system under the present experimental conditions.

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Clinical and Experimental Health Sciences

Evaluation of Antidiabetic Activities of Scorzonera Species on Alloxan-Induced Diabetic Mice

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ABSTRACT

Objective: In Turkish folk medicine, different species of *Scorzonera* (Asteraceae) have been used in the treatment of various disorders. The study aimed to investigate antidiabetic activity of *Scorzonera* species and if rutin is the primary component responsible of this activity.

Methods: The extracts of aerial parts of *Scorzonera tomentosa*, *S. mollis ssp. szowitsii, S. suberosa ssp. suberosa, S. eriophora, S. acuminata, S. sublanata and S. cana var. jacquiniana* were used in the experiment. Additionally, rutin, which has been analysed and detected by using HPLC qualitatively and quantitatively in some *Scorzonera* species, was also tested for its antidiabetic activity in the same conditions. An alloxan-induced diabetic mice test model was used in order to verify antidiabetic activity. Antidiabetic activities of the 9 groups (n=5; each) were measured at four different times: before *Scorzonera* extracts and rutin treatment and after 1, 2 and 4 hours of treatments.

Results: *S. sublanata* extract exhibited the highest antidiabetic activity and at 100 mg/kg dose, it significantly reduced blood glucose levels measured after 1, 2 and 4 hours of treatments when compared to isotonic saline solution group (diabetic control group). *S. cana* var. *jacquiniana* extract also displayed notable decrease after 4 hours of treatment. Significant lowering effect on blood glucose level was also observed by treatment with rutin in all tested times at 100 mg/kg i.p. injection. According to the HPLC analyses the highest rutin content was determined in the *S. acuminata* aerial parts.

Conclusion: Rutin content and the antidiabetic activity of the plant extracts were not correlated as displayed in this present study. Further studies should be performed to reveal responsible compunds for antidiabetic activity.

Keywords: Alloxan, antidiabetic activity, diabetes, rutin, *Scorzonera*

1. INTRODUCTION

Diabetes is a chronic metabolic disorder characterized by stable high levels of blood glucose, which is known as the seventh leading cause of death in the world and affects 100 million people annually. Factors such as aging, obesity, physical inactivity, population growth and urbanization gradually lead to a steady increase in the number of patients with diabetes. In the year 2000, the prevalence of diabetes worldwide among adults was estimated as approximately 171 million. This number increased to 422 million in 2014, and it is expected that in year 2030, approximately 366 million people will suffer from diabetes worldwide (1, 2).

Diabetes is closely associated with other diseases such as hypertension, cardiovascular diseases, atherosclerosis, peripheral vascular diseases, and insufficient control generally results in many complications in the vascular system, kidneys, retinas, lenses, peripheral nerves and skin as well as overall quality of life (3, 4). Therefore, still there has been a growing interest in drug development for diabetes, especially phytochemicals derived from plants (5).

Scorzonera L. genus belonging to the Asteraceae family is widespread in more arid regions of Eurasia and northern Africa with about 160 species. *Scorzonera* species grow naturally by 52 species in Turkey and 31 of which are endemic (6). *Scorzonera hispanica*, the most common species of *Scorzonera* in Europe, which was consumed by the ancient Romans and Greeks has been cultured since the sixteenth century for medicinal purposes and as a vegetable. In the middle age, this plant was used as a tonic as well as for treatment of snakebites. Nowadays, especially in Belgium, France and Holland, it is cultured for consumption as a vegetable. *Scorzonera* species are used in the treatment of various diseases, including the common cold, appetite and pectoral problems, as a mucolytic, diuretic, antipyretic and diaphoretic (7–10). There is also information about its use for digestive problems as well as in the treatment and

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prevention of diabetes (11, 12). In Turkey, the young shoots and the leaves of some *Scorzonera* species are consumed either raw or cooked. In addition, they are recorded in Turkish folk medicine as treatments for atherosclerosis, high blood pressure, rheumatoid arthritis, kidney diseases and diabetes (7). Some of the species belonging to this genus are also used for their antidiabetic activities in Turkish folk medicine. These include *Scorzonera mollis* M. Bieb. subsp. *szowitzii* (DC.), *S. semicana* DC., *S. cinerea* Boiss., *Scorzonera latifolia* (Fisch. & C.A.Mey.) DC. var. *latifolia* (13–15). Although, there are various studies revealing the antidiabetic effect of rutin (16, 17), none of these studies has been found to be related to the antidiabetic activities of *Scorzonera* species selected for this present study.

As diabetes is a progressive disease, besides insulin, there is still a need to find effective compounds for the treatment. Medicinal plants are good sources of new drugs and many of the currently available drugs have been derived directly or indirectly from them (1, 2, 5). In light of the traditional usage of *Scorzonera* species for the treatment of diabetes, this study investigated aerial parts of some *Scorzonera* species including *S. acuminata, S. cana* var. *jacquiniana, S. eriophora, S. mollis* ssp. *szowitsii, S. sublanata, S. suberosa* ssp. *suberosa, S. tomentosa* for their potential antidiabetic activity. Moreover, this study tried to establish rutin as the responsible component for antidiabetic activity.

2. METHODS

2.1. Plant Material

Scorzonera species were collected from different parts of Anatolia, Turkey in their flowering time. Prof. Hayri Duman, a plant taxonomist from the Gazi University, confirmed the taxonomic identification of the plants. Voucher specimens are kept in the herbarium of Ankara University, Faculty of Pharmacy (Table 1).

Table 1. *List of Scorzonera species collected for the study*

Species	Collection Place	Herbarium number
S. acuminata Boiss.	Yumakli village, Cankiri, 2010	AEF 25938
S. cana (C.A. Meyer) Hoffm. var. *jacquiniana* (W. Koch) Chamberlain	Camlidere town, Ankara, 2008	AEF 23834
S. eriophora DC.	Cubuk town, Ankara, 2007	AEF 23832
S. mollis Bieb. subsp. *szowitsii* (DC.) Chamberlain	Kizilcahamam town, Ankara, 2006	AEF 23844
S. suberosa C. Koch subsp. *suberosa*	Pinarbasi town, Kayseri, 2006	AEF 23843
S. sublanata Lipschitz	Kizilcahamam town, Ankara, 2010	AEF 25937
S. tomentosa L.	Akdagmadeni town, Yozgat, 2005	AEF 23841

2.2. Preparation of the Extract

Dried and powdered aerial parts of the plant material were extracted with methanol:water (80:20 *v/v*) mixture at room temperature through 8 hours by continuous stirring which was followed by maceration during 16 hours for 3 days. Each extract was filtered from filter paper and concentrated to dryness under reduced pressure and low temperature (40–50°C) on a rotary evaporator to yield crude extracts.

2.3. Isolation of Rutin

Rutin was isolated from *S. acuminata* aerial parts. *S. acuminata* aerial parts (475.79 g) were extracted with methylalcohol at room temperature for 24 hours followed by extraction in ultrasonic bath for 1 hour. After 5 times this extraction techniques were applied, all extracts were filtered and evaporated under vacuum (40–50 °C) on a rotary evaporator. Obtained crude extract (72.74 g) suspended in water and extracted with petroleum ether and ethylacetate respectively by using liquid-liquid extraction techniques. The ethylacetate part (10.56 g) was subjected to column chromatography on silica gel and eluted with ethylacetate: methanol:water (100:13.5:10) mixture to obtain 52 fractions. Fraction 35 gave a yellow amorphous precipitate. After filtration, compound purity was checked with HPLC and MS, as well as with NMR (^{1}H – and ^{13}C-NMR), analyses were conducted for structure elucidation (Table S1). For HPLC analysis as well as antidiabetic activity assays, isolated compound which was detected in enough purity was used.

2.4. HPLC Analysis

HPLC analyses were carried out using Agilent LC 1100 model chromatograph (Agilent Technologies, California, USA). The diode array detector (DAD) was set at a wavelength of 254 nm and peak areas were integrated automatically by computer using Agilent Software. The chromatograms were plotted and processed using Agilent software. Separation was carried out using a Supelcosil (250 mm × 4.6 mm; 5 µm) column. The mobile phase was made up of acetonitrile (A) and water (B) in gradient elution: initial 0 min, A–B (8:92, v/v), then 0–10 min, linear change from A–B (8:92, v/v) to A–B (18:82), 10–20 min, there is isocratic flow A–B (18:82) and the linear gradient elution is from A–B (20:80) to A–B (22:78) with the range of 20–45 min. This was followed changing A–B (22:78) from 45 min to 55 min. The flow rate was 0.7 mL/min and column temperature were maintained at 40 °C. The sample injection volume was 10 µL.

2.5. Preparation of Standard Solutions and Calibration

Standard stock solution for rutin was prepared as 1 mg/mL. Rutin was weighed in 10 mL volumetric flask, dissolved in methanol:water (80:20) mixture and adjusted to the final volume separately. Six different concentration levels (0.01 mg/mL, 0.02 mg/mL, 0.05 mg/mL, 0.1 mg/mL, 0.2 mg/mL, and 0.5 mg/mL) were prepared by diluting the stock solution. Triplicate 10 µL injections were performed for each

standard solution. Peak area of each solution was plotted against the concentration to obtain the calibration curves.

2.6. Validation Procedure

2.6.1. Limit of detection and quantification

Limit of detection (LOD) and limit of quantification (LOQ) were established at a signal to noise ratio (S/N) of 3 and 9, respectively. LOD and LOQ concentrations were experimentally verified by 6 injections of rutin. LOD and LOQ levels were also determined as 0.13 and 0.45 µg/mL respectively.

2.6.2. Precision

Precision tests were performed by the evaluation of intra-day variations of the same standard solution of rutin at the LOQ level. The intra-day precision was determined by analyzing the same samples six times in a single day. The inter-day precision was determined three different days in triplicates of three different concentration injections. The results of precision tests were expressed as the relative standard deviations (RSDs) of the retention time (R_t) and peak area (P_a) for rutin.

2.7. Animals

Adult female Balb/C strain mice (22-30 g) obtained from Istanbul Medipol University Regenerative and Restorative Medical Center (REMER), were used for this experiment. The animals were housed in standard cages (48 cm × 35 cm × 22 cm) at room temperature (22±2 °C), with artificial light from 7.00 am to 7.00 pm, and provided with pelleted food and water ad libitum. Ethics Committee of Istanbul Medipol University approved the study protocol (01/02/2017-03).

2.8. Antidiabetic Activity Assay

An alloxan-induced test model was used to evaluate antidiabetic activity (3, 18, 19). Mice were kept without food for 18 hours before the alloxan treatment. Alloxan was applied in an isotonic saline solution by i.p. (intraperitoneal) administration (150 mg/kg of body weight) three times over a 48-hour period. Mice were kept without food following the last alloxan treatment, and blood glucose levels were measured. Previous studies have shown that blood glucose of healthy mice should be below 125 mg/dL (20, 21). In this study mice with blood glucose levels of 200 mg/dL and higher were subjected to further studies as diabetic animals. Diabetic mice were divided into nine groups in order to determine the antidiabetic activities of *Scorzonera* extracts (all extracts were dissolved in an isotonic saline solution). Animals were assigned to the following groups (n=5, for each): Group 1, the control group, received isotonic saline solution (ISS) 0.1 mL; other groups received *S. tomentosa, S. mollis* ssp. *szowitsii, S. suberosa* ssp. *suberosa, S. eriophora, S. acuminata, S. sublanata, S. cana* var. *jacquiniana* extracts 100 mg/kg of body weight i.p., respectively. This procedure was followed by rutin investigation (in total 9 animal groups [n=5, for each] was evaluated). Rutin was dissolved in an isotonic saline solution and administered 100 mg/kg of body weight i.p. The dosage of the plant extracts (22,23) and rutin (17) were determined according to the results of previous studies. Animals were sacrificed in the end of the study.

Following the administration of the tested extracts and rutin, blood samples collected from the tail at the 1st, 2nd and 4th hours were measured for their glucose concentrations, using an Accu-Check® sugar strip, employing the glucose-oxidase-peroxidase method.

2.9. Statistical Analyses

Statistical analysis was carried out using SPSS 22.0 software. The results were reported as mean ± standard error of mean (SEM). A one-way analysis of variance (post-hoc Dunnett-t test) was used for statistical analyses. Probability levels of less than 0.05 were considered as significant.

3. RESULTS

The aerial parts of *Scorzonera tomentosa*, *S. mollis* ssp. *szowitsii*, *S. suberosa* ssp. *suberosa*, *S. eriophora*, *S. acuminata*, *S. sublanata* and *S. cana* var. *jacquiniana* were tested for their antidiabetic activities for the first time in current study. *Scorzonera* extracts were tested for their potential antidiabetic activities using alloxan-induced diabetic animals. Blood glucose levels were measured at four different times: before *Scorzonera* extracts treatment and after 1, 2 and 4 hours of treatment. All of the measured levels of blood glucose are presented in Table 2. According to the results, *S. sublanata* was associated with significantly decreased blood glucose levels after 1, 2 and 4 hours of treatment; these were determined as follows: 207.20 ± 28.45; 200.80 ± 43.88 and 174.00 ± 38.77 mg/dL, when compared to the isotonic saline group (diabetic control group) ($p<0.05$). Additionally, *S. cana* var. *jacquiniana* caused notable decreasing in blood glucose levels after 4 hours of treatment as shown in Table 2, 232.80 ±49.80 mg/dL respectively when compared to the diabetic control group ($p<0.05$).

A similar research procedure was applied to diabetic mice, which received rutin compound as one of the constituents of some *Scorzonera* species. Rutin was found to have significant antidiabetic activity, and blood glucose levels decreased significantly to 307.20 ± 40.73 after 1 hour, 236.40 ± 50.48 after 2 hours and 190.60 ± 52.69 mg/dL after 4 hours of treatment compared with diabetic control group.

Phytochemical structure of the tested extracts was also investigated by HPLC analysis with this research. Mainly flavonoids as well as chlorogenic acid derivatives have been detected (Figure S1-S7). Furthermore, content of the rutin (Figure 1) which was isolated from the *S. acuminata* aerial parts, of the tested extracts were determined qualitatively and quantitatively (Table 3) to reveal whether there is a relationship between rutin content and antidiabetic activity. Calibration curve of the rutin was exhibited as Figure 2. According to the HPLC results, *S. acuminata* aerial parts contain higher amount of rutin than the other *Scorzonera*

species which was determined as 442.69±24.33 µg/mL. Rutin was also detected as 23.30±0.067 and 53.46 ±1.59 µg/mL for *S. eriophora* and *S. mollis ssp. szowitzii* aerial parts, respectively. *S. sublanata* contains rutin in trace amount while the remaining extracts were found to lack rutin (Table 3).

Table 2. *Blood glucose levels of Scorzonera treated alloxan-induced diabetic mice*

Groups	Blood sugar levels (mg/dL) [Mean ± Standart Error of Mean]			
	Before *Scorzorena* treatment	After *Scorzorena* treatment		
		1. hour	2. hour	4. hour
Control (ISS)	470.60±23.61	472.20±13.94	493.80±15.55	494.20±27.32
S. acuminata	479.20±28.16	466.80±29.13	429.20±42.48	415.60±71.70
S. cana var. *jacquiniana*	361.60±31.80	407.60±30.77	376.40±42.36	232.80±49.80*
S. eriophora	331.00±45.93	372.20±49.38	340.60±54.19	300.20±61.05
S. mollis ssp. *szowitsii*	412.00±27.60	413.00±33.02	430.40±38.63	342.20±55.24
S. suberosa ssp. *suberosa*	418.20±27.06	371.80±70.06	297.00±70.56	308.60±65.19
S. sublanata	312.20±31.27	207.20±28.45*	200.80±43.88*	174.00±38.77*
S. tomentosa	405.00±37.56	361.60±50.73	362.80±59.87	277.00±58.56
Rutin	368.80±42.19	307.20±40.73	236.40±50.48*	190.60±52.69*

*Post-hoc Dunnett-t test; *comparision with saline group (p<0.05)*

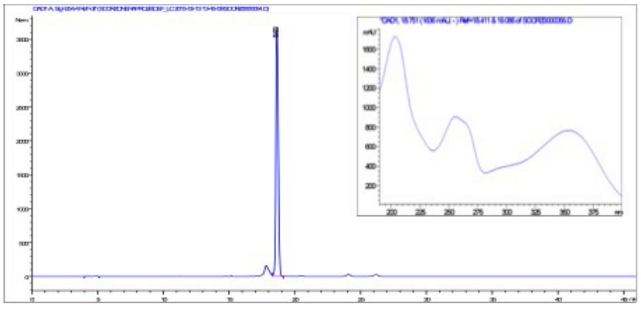

Figure 1. *HPLC chromatogram and UV spectrum of isolated rutin*

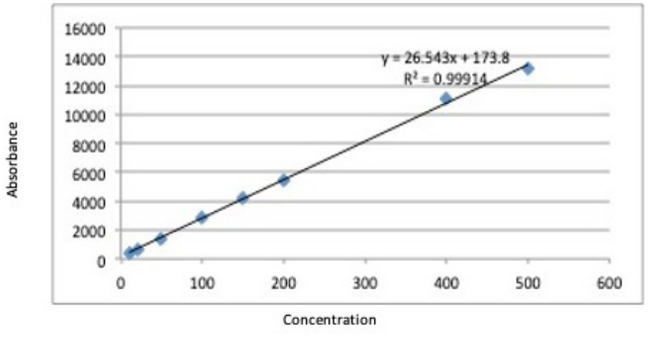

Figure 2. *Calibration curve of the rutin*

Table 3. *Rutin content of the tested Scorzonera species*

Species	Rutin (µg/100 mg plant material) [Mean ± Standart Deviation]
S. acuminata	442.69±24.33
S. cana var. *jacquiana*	-
S. eriophora	23.30±0.067
S. mollis ssp. szowitzii	53.46 ±1.59
S. suberosa ssp. *suberosa*	-
S. sublanata	tr.
S. tomentosa	-

tr: trace (under limit of quantification)

4. DISCUSSION

It is a well-known fact that *Scorzonera* species are beneficial in many medical indications such as, common cold (such as mucolytic, diuretic and antipyretic), wound healing and against acute hepatotoxicity, arteriosclerosis, kidney diseases, hypertension, diabetes mellitus, rheumatism, pain (7,12,22,23). Studies related to Turkish folk medicine have mentioned that many of the *Scorzonera* species have antidiabetic properties (7,13) and a single recent study has been reported *S. cinerea* radical leaves have an antidiabetic effect by inhibiting α-amylase and α-glucosidase and increasing the insulin level in diabetic rats (24). Although all that background a study that examines the antidiabetic properties, with an up-to-date method, of many *Scorzonera* species was not found. In the current study, *Scorzonera* species were evaluated for their antidiabetic potentials on alloxan induced diabetic mice in order to clarify their

traditional usage as antidiabetic medicinal plants. *S. sublanata* and *S. cana var. jacquiniana* were found to have blood glucose lowering effect. Rutin is one of constituents of various *Scorzonera* species which was reported to have potential antidiabetic activity previously (16). In present study antidiabetic activity of rutin was investigated and compared with previous studies. Furthermore, this study was tried to find a relationship with rutin content in *Scorzonera* species and antidiabetic activity.

Rutin is a common flavonoid that is broadly consumed from plant-derived beverages and foods as traditional and in folkloric medicine. Rutin is mentioned to exhibit fundamental pharmacological activities, including anti-inflammation, anti-diabetic, anti-adipogenic and anti-oxidation (25). For example, after rutin treatment to streptozotocin-induced diabetic rats for 45 days at 100 mg/kg resulted in decreasing plasma glucose and increasing insulin levels along with the restoration of glycogen content and increasing activities of carbohydrate metabolism enzymes (26). Histopathological studies have also revealed that rutin has protective effects for the pancreas; expansion of the islets and decreased fatty infiltration of the islets were also observed in treatments with rutin. In the same study, healthy rat parameters were not changed by rutin treatment (17). The underlying mechanism is thought to be decreasing carbohydrate absorption from the small intestine, enhancing insulin secretion by pancreatic *β*-cell stimulation, increasing tissue glucose uptake and inhibition of tissue gluconeogenesis (16).

Type 2 diabetes is the most prevalent form of diabetes mellitus and it is shown that rutin administration to these patients improve significant parametres such as plasma glucose, insulin level and and antioxidant status of liver (27–29). Rutin treatment displayed antidiabetic activity in high fat diet + streptozotosin induced diabetic rats by affecting many cellular events participating in the etiology of type 2 diabetes. Rutin descreased plasma glucose, increased body weight, decreased glycosylated hemoglobin significantly, and its effect is comparable to pioglitazone (a clinically effective new generation antidiabetic drug). Furthermore, rutin reduced intracellular pro-inflammatory cytokines such as IL-6 and TNF-alpha, which are intimately associated with insulin resistance, could result in improved insulin receptor substrate phosphorylation (29).

Diabetic patients with combined dyslipidemia are common; many diabetic patients have elevated low-density lipoprotein (LDL) and triglyceride as well as the reduced level of high-density lipoprotein (HDL) (28). The study that is mentioned above stated that after 3 weeks of rutin administration a significant decrease in plasma total cholesterol, total triglyceride, LDL, very low density lipoprotein (VLDL) and an increase in HDL levels were recorded in high fat diet + streptozotosin induced diabetic rats (29). Likewise, in another study after 3 weeks of oral administration of rutin to streptozotocin-induced diabetic rats, reduced levels of lipids in plasma and tissues, increased HDL-cholesterol, decreased LDL- and VLDL-cholesterol levels of plasma were observed (30). Moreover, atherosclerosis and cardiovascular diseases are more prevalent in diabetic patients (28,31). Studies are claimed that rutin also protect and improve myocardial dysfunction, oxidative stress, apoptosis and inflammation in the hearts of the diabetic rats (27).

In the current study it is shown that rutin has significant antidiabetic activity, this data is consistent with the literature. However, antidiabetic activity was not directly associated with rutin content of the plant extracts. *S. sublanata* and *S. cana* var. *jacquiniana* displayed remarkable antidiabetic activity while they contain rutin in low amount. According to the HPLC results all investigated *Scorzonera* species contain mainly phenolic structures as flavonoid derivatives, some chlorogenic acid derivatives and other constituents. From the chemical point of view, the *Scorzonera* species have been subjected to intensive studies which have led to the isolation of several types of compounds. Previously, a number of compounds such as dihydroisocoumarines, bibenzyl derivatives, flavonoids, lignans, stilbene derivatives, quinic and caffeic acid derivatives, sesquiterpene, sesquiterpene lactones and triterpenes have been isolated from the *Scorzonera* species and many of the isolated compounds have been identified as new structures (8–10,12,22–24,32,33). In current study none of the investigated *Scorzonera* species aerial parts except *S. cana* var. *jacquiniana* (Syn. *Podospermum canum*) have been analysed for their chemical contents. *S. cana* var. *jacquiniana* contains phenolic compounds and flavonoids as following; arbutin, 6´-*O*-caffeoylarbutin, cichoriin, 3,5-dicaffeoylquinic acid methyl ester, apigenin-7-*O*-*β*-glucoside, luteolin-7-*O*-*β*-glucoside, apigenin-7-*O*-*β*-rutinoside, isoorientin, orientin, vitexin, procatechuic acid, and 4-hydroxy-benzoic acid 4-(6-*O*-*α*-rhamnopyranosyl-*β*-glucopyranosyl) benzyl ester in its aerial parts (28). According to the phytochemical investigation results of the current study, also previous researches on *Scorzonera* species it could be suggested that the antidiabetic activities of these extracts based on synergistic effect of the phenolics, and especially flavonoids with other constituents.

Our findings should be interpreted in light of several limitations. The main limitation of the study that should be mentioned was the lack of healthy control and positive control groups. Since it is the first study that investigate antidiabetic activity for many evaluated Scorzonera species, authors have only planned to compare with diabetic control group. Moreover, antidiabetic activity was only evaluated with single parameter such as prolonged effect of the Scorzonera species on blood glucose was not evaluated, this can be counted as a limitation of the study. Regarding to preliminary results of this study, active extracts and possible other active contents will be evaluated in further detailed studies.

5. CONCLUSION

In conclusion, the antidiabetic usage of the *Scorzonera* species in Turkish folk medicine has been indicated by the current study. *S. sublanata* displayed the highest antidiabetic

activity, followed by *S. cana* var. *jacquiniana* aerial part extracts. Rutin, also exhibited the significant reducing effect on blood glucose levels in alloxan diabetic mice as one of the constituents of some *Scorzonera* species, in the current study. Rutin content was not found to be correlated with the activity of the tested extracts. On the other hand, HPLC analyses have revealed that the tested *Scorzonera* species contain many phenolic compounds and flavonoids which were not clarified in detail. Therefore, further well-designed studies are needed to enlight the responsible compounds as well as their mechanisms in the treatment of diabetes mellitus.

Conflict of interest

The authors declare that they have no competing interests.

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Clinical and Experimental Health Sciences

Antioxidant and Cytotoxic Activity Studies of Sulfur Containing Glycine Imine Derivatives MCF-7 and DLD-1 Cell Lines

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ABSTRACT

Objective: To investigate the antioxidant and cytotoxic activities of sulfur-containing glycine imine derivatives MCF-7 (human breast adenocarcinoma) and DLD-1 (colorectal adenocarcinoma) cell lines.

Methods: This study examined the antioxidant activities (25-200 µM) of sulfur-containing glycine imine derivatives via the DPPH, metal chelating and reduction methods. Furthermore the cytotoxic activity of MCF-7, MCF-12A (normal breast epithelial) and DLD-1, CCD-18CO (normal colon fibroblast) were examined with MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) and RTCA (Real-time Cell Analysis) assays.

Results: The antioxidant assay of the metal chelating activity showed significant results (71, 77 and 40% respectively) as compared to knowing synthetic antioxidant (trolox; 95.45, EDTA; 97.06 %). Reducing activity was found to be very low compared to the standard compounds. Compounds were shown to be moderated by DPPH (2,2-Diphenyl-1-picrylhydrazyl) activity, and the IC_{50} value ranged from 91 to 150. The IC_{50} values (100 µM) of the MTT and RTCA analyses were similar.

Conclusion: The study showed that the compounds had selective and significant antioxidant activities, and we also found that they had cytotoxic effects on MCF-7 and DLD-1 cells.

Keywords: Sulfur, Glycine Imine, MTT, RTCA, DPPH, Metal chelating

1. INTRODUCTION

According to the World Health Organization the most common types of carcinoma are breast, colon, prostate and lung cancer (1, 2). Breast cancer, which is a critical health problem worldwide, is the most common type of cancer among women and the deadliest cancer after lung cancer (3). The cancer with the third highest mortality rate and fourth highest incidence worldwide is cancer of the colon (4). Colorectal cancer accounts for approximately 10% of cancers in men and women (5).

Human life often involves disease, stressand various other difficulties. In order to protect ourselves against diseases and boost our immune systems, it is necessary for us to take supplemental foods in addition to the basic nourishment required to sustain life. Such protective or inhibitory compounds are called antioxidants. Antioxidants are substances that can suppress or reduce oxidative damage at low concentrations. For many years, antioxidants have been critical in pharmacological studies (6).

Free radicals and oxidants are produced physiologically and metabolically, and play a role in the human body's defence mechanism. These mechanisms depend on cell and tissue type and act antagonistically or synergistically. When produced in large quantities, they can seriously disrupt the structure of biological substrates such as proteins, lipids, lipoproteins and deoxyribonucleic acid (DNA). They have many different potential effects on cells and they easily emerge as anti-cancer. They have an effect on the cell cycle. As a result of this, they prevent cell deathin aging, apoptosis and necrosis and change the anti-cancer mechanism. They cause increased proliferation, angiogenesis and metastasis and suppression of apoptosis. When ROS (reactive oxygen species) increases in concentration, it has a toxic effect and causes chronic diseases. It has been stated that oxidative damage can be prevented by antioxidants in the cell, possibly at low concentrations (7).

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Glutathione (GSH), consisting of glutamic acid, cysteine and glycine, is an antioxidant tripeptide with a reducing form in the cell (8). High oxidative stress and low antioxidant capacity have relationship with glutathione antioxidant systems (9). An important function of glycine is also partially to prevent the antioxidant capacity, and to reduce the oxidative stress which occurs in heavy metals, peroxide or other medicinal toxins (10). Glycine imines with biologically significant activity are also relatively antidiabetic (11).

Glycine suppresses the formation of angiogenesis signals and the growth of tumor cells in endothelial cells. Glycine is seen as a promising addition to targeted cancer treatments used as a standard (12).

Sulfur is a major constituent of biological systems. This element is ordinarily integrated into proteins as a redox active cysteine residue. It is found in the structure of vital antioxidant molecules and glutathione, thioredoxin and glutaredoxin, which are necessary for life. Sulfur exists in all living cells and is a valuable component of many proteins necessary for a healthy life (13).

Many researchers have reported that glycine is protective against hepatotoxicity and nephrotoxicity caused by anoxia, ischemia and various xenobiotics (10, 14).

MSM (Methylsulfonethane) is a sulfur-containing organic compound known to have no toxic properties. MSM has been shown to significantly reduce cell viability in human breast cancer cells (15). Cases have been reported where proliferation in various cancer cells was suppressed in treatments with natural sulfur (16). It has also been reported for the first time that cell proliferation was inhibited by an inorganic sulfur in a study on breast cancer (17).

Here, we report the design and testing of the bis(alkylthio) imines, which are key compounds, used for the antioxidant (18) and anti-lipid peroxidation system (19).

Our total synthesis of (Figure 1) began with the preparation of glycine ethyl ester, the key amino acid fragments required for the synthesis of bis-sulfur substituted glycine ethyl ester via the known procedure (18, 19).

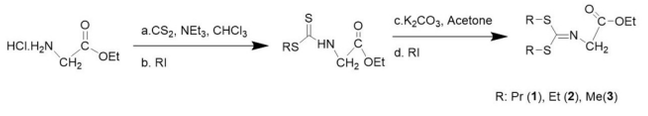

Figure 1. *Synthesis of ethyl 2-((bis(alkylthio)methylene)amino) acetate*

In this study, the antioxidant activities (25-200 µM) of the sulfur-alkyl side chains in glycine imine derivatives were determined via the free radical scavenging, reducing and metal chelating methods.

In addition, the cytotoxic activity of MCF-7, MCF-12A and DLD-1, CCD-18CO was examined by MTT and RTCA assays with varying concentrations (25-100 µM) of compounds for 24hr. The significance of differences between the data sets in the MTT assay was analyzed statistically by ANOVA for four cell lines using the SPSS 20.0 program.

2. METHODS

2.1. Chemistry

All commercial reagents and solvents were purified by standard procedures prior to use. The silica gel (100–200 Mesh) used for column chromatography were supplied and thin layer chromatography used for Merck silica gel 60 F254 precoated plates (0.25 mm). The ^{1}H and ^{13}C – NMR chemical shifts were measured relative to $CDCl_3$ on a Bruker-Spectrospin, Avance spectrometer. Mass spectrums were taken Agilent 7890-GCMS and the mass spectra showed the expected molecular ion peaks.Compounds **I**, **II** and **III** were prepared by known methods (20).

General Synthesis Procedure: Glycine ethyl ester hydrochloride (6 mmol) and carbon disulphide (6.2 mmol)in chloroform (50 mL) were mixed before triethylamine (12.4 mmol) was added dropwise at room temperature. After 1h alkyl iodine (7 mmol) was added, and then the solution was refluxed. After the reaction was completed, first it was extracted with water twice (30 mL), and secondly with ether and water. The residue was dissolved in acetone (20 mL) and alkyl iodine (6 mmol), then potassium carbonate (7 mmol) was added. The reaction solution was refluxed for 1h. The reaction mixture was extracted and residue was evaporated to obtain a light-yellow oil.

Compound I: yield 75%; oil; selected NMR values ^{1}H NMR (250Hz, $CDCl_3$)2.78, 2.82 (s, 6H; – SCH_3), 4.33 (t, *J* 7.4 Hz, 2H, – OCH_2) ppm.**Compound II**: yield 68%; oil; selected NMR values ^{1}H NMR (250Hz, $CDCl_3$) 3.35-3.48 (m, 4H, – SCH_2), 4.25 (t, *J* 7.4 Hz, 2H, – OCH_2) ppm.**Compound III**: yield 70%; oil; selected NMR values ^{1}H NMR (250Hz, $CDCl_3$) 3.37-3.51 (m, 4H, – SCH_2), 4.00-4.12 (m, 2H, – OCH_2)ppm.)

2.2. Antioxidant Activity

Doses of the compounds were studied at concentrations of 25, 50, 100, 150 and 200 µM. In addition, the antioxidant activity of the compounds was determined using free radical scavenging (21), metal chelating (22), and reducing (23).

2.2.1. Free Radical Scavenging (DPPH) Activity Assay

The free radical scavenging activity assay was performed according to (21). Three replicates of the compounds with different concentrations were studied. Butylated hydroxytoluene (BHT), Trolox and Butylated hydroxyanisole (BHA) (25-200 µg/mL) were used as standard. The activities of the compounds at 517 nm absorbance were measured. The free radical scavenging activity (%) = $[(A_0 – A_1) / A_0] \times 100$. ($A_0$ = control absorbance and A_1 = sample solution absorbance).

2.2.2. Metal Chelating Activity (Fe^{+2}Ions Chelating Activity) Assay

The chelate activity assay of the compounds was performed according to (22). Butylated hydroxytoluene (BHT), Trolox and Ethylenediaminetetraacetic acid (EDTA) (25-200 µg/mL) were used as standard. The activities of the compounds at 562 nm absorbance were measured. The metal chelating activity (%) = $[(A_0 – A_1) / A_0] \times 100$. ($A_0$ = control absorbance and A_1 = sample solution absorbance).

2.2.3. Reducing Activity Assay

The reducing activity of the compounds was performed according to (23). EDTA, Trolox and Gallic were used as standard (25-200 µg/mL). The activities of the compounds at 700 nm absorbance were measured and activity results were evaluated by absorbance measurements.

2.3. Cytotoxic Activity

2.3.1. Cell Lines

In *in vitro* cell culture studies MCF-7 (ATCC® HTB22™) (human breast adenocarcinoma), MCF-12A (ATCC® CRL-10782™) (normal breast epithelium), DLD-1 (colon cancer) (ATCC® CCL221™) and CCD-18CO (normal colon epithelium) (ATCC® CRL-1459™) cell lines were used. Cancer cell lines indicated with ATCC numbers were obtained from the cell culture collection at Gebze Technical University. The study did not include human subjects, and ethics committee approval was therefore not required.

2.3.2. Cell Culture

In the study, MCF-7, MCF-12A, DLD-1 and CCD-18CO cell lines DMEM (Dulbecco's Modified Eagle Environment), EMEM (Eagle's Minimum Essential Medium) and RPMI-1640 (Roswell Park Memorial Institute) mediums containing 10 % fetal bovine serum (FBS) and/or horse serum were cultured with a 5 % CO_2 incubator. These were produced by incubation for 24 hours. Cells were grown to 80% saturation, followed by washing with phosphate buffered saline (PBS). 1X Trypsin-EDTA was used to pass the cells (24).

2.3.3. MTT Assay

MTT analysis is a method in which cell proliferation is determined based on the colorimetric measurement of color change occurring in cells incubated with enzymatic activity due to formazan dyes or MTT reduction. The cytotoxic or proliferative effects of any therapeutic agent to be used on this cell can be determined by this method.

The analysis of the possible cytotoxic effect of the sulfur-containing glycine imine derivatives compounds on MCF-7, MCF-12A, DLD-1 and CCD-18CO cell lines was performed according to the manufacturer's instructions for use with the MTT (Sigma). The resulting color change is caused by the reduction of the tetrazolium salt in activated cell mitochondria of yellow-colored formazan salts. The absorbance value of these compounds is proportional to the metabolic activity.

100 µl of RPMI medium was prepared in a 96-well plate ($1x10^4$ / well) 1 day before the MTT assay was applied and the wells were cultured. The microplate was kept in an incubator at 37 °C and 5% CO_2 for 24 h to allow the cells to adhere to the surface. Sulfur-containing glycine imine derivatives compounds prepared in serial dilutions (1.56-3.12-6.25-12.5-25-100 µM) were added to the wells after 24h of incubation. After incubation, 100 µl of MTT (5 mg/mL) solution was added to the cells for 2h and then 100 µL of DMSO (dimethyl sulfoxide) was added to the wells to terminate the reaction. Incubated cells, microplate reader spectrophotometer and 570 nm absorbance value measurements were taken in three replicates. The dose and % cell viability curve were determined with the help of the Microsoft Excel program and 50% suppressive concentration (IC_{50}) was calculated by logarithmic slope graph (Cytotoxicity = test absorbance value / control absorbance average value x 100). Experiments were repeated three times for each compound concentration and solution. According to these results, the cytotoxic effects of sulfur-containing glycine imine derivatives compounds in cell lines were evaluated and the dose-response relationship was defined (25).

Statistics

The significance of the differences between the data for the MCF-7 and MCF-12A, DLD-1 and CCD-18CO cell lines was analyzed statistically with ANOVA using SPSS 20.0 program. The conclusions were indicated as ID50 ± SE (standard error of the mean) for cell lines.

2.3.4. Real-Time Cell Analyzer (iCELLigence) Assay

The study used MCF-7, MCF-12A, DLD-1 and CCD-18CO cells. The mediums of DMEM, EMEM and RPMI-1640 were incubated at 37 °C and 5% CO_2. 10% FBS and/or horse serum was added to the media. $1x10^4$ cells / 400 µL medium were loaded into the E-plates of the iCELLigence RTCA device. Cells were left to adhere to the E-plates for 24 hours, 25-50-100 µM concentrations of the compounds were added to the E-plates at the end of 24 hours and monitored in real time for 24 hours. The viability of the cells was calculated by comparing the 25-50-100 µM concentrations of the compounds according to the negative control (26).

3. RESULTS

3.1. Chemistry

The three bis(alkylthio)imines, ethyl 2-((bis(methylthio) methylene)amino)acetate, ethyl 2-((bis(ethylthio)methylene) amino)acetate, and ethyl 2 ((bis(propylthio)methylene) amino)acetate, which were given the names **I**, **II**, and **III** respectively. were synthesized according to the methods found in the literature (18, 19).

3.2. Antioxidant Activity

The DPPH activity **III** › **II** › **I** and the metal chelating activity **II** › **I** › **III** were determined. The reductive activity results were found to be very low compared to standard compounds. The IC_{50} value is defined as the half inhibition concentration in inhibiting antioxidant activity. Antioxidant activities have an IC_{50} value of 91 to 150 (Table 1-3) (Figure 2-4).

Table 1. *DPPH activity (free radical scavenging) and IC_{50} of compounds* ***I, II and III***

Compounds	Concentration (µM)					
	25	50	100	150	200	IC_{50}
I	36.727	41.467	44.495	51.376	53.975	150.01
II	29.969	51.834	53.975	61.071	63.761	91.733
III	7.4159	46.116	63.180	68.455	78.746	96.986
BHT	61.840	74.570	78.360	85.620	94.690	
Trolox	59.590	70.520	72.540	81.360	90.570	
BHA	50.450	53.970	60.990	76.430	82.470	

Table 2. *Chelating activity of Metal (Fe^{2+} ions chelating activity) of compounds* ***I, II and III***

Compounds	Concentration (µM)				
	25	50	100	150	200
I	42.933	53.069	62.961	67.567	71.662
II	12.043	31.603	42.311	56.298	77.356
III	17.198	26.615	29.500	33.956	40.388
BHT	77.000	81.250	83.090	94.200	97.500
Trolox	54.270	67.840	74.250	81.530	95.450
EDTA	70.850	72.320	79.470	81.090	97.060

Table 3. *Reducing activity of compounds* ***I, II and III***

Compounds	Concentration (µM)				
	25	50	100	150	200
I	0.050	0.080	0.104	0.113	0.123
II	0.137	0.155	0.213	0.212	0.238
III	0.168	0.216	0.274	0.267	0.317
EDTA	2.178	1.503	3.865	3.925	4.355
Trolox	1.506	2.765	2.537	3.689	2.701
GALLİC	1.273	1.511	1.876	3.597	3.756

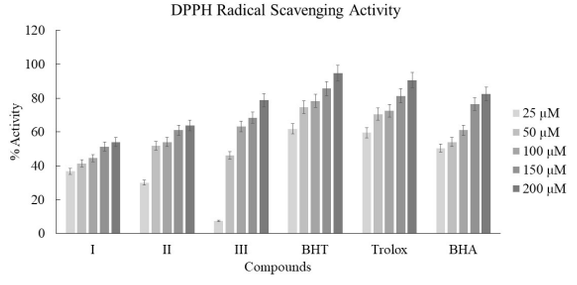

Figure 2. *DPPH activities of compounds and BHT, Trolox and BHA as standards*

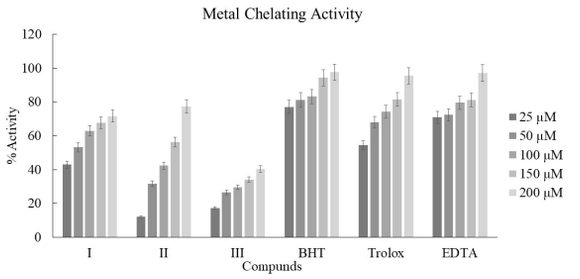

Figure 3. *Metal activity of compounds and BHT, Trolox and EDTA as standards*

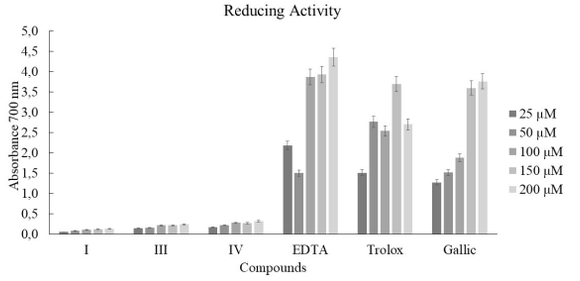

Figure 4. *Reducing activities of compounds and EDTA Trolox and Gallic as standards*

3.3. Cytotoxic Activity

Three compounds were investigated by comparison of two normal and two cancer cell lines. Compounds were evaluated for their cytotoxic effects against MCF-7, DLD-1 cancer cell lines and MCF-12A, CCD-18CO normal cell lines. For this purpose, cells were cultured with compounds (**I-II-III**) at 37 °C for 24 h at concentrations of 25, 50, 100 µM (the best doses obtained in MTT assays). Cell viability was measured at 570 nm, the optical density (OD) value. Cytotoxic activity was calculated using the formula (%) = (average experimental OD value / average control OD value) x 100 %. Values were stated as 50 % inhibitory concentration (IC_{50}). The compounds synthesized in the study are particularly noteworthy as anti-cancer agents.

Based on the MTT assay the cytotoxic activities of the compounds for MCF-7 cell line were determined as **II** › **III** › **I**. The cytotoxic activities of the compounds for DLD-1 cell line were determined as **II** › **I** › **III**. In fact, the cytotoxic activity of the compounds **I** and **III** were approximately the same (Figure 5 and 6).

Based on the RTCA assay the cytotoxic activities of the compounds for MCF-7 cell line were determined as **II** › **III** › **I**. The cytotoxic activities of the compounds for DLD-1 cell line were determined as **II** › **III** › **I**. It can even be said that compound **I** did not affect cell proliferation (Figure 7 and 8).

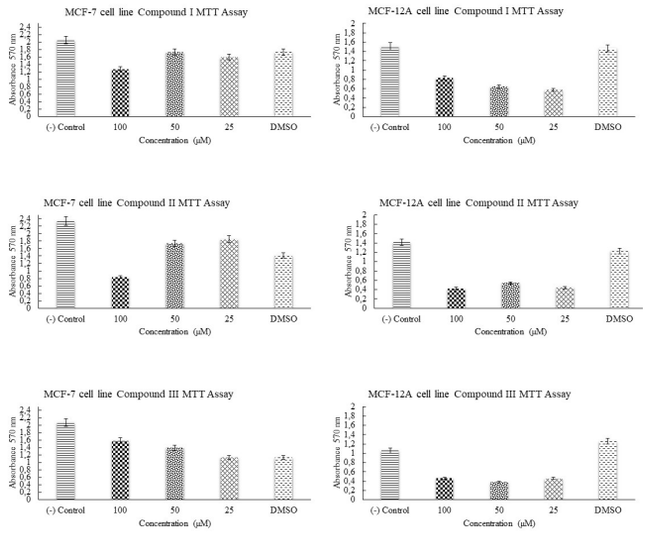

Figure 5. *MTT assay of increasing concentrations of compounds on MCF-7 and MCF-12A cells.*

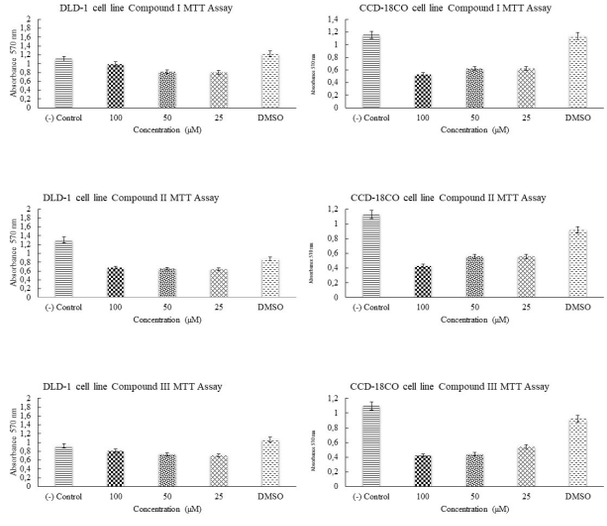

Figure 6. *MTT assay of increasing concentrations of compounds on DLD-1 and CCD-18CO cells.*

Compound **II** exhibited IC_{50} values approximately better than compound **I** and **III** under the same conditions, indicating improved selectivity for cancer cells.

Also, MCF-12A and CCD-18CO were found to affect cell proliferation and reduce cell proliferation in normal cell lines. Compounds **I**, **II** and **III** exhibited moderate cytotoxicity toward the normal cell lines.

In addition, cytotoxic activities (MTT and RTCA assays) of sulfur-containing glycine imine derivatives were compared with antioxidant activities, and the cytotoxic activities were found to be similar to the antioxidant activity results.

The MTT assay concentrations of compounds **I**, **II** and **III** in four cell lines were compared. As a result of ANOVA analyses (SPSS 20.0), there was a significant difference between the compounds and concentrations. Post hoc analyzes were done to understand the relationships between the groups. First, the homogeneity of the variance test result was examined and tests were applied depending on these results. Games-Howell and Tukey tests were used for multiple comparisons of compounds **I, II** and **III** in MCF-7 and MCF-12A, DLD-1 and CCD-18CO cell lines *(p <0.005)* (Table 4 and 5) (Figure 9 and 10).

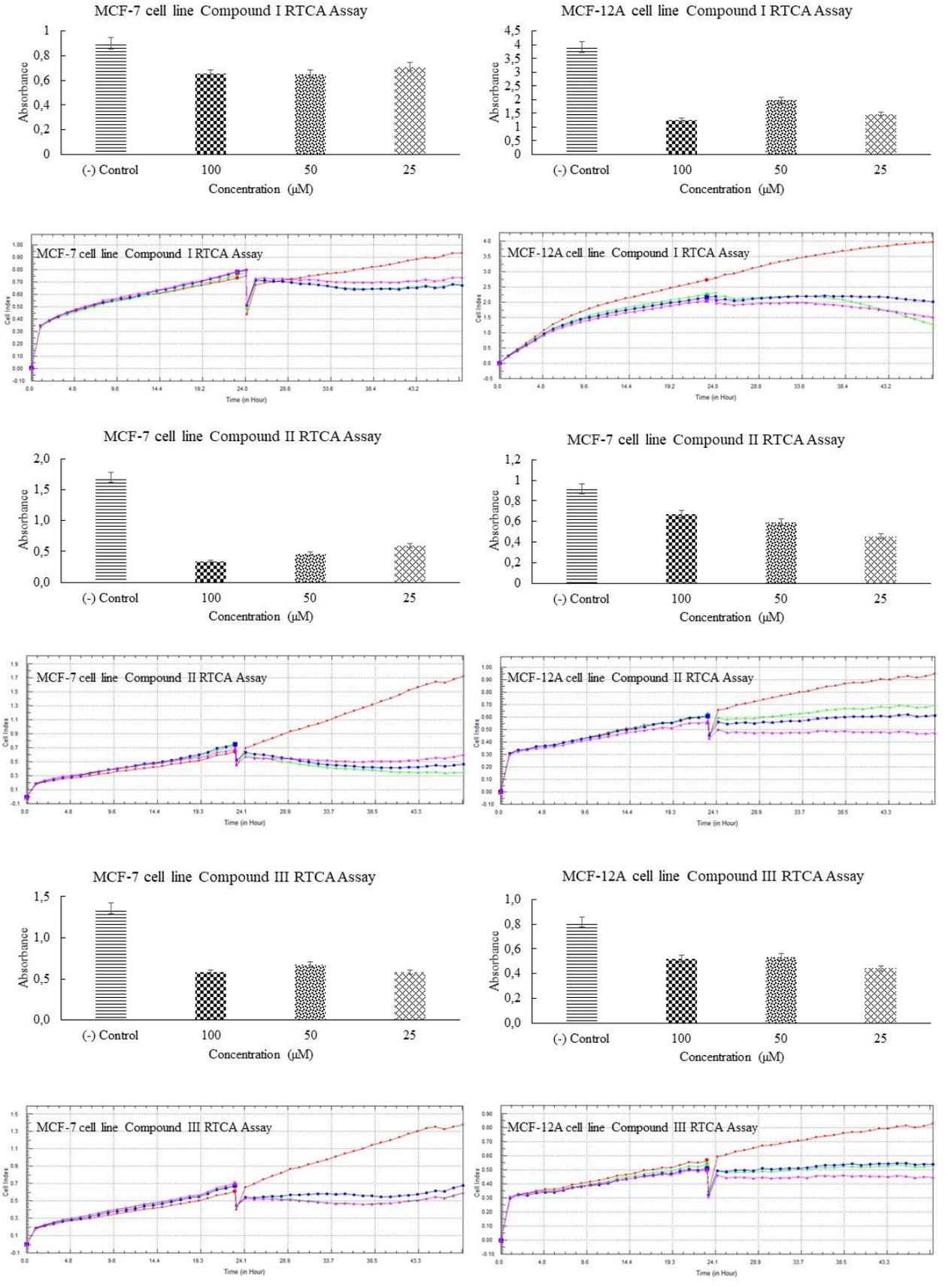

Figure 7. *Real-time cell analysis of increasing concentrations of compounds on MCF-7 and MCF-12A cells. (Red; Negative control, Green; 100 µM, Blue; 50 µM, Pink; 25 µM).*

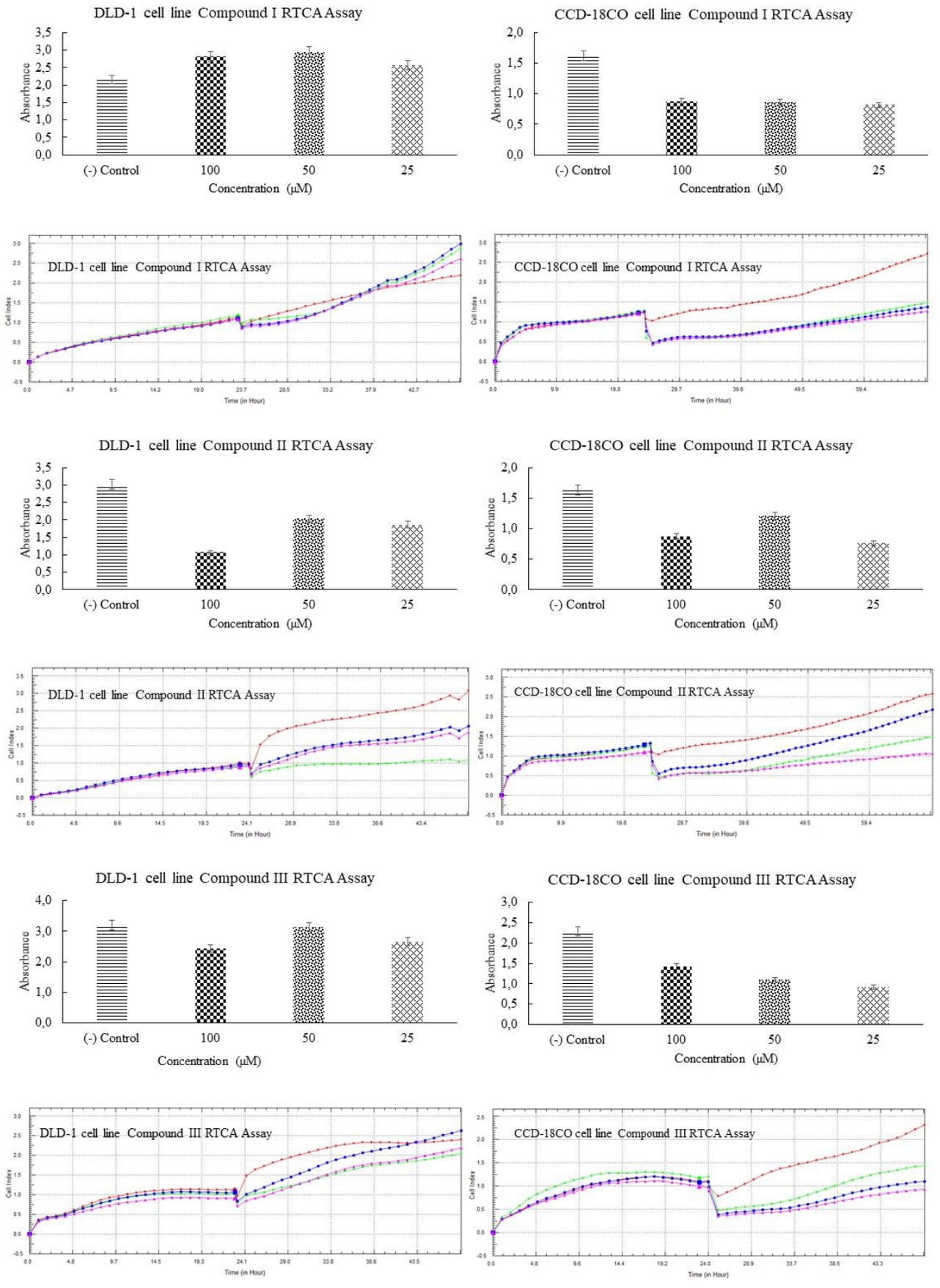

Figure 8. *Real-time cell analysis of increasing concentrations of compounds on DLD-1 and CCD-18CO cells. (Red; Negative control, Green; 100 µM, Blue; 50 µM, Pink; 25 µM).*

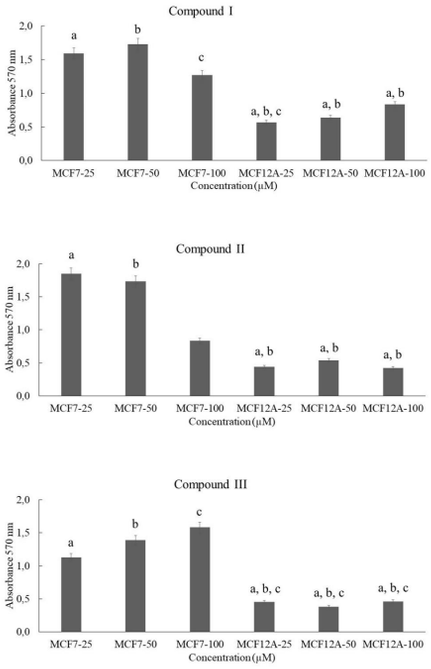

Figure 9. *Comparison between MCF-7 and MCF-12A cell lines in terms of cell viability. a, b, c indicate significant difference for compounds*

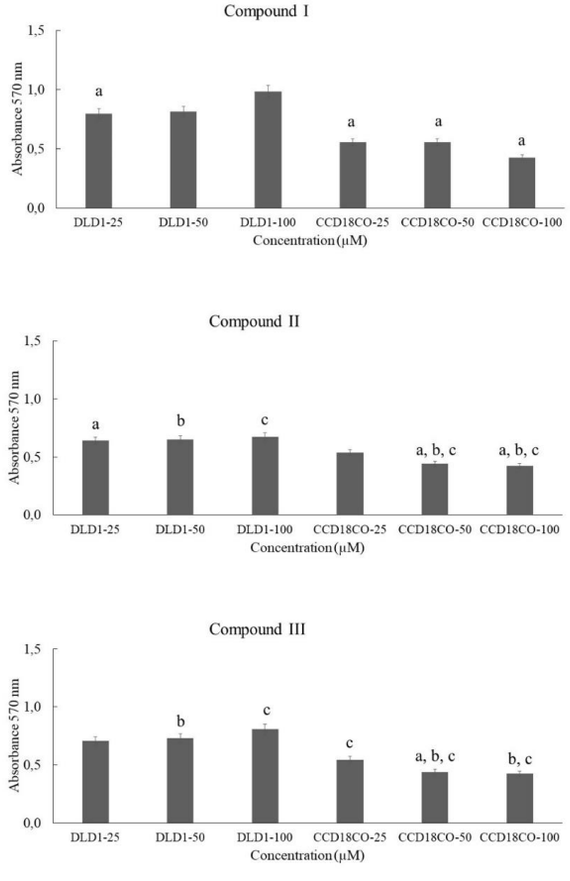

Figure 10. *Comparison between DLD-1 and CCD-18CO cell lines in terms of cell viability. a, b, c indicate significant difference for compounds*

Table 4. *In vitro cell viability of the cell lines were investigated by MTT assay after treating MCF-7 and MCF-12A cell lines with varying concentrations of compounds for 24 hr. The acquired data were evaluated using SPSS 20.0 analysis and defined as IC_{50} values.*

Compounds	Concentration (µM)	Cell lines ID50 [µm] ± SE	
		MCF-7	MCF12A
I	25	1.592±0.143	0.568±0.095
	50	1.731±0.123	0.64±0.058
	100	1.272±0.255	0.832±0.129
II	25	1.848±0.243	0.439±0.014
	50	1.733±0.040	0.539±0.025
	100	0.835±0.046	0.424±0.008
III	25	1.130±0.179	0.454±0.015
	50	1.391±0.202	0.381±0.010
	100	1.583±0.214	0.462±0.018

Table 5. *In vitro cell viability of the cell lines were investigated by MTT assay after treating DLD-1 and CCD-18CO cell lines with varying concentrations of compounds for 24 hr. The acquired data were evaluated using SPSS 20.0 analysis and defined as IC_{50} values.*

Compounds	Concentration (µM)	Cell lines ID50 [µm] ± SE	
		DLD-1	CCD-18CO
I	25	0.799±0.022	0.556±0.037
	50	0.816±0.096	0.556±0.029
	100	0.985±0.075	0.427±0.004
II	25	0.641±0.057	0.537±0.008
	50	0.649±0.065	0.441±0.006
	100	0.673±0.039	0.423±0.003
III	25	0.706±0.076	0.544±0.003
	50	0.731±0.032	0.440±0.003
	100	0.809±0.031	0.423±0.011

4. DISCUSSION

A study was conducted on the design, characterization and biological activity of the glycine derivatives, and the results showed cytotoxicity to 293T (27). In the current study, three different compounds derived from glycine imine containing sulfur were synthesized. Antioxidant and cytotoxic activity studies of the compounds were designed and carried out using five different methods. The compounds were shown to exhibit high DPPH and Metal chelating activity and high cytotoxic activity in MCF-7 and DLD-1 cells. It has been reported that glycine has protective properties against the state of shock caused by blood loss or endotoxin, reduces alcohol levels in the stomach and provides healing against alcohol-induced hepatitis. It has also been determined that it reverses the liver damage caused by hepatotoxic drugs, suppresses programmed cell death (apoptosis) and reduces the nephrotoxicity caused by the drugs, and prevents hypoxia and the formation of free radicals in the kidney (28). In the current study, three different antioxidant activity studies and two different cytotoxic activity studies were performed using two different cell lines. It was observed that glycine derived compounds exhibit anti-cancer activity in MCF-7 and DLD-1 cells. Apoptotic activity studies of glycine derived compounds are planned as the next step. MSM, a natural compound containing sulfur, has been reported to significantly reduce

human breast cancer cells (15). In the current study, the anti-tumor effects of the compounds were investigated using breast and colon cancer cells. It was determined that the compounds show high cytotoxic activity in breast and colon cancer cells. It has been reported that many patients with cancer have been given antioxidant supplements during cancer treatment to alleviate toxic effects and that these supplements contribute to an improvement in their condition, even if only to a small extent (29). Given that antioxidant and cytotoxic activity studies form the basis of cancer studies, the original compounds and methods used in this study confirm this purpose.

The information in the literature on synthesized compounds and biological activity studies is limited. Therefore, studies of compounds and activity are rare. The current study shows that sulfur-containing compounds in MCF-7 and DLD-1 cell lines have both antioxidant and cytotoxicity activities. In terms of comparing the compounds' antioxidant and cytotoxic activities, it was found that the compounds gave parallel results. Compound **II** had prominent selective properties in both the cytotoxic activity studies and the antioxidant studies.

5. CONCLUSION

We evaluated the antioxidant activities as DPPH, metal chelating, and cytotoxic activity using the MTT and RTCA methods of methyl, ethyl, propyl analogs of sulfur-containing glycine imine derivatives for describing the effect of a sulfur-linked alkyl group.

As a result of the study, it is thought that compound **II** has anti-oxidative and cytotoxic properties, preventing cell proliferation and reducing oxidative damage.

It is difficult to find information or studies about sulfur-containing glycine imine derivatives in the literature. This study is specific both for derivatives of compounds and cancer cells.

We concluded that this new drug candidate could reduce oxidative damage and had an effect on MCF-7 and DLD-1 cancer cells.

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Clinical and Experimental Health Sciences

Diagnostic Value of Minor Salivary Gland Biopsy: A Retrospective Study

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ABSTRACT

Objective: Minor salivary gland biopsy is an adjunctive diagnostic method which is widely used and its diagnostic value is still controversial. It is mainly used in sarcoidosis and Sjögren's Syndrome. This study aims to identify the contribution of histopathological diagnosis to clinical diagnosis and to supply information to literature.

Methods: Minor salivary gland biopsy was carried out on the patients with early diagnosis of sarcoidosis or Sjögren's Syndrome who were referred from the hospital clinics of Istanbul University Faculty of Medicine, to the Oral and Maxillofacial Surgery Department of the Faculty of Dentistry. 100 patients between the ages of 18-65 were performed lower lip MSGB, then patients were called, the questions were asked and the answers were evaluated.

Results: The presence of dry mouth was statistically higher in patients with Sjögren's Syndrome than histopathologically normal patient and sarcoidosis patients. In patients with Sjögren's syndrome, the presence of dry eyes was found to be statistically significantly higher than the others. A statistically significant difference of clinical diagnosis distribution was observed between the patients with normal histopathological findings and patients with mild inflammation infiltration, granulomatous sialadenitis and lymphocytic sialadenitis.

Conclusion: Minor salivary gland biopsy is an easy and trusted method for establishing sarcoidosis and Sjögren's Syndrome diagnosis and follow-up. Studies reveal that as more knowledge about disease immunology is collected and more sensitive techniques for interpretation of saliva and other serological markers are developed, less invasive or noninvasive techniques will come into question. Contemporarily, it will continue to be one of the main diagnostic tests for adjunctive diagnostic of multisystemic chronic diseases.

Keywords: minor salivary glands, biopsy, sarcoidosis, amiloidosis, Sjogren's Syndrome

1. INTRODUCTION

Minor salivary gland biopsy (MSGB) is an adjunctive diagnostic method which is widely used in internal medicine, rheumatology, thoracic medicine and ophthalmology. The MSGB technique was first used in 1966 for the diagnosis of Sjögren's Syndrome; but with Chisholm and Mason's publication in 1968, it began to be used routinely (1). After that publication, MSGB has been included in almost all classification criteria due to identification of lymphocytic infiltration of the salivary glands. With the introduction of the American European Consensus standards in 2002, the value of MSGB has risen (2,3).

The main diseases that may require minor salivary gland biopsy are sarcoidosis, Sjögren's Syndrome, amyloidosis and other infiltrative diseases. Despite the simplicity of the technique, MSGB is not preferred in daily practice because it is considered as an invasive technique and has variable complication rates according to the technique that is used (4). Although it is a controversial technique, it is among the diagnostic criteria of certain diseases. Our purpose in this article is to contribute to defining the role of MSGB in future diagnostic criteria.

2. METHODS

Between 01.06.2017 and 01.06.2018, lower lip MSGBs were conducted on 100 patients between 18-65 years old with early diagnosis of sarcoidosis or Sjögren's Syndrome referred from the hospital clinics of Istanbul University Faculty of Medicine to the Oral and Maxillofacial Surgery Department of the Faculty of Dentistry. Evaluation of MSGB findings is done by expert histopathologists in the Department of Pathology, Istanbul Medicine Faculty, Istanbul University. The stitches were removed after 7 days, and a retrospective analysis was made with a pre-prepared survey. This project

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has been reviewed and approved by the Ethical Committee of Istanbul University, Faculty of Dentistry (2017/13).

2.1. Operation Procedure

Prior to the MSGB procedure, the patient's lower lip is palpated for the appropriate incision area. In order to prevent damage to the structure of the samples to be taken, local anesthesia is performed as a ring blockage. The lip ruled out and, an incision (approximately 1 cm) is made superficially on the mucosa in order not to damage the deep tissues, including nerves and muscles. Since it is important to collect enough salivary glands for diagnosis, five minor salivary glands are taken and kept in 10% formaldehyde solution.

2.2 Histopathologic Evaluation

The common findings in MSGBs focal lymphocytic sialoadenitis, with a focus score >1, defined as the number of lymphocytic foci (which are adjacent to normal-appearing acini and contain >50 lymphocytes) per 4 mm^2 of glandular tissue.

2.3. Statistical Evaluation

In this study, statistical analysis was done by NCSS (Number Cruncher Statistical System) 2007 Statistical Software (Utah, USA) package program. In addition to descriptive statistical methods (mean, standard deviation), one-way analysis of variance between groups, Tukey multiple comparison test for sub-group comparisons, independent t-test for comparison of dual groups, and chi-square test for comparison of qualitative data were used. The results were evaluated at $p<0.05$ level.

3. RESULTS

There was no statistically significant difference between the mean age and sex of patients with normal histopathological diagnosis and with sarcoidosis and Sjögren's Syndrome ($p>0.05$). There was no statistically significant difference between the patients with normal histopathological diagnosis and patients with sarcoidosis and Sjögren's Syndrome in terms of allergy, smoking, presence of rheumatic disease, presence of nodules in the lung, incision design, and presence of oral aphthae ($p>0.05$).

The presence of dry mouth in the group with histopathologic diagnosis of Sjögren's Syndrome was statistically higher than groups with normal histopathology and sarcoidosis ($p=0.036$) and the presence of dry eyes in the group with histopathologic diagnosis of Sjögren's Syndrome was statistically higher than groups with normal histopathology and sarcoidosis ($p=0.0001$) (Figure 1). Mild inflammatory infiltration was found in patients with normal histopathological diagnosis and lymphocytic sialenitis was significantly higher in the Sjögren's Syndrome group.

Histopathological results of the minor salivary glands were classified as normal, mild inflammatory infiltration, granulomatous sialadenitis and lymphocytic sialadenitis. There was no statistically significant difference between the mean age and gender distribution of these patients ($p>0.05$). No statistically significant difference was observed between patients with normal histopathology, mild inflammatory infiltration, granulomatous sialadenitis and lymphocytic sialadenitis in terms of allergy, smoking, dry mouth, dry eyes, rheumatism, presence of nodules in the lung, presence of oral aphthae and incision distribution ($p> 0,05$).

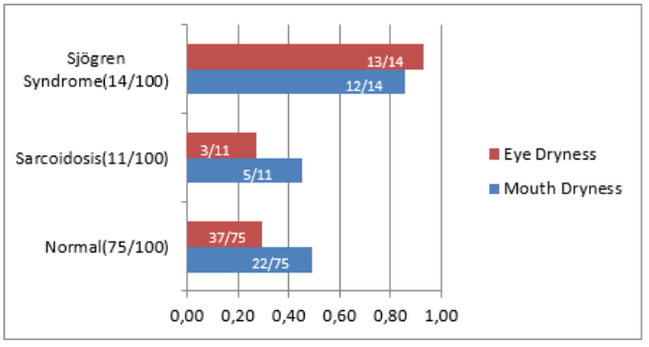

Figure 1. *Eye and Mouth Dryness Ratio in Patient Groups*

The histopathological diagnosis of minor salivary gland biopsies revealed that 11% of the patients had sarcoidosis, 14% had Sjögren's syndrome and 75% had normal histopathological results (Figure 2).

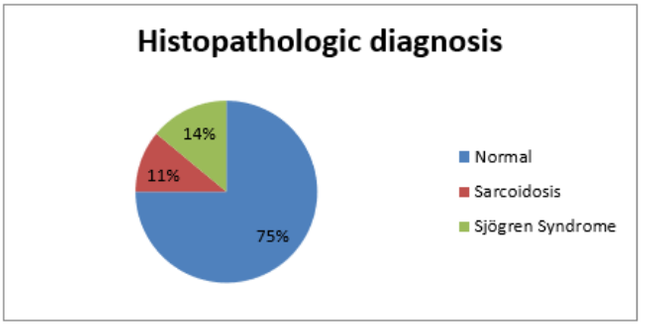

Figure 2. *Histopathologic Diagnosis of Patient Groups*

It was asked if the patients were under regular control and 50% of the patients with Sjögren's Syndrome were under regular control, significantly higher than other two groups (Figure 3).

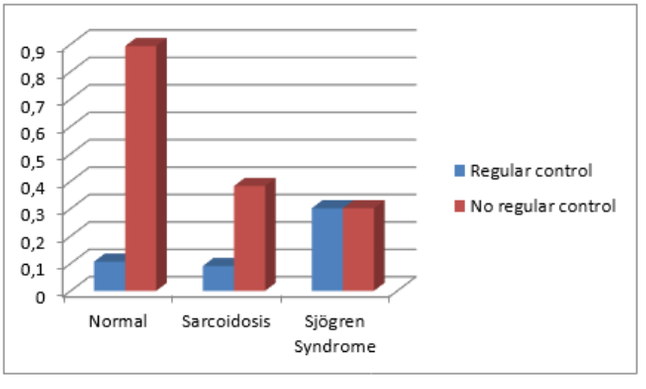

Figure 3. *Percentage of Patients with and without Regular Control*

4. DISCUSSION

The MSGB technique was first used in 1966 for the diagnosis of Sjögren's Syndrome; but with Chisholm and Mason's publication in 1968, it began to be used routinely in diagnosis of various other connective tissue diseases including rheumatoid arthritis, osteoarthritis, Reiter's disease, psoriatic arthritis, scleroderma (1).

Before Chisholm and Mason's publication, some authors advocated that biopsies should be taken from the major salivary glands; however, because of the buccal fat and the buccal muscle deep dissection, biopsies from the parotid gland may cause complications such as sialocele and facial nerve injury due to trauma (5-7). Besides that, in one study, based on the US-European Union diagnostic criteria (AECG) for diagnosis of Sjögren's Syndrome, both lip biopsy and parotid biopsy were performed simultaneously from 35 patients and the same specificity and sensitivity values were obtained in both procedures (8). According to these studies, MSGB is more advantageous in terms of both reducing the risk of complications and ease of applications. The only long-term complication reported was continuous numbness of the lower lip in 0-6% of patients (8-10).

One of the reported major disadvantages of the technique is that it may be difficult to obtain sufficient number of minor salivary gland tissue to establish a histopathological diagnosis. Although this technique is referred to as lip biopsy, it does not include mucosal tissue for diagnosis. Instead, approximately 5 minor salivary gland tissues are required (7). In this study, no patient had insufficient number of salivary glands.

Caporali et al. stated that MSGB can be performed easily and provides sufficient material for histopathological studies and it is safe. Moreover, mentioned in same study, only one patient had long-term paresthesia. It is underlined that this technique is acceptable for patients and the possibility of re-performation during the follow-up period is a huge advantage (11). In our study, paresthesia was not observed during the post biopsy controls.

Some studies report problems with histopathological evaluation of MSGB. Vivino et al. reported false positivity in almost half of the initial diagnosis of 60 MSGBs. It is highlighted that these variabilities can be minimized with the exact use of Chisholm criteria (12). Bamba et al. questioned the contribution of MSGB to the diagnosis and its necessity and concluded that MSGB is an invasive method which can cause false negativities and false positivities (13). Since it is included in Consensus Criteria (AECG), it continues to be used in the diagnosis of Sjögren's Syndrome. It has been reported be helpful diagnosis in patients with nonspecific clinical findings, extraglandular involvement and autoantibody negativity (14). In this study there were no false negative or positive histological results in any case. All diagnoses matched with other clinical findings.

The fact that this technique is invasive has led to the search for alternative methods that can replace it. Efforts have been made to improve the accuracy of diagnostic criteria using new methods such as ultrasonography and B cell subpopulations (15-18). Two studies have suggested that serological markers have better results than lip biopsies (19,20). Bamba et al. also stated that 53% of samples are accompanied by normal anti-SSA and anti-SSB antibody levels but a positive lip biopsy and according to that MSGB is not an excellent marker. Nevertheless, considering both the symptoms of the disease and the serology will produce a more accurate clinical picture (13). One of the limitations of our study was that we only evaluated the biopsy results and the answers given to the questions in the questionnaire. As the biopsy unit, there was no chance to obtain serological and immunological markers in our study. We suggest it would be useful to examine serologic and immunological markers in multidisciplinary studies, as well.

Although it has not yet been accepted among the diagnostic criteria, salivary gland scintigraphy is another alternative test which has been evaluated as a less invasive method in recent years. In a study conducted by Aquilera et al., 61 healthy volunteers, 66 patients with Sjögren's Syndrome and 18 patients with a diagnosis of fibromyalgia were performed both a minor salivary gland biopsy and a salivary gland scintigraphy. As a result, it was reported that salivary gland scintigraphy, which is evaluated at normal limits, would not clarify the diagnosis of Sjögren's Syndrome, mild changes in saliva flow should be evaluated as nonspecific, but moderate and severe changes support the diagnosis of Sjögren's Syndrome. Therefore, it was concluded that salivary gland scintigraphy cannot replace minor salivary gland biopsy (21).

Again, the ultrasound scoring system, which was claimed as a non-invasive diagnostic method, was compared with scintigraphy and biopsy on 107 patients and 28 healthy people in a study group of by Milic et al. and the specificity and sensitivity of scintigraphy was measured as 90% and 87%, respectively (22).

Another method recommended instead of salivary gland scintigraphy in the differential diagnosis of Sjögren's Syndrome is magnetic resonance (MR) sialography. Tonami et al. performed minor salivary gland biopsy, MR sialography and salivary gland scintigraphy in 130 patients with suspected Sjögren's Syndrome. MR sialography and salivary gland scintigraphy results of 80 patients, whose small salivary gland biopsy findings proved Sjögren's Syndrome histopathologically, have been compared. As scintigraphy for Sjögren's Syndrome shows high sensitivity, MR sialography has been reported to have high specificity, in addition it has also been reported that minor salivary gland biopsy is the main diagnostic method (23).

Molecular basis of autoimmune diseases has been investigated in recent years. Salivary gland biopsy materials are also evaluated in terms of many criteria such as DNA microarray, IL-22 producing cell level, presence of monocyte chemotactic protein-1 receptor, IL-17, IL-23 and the expression of their receptors (24-27). Another reason why MSGB is indispensable is that besides the routine histopathological evaluation, it

makes possible to determine the presence and number of ectopic germinal centers, which is an important criterion for lymphoma (28). Beside, Jonsson et al. reported that the presence and absence of germinal center-like structures could be used to determine the serologic profiles of patients and thus to identify subgroups that would help predict their prognosis (29). Studies have shown that lymphocytic evaluation of lymphocytic infiltration, which can be used as a positive finding for Sjögren's Syndrome, is related to the diagnosis (2,11,30). Our study supports this information ($p = 0.0001$).

5. CONCLUSION

As more information is collected about the immunology of Sjögren's Syndrome, amyloidosis and sarcoidosis, and more sensitive techniques are developed for the interpretation of histopathological, serological and immunological markers, less invasive or noninvasive methods will be preferred. Today, MSGB continues to be one of the tests used as an adjunctive diagnostic method in the diagnosis and treatment of chronic and multi systemic diseases such as Sjögren's Syndrome, amyloidosis and sarcoidosis.

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Clinical and Experimental Health Sciences

The Effect of Simulation-Based Training About Emergencies in and Approaches to Delivery Given to Emergency Personnel

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ABSTRACT

Objective: This study was carried out to determine the effect of the medium level simulation-based training about "Emergencies in and Approaches to Delivery" given to the personnel working in emergency health services on the change in their current knowledge about emergency interventions in delivery.

Methods: The study used a quasi-experimental design. It was carried out with 232 personnel working in emergency health services. Data were collected using "Information Form" and "Knowledge Test for Emergencies in Delivery (ED)". The study included the teaching of the relevant topics to the participants, administration of the knowledge test, the application of the subjects taught using a simulator in the laboratory.

Results: Of the emergency health service personnel participating in the study, 68.5% were found to not have adequate knowledge for emergencies in delivery. As a result of the training given, the mean of the total pretest score obtained from the ED was 37.17±10.36, and the mean of the total posttest score was 70.14±11.33, and the post-training difference was found to be statistically significant.

Conclusion: According to the findings, it can be said that the training given to the emergency health service personnel increased their knowledge about emergencies in and approaches to delivery significantly and that the education was effective.

Keywords: Delivery, emergencies during delivery, emergency health service personnel, midwifery, simulation training, management of delivery emergencies.

1. INTRODUCTION

Emergency healthcare is one of the important components of primary healthcare services, which is defined as a discipline that includes rapid intervention and decision-making necessary to prevent further harm or death of patients by providing emergency care from the reporting of an injury or disease to the provision of definitive treatment (1-3). Another of these components is emergency obstetric care services (EMOC). EMOC describes the services provided in cases that occur in the 42-day period during pregnancy, birth and postpartum, threatening the life of the mother and baby and requiring emergency intervention. These services are extremely effective in reducing maternal and neonatal mortality rate, which is the most important indicator of development level of a country in worldwide (1).

Nearly half of child deaths and about one-third of maternal deaths occurring from the year 2000 to the present day have decreased. It is getting more difficult to reach better values than this level. Many factors such as economic conditions (e.g. lack of financial opportunities for transportation or health care), cultural aspects (e.g. lack of value for woman's life), and geographic conditions (e.g. distance from health institution, the inappropriateness of roads) prevent women from accessing healthcare services, which results in the current situation. Regardless of the conditions, any delay in a woman's access to any service can, unfortunately, cost her life (4-8). According to the results of the National Maternal Mortality Study conducted to determine the rate and cause of maternal deaths in Turkey (NMMS), 10.4% of maternal deaths occur on the road during the transfer or referral of women to a health institution (9). For this reason, personnel working in emergency health services equipped to provide EMOC can prevent most maternal deaths. To accomplish this, health institutions should provide health personnel with the highest level of knowledge and skills related to the services to be provided in emergencies that may occur frequently in obstetrics, as well as replenishing the deficiencies of medical equipment and infrastructure. It should be remembered that first aid services that stabilize the health status of women before being referred are life-saving (10,11).

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Prolonged delivery, cord prolapse, shoulder dystocia, placental anomalies, hemorrhage, preeclampsia-eclampsia, HELLP syndrome, position and presentation anomalies, premature rupture of membranes, and premature birth threat are among common obstetric complications (12). Shoulder dystocia development in 0.54% of vaginal deliveries was reported by Sukgen et al. (2020); in the study of İngeç et al. (2005) the rate of eclampsia in pregnant women was found as 1.2%, Queenan et al. (2012) demonstrated approximately 1-2% of all births have placental retention; and Su et al. (2012) reported that uterine atony encountered in every twenty births. Each of these complications are important problems that threaten the life of the mother and baby. Determining the possible condition without delay and administering the appropriate intervention will be life-saving.

Administration of the quickest and appropriate intervention by healthcare personnel or the prompt referral of the patient in line with procedures in cases where necessary makes it possible to prevent maternal mortality and morbidity in an emergency obstetric case. The first condition for quick decision-making requires an awareness of the complications, signs, and symptoms that may occur during pregnancy, delivery, and the postpartum period, and also the knowledge of how to administer appropriate interventions in the correct sequence and in an appropriate way (9, 19-22).

The role of health personnel is very important in the provision of emergency health services. Ambulance services, which are important members of this team, have the same significance (23). The medical personnel serving in the ambulance are not midwives; they are often paramedics or emergency medical technicians (EMT). Therefore, these professionals are expected to have the current medical knowledge and skills necessary for issues related to emergencies during delivery. Otherwise, mothers and babies may experience health problems or even lose their life related to the delivery process (3). In this context, the present study is thought to be highly significant in terms of providing midwives, nurses, health officers, EMT, paramedics, and physicians working in the stations of Health Services in Emergencies and Disasters Department of Health Directorate with up-to-date and evidence-based information and skills to meet the needs of women with pregnancy during delivery and emergencies. There are research results in the literature that support this. Walker et al. (2014) determined that there was an increase in team performance and self-efficacy levels of midwives and obstetricians who participated in simulation-based training. Calvert et al. (2013) found that the team participating in the simulation training was more successful in intervening in the eclampsia crisis. Draycott et al. (2000) determined that the rate of injuries in the baby due to shoulder dystocia decreased from 9.3% to 2.3% after simulation-based training. Besides, Merien et al. (2010) found that midwives and obstetricians who completed the simulation training carried out the delivery within 5 minutes in cases of shoulder dystocia delivery. Dayal et al. (2009) performed a study with medical students and reported that in the experimental group in which the simulation method was applied, the performance of the students in vaginal birth maneuvers, their participation in clinical applications and their self-confidence were higher than the control group.

The present study was conducted to evaluate the effect of training about 'Emergencies and Approaches during Delivery' given to all personnel working in the stations of Health Services in Emergencies and Disasters Department in a province in the Central Black Sea Region on the changes in their current knowledge related to emergencies during delivery.

The study's hypotheses are:

H0: Simulation-based training given to emergency personnel about emergencies and approaches in delivery has no effect on their knowledge level.

H1: Simulation-based training given to emergency personnel about emergencies and approaches in delivery has effect on their knowledge level.

2. METHODS

2.1. Study Type

The study used a pretest-posttest, non-control group, quasi-experimental design.

2.2. Sample

The study consisted of 295 personnel (EMT, paramedics, physicians, health officers, nurses, and midwives) working in the stations of Health Services in Emergencies and Disasters Department in a province in the Central Black Sea Region between (01 March 2017 and 31 December 2017). The study was carried out with 232 participants due to the personnel on leave, moving to another institution, on breastfeeding leave, or assignment to a different post.

2.3. Data Collection Tools

A 13-item "Information Form" for the evaluation of the training and a "Knowledge Test for Emergencies in Delivery (ED)" with 25 multiple-choice items for emergencies in and approaches to labor were used.

2.4. The Information Form

The form designed by the training coordinators consisted of 13 items aiming to collect data about socio-demographic characteristics, education status, and professional status of the participants, and their views about delivery. This form was administered to the participants only once before the training.

2.5. ED Knowledge Test

This form, which aimed to measure the level of knowledge of the staff receiving the training, was developed by the researchers based on a literature review (9, 29-32).

The knowledge test consisted of items including delivery and risky situations that can be encountered most frequently during delivery. The distribution of the items by topics was normal delivery (6 questions), umbilical cord anomalies (4 questions), placental anomalies (4 questions), shoulder dystocia (4 questions), breech delivery (3 questions), preeclampsia-eclampsia (2 questions), amniotic fluid anomalies (1 question), and postpartum hemorrhage (1 question). The test was made up of 25 multiple choice questions, each of which had 5 options. Each question had only one correct option and was evaluated over 4 points. The minimum and maximum scores that could be obtained from the knowledge test ranged between 0 and 100 points.

For the content validity of the knowledge test questions, the test was submitted to the opinions of 5 experts who had already been studying in the field of risky delivery management and simulation education. Lawshe technique was utilized to evaluate the content validity of the items in the test. According to the Lawshe technique, the minimum fit criterion for five experts was evaluated as 0.99 (33). No items with a content validity ratio below 0.99 were detected in the test. To test the comprehensibility of the questions, the test was piloted to 10 personnel who did not know about the content of the test, and the test was finalized.

The pretest application was carried out after the first meeting with the participants, just before the training. Posttests were administered after the completion of the training. The tests were evaluated immediately after the 45-minute response time given for the tests. After evaluating the tests, the participants were given the correct answers, and necessary explanations about the questions were made.

2.6. Applied Training for Emergencies and Approaches during Delivery

The training was given in the applied training room and birth simulator application laboratories of the Health Sciences Faculty of a University in the Central Black Sea Region. The training sessions were given once or twice a month, in a total of 8 hours on a working day by two faculty members who worked in the Midwifery Department of the Faculty of Health Sciences and who were experts in the field. The pretest and posttest administration times of the information form were not included in the total training time. In the training held with 12 groups in total, the group sizes ranged between 16 and 25 people.

The training topics included emergencies that might be encountered during delivery such as normal delivery, breech delivery, placental anomalies, shoulder dystocia, umbilical cord prolapse, preeclampsia-eclampsia, and the management of hemorrhage. The topics were first taught in the classroom environment using teaching methods and materials such as lecturing, slide shows, videos, question-answer, and demonstrations (4 hours theoretically). Then, a 4-hour long session was performed on a birth simulator in the laboratory (Figure 1). The applications were first carried out by the trainers on a low-fidelity simulator in the delivery laboratory. Then each participant performed each application at least once under the supervision of the trainers.

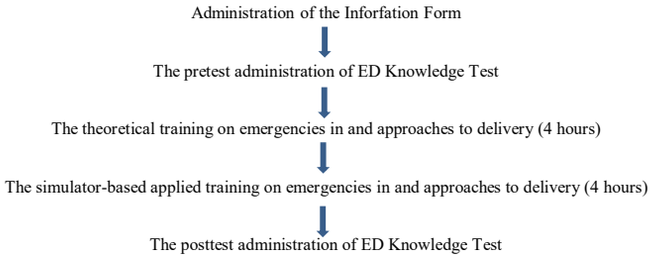

Figure 1. *Flow diagram of the study*

TOPİC	PRESENTATİON FORMAT	HOUR
Normal delivery	Slide shows, videos, question-answer, and demonstrations	08:00-08:30
Breech delivery		08:45-09:15
Shoulder dystocia		09:30-10:00
Placental anomalies, umbilical cord prolapse		10:15-10:45
Preeclampsia-eclampsia		11:00-11:30
Management of hemorrhage		11:45-12:15
Normal delivery practice	Simulation training (Delivery simulator)	13:15-13:45
Breech delivery practice		14:00-14:30
Application of shoulder dystocia maneuvers		14:45-15:15
Cord prolapse flow chart application		15:30-16:00
Bleeding management flow chart application		16:15-16:45

Figure 2. *Group training content*

2.7. Ethics of the Study

In the study, necessary permissions were taken for the personnel (EMT, paramedic, physician, health officer, nurse, and midwife) working in the Emergency and Disasters Health Services of the Health Directorate for the day when the study would be carried out from the Public Health Directorate of the province where the study was conducted.

Ethical approval was obtained before starting the study. The written institutional permission was obtained from the Faculty of Health Sciences of the university where the study was conducted. Informed consent of the participants who agreed to participate in the study was obtained. Ethical approval of Cumhuriyet University Non-Interventional Clinical Research Ethics Committee (Decision no: 2016-12701) was obtained before starting the study.

2.8. Data Analysis

Descriptive analyses were done to provide information about the general characteristics of the data. Data of continuous variables were expressed as mean ± standard deviation values, while categorical variables were presented as n (%). The comparison of the means of the quantitative variables

between the groups was conducted using the significance of the difference between two means test and one-way analysis of variance test. A chi-square test was employed to evaluate the relationship between qualitative variables. The statistical significance was accepted as $p<0.05$. Analyzes were performed on the SPSS statistical software package (IBM SPSS Statistics 19, SPSS Inc., An IBM Co., Somers, NY).

2.9. Limitations of the study

We were able to only evaluate the knowledge aspect of the training provided, but the application part could not be evaluated. With the provision of necessary conditions, the evaluation of the application in the hospital environment during the actual delivery process for each staff was planned as the second step of the study. Despite the abundant number of topics to be addressed about emergencies in and approaches to delivery, we were able to handle only 8 main topics since the training program was limited to an 8-hour.

3. RESULTS

Table 1 can be seen in, the socio-demographic characteristics of the emergency personnel included in the study.

Table 1. *The distribution of the socio-demographic characteristics of the emergency personnel*

Characteristics (n:232)*		n	%
Gender	Male	91	39.2
	Female	141	60.8
Professional Title	EMT	162	69.8
	Paramedic (AECT)	50	21.6
	General practitioner	9	3.9
	Other (Health officer, nurse…)	11	4.7
Education	Health Vocational High School	216	93.1
	University	16	6.9
Marital Status	Married	155	66.8
	Single	77	33.2
Place of residence	Village	7	3.0
	County	116	50.0
	Province	109	47.0
Another healthcare personnel in the family	Yes	98	42.2
	No	134	57.8
Professional title of the second healthcare worker in the family	Midwife	11	11.0
	Nurse	24	24.0
	Doctor	7	7.0
	Health officer	26	26.0
	Other	40	40.0
*Mean age (Year)	: 28.77±4.73 (Min.:20 – Max.: 46)		

According to the distribution of the knowledge of emergency personnel on emergencies in delivery in Table 2, of the participants, 68.5% had enough knowledge about the emergency in delivery, 81% saw a vaginal delivery beforehand, 60.6% of those who had seen a vaginal delivery before saw it during school practice as students, 49.6% actively participated in a vaginal delivery, 40% participated in a delivery in the hospital, and the active role of 63.5% in this delivery was to assist the midwife/physician who administered the delivery.

Table 2.*The distribution of some of the knowledge of the emergency personnel regarding emergencies during delivery (n=232)*

Characteristics	n	%
Having adequate knowledge of emergencies during delivery		
Yes	73	31.5
No	159	68.5
Have you observed a vaginal delivery before?		
Yes	188	81.0
No	44	19.0
Where did you see a vaginal delivery? (n=188)		
In the hospital practice when I was a student	114	60.6
In the media	11	5.9
While working in a hospital	10	5.3
During the delivery in the ambulance	7	3.7
In more than one places	46	24.5
Did you actively participate in the delivery?		
Yes	115	49.6
No	117	50.4
Where did you actively participate in the delivery? (n= 115)		
Hospital	46	40.0
Ambulance	45	39.1
More than one place*	24	20.9
What was your active role in the delivery? (n=115)		
Managing the delivery	42	36.5
Assisting the midwife/physician managing the delivery	73	63.5
*Hospital, healthcare center, ambulance, home		

Table 3 shows the distribution of the responses of the emergency personnel to the pretest and posttest administration of the ED knowledge test questions. The majority of the emergency personnel were found to give incorrect answers to the questions in the following topics in the pretest: care for umbilical cord prolapse (93.1%), the application of the Pinard maneuver (86.6%), maternal risk factors for shoulder dystocia (85.3%), umbilical cord clamping distance (84.5%), the application of McRobert maneuver (84.1%), the identification of the placenta previa (84.1%), and the rupture time of the amniotic fluid sac (80.2%). There was a significant increase in the rate of correct responses given by the emergency personnel in the posttest, whereas there was no increase in the correct responses to the question about "knowing the use of MgSO4". Especially, the rate of correct responses given by the emergency personnel to the questions about the following topics was observed to be quite high: umbilical cord clamping distance (15.5-94%), the application of the McRobert maneuver (15.9 – 80.2%), the indication of the Turtle sign (22.4 – 80.2%), the application of the Pinard maneuver (13.4 – 76.3%), identification of the Placenta Previa (15.9 – 74.1%) and the position taken in the shoulder dystocia (27.2 – 87.5%).

Table 3. *The distribution of the responses of the emergency personnel to the pretest and posttest administration of the ED knowledge test questions*

QUESTIONS	ED knowledge test							
	Pretest				Posttest			
	Correct		Incorrect		Correct		Incorrect	
	n	%	n	%	n	%	n	%
The characteristics of vaginal delivery	101	43.5	131	56.5	207	89.2	25	10.8
Fetus-related factors at delivery	190	81.9	42	18.1	206	88.8	26	11.2
Signs of the onset of delivery	174	75	58	25	224	96.6	8	3.4
Purposes of protecting the perineum in delivery	95	40.9	137	59.1	129	55.6	103	44.4
Features of real delivery contractions	146	62.9	86	37.1	191	82.3	41	17.7
Rupture of time of the amniotic fluid sac	46	19.8	186	80.2	115	49.6	117	50.4
Symptoms of the separation of the placenta	165	71.1	67	28.9	207	89.2	25	10.8
Symptoms at the stage of delivery	138	59.5	94	40.5	149	64.2	83	35.8
Applications to puerperant with postpartum hemorrhage	210	90.5	22	9.5	216	93.1	16	6.9
Position to be taken in case of cord prolapse	86	37.1	146	62.9	185	79.7	47	20.3
Position to be taken in shoulder dystocia	63	27.2	169	72.8	203	87.5	29	12.5
The application of McRobert maneuvering	37	15.9	195	84.1	186	80.2	46	19.8
Clamping distance of the umbilical cord of the newborn	36	15.5	196	84.5	218	94	14	6
Definition of the cord prolapse	83	35.8	149	64.2	136	58.6	96	41.4
Emergency care principles of the cord prolapse	77	33.2	155	66.8	147	63.4	85	36.6
Definition of Placenta Previa	37	15.9	195	84.1	172	74.1	60	25.9
Maternal risks for shoulder dystocia	34	14.7	198	85.3	78	33.6	154	66.4
Care for Cord Prolapse	16	6.9	216	93.1	21	9.1	211	90.9
Management of placental anomalies	59	25.4	173	74.6	184	79.3	48	20.7
The application of Mauriceau-Smellie-Veit maneuver	71	30.6	161	69.4	198	85.3	34	14.7
Principles for MgSO4 use	56	24.1	176	75.9	50	21.6	182	78.4
The indication of the Turtle sign	52	22.4	180	77.6	186	80.2	46	19.8
Responsibilities in the care and follow-up of the placenta previa	90	38.8	142	61.2	124	53.4	108	46.6
Getting fetal heart sounds in breech presentation	63	27.2	169	72.8	159	68.5	73	31.5
The application of the Pinard maneuvering	31	13.4	201	86.6	177	76.3	55	23.7

Table 4 shows the comparison of the mean scores of the emergency personnel regarding the pretest and posttest administration of the ED knowledge test. The mean of the total score obtained from the pretest was 37.17±10.36, and the mean of the total score obtained from the posttest was 70.14±11.33. The difference between the two was found statistically significant (p <0.001).

Table 4. *The comparison of the mean scores of the emergency personnel obtained from the pretest and posttest administration of the ED knowledge test*

ED Knowledge Test	Mean ± SD	Min	Max.	t	p
Mean Pretest Total Score	37.17±10.36	12.00	76.00	40.858	<0.001
Mean Posttest Total Score	70.14±11.33	36.00	96.00		

x^2: chi – square test, p: significance (p< 0.05)

As shown in Table 5, in the comparison of the mean pretest-posttest and total scores of the emergency personnel obtained from the ED Knowledge test with socio-demographic features, the intra-group comparisons of all variables were determined to be statistically significant (<0.001). In the inter-group comparison of the variables, the mean of the pretest, posttest, and total scores for gender, profession, and educational status were found to be statistically significant (p <0.05).

As shown in Table 6, in the comparison of the mean pretest-posttest and total scores of the emergency personnel obtained from the ED Knowledge test with some properties related to the emergencies in delivery, the intra-group comparisons of all variables were determined to be statistically significant (<0.001). In the inter-group comparisons, a significant difference was found between "the status of actively participating in vaginal delivery" and the pretest-posttest scores and means of the total scores, and between "the place of active participation" and the posttest score and means of total scores (p <0.001).

Table 5. *The comparison of the mean pretest-posttest and total scores of the emergency personnel obtained from the ED Knowledge test with socio-demographic features*

Variable	n	ED Knowledge Test			p_1
		Pretest	Posttest	Total	
		Mean ± SD	Mean ± SD	Mean ± SD	
Gender					
Female	91	34.64±9.86	67.03±11.06	50.84±0.91	<0.001
Male	141	38.81±10.38	72.14±11.08	55.48±0.73	<0.001
p_2		<0.001	0.011	<0.001	
Marital status					
Married	155	37.26±10.04	70.09±11.21	53.68±8.61	<0.001
Single	77	36.99±11.05	70.23±11.65	53.61±9.65	<0.001
p_2		0.848	0.928	0.957	
Place of residence					
Village	7	37.14±8.23	68±10.07	52.57±7.55	<0.001
County	116	37.14±8.98	70.07±10.91	53.6±7.67	<0.001
Province	109	37.21±11.83	70.35±11.92	53.78±10.27	<0.001
p2		0.999	0.999	0.939	
Profession					
EMT	162	36.57±9.04[b]	69.53±10.57[b]	53.05±7.5[b]	<0.001
Paramedic	50	36.56±9.86[b]	70±12.32[b]	53.28±9.54[b]	<0.001
Physician	9	46.67±16[a]	81.33±11.49[a]	64±13.11[a]	<0.001
Health officer, Nurse, Midwife	11	41.09±19.44[ab]	70.55±14.12[ab]	55.82±15.89[ab]	<0.001
p2		0.019	0.025	0.003	
Level of education					
University	16	43.5±11.49	77.25±10.9	60.38±10.15	<0.001
Health vocational high School	216	36.7±10.15	69.61±11.21	53.16±8.68	<0.001
p2		0.011	0.009	0.002	

Two-way repeated measures ANOVA was used for repeated measures. P: significance (P<0.05). p_1: Intra-group comparison p2: Inter-group comparison (abc: common letter refers to statistical insignificance)

Table 6.*The comparison of the mean pretest-posttest and total scores of the emergency personnel obtained from the ED Knowledge test with some properties related to the emergencies in delivery*

Some properties related to the emergencies in delivery	n	ED Knowledge Test			p_1
		Pretest	Posttest	Total	
		Mean ± SD	Mean ± SD	Mean ± SD	
Knowledge of emergencies during delivery					
Yes	73	37.59±10.51	69.7±10.54	53.64±8.86	<0.001
No	159	36.98±10.32	70.34±11.7	53.66±9.02	<0.001
p_2		0.679	0.690	0.990	
Status of active participation in a vaginal delivery					
Yes	113	38.97±9.75	69.95±11.2	54.46±8.46	<0.001
No	119	35.46±10.67	70.32±11.5	52.89±9.37	<0.001
p2		0.010	0.803	0.183	
Place of the active participation					
Hospital	46	39.65±9.5	74.61±8.53[a]	57.13±7.24[a]	<0.001
Ambulance	45	39.47±7.8	65.6±10.7[b]	52.53±7.52[b]	<0.001
More than one place	24	35.67±13.13	68.83±13.24[ab]	52.25±10.8[ab]	<0.001
p2		0.225	<0.001	0.013	
A second healthcare worker in the family					
Yes	98	37.88±11.3	69.22±12.08	53.55±9.74	<0.001
No	134	36.66±9.63	70.81±10.75	53.73±8.37	<0.001
p_2		0.377	0.295	0.880	
Active role in the delivery					
Management of the delivery	42	39.33±9.35	68.1±10.58	53.71±8.12	<0.001
Assisting midwife/physician managing the delivery	73	38.41±10.13	70.9±11.44	54.66±8.67	<0.001
p_2		0.630	0.195	0.567	

Two-way repeated measures ANOVA was used for repeated measures. P: significance (P<0.05). p_1: Intra-group comparison p_2: Inter-group comparison (abc: common letter refers to statistical insignificance)

4. DISCUSSION

This study discussed the outcomes of the training given to personnel working in emergency health service units to update their knowledge and skills about the methods of treatment and intervention in emergencies during delivery.

There is limited research abroad and in our country into the knowledge and skills of the personnel working in health services about the management of emergencies during delivery (3, 24,25,27,34,35). In this respect, our study is of significance as an original study. Also, we think that the scarcity of scientific studies related to this topic and the sample group in emergency health services is a limitation of the discussion. Considering that 40% of the participants who participated in an active delivery participated in a delivery in the ambulance, and also, 36.5% of them managed the delivery actively, it will be again emphasized that emergency personnel should have knowledge and skills about the emergencies in delivery.

According to 2013 data, 2.3% of maternal deaths occurred during referrals to hospitals. Therefore, improving the referral system is one of the most important steps in reducing maternal mortality. To enhance the system, it is very important to improve the knowledge and skills of health personnel providing the service, as well as replenishing the medical equipment and infrastructure deficiencies of health institutions (9). In our study, the majority of the personnel were found to not have sufficient information about emergencies during delivery and their role was determined to be limited to assisting the midwife/physician during delivery (Table 2). These data indicated that providing this training to emergency service personnel was quite an accurate decision.

According to the findings of NMMS, factors related to healthcare providers such as incomplete diagnosis, lack of knowledge, and the inability of health personnel to cope with the problem played a role in 13.7% of maternal deaths (9). Therefore, it is important for team members to know their duties and how they will function most effectively in emergencies when an obstetric emergency is encountered. To do this, they need to be familiar with clinical conditions, diagnoses and treatments, medications, the use, administration, and side effects of medications, and emergency equipment and their functions.

When the pretest-posttest results of the personnel were examined, a significant increase was observed in the level of knowledge (in the rate of correct responses to the questions) (Table 3).On the other hand, a decrease was determined in the rate of correct responses to the question about “knowing the use of MgSO4” with a decrease from 24.1% to 21.6%. The low success rate relating to this question may have been because it was not understood or learned adequately. The rate of correct responses to the questions in the following areas was determined to increase especially in the posttest considerably: the umbilical cord clamping distance, the application of the McRobert and Pinard maneuver, the Turtle sign symptom, the identification of the Placenta Previa, and the position taken in shoulder dystocia. This result suggested that the personnel were adequately informed about these issues and that they learned these topics better. Also, the fact that questions with high correct answers were particularly related to applied areas showed the necessity and effectiveness of simulation application in education. Findings of the rate of simulation-based training were also observed to be high in studies in the literature, which supported the findings of our study. Walker et al. (2014) determined that there was an increase in team performance and self-efficacy levels of midwives and obstetricians who participated in simulation-based training. Calvert et al. (2013) found that the team participating in the simulation training was more successful in intervening in the eclampsia crisis. Draycott et al. (2000) determined that the rate of injuries in the baby due to shoulder dystocia decreased from 9.3% to 2.3% after simulation-based training. Besides, Merien et al. (2010) found that midwives and obstetricians who completed the simulation training carried out the delivery within 5 minutes in cases of shoulder dystocia delivery. Dayal et al. (2009) performed a study with medical students and reported that in the experimental group in which the simulation method was applied, the performance of the students in vaginal birth maneuvers, their participation in clinical applications and their self-confidence were higher than the control group.

Obstetric emergencies are special situations that can affect both the health of the mother and the fetus at the same time, requiring team collaboration and highlighting fast and correct intervention (36). In the present study, when the means of the total scores of the emergency personnel obtained from the pretest and posttest administration of the ED Knowledge test were compared, a significant increase was observed in the posttest mean scores and the difference was statistically significant ($p < 0.001$, Table 4). This result showed that the training provided significantly increased the knowledge of the personnel regarding emergencies in and approaches to delivery, and the effectiveness of the training was good. The reason for success can be attributed to the following aspects: the willingness of the personnel for simulation training, the expertise of the trainers in their subjects, the presentations which were prepared using visual content, the use of simulation with active learning methods, and the increase in its effectiveness. The results of the basic module training given to personnel by Çalışkan et al. (2016), Ülger et al. (2013), and Söğüt et al. (2008) were found to be significant and similar to those of our study in terms of the comparison of mean pretest-posttest total scores (3,11). Training conducted using medium level simulation has a higher benefit than training conducted using low-level simulation. In a systematic review by İldan Çalım and Öztürk (2018), the simulation-based training about before, while, and after delivery care given to midwifery students proved effective in ensuring readiness for the clinical setting. As for our study, we can say that the training conducted with medium level simulation significantly increased the knowledge of the personnel and that it was effective. Apart from this, the low or high fidelity sensitivity of the simulation

model may affect the outcome of the training. In a study with 45 doctors and 35 midwives, the shoulder dystocia training provided by a high fidelity simulation method was found to be more successful than training with low fidelity simulation in delivery (39). Although the delivery simulation model used in our study was a medium sensitive simulator, it could be said to have an important contribution to increasing the success in the practical part of the training.

When the pretest and posttest scores and mean total scores of the emergency personnel were compared with their socio-demographic characteristics, the intra-group comparisons of all variables were found to be statistically highly significant (<0.001). In the inter-group comparison of the variables, the relationship between the mean pretest and posttest total scores and gender, occupation, and education level was determined to be statistically significant ($p < 0.05$, Table 5). Unlike our study, the study of Ülger et al. (2013) found no difference between professions in terms of the pretest and posttest scores in the training they gave to ambulance personnel. In a study with physicians group, Kavalci et al. (2009) and Eryilmaz et al. (2007) attributed the high scores obtained from the training to the fact that the trainees were able to easily grasp the subjects because they were physicians, they had practical experience related to the subject, and that they had knowledge about the training content. In our study, the high success rates in the physician group can be attributed to similar reasons compared to other occupational groups.

In our study, university graduates created a difference in all mean scores compared to health vocational high school graduates. This indicated that the high level of education was important in terms of contributing positively to the effectiveness of the basic professional knowledge of individuals, and the positive increase in learning and understanding of the information provided in the current course.

When the pretest and posttest scores and mean total scores of the emergency personnel were compared with their findings regarding emergencies during delivery, intra-group comparisons of all variables were found to be statistically highly significant ($p < 0.001$). The inter-group comparisons, on the other hand, indicated that there was a statistically significant difference between the status of "*active participation in a vaginal delivery*" and the pretest, posttest scores and means of total scores and between the "*place of the active participation*" and the posttest score and means of total scores ($p < 0.05$, Table 6). The posttest scores of the participants who did not actively participate in a vaginal delivery were higher than those who did not. Also, the posttest scores of the participants who observed the delivery during internship had higher scores. In the study conducted with paramedic students by Tuygar (2016), 85.2% of the students were found to think that the healthcare workers working at the places they did their internship helped them gain professional experience.

In a study conducted by Göllüce et al. (2017) with paramedic students, 31.7% of the students stated that they needed to update their knowledge and skills to specialize in their job. The findings of Göllüce et al. (2017) and the findings of this study pointed out the necessity of the training given.

5. CONCLUSION

In conclusion, the findings laid out that the training given increased the knowledge of the emergency health service personnel about emergencies and approaches during delivery and that it was effective. In light of the findings, we recommend that future studies should measure and evaluate the applied skills development dimension of this training, as well as assessing the pretest-posttest scores; this type of training events should be repeated periodically for healthcare personnel working in emergency health service units; both theoretical and practical course content topics such as normal delivery, obstetric emergencies, and approaches to these areas should be added to the undergraduate educational syllabus of the personnel working in emergency health service units; larger sample sizes should be used to assess the efficiency of this type of training; and that the effect of these findings on the health indicators of the region where the study is conducted should be reported.

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Clinical and Experimental Health Sciences

The Impact of Socioeconomic Factors and Oral Hygiene Habits on Knowledge Level of Oral Health and Procedures: The Questionnaire Based Research

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ABSTRACT

Objective: The purpose of this study is to describe oral health behaviour of the individuals and determine the relationship level of knowledge of the participants about dental health and procedures and their personal variables.

Methods: The total number of participants who answered the questionnaire was 575. Oral health behaviours were assessed according to tooth brushing frequency, dental floss or interdental brush use, when was the last time he/she went to dentist and their reason for going to the dentist. Also, the eight dental health knowledge items were selected to determine the individual's dental health knowledge status.

Results: There was no statistical difference between the ages of the participants and the knowledge levels of dental health and procedures ($p=0.089$, $p>0.05$). There was statistical difference between level of education and the knowledge levels of dental health and procedures ($p=0.001$, $p<0.05$). There was statistical difference between level of family income and the knowledge levels of dental health and procedures. ($p=0.001$, $p<0.05$). There was statistical difference between dental floss or interdental brush using and the knowledge levels of dental health and procedures ($p=0.001$, $p<0.05$).

Conclusion: The findings of the present study showed that Oral hygiene habits and oral and dental health knowledge level is insufficient in our society. Preventive and educational programs should be developed in order to both improve and inform individuals about oral care.

Keywords: Knowledge, oral health, socioeconomic factors, tooth brushing

1. INTRODUCTION

Dental problems are common in both primary and permanent teeth reaching about four million people worldwide (1). It is accepted that the most important etiological factor that impairs oral and dental health is microbial dental plaque (2). Although microbial dental plaque is accepted as the primary factor in etiology, it has been shown that individuals' factors such as age, gender, race, habits, systemic disease, socioeconomic and cultural conditions also affect oral and dental health (3-5). The maintenance and improvement of oral health status depends on correctional care behaviours (6). Advanced oral health behaviour is known to provide a better oral health status (7-9). Therefore, oral health behaviours certainly must be improved.

The impact of socioeconomic status on oral health has been investigated for a long time. Socioeconomic impossibilities can reduce oral and dental care given by individuals. Also, investigating and determining of social inequalities is fundamental to obtain knowledge about population differences in oral health care needs, conservative application and oral health recruitmenting (8,9).

Oral health behaviour in adults is known to be associated with various factors, such as age, sexual differences (10-11), educational and socioeconomic status (12-14). Family income and education status are the most widely used markers for socioeconomic status assesment in epidemiologic studies (15-19).

Earlier researches have reported that socioeconomic status is adversely related with poor oral health behaviour and dental disease. The researches demonstrated that people with higher socioeconomic level are more concerned about oral health and less experience dental diseases (15-17). Also families with better socioeconomic level are more aware of oral and dental health. As a result of this statement, they can guide their children about oral and dental care. It has been observed that families with high education level and monthly income perform more frequently oral and dental health applications and their knowledge level is better (20). It is stated that families who take more seriously the oral and dental care habits of their children and their routine dental controls, have higher awareness about dental procedures

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and preventive methods. Also it has been reported that their children have higher dental knowledge than the children of other families (21).

In our study, different from these studies, we want to evaluate the effect of socioeconomic conditions on knowledge of dental procedure terms as well as oral health behavior. In addition, we aimed to determine that knowledge of the participants about pedodontic procedures.

In the present study, we examined a sample of adults aged 18–80 living in. The purpose of this study is to describe their oral health behaviour; and determine the relationship between dental health and procedure knowledge levels and their personal variables (such as age, gender, socio-economic status, education status, toothbrushing frequency, using dental floss, and reason and frequency of dental check-ups etc).

2. METHODS

This research is based on data collected from a questionnaire that assessed dental health knowledge and oral health behaviour of dental patients who applied to Karabük Oral and Dental Health Hospital from Karabük and neighboring provinces from February to April, 2020. All participants were informed before completing the questionnaire and signed the informed consent forms. This study was approved by the Ethics Committee of Karabuk University (2020/170). This study was conducted in compliance with the Helsinki Declaration.

2.1. Participants

The total number of questionnaires distributed patients who applied to Karabük Oral and Dental Health Hospital from Karabük and neighboring provinces was 750 of which the total number of participants who answered the questionnaire was 575. Therefore, the participation rate was calculated as 76.66%.

The participants are individuals older than 18 years, which is considered that have the ability of assesing their dental health behaviour and knowledge. The inclusion criteria were as follows: agreement to participate in the study, aged older than 18 years and having cognitive ability to respond the questions in the survey. The exclusion criteria were as follows: not willing to participate in the study, aged younger than 18 years and individuals lacking cognitive ability to respond the questions in the questionnare.

2.2 Questionnaire

Socioeconomic variables were assesed according to age group (18-30, 31-44 and 45-80), gender, level of education (primary school, high school, university, post graduate), and family income (<minimum wage, minimum wage, minimum wage-3000 TL, 3000-4000 TL, 4000-6000 TL, above 6000 TL, whether she/he has a child and general health condition (sistemic disease state and medication continuously).

Oral health behaviours were assessed according to toothbrushing frequency (never, sometimes, once a day, twice or more in a day), dental floss or interdental brush use (use, not use), when was the last time he/she went to dentist (in the past three months, 3-6 months ago, 6-month 1 year ago, dont remember) and their reason for going to the dentist (for dental check-up, because of toothache, for treatment).

The eight dental health knowledge items (calculus, prosthesis, fissure sealant, gingival recession, fluor, implant, space maintainer and panoramic radiography) were selected to determine the individual's dental health knowledge status. Responses to items were 'know the meaning' and 'dont know the meaning'. Total number of 'know the meaning' responses were collected and then collapsed into three categories of low (0-2), middle (3-5) and high (6-8).

2.3 Statistical Analysis

The statistical analysis of the data was performed using MiniTab 17 Statistical Software (Statistical Software Release, Version 17.3.1. Minitab Inc. USA). Descriptive statics were obtained in frequency and percentage. T test was used for multiple comparisons of normally distributed variables with continuous variation. Kruskal Wallis test was used for non normally distributed variables. Mann-Whitney test and Chi-square test were used in the evaluation of the binary comparisons. P values <0.05 were used for indication of statistical significance for all tests.

3. RESULTS

The mean age of 575 participants was 34.7±11.52. The demographic data of the participants are shown in Table 1 and oral health behaviours of the participants are shown in Table 2. The participants included in the study were divided into 3 groups by age such as 18-30, 31-44 and 45-80. There was no statistically difference between the ages of the participants and the knowledge levels of the about dental health and procedures (p=0.089, p>0.05) (Table 3).

335 of the 575 participants were women and 240 were men. There was no statistically difference between the gender of the participants and the knowledge levels about dental health and procedures (p=0.520, p>0.05) (Table 3).

171 of the participants were primary school (E1), 171 were high school (E2), 219 were university (E3), and 14 were post graduates (E4). There was statistical difference between level of education and the frequency of tooth brush. It was found that there was no significant difference in terms of frequency of teeth brushing only between the participants with university and postgraduate education levels (p=0,3712), while there was a significant difference between the participants with other education levels (E1-E2, p=0,0236; E1-E3, p=0; E1-E4, p=0,0023; E2-E3, p=0,0014) (Table 4). The frequency of tooth brushing increased as the education level increased. There was statistical difference between level

of education and the knowledge levels of the about dental health and procedures (p=0.001, p<0.05) (Table 3). As the level of education increases, the knowledge levels of the about dental health and procedures increase significantly. Binary comparisons of education levels among themselves are shown in Table 3.

Table 1. *The demographic data of the participants*

		Number	Percent (%)
Gender	Male Female	240 335	41.7 58.3
Age	18-30 31-44 45-80	227 234 114	39.5 40.7 19.8
Education level	primary school high school university post graduate	171 171 219 14	29.7 29.7 38.1 2.5
Family income	<minimum wage minimum wage minimum wage-3000 3000-4000 4000-6000 above 6000	219 118 85 67 70 16	38.1 20.5 14.7 11.7 12.2 2.8
Whether she/ he has a child	Yes No	351 224	61.1 38.9
Sistemic disease state	Yes No	95 480	16.5 83.5
Medication continuously	Yes No	89 486	15.5 84.5

Table 2*. Oral health behaviours of the participants*

		Number (n)	Percent (%)
Toothbrushing frequency	never sometimes once a day twice or more in a day	10 108 272 185	1.8 18.8 47.3 32.1
Dental floss or interdental brush use	use not use	116 459	20.1 79.9
When was the last time he/she went to dentist	in the past three months 3-6 months ago, 6-month 1 year ago, dont remember	246 95 107 127	42.8 16.5 18.6 22.1
Reason for going to the dentist	for dental check-up because of toothache for treatment	73 312 190	12.6 54.3 33.1

There was statistical difference between level of family income and the knowledge levels of the about dental health and procedures (p=0.001, p<0.05) (Table 3). As the amount of family income increases, the knowledge levels of the about dental health and procedures increase significantly. Binary comparisons of family income amounts among themselves are shown in Table 3. Also it was found that there was no significant difference in terms of frequency of teeth brushing between the participants with FI1 and FI2 (p=0,0751), and FI5 and FI6 (p=0.3838). There was a significant difference between the participants with other family incomes (p<0.05) (Table 5). Apart from the participants with the lowest and the two highest income levels, the frequency of brushing increases significantly as the income level increases.

When the participants who participated in our study were evaluated in terms of their general health conditions, it was determined that 95 of them had systemic disease and 89 of them constantly take medication. There was no statistically difference between the general health conditions (p=0.213), constanly taking medication (p=0.09) of the participants and the knowledge levels about dental health and procedures (p>0.05) (Table 3).

Table 3. *The dental knowledge levels of the participants*

		low middle high	p
Gender	Male Female	72 128 40 105 155 75	0.520[1]
Age	18-30 31-44 45-80	81 107 39 65 120 49 31 56 27	0.089[1]
Education level	primary school high school university post graduate	86 77 8 48 95 28 43 107 69 0 4 10	**0.001***[2]
Family income	<minimum wage minimum wage minimum wage<3000 3000-4000 4000-6000 above 6000	84 100 35 52 48 18 24 50 11 12 39 16 5 36 29 0 10 6	**0.001***[2]
Whether she/ he has a child	Yes No	116 165 70 61 118 45	0.295[1]
Sistemic disease state	Yes No	34 45 16 143 238 99	0.213[1]
Medication continuously	Yes No	33 43 13 144 240 102	0.09[1]
Toothbrushing frequency	never sometimes once a day twice or more in a day	8 2 0 60 39 9 81 140 51 30 100 55	**0.001***[2]
Dental floss or interdental brush use	Use not use	17 56 43 163 225 71	**0.001**[1]
When was the last time he/she went to dentist	in the past 3 months 3-6 months ago 6-month-1year ago no remember	76 124 46 21 49 25 24 54 29 59 54 14	**0.001***[2]
Reason for going to the dentist	dental check-up toothache treatment	15 27 31 114 154 44 51 100 39	**0.043***[2]

[1] *Chi-square test,* [2] *Mann Whitney U test*

***Table 4**. The relationship between education level and tooth brushing frequency*

		Never	Sometimes	Once a day	Twice or more in a day	p value*
Primary School (E1)	n	2	49	86	34	E1-E2; **0.0236**
	%	1,16	28,65	50,29	19,88	
High School (E2)	n	2	35	84	50	E1-E3; **<0.001**
	%	1,16	20,46	49,12	29,23	
Universtiy (E3)	n	2	26	96	95	E1-E4; **0.0023**
	%	0,91	11,87	43,83	43,37	
Post Graduate (E4)	n	0	0	7	7	E2-E3; **0.0014**
	%	0	0	50	50	E3-E4; 0.3712

**Mann Whitney U test*

***Table 5**. The relationship between family income and tooth brushing frequency*

		Never	Sometimes	Once in a day	Twice in a day	p value*
< minimum wage (FI1)	n	2	58	113	46	FI1-FI2; 0.0751
	%	0,91	26,48	51,59	21	
minimum wage (FI2)	n	2	25	54	37	FI1-FI3; **0.0251**
	%	1,69	21,18	45,76	31,35	
minimum wage-3000 TL (FI3)	n	0	10	36	39	FI2-FI3; **0.0452**
	%	0	11,76	42,35	45,88	
3000-4000 TL (FI4)	n	2	9	35	21	FI1-FI4; **0.0357**
	%	2,98	13,43	52,23	31,34	
4000-6000 TL (FI5)	n	0	8	28	34	FI4-FI5; **0.0341**
	%	0	11,42	40	48,57	
above 6000 TL (FI6)	n	0	0	7	9	FI5-FI6; 0.3838
	%	0	0	43,75	56,25	FI1-FI6; **0.0006**

**Mann Whitney U test*

The participants were evaluated in terms of oral health behaviours such as tooth brushing frequency (never, sometimes, once a day, twice or more in a day), using dental floss or interdental brush (use, not use), when was the last time he/she went to dentist (in the past three months, 3-6 months ago, 6-month 1 year ago, more than 1 year, dont remember) and their reason for going to the dentist (for dental check-up, because of toothache, for treatment). Descriptive statistics are summarized in Table 3. There was statistically difference between tooth brushing frequency and the knowledge levels about dental health and procedures (p=0.001, p<0.05). There was statistically difference between "never" and "sometimes" groups in terms of the knowledge levels about dental health and procedures (p=0.001, p<0.05) (Table 3). Also there was statistically difference between "once a day" and "twice or more in a day" groups in terms of the knowledge levels about dental health and procedures (p=0.0002, p<0.05) (Table 3).

There was statistically difference between using dental floss or interdental brush and the knowledge levels about dental health and procedures (p=0.001, p<0.05) (Table 3). In brief; as the frequency of brushing and flossing increase, the knowledge levels about dental health and procedures increase.

There was statistical difference between when was the last time he/she went to dentist and the family income of the participants (p=0,0004) (Table 6). There was statistical difference between when was the last time he/she went to dentist and the education level of the participants (p=0,024) (Table 7). It has been concluded that individuals with low socio-economic level and low education level have longer periods to go to the dentist. Also there was statistical difference between when was the last time he/she went to dentist and the knowledge levels of the about dental health and procedures (p=0.001, p<0.05) (Table 3). There was statistical difference between "dont remember" and other groups (in the past three months, 3-6 months ago, 6-month 1 year ago, more than 1 year) in terms of the knowledge levels of the about dental health and procedures (p=0.001, p<0.05) (Table 3).

***Table 6.** The relationship between the family income and when was the last time he/she went to dentist*

		In the past three months	3-6 months ago	6-month 1 year ago	Don't remember
< minimum wage	n	79	28	46	66
	%	36,07	12,78	21	30,13
minimum wage	n	51	25	19	23
	%	43,22	21,18	16,10	19,49
minimum wage-3000 TL	n	33	20	15	17
	%	38,82	23,52	17,64	20
3000-4000 TL	n	39	11	9	8
	%	58,2	16,41	13,43	11,94
4000-6000 TL	n	38	8	12	12
	%	54,28	11,42	17,14	17,14
above 6000 TL	n	6	3	6	1
	%	37,5	18,75	37,5	6,25
					p= **0,0004***

**Mann Whitney U test*

There was statistically difference between reason for going to the dentist and the knowledge levels about dental health and procedures (p=0.043, p<0.05) (Table 3). Participants who went to the dentists for dental check-up – although their number is the least (73) – had significantly higher knowledge levels about dental health and procedures than other groups (because of toothache (312), for treatment (190) (Table 3).

The participants' knowledge levels about dental health and procedures did not show a statistically difference in terms of whether they have children or not (p=0.295 p>0.05) (Table 3). In addition, we investigated the knowledge levels of the participants with and without children about the terms concerning pediatric dentistry (fluor, fissur sealant and space maintaner). The results were surprising, in general, the knowledge levels of this terms was very very low, and no significant difference was found between those with and without children. In addition, when participating parents were evaluated in terms of knowing the terms of pediatric dentistry, no significant difference was found between the genders (p>0.05) (Table 8).

Table 7. *The relationship educational level and when was the last time he/she went to dentist*

		In the past three months	3-6 months ago	6-month 1 year ago	Don't remember
Primary school	n	65	21	33	52
	%	38,01	12,28	19,29	30,4
High school	n	66	32	33	40
	%	38,59	18,71	19,29	23,39
University	n	110	40	35	34
	%	50,22	18,26	15,98	15,52
Post graduate	n	5	2	6	1
	%	35,71	14,28	42,85	7,14
					p= **0,024***

**Mann Whitney U test*

Table 8*. The knowledge levels of the participants with and without children about the terms concerning child dentistry*

	Male/ knowledge(n)	Female/ knowledge(n)	P
flüor	138/36	215/70	0.19[1]
fissur sealant	138/11	215/15	0.72[1]
space maintaner	138/19	215/27	0.74[1]

[1]*Chi-square test*

4. DISCUSSION

Epidemiological studies have always been useful in determining the importance given to oral and dental health by individuals and their level of knowledge (22). It is known that oral hygiene education and preventive programs have an important role in reducing the prevalence of dental caries and periodontal disease in children and young people. It was reported that the role of the teacher and friends was more important than the dentists as the age grew older, while the effect of the family was dominant in gaining oral hygiene habits at a younger age (23). In elderly adults, dentists play an active role in informing the community about oral and dental care.

In our study, data was collected with a questionnaire evaluating the oral hygiene habits and dental knowledge levels of patients who applied to Karabük Oral and Dental Health Hospital from Karabük and neighboring provinces, and the deficiencies in the region were tried to be eliminated in the light of the data obtained.

The frequency of tooth brushing in our study was found 1.8% in those who never brush, 18.8 % who brush sometimes, 47.3% who brush once a day and 32.1% who brush two or more time in a day. In a study conducted by Koçak N, similarly to our study, it was reported that 34.6% of individuals brushed their teeth two or more times a day and 63.9% brushed their teeth rarely (24). Also in another study conducted in Brazil, it was reported that 96.6% of participians who are students aged 14-19 years, brush their teeth everyday. It means they brush teeth more than our society (25). On the other hand in a study investigating the oral and dental health knowledge among African Americans, it was reported that 50% of the participants brushed their teeth every day, that means African Americans brushing frequency lower than us (26).

In our study, in accordance with other studies, tooth brushing frequency was found higher in female sex (27-29). However, when the relationship between gender and dental knowledge levels of patients was examined, no difference was found between female and male in terms of dental knowledge level. This situation can be explained by the fact that the rate of informing women and men by dentists is similar, but due to the fact that females' social and aesthetic concerns are higher than males, females pay more attention to oral care (28, 30, 31).

There was no statistically difference between the ages of the patients and tooth brushing frequency. Also there was no diffrence in dental knowledge levels of patients with age. This situation may ocur because of the majority of patients participating in the study may have applied to our hospital with the need for dental treatment regardless of age group. During their treatment, dentists may be increasing this rate in all ages groups, both by mentioning the importance of brushing teeth and informing patients about treatments.

In previous studies, it is stated that one of the important markers of oral and dental health is the socioeconomic status of individuals. The ability of individuals to purchase equipment necessary for oral and dental care and the ability to go to routine dental controls are associated with economic conditions (30-33).

Peltzer K et al. (30) reported that the frequency of brushing teeth is higher in individuals living in better economic conditions. Also Küçükeşme et al. (34) reported that low socio-economic situation negatively affects oral hygiene and nutrition, increases the risk of caries and reduces the importance given to oral hygiene. In accordance with their study, it was observed that there was a significant relationship between the monthly income level of the families and their brushing frequency in our study. The frequency of tooth brushing significantly increases as the family income level increases.

In our study it was found that, there was statistically difference between tooth brushing frequency and the dental knowledge levels. Also there was statistical difference between dental floss or interdental brushing and the dental knowledge levels. In brief; as the dental knowledge level increases, the frequency of brushing and using floss will increase.

In the results of our study, it was found that there was a significant relationship between using dental floss and monthly family income and education levels. Participants with higher income and education level used dental floss more. In accordance with our study, Freddo et al. (31) reported that, individuals with higher socioeconomic levels had higher frequency of dental floss use than those with lower socioeconomic levels. It was suggested that the reason for this situation might be high floss prices or low dental knowledge levels of patients. In another study conducted by Bordin D et al. (27) was stated that the level of education is more effective than the purchasing power of individuals and that it is more effective on the level of dental knowledge.

In our study, a hypothesis was established that the level of dental knowledge may be better due to the fact that individuals with systemic diseases are found more in hospital settings. However, no significant difference was found between dental knowledge levels of those with and without systemic disease. It is thought that the reason for this situation might be, weak comminication between patient and medical doctors about the relationship between dental diseases and systemic diseases. Therefore, patients with systemic disease do not care about oral and dental care more than others. Also other studies have reported that the relationship between systemic diseases and oral health is not fully known by their patients (26, 35).

According to the findings obtained in our study, dental knowledge levels of the patients were found to be 30.8% low, 49.2% medium, and 20% high. The reason for the number of patients with a high level of knowledge is less, may be due to the fact that dentists are too busy in daily practice, so that they do not have time to provide detailed information about oral and dental care and treatment methods to be applied. The habit of using dental floss/interdental brush with tooth brushing has been accepted as oral health behavior. When we compare oral health behavior and dental knowledge levels of patients; it was observed that as the dental knowledge level of the patients increased, the frequency of tooth brushing and the use of dental floss/interface brush increased.

When the reasons of the patients going to the dentist were examined, it was seen that the number of patients who went to dentist just dental check up lower than the others. However, when the dental knowledge levels of these patients were evaluated, it was seen that their knowledge level was quite high compared to the patients who went for dental pain or routine treatments. It is considered as the main reason for this situation that individuals with high awareness of oral and dental health know the importance of routine controls, which have an important role in maintaining oral and dental health.

Although the periods and causes of patients going to the dentist are part of their oral and dental health habits, they are also thought to be related to the dental knowledge levels of individuals. In our study, dental knowledge levels of patients who went to the dentist with large intervals were found to be lower than others. In parallel with our study, in earlier studies, it has been observed that individuals with low socio-economic level and low education level have longer periods to go to the dentist (28-30). Similar to our study, Woolfolk et al. (36) stated that the frequency of dental check-ups is also related to the monthly income levels of individuals.

While planning the study, it was emphasized that individuals with children may have more information about space maintainer, fluor and fissure sealant terms related to pediatric dentistry. However, no significant difference was found between families with or without children in terms of their level of knowing these terms related to pediatric dentistry. The reason for this situation may be that the pedodontist have just started in the hospitals where the study is carried out or the dentists who have been treating children before did not inform the families much about this topic.

The limitations of this study are as following;

1. The interpretation of our results should take into account the methodological limitation that the sample is not representative of the population in general. Because our study was carried out in a public hospital.
2. Also evaluating of socio-economics statuations of participiants just based on monthly income and education level. These criteria may not be enough to evaluate indivudials socioeconomis situation.
3. It is possible that other types of conceptual knowledge, such as recommended self-care behaviors and previous dental treatments could have been associated with the oral health knowledge levels. But we evaluate participians level of knowledge regardless of previous treatments and recommendations of their dentist about their oral care behaviours.

5. CONCLUSION

The findings of the present study showed that oral hygiene habits and dental knowledge levels are not sufficient in our society. Preventive and educational programs should be developed in order to both improve and inform individuals

about oral care. In such a case, dentists have important responsibilities to inform their patients adequately and guide the planning of preventive oral programs.

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Clinical and Experimental Health Sciences

The Effect of Finger Puppets on Postoperative Pain in Children: A Randomized Controlled Trial

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ABSTRACT

Objectives: This study was conducted to determine the effect of finger puppet plays on the postoperative pain relief in children.

Methods: This study was conducted with 90 children who were aged between 1-5 years and who underwent surgery in 2016 in Turkey. The children were randomly divided into three groups. The control group (n=30) was given routine treatment (analgesic treatment), intervention group 1 (n=30) was played finger puppet by nurse, and intervention group 2 (n=30) was played finger puppet by parents. Data collection instruments were "Child and Parent Assessment Form", "Children's Hospital of Eastern Ontario Pain Scale" and "PedsQL Health Care Parent Satisfaction Scale". After the intervention, the pain of children was evaluated by "Children's Hospital of Eastern Ontario Pain Scale" and the satisfaction of the parents was evaluated by "PedsQL Health Care Parent Satisfaction Scale".

Results: Mean score of the pain scale in the control group was found higher than the intervention groups 1 and 2 ($p<0.001$). Mean score of satisfaction in control group was found lower than intervention group 1 and 2 ($p<0.001$).

Conclusion: This study highlights that finger puppet plays can be used to decrease postoperative pain by the nurses as an independent role.

Keywords: Children, postoperative pain, pediatric nursing.

1. INTRODUCTION

Pain is one of the most important factors affecting life quality during postoperative period. Despite a large number of studies on postoperative pain management in children, no clear results have been obtained for pain relief (1–3). Children still experience moderate to severe postoperative pain (4). Thus, more studies are needed to find new and effective techniques for pain relief in children (5).

Distraction techniques constitute an independent part of a nurse's roles (6). Recent studies have shown that play is a perfect choice as a distraction technique for children. Play can be used as a distraction method helping children to cope with pain, anxiety and fear (7–10). Play can be highly important for nurses since they provide an opportunity to perform atraumatic care and family-centered care that constitutes the philosophy of pediatric nursing (7,8,10).

Puppets are used as a part of playing activities for children in the hospital. They play an important role as a supportive factor for children having chronic diseases and for children in case of acute conditions. Puppets were found to be effective in decreasing aggression in children with attention deficit disorder and hyperactivity (11), in effective management of the disease in children with type 1 diabetes (12), in coping with disease and expression of feelings and thoughts for the disease in children diagnosed with cancer (13).

In recent years, research on distraction and preparation for children has been advanced (7–10). However, in the literature, no study was found reporting its use during postoperative period among children. Therefore, this study was carried out to determine the effect of distraction techniques (finger puppet plays) on the elimination of postoperative pain among children. It is important nursing research on pediatric surgical care, especially pain management, since it is difficult to evaluate pain in children.

2. MATERIAL AND METHODS

2.1. Study Design and Sample

This randomized controlled study was carried out in Pediatric Surgery Service of Zonguldak Obstetrics and Pediatrics

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Hospital in Turkey. The number of children within each group was determined as 26 at least based on the calculations made in G-Power 3.1 Demo package program for an effect size of 0.8 and a power of 80%. However, it was decided to include 30 cases in each group due to the probability of any withdrawals from the study by any parents and children. Inclusion criteria of the study were the following: 1 – The child should be operated, 2 – The child should be aged between 1-5 years old, 3 – The child should not have a disability impairing facial expression, 4 – The child and the parent should be voluntary to participate in the study. Children were assigned to groups randomly based on their order of hospitalization. This study was conducted with 90 children who were aged between 1-5 years old who underwent elective surgery. The patients were randomly divided into a control group and two intervention groups as shown in consort flow diagram (Figure 1).

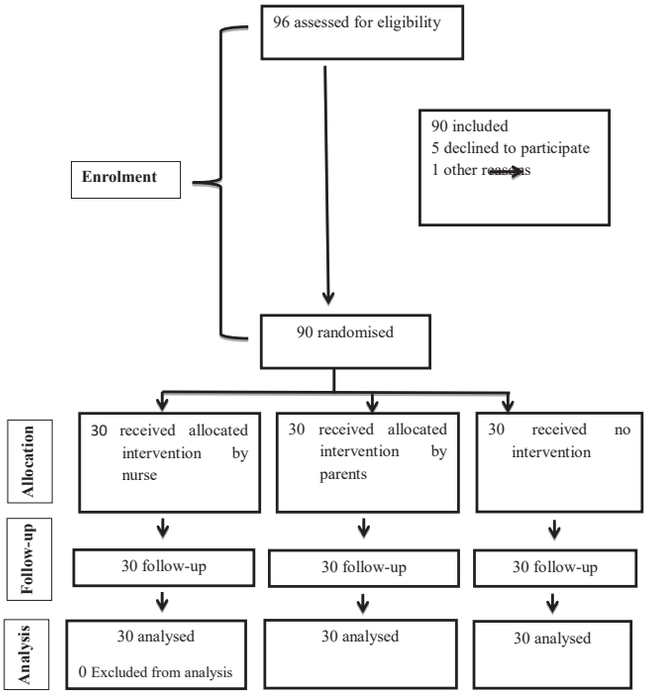

Figure 1. *Flow diagram of the progress through the phases of the study*

2.2. Data Collection Instruments

Child and Parent Assessment Form: This form included some questions about the socio-demographic characteristics of the child and the family, the name of the child's surgery, the presence of other illnesses, the number of children and the type of admission to the hospital.

Children's Hospital of Eastern Ontario Pain Scale in Young Children (CHEOPS): This scale was developed by Patrich J. McGrath et al. in order to evaluate postoperative pain among children; and consisted of 6 items including cry, facial expression, verbal responses, torso, wound evaluation and legs. Item 1 (cry) is evaluated with a minimum score of 1 and a maximum score of 3 points; items 2 (facial expression) and 3 (verbal responses) are evaluated with a minimum score of 0 and a maximum score of 2 points and items 4 (torso), 5 (wound evaluation) and 6 (legs) are evaluated with a minimum score of 1 and a maximum score of 2 points. The lowest total score of the scale is 4 whereas the highest score of the scale is 13. A high total score obtained from the scale shows that the child has a higher pain during postoperative period (14). The validity and reliability study of this scale in Turkish was conducted by these researchers (15) and the Cronbach's alpha internal consistency coefficient was found as α=0.87 in this study.

PedsQL Health Care Parent Satisfaction Scale (Version 3.0): This is a questionnaire including 25 questions which was developed by James W Varni in 1999. The questions included in this inventory are for measuring satisfaction from healthcare services provided. The scale is composed of six subscales including information, inclusion of family, communication, technical skills, emotional needs and overall satisfaction. Its adaptation to Turkish was conducted by Ulus and Kubilay (16). Internal consistency analysis of the inventory was performed in the context of reliability studies of the scale; and Cronbach's alpha internal consistency coefficient was found as α=0.93 (16).

2.3. Procedure

The sample of the study was composed of children who underwent surgery between May and August 2016 and who were aged between 1-5 years and had no any chronic diseases. The children included in the study were randomly assigned to three groups by the researchers according to the order of hospitalization. Among the groups, first one (control group) was given routine treatment (analgesic treatment), the second one (intervention 1 – no analgesic treatment) was played finger puppet plays by a nurse (that nurse was one of the researchers and working in that pediatric unit where this study was conducted), and the third group (intervention 2 – no analgesic treatment) was played finger puppet plays by the parents. The children underwent surgery with general anesthesia and the children for intervention groups didn't use analgesic treatment by the decision of the operating surgeon. The children for control group received acetaminophen (10-15 milligrams per kilogram) for an analgesic treatment. The interventions and evaluations by the scales were conducted an hour later the surgery when the children were totally awake and needed an analgesic treatment.

Puppets are used as a part of playing activities for children in the hospital. They play an important role as a supportive factor for children having chronic diseases and for children in case of acute conditions. Despite playing the puppet, the nurse or person is invisible in the environment due to the child's focus on the puppet. Therefore, the child does not have a fear of nurses. The nurse transmits her own knowledge to the child, hidden (17). The puppets used in the study are made of felt by the researcher-nurse (Figure 2). Since these puppets made of felt are washable and easy to

reconstruct if desired, the risk of carrying infections is tried to be minimized.

The texts used for puppet plays were written by a child development expert in accordance with the development of children aged between 1-5 years. At the same time, the researcher-nurse (AK) had training on puppet plays from a child development expert. The parents were trained by the researcher nurse on how to play puppet plays one day before the surgery (first day of hospitalization). The training was planned for 15-20 minutes but that time was extended with the questions of the parents. After the intervention, the pain of children was evaluated by "Children's Hospital of Eastern Ontario Pain Scale" and the satisfaction of the parents was evaluated by "PedsQL Health Care Parent Satisfaction Scale".

Figure 2. *Examples of Finger Puppets that were used for Puppet Plays*

2.4. Data Analysis

G-power 3.1 Demo package program was used to determine sample size. "SPSS for Windows 16.00" statistical program was used to analyze data. Intraclass correlation analysis was performed to assess pre-treatment of the study. Percentage distribution and Pearson chi-square test were used in the analysis of socio-demographic characteristics of the children and their parents. The differences between groups based on the variables were tested by one way analysis of variance (ANOVA) and complementary post-hoc analysis was used to determine the sources of the differences between groups.

2.5. Ethical Consideration

Ethics committee approval for the study was obtained from Kocaeli University Non-Interventional Clinical Research Ethics Committee (Date: 25/05/2016 and approval No. 2016/12). The written permission was obtained from the administration of the Zonguldak Bülent Ecevit University Institution of Health Sciences (Date: 27/06/2016 and approval No. 2016/18). Data collection was performed based on the voluntary participation of the individuals enrolled in the study. Parents were informed about the aim of the study and the confidentiality of all data, and their written consents were received. The research conforms to the provisions of the Declaration of Helsinki in 1995 (as revised in Brazil 2013).

3. RESULTS

This research was carried out on 90 patients aged 1-5 years who underwent elective surgery. The patients were randomly divided into a control group and two intervention groups. In the control group, 27 of the children (90.0%) were male, 9 (30.0%) were 3 years old and 27 (90.0%) had circumcised. Twelve (40.0%) of the children's mothers and 15 (50.0%) of the fathers graduated from high school. In the intervention 1 group, 27 (90.0%) of the children were male, 9 (30.0%) were 3 years old and 26 (86.7%) were circumcised. Thirteen (43.3%) of the children's mothers and 15 (50.0%) of the fathers graduated from high school. In the intervention 2 group, 28 (93.3%) of the children were male and 5 (16.7%) were 3 years old and 28 (93.3%) were circumcised. Sixteen (53.3%) of the children's mothers and 13 (43.3%) of the fathers graduated from high school (Table 1). There were no statistically significant differences between the three groups according to the descriptives as shown in Table 1 ($p>0.05$). Children's pain and parental satisfaction do not make a difference according to sociodemographic data.

3.1. Comparison of Pain Levels of Children between the Groups

Analysis of variance (ANOVA) results considering pain scores of children in control, intervention 1 and intervention 2 groups were given in Table 2.

CHEOPS scores of the children in control group (9.470±1.925) were found to be higher than the children in intervention group 1 (7.130±1.871) ($p<0.001$). CHEOPS scores of the children in the control group (9.470±1.925) were found to be higher than CHEOPS scores of the children in intervention group 2 (6.470±1.978) ($p<0.001$) (Table 2).

3.2. Comparison of Anxiety Levels of Parents between the Groups

In Table 3, analysis of variance (ANOVA) results considering PedsQL scale scores of parents in control, intervention group 1 and intervention group 2 were given.

PedsQL scores of the parents in intervention group 1 (3.283±0.401) were found to be higher than the parents in control group (2.121±0.360) ($p<0.001$). PedsQL scores of the parents in intervention group 2 (3.288±0.433) were found to be higher than the parents in control group (2.121±0.360) ($p<0.001$) (Table 3).

Table 1. *Descriptives and chi-square analysis of sociodemographic factors among the children in control, intervention 1 and 2 groups*

		Control		Intervention1		Intervention2		p
		n	%	n	%	n	%	
Sex	Female	3	10.0	3	10.0	2	6.7	X^2=0.274 p=0.872
	Male	27	90.0	27	90.0	28	93.3	
Age (years)	1	6	20.0	5	16.7	7	23.3	X^2=2.798 p=0.946
	2	6	2.0	5	16.7	5	16.7	
	3	9	30.0	9	30.0	5	16.7	
	4	5	16.7	5	16.7	6	20.0	
	5	4	13.3	6	20.0	7	23.3	
Type of surgery	Lingual phrenectomy	1	3.3	1	3.3	1	3.3	X^2=4.874 p=0.560
	Inguinal hernia	2	6.7	3	10.0	0	0.0	
	Circumcision	27	90.0	26	86.7	28	93.3	
	Umbilical hernia	0	0.0	0	0.0	1	3.3	
Education level of mother	Literate	1	3.3	3	10.0	2	6.7	X^2=4.365 p=0.627
	Primary school	12	40.0	10	33.3	15	50.0	
	High school	12	40.0	13	43.3	7	23.3	
	University	5	16.7	4	13.3	6	20.0	
Education level of father	Literate	0	0.0	1	3.3	0	0.0	X^2=2.926 p=0.818
	Primary school	10	33.3	8	26.7	11	36.7	
	High school	15	50.0	16	53.3	13	43.3	
	University	5	16.7	5	16.7	6	20.0	

X^2: Chi-square test

Table 2. *Analysis of variance (ANOVA) results considering pain scores of children in control, intervention 1 and intervention 2 groups*

	Control (1)		Intervention 1 (2)		Intervention 2 (3)		F	p	Comparison
	M	SD	M	SD	M	SD			
Pain Scores of Children (CHEOPS)	9.470	1.925	7.130	1.871	6.470	1.978	20.089	**0.000***	**1 > 2** **1 > 3**

*CHEOPS: Children's Hospital of Eastern Ontario Pain Scale, *p<0.001*

Table 3. *Analysis of variance (ANOVA) results considering PedsQL scale scores of parents in control, intervention group 1 and intervention group 2*

	Control (1)		Intervention 1 (2)		Intervention 2 (3)		F	p	Comparison
	M	SD	M	SD	M	SD			
PedsQL Scale Scores of Parents	2.121	0.360	3.283	0.401	3.288	0.433	85.092	**0.000***	**2 > 1** **3 > 1**

**p<0.001*

4. DISCUSSION

There has been an ongoing research for evaluating the effect of play, which are one of the distraction techniques that are often used in the hospitals, on the children during postoperative period (7,9,18). Nurses play a key role in the management of postoperative pain of children in the hospital (2,19–21). Yun et al. (9) reported that children experienced less anxiety and less pain during postoperative period after having a training on pre – and postoperative periods from clown nurses. Moreover, Fincher et al.(7) reported that training given through play by game-trained experts was effective in eliminating postoperative pain in children. He et al.(18) found that children and parents, who had a one-hour therapeutic game intervention, experienced anxiety more than the ones who did not. In the same study, it was also reported that children having less anxiety experienced less postoperative pain. At the end of this study, children in control group were found to experience more pain compared to the children in the first intervention group (the group

which was played finger puppet game by the nurse). Thus, results are similar to the relevant data in the literature.

Parents may be one of the most important factors affecting pain of children during postoperative period (8,22–24). Parents have significant roles in the management of pain at home during postoperative period, and they may feel themselves desperate since they may become incapable of adjusting analgesic drug doses (22,24–26). Chorney et al.(27) emphasized that parents wanted to be trained especially on distraction techniques in postoperative pain management of their children. Therefore, family should be included in postoperative pain management of children (22,28). Ullan et al.(8) divided 95 children aged between 1-7 years old into two groups during postoperative period; and one group was played a game using a toy by the parents and the other group did not undergo any intervention. In conclusion, it was reported that children in the group which played game with parents experienced less pain compared to the children who did not play.

When the effect of the inclusion of parents during postoperative period on the children was examined in the literature, positive effects have been observed. At the end of this study, it was found that children in control group experienced more pain compared to the children in the second intervention group where parents played finger puppet plays.

Besides the studies reporting that parents have a positive effect on the children during postoperative period, there are also studies indicating some negative effects (29). Scalford et al.(29) stated that children, who were kept together with their parents during early postoperative period, experienced more pain. The reason of this was considered to be the fact that parents might reflect their own anxiety to the child and make them feel more anxious; and thus, have more pain. Parents having less anxiety, a higher level of knowledge and whose child was provided comfort were satisfied with the healthcare given about postoperative pain management (7). In this study, it was seen that parents in both intervention groups were more satisfied with the healthcare given to their children compared to the parents in the control group. These results were also found to be consistent with literature data.

No significant difference was found between the parents in both study groups based on satisfaction. Parents see the experience of pain during postoperative period as an expected situation (30). Therefore, it can be stated that they were satisfied with the interventions made for their children (finger puppet plays played by themselves as well as nurses) and the interest shown to them.

5. CONCLUSION

Finger puppet plays effectively decreased postoperative pain in children. Finger puppet plays can be used to decrease postoperative pain by the nurses as an independent role. This study highlights that puppet plays can be used by the nurses and parents for pain relief in children as a non-pharmacological method. Also, inclusion of parents in the plays has a positive effect on postoperative pain in children and on parental satisfaction. Pediatric nursing care for pain management should include distraction techniques and inclusion of parents. However, nurses may not be willing to use these distraction techniques and their attitudes towards parent inclusion in postoperative pain relief in children can be negative. Further nursing education programs addressing non-pharmacological techniques such as distraction techniques and parent inclusion for pain relief can be beneficial for children.

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Clinical and Experimental Health Sciences

The Evaluation of the Nutritional Status in Patients with Irritable Bowel Syndrome

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ABSTRACT

Objective: Irritable bowel syndrome (IBS) is one of the most common chronic functional diseases of the gastrointestinal tract. There is no organic disorder in IBS. The pathogenesis of IBS is not fully understood. Although the symptoms of IBS patients are mostly diet-related, there are not many studies evaluating nutrient intake. In this study, it was aimed to determine nutrient intake and nutrition habits of IBS patients.

Methods: A total of 70 IBS patients, 52 females and 18 males, were included in the study. Three-day food records were compiled. Besides, foods that reduced symptoms of IBS and foods that caused sitophobia were interrogated in the study.

Results: The mean age of the patients was 37.8±10.03 years and 40% of them were IBS-mixed subtype. Daily intake of energy and some nutrients were found to be similar to the recommended levels. The mean intake of energy, protein, carbohydrate, saturated fatty acid, vitamin B1, vitamin B2, vitamin B6, vitamin B12, calcium, magnesium, phosphorus, iron, copper ($p<0.001$), total folic acid, carotene, sodium, and potassium ($p<0.05$) showed statistically significant differences according to gender. It was determined that 85.7% of the patients had sitophobia. In both genders, apricot was the most reported food to have reduced IBS symptoms. The foods causing sitophobia were raw vegetables-fruits (35.7%), spicy foods (34.3%), and legumes (27.1%).

Conclusions: Although IBS avoids consuming certain foods for fear of increasing their symptoms, energy, and nutrient intake is found to be similar to generally recommended levels.

Keywords: diet, gastrointestinal diseases, irritable bowel syndrome, nutrition, nutrition habits

1. INTRODUCTION

Irritable bowel syndrome (IBS) is a chronic functional bowel disease without a clinically organic pathology. It is characterized by lower and upper gastrointestinal symptoms such as a change in bowel habits (constipation/diarrhea), abdominal pain, dyspeptic complaints (gas, bloating, and mist). However, the etiology of IBS is not fully understood (1). While IBS is often seen in young adults, it may be encountered in different age categories. Irritable bowel syndrome, which is one of the reasons for applying to a physician in primary health care institutions, is also a frequent disease in gastroenterology polyclinics. As per epidemiological data, the frequency of the disease is higher in females than in males. The majority of IBS patients are between 20 and 40 years of age. It has been reported that the frequency of this disease in the general population globally is 5-20% (2). The prevalence in Western populations is 8-23%, 60-70% of which are female. It is most commonly seen between the ages of 30-50 (3-5). The country with the lowest prevalence rate is Singapore (2.3%), while the country with the highest prevalence rate is reported to be Niger (30%). In Asian countries, 1% to 10% of the population has symptoms consistent with IBS (4). In studies carried out in different regions of Turkey, IBS frequency was determined as 2.7 – 19.1% (6-8).

Although IBS may not be fatal, it seriously lowers the quality of life (QoL) of patients and increases the levels of depression and anxiety. In recent studies, it has been shown that the low fermentable oligo-, di-, and monosaccharides and polyols (FODMAP) diet administered in intestinal diseases reduces the symptoms of illness (especially inflammatory bowel disease and IBS) and also changes intestinal motility and secretion. FODMAP includes fructans, galacto-oligosaccharides – oligosaccharides, lactose-disaccharide, fructose-monosaccharide especially over glucose, mannitol, sorbitol, maltitol, and xylitol-polyols. Observational cohort studies in the 1980s and 1990s found that the low FODMAP diet reduced symptomatic

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complaints. Current studies confirm this finding. Looking at the historical process of the term FODMAP, it was used by a group of Australian researchers in 2005 and is frequently mentioned in the scientific literature (9, 10). On the other hand, there are some different diet models for IBS such as a gluten-free diet. Although diet has an important role in both IBS pathophysiology and treatment management, it is recommended that a sustainable diet that patients can adapt to in the long term should be arranged according to nutritionists and the patient's nutritional habits.

The low FODMAP diet is based on the principle of reducing the fermented oligosaccharides, disaccharides, monosaccharides, and polyols in the diet. FODMAP expresses all short-chain carbohydrates. These carbohydrates are thought to increase the symptoms because they are known to be absorbed poorly and ferment rapidly in the intestine (11). Diets containing low FODMAP have been shown to reduce gastrointestinal symptoms when compared to diets containing high FODMAP (24). Also, diets containing low FODMAP improve the QoL of patients, positively affect intestinal microbiota and gastrointestinal endocrine cells (12-14).

On investigation of studies to date, insufficient data is encountered about the dietary changes of IBS patients assessed, the effects of nutrient deficiencies, and the dietary changes on the symptoms of patients. This study aims to evaluate the nutritional status of IBS patients and shed light on providing novel recommendations for them.

2. METHODS

2.1.Study Subjects

The data were collected from 70 volunteers (52 females, 18 males) between 20-55 years who were admitted to Ankara Numune Training and Research Hospital Gastroenterology Polyclinics and diagnosed with IBS according to Rome III Criteria. This study was conducted between February and September 2016.

Patients who were able to choose nutrients on their own, who did not have perception impairment and communication problems were included in the study. Patients with inflammatory bowel disease, celiac disease, who are known to have lactose intolerance, advanced cardiac, respiratory, renal, hepatic disorders, malignancy, major psychiatric disorder, and narcotic/alcohol use were not included in the study. Apart from uncomplicated appendicitis, patients who underwent previous abdominal surgery, pregnant and lactating patients were excluded from the study.

2.2.Anthropometric Measures

Anthropometric measurements (body weight (kg), height (cm), waist circumference (cm), hip circumference (cm)) of the subjects participating in the study were measured by the researcher. Bodyweight measurements were analyzed using the Tanita BC 532 bioelectrical impedance device in an empty stomach in the morning with minimal clothing on the individual. Height was measured on the head Frankfurt plane with the individuals' feet together. The body mass index (BMI) was calculated as the bodyweight/height^2 (kg/m^2) (15). Prior to waist circumference measurements, the patients were asked to took off the clothing and objects that might prevent measurement. The measurement was taken at the position where the abdomen was loose while the arms stood on two sides and the feet placed side by side facing the person recording the measurements. Between the lowest rib bone and the crista iliaca, the circumference passing through the middle point was measured by a non-stretching tape. Measurements were recorded in cm and with a sensitivity of 0.1 cm. Measurement of the hip circumference was taken from the widest region of the hip (16).

2.3.Dietary Assessment

To determine dietary energy and nutrient intake of the individual, three-day foods records (three consecutive days one day at the end of the week) were compiled by dieticians so that individuals could take the changes into account in weekly nutrient consumption. The daily intake of energy and nutrients were evaluated using a Nutrient Data Base Program (BeBiS, version 7.0; Pasifik Company, Istanbul, Turkey), adapted for Turkish individuals (17).

To evaluate the eating habits of the patients, the consumption frequency of different foods was examined before and after the diagnosis of IBS. The questionnaire interrogated the frequency of consumption of 43 food types (dairy products, fruits, vegetables, drinks, etc.) over the previous 12 months. Participants had six choices to indicate the frequency of consumption of each of the 43 foods from "never" to "per day". Foods that aggravated the symptoms (raw vegetables-fruits such as potato, carrot, citrus; dairy products such as milk, yoghurt, drinks, legumes, etc.) in IBS patients were given wide coverage in food frequency questionnaire based on the studies conducted with IBS patients prior to this study. In addition, IBS symptoms were taken into account when food was grouped. For example, fruits were grouped as follows: citrus; apples, peaches, quince and bananas; other fruit and dried fruits. In this way, it was aimed to evaluate whether there was any change in the frequency of food consumption before and after the diagnosis of IBS. Besides, the foods that the individuals thought alleviated or aggravated their symptoms were questioned. The questions in this section were asked in open-ended fashion so that patients would not be biased.

2.4.Statistical analysis

Statistical Package for the Social Sciences (SPSS) 22.0 package program was used to evaluate the data (18). For descriptive analyses, frequencies and percentages were used for qualitative observations and Chi-square analyses

were utilized for interpretations. Mann Whitney U test was also employed in the two groups considering the number of observations for quantitative observations. P-values less than 0.05 were considered statistically significant.

3. RESULTS

Participant characteristics were shown in Table 1. In total, 70 subjects with IBS participated in the study, 26% of whom (18) were males and 74% (52) were females. The mean age of the subjects was 37.8±10.03 years, the mean diagnosis time of IBS is 6.5±17.47 years. There was no significant difference between gender in mean age and diagnosis time. The percent of alternating IBS symptoms (IBS-M) and constipation-predominant symptoms (IBS-C) was approximately the same (37.1% and 40.0%, respectively), while diarrhea-predominant symptoms (IBS-D) ratio was 22.9%. Although IBS-M was seen more frequently in males, in females, IBS-C and IBS-M were more frequent than the other type. Male subjects had a mean body mass index (BMI) of 27.1±2.97 kg/m^2 and were not significantly different from females (26.6±5.81 kg/m^2). Most of the individuals were married (%68.6) and 35% of them were primary school graduates. More than half of the participants (57.1%) had at least 8 years of formal education. There was no significant difference between males and females in terms of education status. While 44% of the male subjects were smokers, only 13.5% of females smoked (p<0.001). Moreover, a small proportion of participants consumed alcohol (%8.6).

***Table 1. T**he characteristics of participant*

Variables	Male (n=18)	Female (n=52)	Total (n=70)	p*
Age, years (mean±SD)	40.2±10.91	37.0±9.69	37.8±10.03	0.250
Body weight, kg (mean±SD)	81.1±9.34	68.6±13.89		
BMI, kg/m^2 (mean±SD)	27.1±2.97	26.6±5.81		
Waist circumference, cm (mean±SD)	97.1±11.06	88.8±14.56		
Hip circumference, cm (mean±SD)	106.7±11.84	105.1±11.49		
Marital status, % Married Single	 66.7 33.3	 69.2 30.8	 68.6 31.4	
Diagnosis time of IBS, month	2.3±2.93	7.9±20.04	6.5±17.47	0.385
IBS-C, %	33.3	38.5	37.1	χ2=0.219 0.896
IBS-D, %	22.2	23.1	22.9	
IBS-M, %	44.4	38.5	40.0	χ2=14.914 ***0.000***
Smokers, %	44	13.5	21.4	
Alcohol drinkers, %	16.7	5.8	8.6	χ2=2.026 0.158

BMI: Body mass index; IBS: Irritable bowel disease; C: constipation, D: diarrhea, M: mixed
**Mann Whitney U and Chi-Square Test*

The mean daily intake (±SD) for energy, macronutrients, and selected micronutrients for males and females with IBS were presented in Table 2. The mean intake of energy, protein, carbohydrate, saturated fatty acid, vitamin B_1, vitamin B_2, vitamin B_6, vitamin B_{12}, calcium, magnesium, phosphorus, iron, copper (p<0.001), total folic acid, carotene, sodium, and potassium (p<0.05) showed statistically significant differences according to gender. The mean energy intake was 2302.5±895.6 kcal/day in male patients, whereas, 1485.5±513.92 kcal/day in female patients (p<0.01). The mean protein intake was 88.1±34.5 g/day in male patients and 47.2±16.72 g/day in female patients (p<0.001). The average daily intake of nutrients as carbohydrates, saturated fatty acids, vitamin B_1, vitamin B_2, vitamin B_6, vitamin B_{12}, calcium, magnesium, phosphorus, iron, copper, total folic acid, carotene, sodium, and potassium was found to be higher in males as compared to females. The mean intake of the other nutrients has been given in Table 2 according to gender. There is no significant difference in terms of gender (p>0.05). Also, there was no significant difference in the dietary intake between the 3 IBS subtypes (no IBS subtypes specific data were shown).

Table 2. *Daily energy and nutrient intake of the study population (mean±SD)*

Total daily intakes	Male (n=18)	Female (n=52)	P
Energy (kcal)	2302.5±895.6	1485.5±513.92	<0.001***
Total protein (g)	88.1±34.5	47.2±16.72	<0.001***
Total protein (%)	16.2±6.0	13.4±2.86	0.142
Total fat (g)	88.2±37.7	65.3±26.25	0.353
Total fat (%)	35.1±7.10	39.9±9.19	0.054
Carbohydrate (g)	284.5±135.6	170.4±72.84	0.002***
Carbohydrate (%)	48.7±10.5	46.8±9.46	0.282
Fiber (g)	21.8±10.7	14.6±5.39	0.015
Saturated fatty acid (g)	29.3±8.02	23.2±9.69	0.008***
MUFA (g)*	29.5±11.81	22.1±9.29	0.135
PUFA (g)**	23.4±21.43	15.7±10.03	0.135
Omega6/Omega3	17.5±10.3	13.9±7.41	0.135
Cholesterol (mg)	295.9±156.10	202.2±135.45	0.013
Vitamin A (mcg)	1927.6±3582.38	844.6±462.03	0.135
Vitamin D (mcg)	0.7±0.84	0.7±0.67	0.337
Carotene (mg)	3.5±3.55	2.8±2.07	0.018****
Vitamin E (mg)	19.3±20.82	13.9±7.11	0.135
Vitamin B_1 (mg)	1.0±0.41	0.6±0.20	<0.001***
Vitamin B_2 (mg)	1.5±0.79	0.9±0.33	<0.001***
Niacin (mg)	17.6±11.7	16.6±7.30	0.080
Vitamin B_6 (mg)	1.5±0.56	1.1±0.36	0.001***
Vitamin B_{12} (mcg)	7.6±12.95	2.4±1.67	0.009***
Total Folic Acid (mcg)	366.8±181.59	240.5±85.59	0.012****
Vitamin C (mg)	59.7±41.67	85.0±45.86	0.053
Sodium (mg)	2253.7±1235.97	1451.7±722.34	0.014****
Potassium (mg)	2173.7±719.62	1757.8±641.75	0.020****
Calcium (mg)	593.8±220.63	452.3±183.59	0.010***
Magnesium (mg)	227.9±76.63	175.9±71.57	0.004***
Phosphorus (mg)	1246.4±346.27	776.1±263.77	0.000***
Iron (mg)	12.8±5.93	8.4±2.99	0.004***
Zinc (mg)	12.1±5.43	6.6±2.27	0.064
Copper (mg)	1.7±0.89	1.1±0.41	0.004***

**Monounsaturated fatty acid ** Polyunsaturated Fatty Acid ***p<0.01. ****p<0.05. Mann Whitney U*

The amount of consumption of food groups was shown in Table 3 according to gender. Consumption of fats, poultry, nuts and seeds, and citrus fruits were significantly higher in males as compared to females (p<0.05). The consumption amounts of other food groups according to gender were given in Table 3 and no significant difference was found between the groups (p>0.05).

The distribution of nutrients causing sitophobia in individuals has been given in Table 4. It was determined that 85.7% of the patients had sitophobia and this ratio was 66.7% in males and 92.3% in females. The foods causing sitophobia in the males were spicy foods (38.9%), raw vegetables-fruits (27.8%) and legumes (27.8%) and in females, it was raw vegetables-fruits (38.5%), piquant foods (32.7%), legumes (26.9%) and dairy products (25.0%), respectively. It was determined that 35.7% of all the individuals were uncomfortable with raw vegetables-fruits, 34.3% with spicy foods and 27.1% with legumes.

More than half of the individuals (62.9%) stated that no food alleviated the symptoms of IBS. Among all foods, apricot was considered to be effective in alleviating the symptoms in both genders followed by yoghurt 8.6%; milk 7.1%; olive oil 4.3%; apple 2.9%; dry plum 2.9%; dried fig 1.4%, leek 1.4%, watermelon 1.4% and potato 1.4% (Table 5). In addition, pre-and post-diagnosis food frequency questionnaire of the participants was evaluated. There were no significant changes in male pre-and post-diagnosis food frequency questionnaire except for yoghurt. At the post-diagnosis period, females participants' consumption of yoghurt, dried fruit, whole-grain bread, and bran bread increased, whereas consumption of milk, citrus fruits, apples, peaches, quince, bananas, fries, sugar, coffee, carbonated drinks, and white bread decreased (No food frequency questionnaire specific data were shown).

Table 3. *The amount of the distribution of food consumption regarding the individuals' gender*

	Male	Female	
Food groups	**X̄±SD**	**X̄±SD**	**p**
Meat group (g)			
Red meat	45.9±98.90	45.9±55.26	0.360
Poultry	40.4±36.94	36.3±77.74	0.011*
Fish	2.8±11.79	1.1±4.53	0.987
Egg	28.3±29.46	20.2±28.44	0.082
Legumes	11.7±14.48	9.5±15.58	0.308
Oil seeds	8.9±16.03	3.5±11.29	0.023*
Milk group (g)			
Milk, yoghurt	101.8±94.24	97.3±92.91	0.674
Cheese	32.4±26.62	46.3±34.32	0.128
Cereals (g)			
White bread	131.2±129.6	128.0±149.93	0.535
Brown bread	15.2±24.89	38.9±51.65	0.172
Other cereals	48.1±37.22	54.1±75.27	0.691
Fruits (g)			
Citrus	20.2±41.09	0.25±1.27	0.000*
Apple, peach, quince, banana	83.4±77.76	169.2±201.89	0.364
Vegetables (g)			
Dark green leafy vegetables	60.7±51.63	53.9±66.94	0.255
Potatoes, zucchini, carrots etc.	201.4±115.99	291.2±181.51	0.064
Oils (g)			
Vegetable oils	32.3±16.17	21.6±20.77	0.002*
Animal fats	3.6±4.05	3.1±5.68	0.198
Sugar and similar foods (g)	28.6±31.42	27.2±42.70	0.225

**p<0.05. Mann Whitney U test*

Table 4. *The distribution of the foods causing sitophobia in individuals*

	Male (n=18)		Female (n=52)		Total (n=70)	
Sitophobia status	**N**	**%**	**N**	**%**	**N**	**%**
Yes	2	66.7	48	92.3	60	85.7
No	6	33.3	4	7.7	10	14.3
The foods causing sitophobia	**N**	**%**	**N**	**%**	**N**	**%**
Dairy products	2	11.1	13	25.0	15	21.4
Raw fruits and vegetables	5	27.8	20	38.5	25	35.7
Cereals	2	11.1	9	17.3	11	15.7
Legumes	5	27.8	14	26.9	19	27.1
Sugar and sweets	1	5.6	6	11.5	7	10.0
Fat and fatty foods	2	11.1	12	23.1	14	20.0
Fizzy drink and caffeinated beverages	3	16.7	5	9.6	8	11.4
Spicy foods	7	38.9	17	32.7	24	34.3
Spices	3	16.7	5	9.6	8	11.4

**Individuals responded to the question more than one and the percentages were calculated according to "n" numbers.*

Table 5. *The distribution of the participants regarding the foods that they think reduce their symptoms*

	Male (n=18)		Female (n=52)		Total (n=70)	
Foods reducing symptoms	**N**	**%**	**N**	**%**	**N**	**%**
Yes	7	38.9	19	36.5	26	37.1
No	11	61.1	33	63.5	44	62.9
Foods	**N**	**%**	**N**	**%**	**N**	**%**
Dried apricots	3	16.7	6	11.5	9	12.9
Yoghurt	2	11.1	4	7.7	6	8.6
Milk	2	11.1	3	5.8	5	7.1
Olive oil	1	5.6	2	3.9	3	4.3
Apple	-	-	2	3.9	2	2.9
Dried plum	1	5.6	1	1.9	2	2.9
Dry fig	-	-	1	1.9	1	1.4
Leek	-	-	1	1.9	1	1.4
Watermelon	1	5.6	–	-	1	1.4
Potato	1	5.6	–	-	1	1.4

4. DISCUSSION

Irritable bowel syndrome is a chronic functional bowel disease characterized by lower and upper gastrointestinal symptoms such as a change in bowel habits (1). Although there are many studies on IBS internationally, there is no large-scale research on adult IBS patients in Turkey. This is a cross-sectional study evaluating the dietary intake of patients with IBS and the dietary habits associated with the symptoms.

In adult individuals with IBS, whereas the age of the sample group varies between 20-62 years, the mean age was reported as 39.3±13.1 years (19), 46.9±13.4 years (20), 33.3±13.9 years (21). On the other hand, the mean age of the individuals is 37.8±10.03 years in this study, which is similar to the populations in other studies (19-21). Besides, studies reported the prevalence of IBS differs according to gender, there are other research stating that IBS is seen more frequently in females than males. In particular, there is a strong relationship between gender and IBS-C, seen higher in female patients (22). Stress is known to be an important factor in the pathophysiology of the disease and the more common occurrence of IBS in females can be explained by this situation. In studies conducted on IBS patients, the ratio of female to male patients was 2/1, 3/1, 5/1 and 8/1. The female/male ratio of this study is 3/1 and has a similar distribution with other studies (21, 23, 24).

Irritable bowel syndrome is usually divided into 3 subgroups, which are IBS-C, IBS-D, and IBS-M. In a study conducted with IBS patients, it was determined that 44.8% of the patients were IBS-M, 28.6% were IBS-D and 26.5% were IBS-C. In another study, a total of 56 individuals were examined and as a result, it was stated that 61% of these individuals were IBS-M. It has been stated that IBS-M subtype is seen more frequently and the rate of occurrence varies in the case of IBS-C and IBS-D. IBS subtypes may vary depending on environmental and individual factors (24, 25). In this study, 40.0% of the individuals are IBS-M, 37.1% of IBS-C and 22.9%

of IBS-D. The obtained data were found to be consistent with the literature. The higher incidence of IBS-M subtype may be due to the fact that the symptoms vary depending on mood and seasonal differences.

The mean BMI of the individuals was calculated as 27.1±2.97 kg/m^2 for males and 26.6±5.81 kg/m^2 for females, respectively. When the obtained data were compared with the mean values obtained in Turkey, the BMI was higher in male subjects while it was found to be lower in female subjects. This may be explained by the fact that the individuals involved in the study did not have adequate knowledge about balanced nutrition and did not restrict the amount of some foods even if they aggravated their IBS symptoms.

The assessment of the nutritional status of individuals is an indication of the extent to which the nutritional requirements are met. Providing a balance between nutrient intake and nutrient requirements is important for optimal health (26). In addition to providing adequate and balanced nutrition in GIS diseases such as irritable bowel syndrome, the importance of medical nutrition therapy is a major factor in increasing QoL. In this context, a low FODMAP diet has been applied in the medical nutrition therapy of IBS in recent years. The low FODMAP diet is based on the principle of reducing fermented oligosaccharides, disaccharides, monosaccharides, and polyols in the diet. Therefore, it refers to all short-chain carbohydrates. Short-chain carbohydrates have low intestinal absorption and they increase symptoms of IBS as they are fermented fast (10, 11).

In the current study, the percent of energy from the macronutrients in male and female subjects was as follows: carbohydrate 48.7±10.5% and 46.8±9.46%, protein 16.2±6.0 and 13.4±2.86%, fat 35.1±7.1% and 39.9±9.19%, respectively. Böhn et al. reported that the distribution of macronutrient percentage in the diet of IBS patients was as follows; 47±8% carbohydrate, 17±3% protein, and 35±7% fat (27). In both studies, the patients with IBS had a high fat intake while the carbohydrate intake was low and protein intake was close to the recommended level. When examined the food frequency questionnaire of the individuals in this study, it was seen that the fat sources such as butter and fried foods are frequently consumed which resulted in a high-fat ratio. When studies in the literature were examined, it was seen that IBS patients generally have adequate energy intake and nutrients, as well as energy and nutrient intake, were similar when compared to healthy groups (21, 28).

As much as the percentage of energy from fat, the fatty acid composition of the diet is also important. When compared to saturated fat, unsaturated fatty acids such as monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) can reduce total cholesterol and low-density lipoprotein-cholesterol (LDL-C) (29). In this study, it was determined that the total amount of fat, saturated fat, MUFA, and PUFA intake of male subjects was higher than that of the females. In line with this, it was revealed that males consumed animal-derived foods such as butter, red meat, vegetable oils such as sunflower oil, and oilseeds more than females.

Dairy products, an item restricted in the FODMAP diet due to lactose content, frequently increase complaints in IBS patients. However, the frequency of lactose intolerance in IBS patients was found to be similar to the healthy population. Lactose increased gas production in the intestine and aggravated symptoms in IBS patients. On the other hand, the consumption of dairy products containing probiotics is thought to have positive effects on IBS patients. In the studies, the intake of calcium and magnesium in IBS patients was found to be lower than the Recommended Dietary Allowance (RDA) (30-32). Similarly, in the present study, calcium and magnesium intake was below the recommended value. When the food consumption of the patients was examined, it was seen that calcium and magnesium sources such as milk, green leafy vegetables and whole grains were consumed insufficiently. With an inadequate intake of calcium and magnesium, patients with high phosphorus uptake may experience mineral homeostasis and deterioration in bone structure (28).

In a study conducted in Norway, it was reported that IBS patients consumed a daily mean of 86.0 g fresh fruit, 150.5 g fresh vegetables, 123.5 g potatoes, 2.2 portions of milk and dairy products, 180 mL carbonated beverages, 420 mL coffee and 170 mL of tea (23). When compared with the results of this study, it was found that fresh vegetable consumption amount was similar, the consumption of fresh fruit was higher whereas the consumption of milk and dairy products was lower. As the regional and cultural differences in nutrition differ from society to society, the consumption amounts can be different depending on this.

Studies have shown that the intake of dietary fiber of IBS patients was below the recommended values (27, 28). In this study, it was also found that IBS patients had below the recommended amount of dietary fiber intake. Since patients did not consume sources of fiber such as fresh vegetables and fruits, legumes, whole grain products, etc. in sufficient quantities, the intake of dietary fiber was low.

Besides, the majority of the participants (72.9%) did not adequately consume fiber sources (e.g. dry legumes) due to worsening of their IBS symptoms. There is no specific fiber recommendation for IBS patients. The amounts determined according to the DRI are also true of IBS patients (38 g/day for adult males and 25 g/day for adult females) (33). Although there is no clear recommendation for soluble and insoluble fiber, it was stated in a report of the American Dietetic Association in 2008 that the intake of 2-8 g/day of soluble fiber had beneficial effects on health (34).

Although soluble fiber is known to have a beneficial effect on IBS, insoluble fiber such as whole-grain wheat products and wheat bran worsens the symptoms. For this reason, insoluble fiber sources are only recommended for IBS-C patients. Other than IBS patients with acute diarrhea, it is suggested that fiber sources should be included in diets to

meet the recommended amounts for other IBS patients, and fiber supplementation should be provided in cases that cannot be met by dieting (35-37). FODMAPs draw water into the intestine, that is, they are osmotic. Therefore, when foods containing high FODMAP are consumed, they can be fermented by bacteria in the intestinal system, depending on problems such as poor digestion or poor absorption. This fermentation causes such problems as gas, abdominal pain, cramps, diarrhea, etc. In recent years, it has been suggested that foods rich in FODMAP such as legumes, wheat, rye, apples should be removed from the diet of IBS patients (11, 38).

Although there is no information in the literature on the presence of sitophobia in patients with irritable bowel syndrome, it was reported that in some intestinal diseases, it could decrease food consumption and result in loss of body weight (39). In this study, the sitophobia status of the patients was questioned and it was determined that 85.7% of the individuals complained of the problem. Foods causing sitophobia were raw vegetables-fruits (35.7%), piquant foods (34.3%), and legumes (27.1%). When the food consumption was examined, it was seen that individuals continued to consume some foods even though they cause sitophobia.

Crohn's & Colitis Foundation of America reported that probiotics and prebiotics taken with dietary sources (fermented dairy products like yoghurt, non-fermented dairy products) and supplements have beneficial effects in IBS and inflammatory bowel disease but there is no strong evidence on this topic (40). In this study, the participants were asked if there were any foods which they thought alleviated their symptoms and 37.1% of them replied in positive. The ones who provided a positive reply stated that mostly dried apricots, yoghurt, milk and olive oil reduce their symptoms. As the symptoms and findings of IBS patients vary from person to person, there is no definitive information about the foods that reduce or recover the symptoms (27).

When the studies conducted on IBS patients in different societies are examined, they do not include evidence-based data. The general nutritional recommendation for IBS is as follows: reducing lactose, FODMAPs, fat and fat sources, and foods containing gas constituents, and increasing or decreasing dietary fiber according to IBS subtype. It is known that patients often have various restrictions depending on their symptoms and medical nutrition therapy. However, it was reported that there was not much difference between healthy individuals and IBS patients in terms of dietary intake. On the other hand, it was stated that the reason for higher energy and nutrients intake of IBS patients was to consume the foods which did not influence their symptoms more to compensate for the foods that increase their symptoms (21, 41, 42).

5. CONCLUSION

In this study, the energy, macro, and micronutrient intake of IBS individuals were assessed using a 3-day food intake record. Besides, foods that increase the symptoms of individuals and the foods that reduce their symptoms were questioned. This study is considered as unique as no studies are evaluating IBS patients in Turkish society in this way. The intake of energy and nutrients of the patients with IBS are similar to those of Turkish society in general. However, folate, folic acid, potassium, calcium and magnesium intake is low in the majority of the patients. Furthermore, there was sitophobia in the majority of individuals, and half of them stated that there was no food that reduces their symptoms. Future research should be planned with a larger number of samples and foods could be questioned more comprehensively. In this way, the nutrient-symptom mechanisms can be understood more clearly.

Ethics Committee Approval: This study protocol was examined by Gazi University Ethics Commission and approved by report number 77082166-604.01.02 – on 13.01.2016. Besides, necessary permissions were obtained from the General Secretariate of the 1st Regional Public Hospitals Association of Ankara and Ankara Numune Training and Research Hospital to carry out the study. All the patients who participated in the study were informed about the research and they signed Gazi University Non-Interventional Clinical Trials voluntary form.

Author contributions: Yılmaz B and Akbulut G contributed equally to planning the research analyzing the data and writing the manuscript. Yılmaz B collected the data and Akbulut G reviewed the manuscript with constructive criticisms.

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Clinical and Experimental Health Sciences

The Changes in Static Balance During Pregnancy: A Prospective Longitudinal Study

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ABSTRACT

Objective: The aim of this study was to examine the changes in static balance during the three trimesters of pregnancy.

Methods: Nineteen pregnant women who were in the first trimester of pregnancy were included in the study. The static balance assessment of the pregnant women was performed with Biodex-BioSway™ Balance System. The balance was assessed with the Postural Stability Test, Limits of Stability Test (LOS) and Modified Clinical Test of Sensory Integration and Balance (mCTSIB). Measurements were repeated during the 1st trimester (10-12 weeks), the 2nd trimester (22-24 weeks) and the 3rd trimester (34th week) of the same pregnant women.

Results: There was no significant difference between trimesters in terms of the postural stability test ($p>0.05$). It was determined that there was a difference between the three trimesters in the LOS scores of the pregnant women ($p<0.05$). There was a significant increase in the LOS in the last trimester compared to the first trimester. According to the mCTSIB, there was only a difference between the three trimesters in the eyes closed firm surface parameter ($p<0.05$). Oscillations were higher in the third trimester than in the second trimester.

Conclusion: As a result of this study, which underlines the importance of evaluating static balance by follow-up of the same pregnant women during three trimesters, it is seen that there are changes in different parameters of balance. Considering the differences in the sub-parameters of balance in pregnant women; multi-directional evaluation involving different parameters may be beneficial when evaluating balances.

Keyword: pregnant, static balance, postural stability, limit of stability, modified sensory test

1. INTRODUCTION

During pregnancy, the body of the pregnant woman undergoes important anatomical and physiological changes in order to nurture and accommodate the developing fetus (1). These physiological changes may lead to alterations in the biomechanical structure of the pregnant woman resulting in changes in balance and postural control (2). The increase in body weight which occurs to meet the needs of the baby and to maintain the health of the mother, is one of the most important changes that occurs during pregnancy. An average increase of 11-16 kg is considered normal (3). Abdominal morphology changes from the second trimester, with the increasing size of the uterus and weight of the fetus, and there is a 30% increase in abdominal mass (4). The increase in abdomen size is related to decrease in static stability (5) and adaptive changes occurring in spinal curvatures which would compensate the anterior displacement of the center of gravity to maintain postural balance (6). It has been reported that gestational weight gain and the asymmetrical distribution of weight mainly in the anterior abdominal region (4), the adaptive postural changes necessary for readjustment of anterior-posterior center of gravity location (7), and increased joint laxity (8) may lead to changes in the static stability of a pregnant woman (5). Adaptations occurring in soft tissue, joints, and posture are important, both due to associated discomfort and pain, and due to changes in postural balance which increases the risk of falling (9). It is also stated that pregnant and postpartum women often suffer from sleep deprivation because they do not get enough sleep and this may also have an effect on postural stability (10). In pregnant women, it is very important to evaluate the changes in postural stability. Good postural control is essential for maintaining balance. In static posturography, increased sway of the human body during quiet standing may indicate a decrease in postural stability. An unstable posture may prevent optimal motor activity and cause falls (5).

It is stated that the above mentioned anatomical and physiological changes may cause postural instability during pregnancy. However, in the literature, there is no consensus

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among the studies evaluating pregnant women in terms of these parameters. Although there are studies evaluating pregnant women in terms of postural sway, to the best of our knowledge we believe that there is no study in literature evaluating all sub parameters of static balance in pregnant women during three trimesters. Based on the assumption that the static balance of pregnant woman who are followed up for three trimesters will decrease, the aim of this study is to examine the changes that occur in static balance during pregnancy.

2. METHODS

2.1. Individuals

This study was carried out in Gazi University Faculty of Health Sciences, Physiotherapy and Rehabilitation Department and Gazi University Faculty of Medicine Obstetrics and Gynecology Department. Approval was obtained from the Ethics Committee of Gazi University under the number 77082166-604.01.02-6834. Pregnant women were informed about the study in detail and written consent was obtained from each participant. Guidance and medical examinations of the pregnant women were carried out by an Obstetrics and Gynecology specialist at Gazi University.

Pregnant women between the ages of 18-40, who were in the first trimester of pregnancy (10-12) with a singleton live fetus, and without a pregnancy risk were included in the study. Exclusion criteria were; fetal developmental delay or anomalies, maternal problems such as; chronic diseases (hypertension, cancer, diabetes, vasculopathy), obesity in the first trimester (Body mass index (BMI)> 30), those who had neuromuscular, visual or orthopedic problems, and any problem which could affect balance were not included in the study. As a result, 19 pregnant women who met the criteria and agreed to participate in the study were included (Figure 1. Flow Diagram).

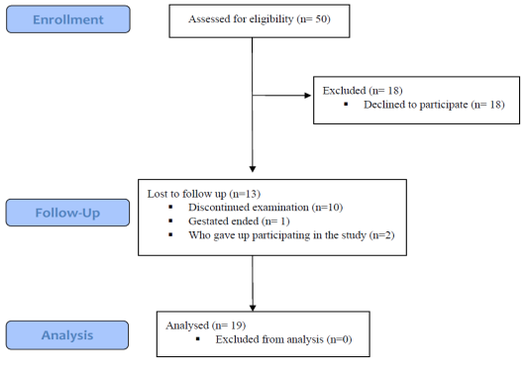

Figure 1. *Flow diagram illustrating the participants in the study*

The measurements were repeated 3 times in the same pregnant women; for 10-12 weeks (in the 1st trimester), 22-24 weeks (in the 2nd trimester) and 34th week-delivery (in the 3rd trimester).

Static balance was evaluated with Biodex-BioSway™ Balance System (BBS) (SD 950-340, Biodex Medical Systems, Inc., Shirley, NY, USA) (11). This device has 3 separate tests and 6 separate training programs and provides valid, reliable, and repeatable objectives measures of a patient's ability to balance on a firm and/or foam surface. Balance assessment with BBS was performed with the Postural Stability Test, Limits of Stability (LOS) and Modified clinical test of sensory integration and balance (mCTSIB).

The Postural Stability Test was used to evaluate the pregnant woman's ability to maintain center of balance. It was explained that the dot on the screen represents her Center of Gravity (CoG) and that she had to maintain her CoG by moving her body (without moving her feet) and hold it on the target point for 30 seconds. This test was repeated 3 times. As a result of the test, the Overall Stability Index (OSI), Medial-Lateral Stability Index (MLSI) and Anterior-Posterior Stability Index (APSI) were obtained. These scores indicate the amount of deviation from the anterior-posterior (AP) and medial-lateral (ML) axes. The patient's score on this test assesses deviations from center. Low scores indicate that the deviation is low, and the person's postural stability is good, thus a lower score is more desirable than a higher score.

LOS test is defined as the maximum angle a person's body can achieve from vertical without losing balance. The pregnant woman was asked to move the cursor representing her CoG to the targets on the screen (8 target points in the shape of a circle and 1 target point in the center). By shifting her weight, the pregnant woman was instructed to reach the blinking target and back as quickly and with as little deviation as possible. The test was repeated 3 times. As a result of the test, the "overall" score of the individual was obtained. A low "actual" score indicates that the person's LOS are decreased.

The mCTSIB test was used to evaluate the standing balance in different situations (eyes open / closed, firm support surface / dynamic support surface) that the individual may encounter in daily life. To reduce the effect of visual feedback on balance, the dot representing the center of gravity is not visible on the screen during the mCTSIB. This test is performed in four different situations, with 30 seconds for each test and a 10 second rest period between each test. For the first situation, the pregnant woman was instructed to stand with her eyes open on the firm surface. For the second situation the test was performed on the same surface with the pregnant woman's eyes closed. For the third and fourth situations an foam surface was placed on the platform (to reduce the effect of the surface) and the test was performed with the pregnant woman's eyes open for the third situation and with her eyes closed for the fourth situation. As a result of the test, the body sway was calculated and the sway index scores (SI-Sway Index) for; Eyes Open Firm Surface, Eyes Closed Firm Surface, Eyes Open Foam Surface, Eyes Closed Foam Surface were obtained. A higher sway index score indicates that the person has increased postural sway.

2.2.Statistical analysis

Statistical analyses of the study were carried out with SPSS for Windows 22.0 package program. The normal distribution of the data was analyzed with Tests of Normality and Shapiro-Wilk Test. The non-normal distributed data obtained from the measurements of pregnant women were presented using medians and interquartile range. In comparisons between trimesters, the statistical significance of the change over time for parameters was determined by Friedman test for the non-normal distributed data. Total type-1 error level was used as 0.05 for statistical significance. When necessary, the Bonferroni corrections for pairwise comparisons were evaluated with the Wilcoxon Test.

3. RESULTS

Based on a 96% power analysis, 19 pregnant women were included in this study. The average age of the pregnant women included was 29.31 ± 5.57 and the average height was 162.79 ± 6.3 cm. BMIs of pregnant women are shown in Table 1. When BMI of pregnant women were evaluated, there was a significant difference between trimesters ($p<0.05$, Table 1). According to the Bonferroni correction, there was no significant difference between average pre-pregnancy BMI and 1st trimester BMI ($p>0.008$, Table 1). However, all other binary comparisons were found to be statistically significant ($p<0.008$, Table 1).

The changes in the static balances of pregnant women are given in Table 2. No statistically significant difference was found in the postural stability evaluations of pregnant women in terms of APSI, MLSI and OSI in all three trimesters ($p> 0.05$, Table 2). It was found that pregnant women showed similar postural stability values in all three trimesters.

There was a significant difference between the three trimesters in terms of the pregnant women's LOS scores; ($p<0.05$, Table 2). According to the binary comparisons, it was seen that this difference was due to the 1st and 3rd trimesters. It was found that the LOS score was higher in the third trimester than in the first trimester. Likewise, although there was no statistically significant difference, the LOS score was found to be higher in the 3rd trimester than the 2nd trimester.

In the mCTSIB evaluation, it was found that in the comparison of Eyes Open Firm Surface, Eyes Closed Firm Surface, Eyes Open Foam Surface, Eyes Closed Foam Surface parameters between trimesters, there was only a difference in the Eyes Closed Firm Surface parameter and this difference was due to the difference between the 2nd and 3rd trimesters ($p<0.05$, Table 2). Differences between trimesters in other parameters were not statistically significant ($p>0.05$, Table 2). It was observed that the amount of sway in Eyes Closed Firm Surface was greater in the third trimester compared to the second trimester.

Table 1. *Body mass index changes of pregnant women*

n =19	Before Pregnancy (0)	1.trimester (1)	2.trimester (2)	3.trimester (3)	p	Difference
BMI (kg/m^2)	23,87 (22,35 / 25,75)	24,56 (22,35 / 26,72)	26,8 (24,36 / 27,48)	29,39 (27,12 / 32,44)	**0,000***	**0,012† for 1-2 0,000† for 2-3 0,000† for 1-3 0,000† for 0-2 0,000† for 0-3**

*BMI: Body Mass Index, *p<0.001, †New p value with Bonferroni correction p/6=0.05/6=0.008*

Table 2. *The changes in static balance of pregnant women during three trimesters*

	1.trimester	2.trimester	3.trimester	p	Difference
APSI	0,2 (0,1 / 0,2)	0,2 (0,1 / 0,2)	0,2 (0,2 / 0,2)	0.203	-
MLSI	0,1 (0,1 / 0,2)	0,1 (0,1 / 0,1)	0,1 (0,1 / 0,1)	0.807	-
OSI	0,2 (0,2 / 0,3)	0,2 (0,2 / 0,3)	0,2 (0,2 / 0,3)	0.717	-
LOS	49 (37 / 58)	53 (40 / 68)	63 (45 / 70)	**0.016***	**0.008† for 1-3**
Eyes Open Firm Surface	0,47 (0,36 / 0,54)	0,47 (0,34 / 0,68)	0,54 (0,43 / 0,6)	0.698	-
Eyes Closed Firm Surface	0,55 (0,48 / 0,75)	0,55 (0,48 / 0,75)	0,79 (0,51 / 1,31)	**0.032***	**0.008† for 2-3**
Eyes Open Foam Surface	0,99 (0,79 / 1,16)	0,91 (0,64 / 1,05)	0,87 (0,71 / 1,16)	0.234	-
Eyes Closed Foam Surface	1,79 (1,6 / 2,04)	1,86 (1,55 / 2,23)	2,09 (1,67 / 2,59)	0.076	-

*APSI: Antero-Posterior Stability Index, MLSI: Medio-Lateral Stability Index, OSI: Overall Stabilite Index, LOS: Limit of Stability, *p<0.05, †New p value with Bonferroni correction p/3=0.05/3=0.016*

4. DISCUSSION

The aim of this longitudinal study was to evaluate pregnant women in terms of static balance and reveal differences during three trimesters. Contrary to expectations, it was seen that there was no significant change in postural stability. However, as expected, changes were detected in the last trimester in terms of sway tested in the LOS and in the mCTSIB.

When literature is examined, several studies evaluating postural stability during pregnancy were encountered. Ribas and Guirro stated that even though pregnant women in the third trimester showed greater AP displacement than those in the first trimester, there was no difference in ML displacement (12). In the mentioned study, different pregnant women were evaluated for each trimester and this may have led to different results from our study. Jang et al. evaluated pregnant women at 0, 4, 8, 12 weeks and at 6 months postpartum with the stabilogram diffusion analysis and compared the results with the control group. They found that postural sway in the AP direction increased during pregnancy and decreased after delivery. They also reported that sway in the ML direction varied little during pregnancy, but it tended to increase after delivery, and compared to the control group, these values were higher in pregnant women (2). In their review, Ribeiro et. al. put forth that postural stability decreased during pregnancy due to possible reasons such as adaptations developed in the spine and lower extremities. They stated that postural stability decreases gradually during pregnancy, this decrease continues for 6-8 weeks after delivery and the reduction in postural stability increases the risk of falls during pregnancy (13). Similar to our study, Mocellin and Driusso, who evaluated postural stability by assessing the same pregnant women over three trimesters, stated that postural control tended to decrease in the first trimester of pregnancy and decreased further in the third trimester, however this decrease was not statistically significant (14). In our study, there was no significant change in postural stability between the three trimesters. Postural control during standing involves the integration of sensory information from the whole body, in particular from mechanoreceptors on the soles of the feet and from specialized receptors which code body position and orientation with respect to the gravitational acceleration, the environment and the body segments. In other words, it is maintained as a result of the central control of visual, vestibular and proprioceptive inputs. Although postural control mechanisms appear to be unaffected during pregnancy, the increase and asymmetric distribution of body mass and posterior body tilt observed during pregnancy may play an important role in modulating the amplitude and frequency of body sway, reflecting certain strategies for maintaining upright standing posture (15). The body of a pregnant woman undergoes significant biomechanical changes in the sagittal plane, such as the posterior body tilt. The pregnant woman's posture adapts with a slight posterior body tilt to reduce muscular energy expenditure and maintain AP stability while standing (5, 7). The increase of mass in the anterior pelvic region in pregnant women is compensated for with the increase of tonic activity of ankle plantar flexors and the increase of ankle stiffness and the duration of body sways increases. Despite the larger amount of tonic ankle activity, women in the later stages of pregnancy would possibly compensate for body sways with modulations of ankle torque at lower frequencies (15). Furthermore, it is stated that pregnant women try to maximize their postural stability by adjusting their step width (14). These mechanisms may explain why postural stability may not have changed between trimesters in our study.

Our results show that the LOS vary between trimesters. LOS is defined as the maximum angle a person's body can achieve from vertical without losing balance. As a result of this study, it was observed that the LOS was increased in the third trimester when comparison to the first trimester. It should be remembered that a low 'actual' score obtained as a result of the LOS test indicates that the person's LOS have decreased. In their study performed to investigate the effects of a maternal support belt, Elena Bey et. al. compared the LOS of pregnant women in different trimesters during pregnancy with and without the belt. Researchers found that without the maternal support belt, the LOS in the first and third trimesters was significantly higher in both anterior and posterior directions than the control group. They stated that the LOS in the second trimester was slightly lower compared to other trimesters but did not differ from the controls. They found that the LOS performance decreased in the early stages of pregnancy before the body mass had increased. They explained that factors such as increased anxiety due to pregnancy could have affected postural stability rather than body mass (16). Our results may have differed from the study by Elena Bey et al, due to the fact that they evaluated different pregnant woman in each trimester. In our study, the reason for the increase in the LOS value may be due to the increase in the size of the abdomen of pregnant women, which enabled them to reach the target points in the test much more easily with less body movement. Furthermore, as it is known, CoG moves forward with the increase in the size of the abdomen. This may have enabled the pregnant women to have better control on the CoG and move it out of the base of support.

mCTSIB evaluates standing balance in different situations. It was seen that during the Eyes Closed Firm Surface test, which is one of the sub-parameters of mCTSIB, sway was increased in the third trimester compared to the second trimester. The main reason for this is the elimination of visual input. According to the sensory organization theory first reported by Nasher, the central nervous system encodes the information provided by visual, vestibular and somatosensory receptors (17). Although the effects of the absence of visual input on upright posture control in normal subjects are controversial, the results of this study propose that pregnant women tend to compensate for the lack of vision by increasing the frequency of sway. In the study carried out by Oliveira et al. healthy pregnant women were evaluated using a stabilometer during different visual situations (eyes open/closed) and in different support base

configuration (feet together/apart). As a result, they found that postural control changed significantly during pregnancy when visual input was suppressed, or the support base was reduced. Therefore, they concluded that the accuracy of visual information and a comfortable support base could be critical for preserving balancing ability in the later stages of pregnancy (15). In their study, Butler et al. measured the eyes open and eyes closed static balance of pregnant women in 3 trimesters and compared the results with the control group. They stated that the second trimester, third trimester and post-pregnancy values were higher than the control group. Thus, they found that postural stability decreased during pregnancy and this decrease continued after delivery. This study also showed the increasing dependence on visual input to maintain balance during pregnancy (18). In their longitudinal study, Opala-Berdzik et al. evaluated pregnant women in their 1st trimester, 3rd trimester and postpartum. The authors concluded that static postural stability was not different between pregnancy and the postpartum period except AP sway, which was tested eyes-closed. This suggests that when visual input is eliminated, women in advanced pregnancy may have reduced static stability compared to non-pregnant women (5). These studies support our study as they show that sway is increased in eyes closed conditions.

Although static balance was evaluated in this study, some studies evaluating dynamic balance in pregnant women showed similar results with our study stating that stability indexes remained the same. McCrory et al. found that there was no difference in timing and magnitude of sway in response to perturbations in the second trimester, however, with progression from the second trimester to the third trimester, it was found that the sway responses decreased in pregnant women (19). Cakmak et al. found that without the support belt, there was no difference between trimesters in terms of OSI, MLSI and APSI, however the fall risk test scores were higher in the third trimester compared to other trimester (20). In their study, Inanir et al. reported that there was no significant difference in terms of OSI, APSI and MLSI between non-pregnant women and pregnant women in the first and second trimesters. They stated that pregnant women in the third trimester had higher OSI, APSI and MLSI scores than non-pregnant controls (21). The lack of difference in OSI, APSI and MLSI scores in these studies supports our study. However, the fact that Cakmak and Inanir evaluated different pregnant women in each trimester differentiates these studies from our study.

When literature is examined, there are different studies providing contradictory results regarding the change of balance in pregnant women. One reason for this may be that there are many different test methods used to assess balance in the clinic. Another reason is that when evaluating between trimesters, some studies included different pregnant women for each trimester, while in longitudinal studies the same pregnant woman were evaluated over three trimesters. The fact that many anatomical and physiological changes occur in pregnant women during pregnancy and these changes are seen in different rates in each pregnant woman may be another reason explaining the variety of results in the studies. Additionally, there is no standardization of evaluation time in these studies. Using different assessment methods at different time intervals may be among the reasons that can explain these conflicting results. Although the results of the studies vary, literature supports the fact that the balance of pregnant women will quite likely be affected due to many reasons mentioned above.

5. CONCLUSION

This study was carried out to examine the changes in static balance in pregnant women during pregnancy. It can be stated that this study contributes to the literature in terms of follow-up of the same pregnant woman for three trimesters and evaluation of static balance with different sub-parameters. It should be considered that balance performance during pregnancy may vary depending on the condition in which it was assessed and the degree of physical difficulty. This may explain why, in most studies, there was no difference between trimesters in a moderately-difficult test performed in a static condition with eyes open. The assessment of postural balance during pregnancy can contribute to the development and application of therapeutic methods to prevent postural instability and falls. Although there is no consensus in literature regarding how balance changes in pregnant women, it is highly probable that the balance of pregnant women will be affected. Pregnant women should be especially educated against the risk of falling when in conditions such as darkness where vision is limited. Therapists should pay attention to changes in the static and dynamic postural control of pregnant women and create an exercise program that aims to improve balance by preventing falls, maintaining muscle strength, and physical well-being throughout pregnancy. In conclusion, we believe that multi-faceted evaluation, using different assessment methods and parameters may be beneficial in evaluating balance and risk of falls, and these factors should be taken into consideration when applying clinical postural tests to pregnant women.

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Clinical and Experimental Health Sciences

Verbal Fluency: An Investigation of Time Variable Among Elderly People

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ABSTRACT

Objective: Little is known about the impact of time on verbal fluency performance among the elderly population. The aims of this study were to (i) examine the verbal fluency (semantic and action fluency) performance across four quarters of 60 seconds and (ii) explore the relationship between demographic (education and age) and time variables on the performance of elderly individuals.

Methods: A descriptive correlational and comparative study of 58 elderly healthy subjects was carried out (range, 60-81 years). Five semantic categories and action fluency were implemented. Participants were stratified into three education groups (Group 1, 2 and 3 including subjects with 5-8 years, 9-11 and more than 12 years of schooling) and each group involved 20, 21 and 17 participants respectively.

Results: The findings revealed mean numbers of words among quarters decreased in a linear fashion. There was significant difference between the production in the first quarter and others. It was seen that age did not correlate with fluency performance across quarters. Significant production differences were found between education groups across all quarters.

Conclusion: This study demonstrates the fluency scores of elderly healthy subjects with different levels of education along with production across four quarters. It is suggested that future studies include neuropathological conditions for the clinical utility of these measures.

Keywords: semantic fluency, action fluency, time, education, age.

1. INTRODUCTION

Verbal fluency (VF) tasks comprise an essential part of neuropsychological assessment. They refer to verbal production within a designated time (usually 1 min) following certain instructions provided (1). Quick and easy to implement, these measures help clinicians assess a wide range of neurological conditions including Alzheimer's disease, Parkinson's disease, primary progressive aphasia, fronto-temporal dementia and post-stroke aphasia (2-5). These tasks are widely used by speech and language therapists (SLTs) and neuropsychologists to assess word retrieval from the lexicon and semantic memory. The VF performances are evaluated through the correct number of words by excluding those that are repeated or out of the category (6).

Three types of fluency tasks exist in the literature: Semantic, action and phonemic. While the first two tasks involve producing either noun or verb categories, the final task is related to uttering words beginning with the same phoneme (most commonly /f/,/a/,/s/). The use of "animal" category is most common among semantic fluency tasks, while "naming things that people do" without adding any inflectional markers (e.g., *sleep* and *sleeping*) is expected in action fluency (7). It is articulated that patients with Alzheimer's disease and semantic dementia hold poorer performance in semantic fluency task as impairment in semantic memory among these conditions is extensively reported (5,8-9). It is also pointed out that action fluency task can be used to assess executive functions among healthy individuals, which is supported by its correlation with other executive function measures (10-11). Action fluency is also reported to hold potential in distinguishing Parkinson's disease with dementia (PDD) from non-demented Parkinson's disease (7), Lewy body dementia and behavioral variant of frontotemporal dementia from Alzheimer's dementia (3,12). Besides its significance in clinical context, the interaction between demographic variables (education, age and gender) and verbal fluency performance has been widely studied among healthy individuals in different languages (1,13).

In addition to these variables, studies including semantic and phonemic fluency tasks investigated the relationship between time and demographic variables among young

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or elderly participants (14-19). These studies divided 60 seconds into two halves (16,18) or four quarters (15,17). The conclusion drawn from the findings of the studies involving elderly participants attached a positive influence of education (14,17-18). They also stated that the production in the first quarter (first 15 s) was significantly more than the ones in other quarters (15,17). They proposed that word production at later quarters became effortful and required a robust executive function (involving self-monitoring abilities, processing speed, etc.) and attention control (15-17). Few studies also asserted that following a semantic fluency task using "Animal" category with a 30-second measure, it was possible to differentiate healthy subjects from patients with Mild Cognitive Impairment (19) and stroke patients with aphasia from those without aphasia (20). Regarding aphasia, a recent study by Bose, Wood and Kiran revealed that the words uttered by people with aphasia (PwA) and control participants in the first 15 s of animal fluency task were more than those in the remaining three quarters, followed by a progressive decrease in production and a final "asymptote" (21). Furthermore, one study investigated the semantic fluency performance of young adults with high-functioning Autism Spectrum Disorders (ASD), and stated that the performance in the first 30 s of the task was significantly lagging behind that of the control group. The authors concluded that their results could refer to deficits in the initiation processes in ASD (22).

VF measures including semantic, phonemic and action were examined among Turkish-speaking individuals (23-29). The "animal" category was the most common. This was utilized both among healthy people (23, 25-26) and those with neurological conditions such as Alzheimer's dementia (AD) (27) and fronto-temporal dementia (28). The other semantic categories included fruits-vegetables, furniture, body parts and clothes (23, 25-26). Moreover, the performances of PwA and healthy participants on the household items and kitchen utensils categories were examined as part of the reliability and validity analyses of Aphasia Assessment Tool (ADD) in Turkish (29). It was concluded that education and age had a significant effect on the semantic fluency performances of healthy participants (23). Two studies examined the action fluency (AF) performances of healthy Turkish-speaking adolescents aged between 15 and 17. The findings of these studies showed that the mean number of correct verbs was 16.26 and there was no statistical difference between the AF performances of participants who were 15, 16 and 17 years old (25-26).

As mentioned, previous studies have examined the performance of semantic and phonemic fluency tasks in different time phases during one minute. In this regard, this study aimed to (i) examine the performance across four quarters of 60 seconds through action and semantic fluency tasks (ii) explore the relationship between demographic (education and age) and time variables on fluency performance of elderly individuals.

2. METHODS

A descriptive correlational and comparative study was carried out. The sample of the study contained 58 elderly neurotypical subjects (28 female, 30 male). The mean age was 65.43 years (range 60-81; SD: 5.51). Participants were stratified into three educational groups: Group 1 (5-8 years of schooling), Group 2 (9-11 years of schooling), Group 3 (more than 12 years of schooling). The groups consisted of 20, 21 and 17 participants respectively. All of the subjects were healthy with no cognitive, neurological or psychiatric complaints and conditions. Mini-Mental State Examination (MMSE) was administered in order to ensure this requirement. All the participants scored more than 24 which was the cut-off score for healthy subjects in MMSE as provided by Güngen et al. for Turkish population (30). It is important to note that the authors included participants with at least 5 years of schooling but did not stratify MMSE scores according to the education profile of the participants in their study. The study received the approval of the Ethical Committee of Anadolu University (Protocol no. 6085). Prior to initiating the tasks, all the participants gave a written consent to take part in the study.

Five semantic fluency categories ("Breakfast Items, Famous People, Food, Beverages and Household Items") and AF test were administered. The instructions were provided as simple as possible. The subjects were asked to name as many breakfast items, famous people, food, beverages and household items as possible. It was stressed that it could be all types of food and beverages, all items for breakfast and house, and famous people known by a large majority of Turkish population. As for AF, the instructions were given in the format presented by Piatt et al. (7): "I would like you to tell me as many different things as you can think of that people do. I do not want you to use the same word with different endings, like eat, eaten, eating. Also, just give me single words such as eat, or run, rather than a sentence. Can you give me one example of something that people do?" As long as this example was correct, they were asked to initiate the task with this example. The administration of all the tasks was conducted in a silent environment by the first author in one session. The order of the administration was the same for all the participants: Breakfast Items, Famous People, Food, Beverages, Household Items (all belonging to Semantic Fluency) and Action Fluency. The phonemic fluency measure was not included in the study; the categorical production was emphasized. Moreover, the semantic categories including "animals" along with "supermarket, fruits-vegetables" were not investigated in this study, albeit their widespread clinical use. This was due to the fact that the effect of time variable on the categories utilized in this study was investigated.

The scores of all the participants were recorded by either using a VoiceRecorder App for Apple Iphone or an MP3 player (Sony NWZ-B173). They were all later transcribed in Microsoft Excel. The time was set to be 1 minute (60 seconds) during fluency assessment. This time limit is frequently used in the literature, even though there are other studies utilizing two minutes

(120 seconds) for categorical fluency measures (18). Excluding those that were repeated and out of the category, the words produced in four quarters were examined (1-15 seconds, 16-30 seconds, 31-45 seconds, 46-60 seconds). The performance between each quarter among all the tasks was examined.

Parametric and non-parametric tests were used in all of the statistical analyses. As for the comparison of performance between quarters, Friedman Test was applied. Regarding the pairwise comparisons, Wilcoxon signed-rank test was employed so that any significant differences between quarters would be explored. Spearman's Rho Correlation Coefficient was calculated in order to analyze the relationship between age and the production across quarters. As descriptive values of mean scores across quarters belonging to all education groups exhibited normal distribution, ANOVA was administered to investigate whether there were production differences between education groups among different quarters. The significance level of .05 was taken into account within all the analyses.

3. RESULTS

58 elderly subjects participated in the study. **Figure 1** reveals mean number of items produced among four quarters. **Table 1** shows the Friedman test results which compared the fluency performance between the quarters. **Table 2** indicates Wilcoxon test results which included pairwise comparisons between items produced in different quarters. **Table 3** shows the correlation between age and production among quarters. Finally, **Table 4** demonstrates both descriptive values and ANOVA results as performance of education groups within all the quarters was examined.

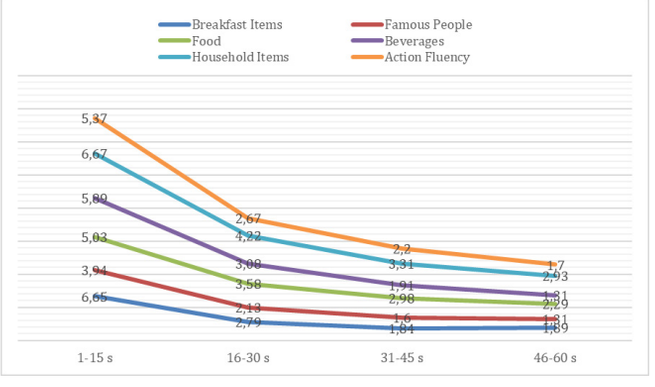

Figure 1: *Mean Number of Items Produced Among Different Quarters*

It was seen that the mean number of words produced among quarters decreased in a linear way (Figure 1). This was statistically significant for all semantic and action fluency measures following the results of the Friedman test (Breakfast Items: $\chi^2(3) = 101.015$, p<.001; Famous People: $\chi^2(3) = 81.226$, p<.001; Food: $\chi^2(3) = 72.473$, p<.001; Beverages: $\chi^2(3) = 129.786$, p<.001; Household Items: $\chi^2(3) = 96.959$, p<.001; Action Fluency: $\chi^2(3) = 109.180$, p<.001) (Table 1).

Table 1. *Mean Number of Items Produced Among Different Quarters and Friedman Test Results*

Categories	n	Mean	SD	χ^2 (df)	p
Breakfast Items					
1st Quarter	58	6.65	2.35	101.015(3)	.000*
2nd Quarter	58	2.79	1.76		
3rd Quarter	58	1.84	1.51		
4th Quarter	58	1.89	1.54		
Total	58	13.18	4.71		
Famous People					
1st Quarter	58	3.94	1.65	81.226(3)	.000*
2nd Quarter	58	2.13	1.53		
3rd Quarter	58	1.60	1.37		
4th Quarter	58	1.31	1.25		
Total	58	9.00	4.15		
Food					
1st Quarter	58	5.03	1.58	72.473(3)	.000*
2nd Quarter	58	3.58	1.53		
3rd Quarter	58	2.98	1.61		
4th Quarter	58	2.29	1.73		
Total	58	13.89	4.62		
Beverages					
1st Quarter	58	5.89	2.10	129.786(3)	.000*
2nd Quarter	58	3.08	1.49		
3rd Quarter	58	1.91	1.58		
4th Quarter	58	1.31	1.09		
Total	58	12.20	4.16		
Household Items					
1st Quarter	58	6.67	2.21	96.959(3)	.000*
2nd Quarter	58	4.22	1.87		
3rd Quarter	58	3.31	1.72		
4th Quarter	58	2.93	1.84		
Total	58	17.13	5.62		
Action Fluency					
1st Quarter	58	5.37	2.20	109.180(3)	.000*
2nd Quarter	58	2.67	1.60		
3rd Quarter	58	2.20	1.47		
4th Quarter	58	1.70	1.52		
Total	58	11.96	5.30		

**p<.001*

Significant difference was found in all the categories between the 1st and 2nd quarters (Z = – 6.288, p<.001 for Breakfast Items; Z = – 5.862, p<.001 for Famous People; Z = – 4.681, p<.001 for Food; Z = – 6.370, p<.001 for Beverages; Z = – 5.876, p<.001 for Household Items; Z = – 6.479, p<.001 for Action Fluency), the 1st and 3rd quarters (Z = – 6.501, p<.001 for Breakfast Items; Z = – 6.322, p<.001 for Famous People; Z = – 5.668, p<.001 for Food; Z = – 6.355, p<.001 for Beverages; Z = – 6.308, p<.001 for Household Items; Z = – 6.392, p<.001 for Action Fluency), and the 1st and 4th quarters (Z = – 6.530, p<.001 for Breakfast Items; Z = – 6.206, p<.001 for Famous People; Z = – 6.075, p<.001 for Food; Z = – 6.586, p<.001 for Beverages; Z = – 6.111, p<.001 for Household Items; Z = – 6.532, p<.001 for Action Fluency) (Table 2). Moreover, significant production differences were seen between the

2nd and 3rd quarters (Z = – 3.458, p<.01 for Breakfast Items; Z = – 2.660, p<.01 for Famous People; Z = – 2.348, p<.05 for Food; Z = – 4.073, p<.001 for Beverages; Z = – 3.534, p<.001 for Household Items; Z = – 2.255, p<.05 for Action Fluency), and the 2nd and 4th quarters (Z = – 3.185, p<.01 for Breakfast Items; Z = – 2.996, p<.01 for Famous People; Z = – 4.435, p<.001 for Food; Z = – 5.597, p<.001 for Beverages; Z = – 3.945, p<.001 for Household Items; Z = – 3.865, p<.001 for Action Fluency) (Table 2). These two findings show that the number of words in the first and second quarters were more in number than the others produced in the last two quarters. The production differences between the 3rd and 4th quarters were significant among Food (Z = – 2.885, p<.01), Beverages (Z = – 2.557, p<.05) and Action Fluency (Z = – 2.487, p<.05). However, contrary findings were seen among Breakfast Items (Z = – .189, p = .850), Famous People (Z = – 1.328, p = .184) and Household Items (Z = – 1.389, p = .165) in this interval. Results also revealed that there was no statistically significant correlation between age and fluency tasks (Table 3).

Table 2. *Results of Wilcoxon signed-rank Test*

Quarters	Breakfast Items		Famous People		Food		Beverages		Household Items		Action Fluency	
	p	Z	p	Z	p	Z	p	Z	p	Z	p	Z
1 x 2	.000***	-6.288	.000***	-5.862	.000***	-4.681	.000***	-6.370	.000***	-5.876	.000***	-6.479
1 x 3	.000***	-6.501	.000***	-6.322	.000***	-5.668	.000***	-6.355	.000***	-6.308	.000***	-6.392
1 x 4	.000***	-6.530	.000***	-6.206	.000***	-6.075	.000***	-6.586	.000***	-6.111	.000***	-6.532
2 x 3	.001**	-3.458	.008**	-2.660	.019*	-2.348	.000***	-4.073	.000***	-3.534	.024*	-2.255
2 x 4	.001**	-3.185	.003**	-2.996	.000***	-4.435	.000***	-5.597	.000***	-3.945	.000***	-3.865
3 x 4	.850	-.189	.184	-1.328	.004**	-2.885	.011*	-2.557	.165	-1.389	.013*	-2.487

**p<.05, **p<.01, ***p<.001*

Table 3. *Results of Spearman's Correlation*

Variables	Quarters	r_s	p
Age x Semantic Fluency	1st quarter	-0.057	0.669
	2nd quarter	-0.170	0.202
	3rd quarter	-0.079	0.556
	4th quarter	-0.187	0.161
	Total	-0.125	0.348
Age x Action Fluency	1st quarter	0.000	0.997
	2nd quarter	0.138	0.300
	3rd quarter	-0.021	0.879
	4th quarter	-0.186	0.163
	Total	-0.015	0.911

Table 4. *Descriptive values (means and standard deviation) and ANOVA results for fluency performance belonging to education groups across quarters, and Post-Hoc results between groups.*

Variables	Quarters	Group 1 (5-8 years of schooling) n=20		Group 2 (9-11 years of schooling) n=21		Group 3 (more than 12 years of schooling) n=17		df	F	p	Post-hoc
		Mean	SD	Mean	SD	Mean	SD				
Education x Semantic Fluency	1st quarter	4.79	1.55	5.56	1.13	6.74	1.46	2	9.110	.000***	Group 1-3
	2nd quarter	2.59	1.16	3.20	1.06	3.80	0.88	2	6.083	.004**	Group 1-3
	3rd quarter	1.74	0.88	2.29	0.78	3.07	0.90	2	11.177	.000***	Group 1-3
	4th quarter	1.49	0.90	1.76	0.88	2.71	0.71	2	10.472	.000***	Group 1-3, 2-3
	Total	10.61	3.78	12.81	2.85	16.32	2.93	2	14.537	.000***	Group 1-3, 2-3
Education x Action Fluency	1st quarter	4.20	1.93	5.61	2.31	6.47	1.77	2	5.932	.005**	Group 1-3
	2nd quarter	2.00	1.89	2.52	1.24	3.64	1.16	2	5.825	.005**	Group 1-3
	3rd quarter	1.30	1.26	2.28	1.14	3.17	1.46	2	9.845	.000***	Group 1-3
	4th quarter	1.30	1.41	1.47	1.28	2.47	1.69	2	3.352	.042*	Group 1-3
	Total	8.80	5.28	11.90	3.78	15.76	4.60	2	10.606	.000***	Group 1-3

**p<.05, **p<.01, ***p<.001*

It was seen that mean numbers of words decreased between quarters within all education groups. The analysis of variance (ANOVA) results revealed that the decrease in production was statistically significant among all quarters. Scheffe's test results for post-hoc comparisons demonstrated that participants with 5-8 years of schooling (Group 1) produced significantly less number of words than those with more than 12 years of schooling (Group 3) in all the quarters within semantic fluency measure ($F(2) = 9.110$, $p<.001$ for 1st quarter; $F(2) = 6.083$, $p<.01$ for 2nd quarter; $F(2) = 11.177$, $p<.001$ for 3rd quarter; $F(2) = 10.472$, $p<.001$ for 4th quarter; $F(2) = 14.537$, $p<.001$ for total). These comparisons also showed that participants in Group 2 scored significantly less than those in Group 3 in the last quarter in semantic fluency ($F(2) = 10.472$, $p<.001$). As for the action fluency, it was observed that participants with more than 12 years of schooling (Group 3) generated a greater number of words than those with 5-8 years of schooling (Group 1) across all quarters ($F(2) = 5.932$, $p<.01$ for 1st quarter; $F(2) = 5.825$, $p<.01$ for 2nd quarter; $F(2) = 9.845$, $p<.001$ for 3rd quarter; $F(2) = 3.352$, $p<.05$ for 4th quarter; $F(2) = 10.606$, $p<.001$ for total).

4. DISCUSSION

The present study revealed production differences among four quarters. It also examined the interaction between demographic and time variables. Regarding the first aim, it was seen that fluency performance of the subjects decreased in a linear way between the quarters. Post-hoc analysis of this decrease demonstrated that mean number of words produced in the first quarter was significantly more than those of other quarters for all semantic categories and action fluency.

Even though different categories were analyzed in other studies, these findings are consistent with previous studies that examined production differences between four quarters (14-15, 17). Crowe (1998) found out that the decrease across quarters were linear and the amount of words produced in the first quarter was statistically more than those in other quarters (15). Brucki and Rocha (2004) reported similar finding regarding the latter result (14). This result complies with the findings of these studies. Though Crowe (1998) included subjects between 18 and 35 years of age, he concluded that as time passes searching for additional words and retrieving them become more challenging after the pool of readily frequent words was eventually exhausted (15). Moreover, Venegas and Mansur (2011) indicated that the performance in the first quartile was significantly different than the one in other quarters in that approximately half of the total words in the semantic fluency task ("animal" category) was produced during the initial 15 seconds (17). In a recent study, Demetriou and Holtzer (2017) concluded that healthy older participants produced significantly more number of words in the first 20 s of semantic fluency measures compared to adults with Mild Cognitive Impairment. They stressed that efficient search processes and monitoring abilities along with fast word retrieval from memory contributed to the performance of healthy older adults (31).

The second aim was to explore whether there is a relationship between demographic variables (education and age) and time on fluency performance. This study revealed no relationship between age and verbal fluency measures (both semantic and action) while the performance in all the quarters was considered. This is consistent with the findings of previous studies (14, 17).

Furthermore, it was found that participants with more than 12 years of schooling (Group 3) produced a higher number of words among all the quarters both in semantic and action fluency tasks compared to the ones generated by the subjects with 5-8 years of schooling (Group 1). The impact of education on verbal fluency performance was articulated substantially in the neuropsychology literature. Regarding semantic fluency, Venegas and Mansur (2011) asserted that the performance pertaining to the first quarter holds potential to reflect the compactness of semantic memory. While this compactness was referred as "semantic sketch resources", the performance in the following quarters (2nd and 3rd quarters) was reported to be influenced by cognitive operations such as planning and monitoring (17). Brucki and Rocha (2004) provided findings in the similar vein: They found out that higher education levels could be associated with higher production across quarters (14).

Previous studies related to action fluency also reported the influence of education. However, as far as the knowledge of the study's authors, there is no other study that investigated the impact of time variable on the action fluency performance. As there is a strong emphasis on the possibility of using action fluency test as a measure of executive function (10-11), it might be useful to investigate the performance across intervals in other languages. Moreover, previous studies reported that performance across time in semantic fluency task could be an indicator of predicting the diagnosis of mild cognitive impairment (19) as well as distinguishing aphasic people from non-aphasic population (20). In reference to these studies, the clinical utility of action fluency task could be explored by further analyzing the performance across intervals within neuropsychological evaluations.

Previous fluency studies that include Turkish-speaking healthy individuals have not examined the effect of the time variable on the fluency performances. However, Kırbaç (2015) reported that the mean number of verbs produced by the participants who were between 15 and 17 years of age was 16.26 (25). The current study showed that the mean number of actions uttered by those who were between 60 and 81 years of age was 11.96. This difference might be remarkable. However, these two studies included different age groups. Moreover, Maviş and Toğram (2009) stated that the highest mean number of correct words in household items category was derived from those who were between 23-44 years of age (mean = 14.2) and received more than 12 years of education (mean = 14). They also reported that the lowest values in the category were obtained from those who

were above 75 years of age and illiterate individuals (mean for both = 8). The current study showed that the mean number of total correct words in the household items category (mean = 17.13; SD = 5.62) was more than those that were presented by Maviş and Toğram (2009) (29).

This study holds some limitations. Initially semantic fluency categories in this study are different from those that are commonly used in the neuropsychology literature. Regarding action fluency, it is recommended that the number of elderly healthy individuals be increased and neuropathological conditions (such as Parkinson's and Alzheimer's disease) be included to establish norms for clinical utility within Turkish context.

5. CONCLUSION

This study investigated the effect of time variable on the semantic and action fluency performances of the elderly people who were between 60 and 81. The production differences within the four time intervals were examined within semantic (breakfast, famous people, food, beverages, household items) and action fluency measures. It is anticipated that this study will contribute to the literature in terms of the production differences between time intervals and the interaction of time and demographic variables such as education and age. Moreover, the action fluency findings reported in this study will provide a reference especially for the studies including patients with Parkinson's disease since action fluency measures hold a particular clinical utility and significance for this population. Regarding the suggestions for future studies, first, various cognitive assessment tools measuring verbal intelligence and executive functions could be utilized. The findings of these tools could be compared with those of the fluency measures. The correlations between these findings could be examined. Second, the participants who are proficient in more than one language could be included to observe whether bilingualism holds any effect on the fluency performance. Third, neuropathological conditions such as Alzheimer's disease, Parkinson's disease, frontotemporal dementia could be included to test the clinical utility of these measures.

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Clinical and Experimental Health Sciences

Does High Self-Efficacy in Adolescents Minimize Cyber Bullying Behaviour?

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ABSTRACT

Objective: This study aimed to explain the moderation role of self-efficacy in the relationship between cyber victimization and cyber bullying.

Methods: A total of 340 high school students participated in the study aged between 14 and 18 years. Research data was obtained by the Cyber Bullying and Internet Aggression Survey Scale, and the Self-Efficacy Scale. Correlation analysis was performed to determine the relationships between variables in the study. Hierarchical regression analysis based on the Hayes method was used to test the moderating effects in the study.

Results: As a result of the research, it was found that 42% of the participants were exposed to cyber bullying and 35% engaged in cyber bullying. The research revealed that there is a moderate, positive and significant relationship between cyber victimization and cyber bullying. As a result of moderating analysis, it was observed that self-efficacy affects the relationship between cyber victimization and cyber bullying.

Conclusion: The results indicate that a decrease in self-efficacy leads to increased cyber bullying behaviour, while an increase in self-efficacy decreases cyber bullying behaviour after cyber victimization. From this point of view, carrying out studies to increase the self-efficacy levels of students in schools will reduce cyber bullying.

Keywords: Cyber victimization, cyber bullying, self-efficacy, moderation

1. INTRODUCTION

The use of new technological tools such as computers, tablets, and smartphones is intensive in education, business and leisure. These tools have benefits such as allowing access to information, communication with other people, for initiating and conducting interpersonal relationships, and organizing leisure activities (1). Adolescents especially use these technological tools for academic activities, establishing friendships and accessing information. Therefore, adolescents may spend most of the day using technological tools. However, this intensive use may negatively affect the spiritual world of adolescents (2).

One of the negative consequences of new technologies is cyber bullying. Cyber bullying is the use of technological tools to harm others (3). The rapidly increasing use of electronic communication tools in the last decade has led to a new form of bullying (4).

Islam et al (5) determined that about 12% of adolescents are involved in cyber bullying. Lapierre and Dane (1) found that 7.4% of adolescents engaged in cyber bullying, while Modecki et al (6) found that about 17% of adolescents were cyber bullying. Hemphil et al (7) reported that 23% of adolescents engage in cyber bullying. Jadambaa et al (8) reported cyber bullying behaviour in 5% of adolescents.

Studies revealed that cyber bullying has various adverse effects on victims, such as depressive mood, high level of loneliness, disappointment and grief, and difficulties with academic learning (9,10). Studies conducted in recent years reveal that people with mental health problems are more likely to engage in cyber bullying behaviour (11, 12). Cyber bullying and cyber victimization were found to be associated with concepts such as depression, anxiety, and self-esteem (13). Moreover, it is stated that cyber bullying affects social cohesion and well-being at school (14).

1.1. Relationship Between Cyber Victimization and Cyber Bullying

Recent research have shown that one of the strongest predictors of cyber bullying behaviour is for someone to experience cyber bullying (15). Numerous studies show strong correlations between cyber victimization and continuation (r = .50 to .60) (16, 17). Leung et al (18) revealed that there

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is a significant relationship between cyber victimization and cyber bullying (r= .65), while Quintana-Orts and Rey (19) reached the same conclusion (r= .57). Hood and Duffy (20) also point to a significant relationship between cyber victimization and cyber bullying (r= .55). An analysis of studies conducted in Turkey show that the relationship between cyber victimization and cyber bullying varies between .27 and .70 (21, r=.27; 22, r= .48; 23, r= .49 for females, r= .52 for males; 24, r= .46; 25, r= .70).

1.2. Self-Efficacy and Cyber Bullying

Self-efficacy is an individual's belief in their capacity to organize actions, cognitive skills, and motivation required to fulfil an undertaking with success (26). According to Bandura (27), self-efficacy is related to a person's own perceptions, and their belief in their capability to organize a result they want, and achieve it with success. The more individuals believe they can treat others offensively, the more probable it is that they will act outrageously and brutally (28). In addition, Erath et al., (29) state that low self-efficacy may cause victimization of individuals. Trompeter et al. (30) revealed that coping self-sufficiency is related to cyber victimization. Bussey et al. (31) showed that cyber bullying, defined as an individual's belief in the competence to engage in cyber bullying, is associated with self-efficacy and cyber bullying. Heiman et al. (32) reported that students exposed to the cyber bullying had lower self-efficacy than students not exposed to cyber bullying.

1.3. Current Study

This study is consistent with the results of previous studies (33, 34), leading to the expectation of a significant relationship between experiencing cyber bullying and cyber bullying behaviour. Furthermore, it is considered that self-efficacy will explain the relationship between cyber victimization and cyber bullying in this study. It is predicted that the positive relationship between cyber-victimization and cyber bullying is going to be weaker for those with high self-efficacy compared to those with low self-efficacy particularly. In line with this, the current research aims to examine the moderating role of self-efficacy in the association between cyber victimization and cyber bullying.

2. METHOD

2.1. Study Group

The sample group in the study consisted of 340 high school students attending four different state schools in Erzurum province, Turkey. Of the students, 45.9% (n=156) were female, and 54.1% were male (n=184). Students participating in the study were aged between 14 and 18 years, with the average age of 15.7 years. Among the students, 24.7% (n=84) were in 9th grade, 32.9% (n=112) were in 10th grade, 28.8% (n= 98) were in 11th grade, and 13.5% (n=46) were in 12th grade. According to the findings of the research, it was determined that 34.6% of the students exhibited cyberbullying behaviors and 42.1% experienced cyber victimization.

The study was conducted between 1-30 April 2020, after ethical approval (30 March 2020). Scales provided via an electronic link. Snowball sampling method was used to determine the participants to participate in the study. The scales prepared online were first given to a student and they were asked to communicate the scale to others using social networks.

2.2. Measures

2.2.1. Self-efficacy scale for children: The Self-Efficacy Scale for Children was developed by Muris (35). Çelikkaleli et al. (35) conducted the adaptation study of the scale into Turkish. The scale consists of 21 items, and 3 sub-dimensions: academic, social, and emotional self-efficacy. As a result of analysis of the scale's internal consistency reliability, the overall coefficient was determined as .86, while the coefficients for academic, social, and emotional self-efficacy were determined as .84, .64 and .78, respectively. Higher scores obtained from the scale signify high levels of self-efficacy in individuals. In this study, the scale was evaluated as the total score.

2.2.2. Cyber bullying and internet aggression survey scale (CIASS): In order to determine the cyber victimization and cyber bullying behaviour of students participating in the study, the scale developed by Hinduja and Patchin (36), and adapted to Turkish by Özdemir and Akar (37) was used. The scale consists of 8 items, and two separate forms: cyber victimization and cyber bullying. The internal consistency reliability coefficient was found to be .79 for cyber victimization, and .94 for cyber bullying. High scores obtained from the scale indicate high levels of cyber victimization and cyber bullying.

2.3. Data Collection Process

As there was no in-person education at schools due to COVID-19, data were collected online. Hence, the online data collection scales were prepared using Google Forms and sent to the participants. Moreover, informed consent was obtained from individuals who accepted participation in the study before they completed the scales. It was ensured that only volunteers were included in the study. Additionally, individuals were informed that they may stop completing the scales whenever they want, and that the results would be kept confidential. The online data collection process was completed within 30 days. The collected data online were analysed in a computer environment. As it is not possible to move to the next question without marking an answer during the online application, there was no incomplete data in the study. As a result, 350 people completed the scales.

2.4. Data Analysis

Before analysis of the data, the levels of discrepancy and normality were examined. In this data set, it was determined that data for 10 individuals violated the parametric conditions and hence these data were removed from the data cluster. In the final stage, Mardia's skewness and kurtosis values were examined using Lisrel 9.0 software to test the multivariate normality of the data set. As a result, it was observed that Mardia's assumptions of multivariate normality were confirmed (p>.05). After all these procedures, it was decided to conduct the analysis process based on data from 340 participants. Pearson correlation analysis was conducted to establish whether self-efficacy is related to cyber victimization and cyber bullying behaviour. Then, regression analysis was performed using the bootstrap method to test the moderating role of self-efficacy in the effect of cyber victimization on cyber bullying. Hayes' (38) Process Macro program was utilized to perform the moderation analysis (Model-1).

2.5. Ethical Approval

The approval of the ethics committee of the study was obtained from the Ethics Committee of Atatürk University Educational Sciences Unit on 30.03.2020.

3. RESULTS

Relationship Between Self-Efficacy and Cyber Victimization and Cyber Bullying, and Descriptive Results

Pearson correlation analysis was conducted to reveal the level of correlation between self-efficacy, cyber victimization, and cyber bullying scores. In addition, descriptive statistical operations related to variables were completed. Both descriptive and correlation results for the variables are presented in Table 1.

Table 1. *Descriptive and correlation results for cyber victimization, cyber bullying, and self-efficacy*

Variables	M	SD	Self-Efficacy	Cyber Victimization	Cyber Bullying
Self-Efficacy	71.63	11.04	1		
Cyber Victimization	9.25	1.88	-.15*	1	
Cyber Bullying	8.77	1.29	-.12*	.55*	1

**p<.001*

Examination of Table 1 shows low level, significant negative correlations between self-efficacy with cyber victimization (*r= – .15*, p<.001) and cyber bullying (*r=-.12*, p<.001). Further, medium and significant level of positive correlation was observed between cyber victimization and cyber bullying (*r= .55*, p<.001).

Moderating *Role of Self-Efficacy*

The effect of self-efficacy in the moderating role between cyber victimization and cyber bullying is shown in Table 2.

Table 2. *Moderating role of self-efficacy*

Variables	b	S.H.	t	LLCI	ULCI
Constant	8.74	.059	149.20	8.6	8.5
Cyber-Victimization (x)	.36	.031	11.62	.30	.43
Self-Efficacy (w)	-.005	.005	-.86	-.02	-.01
x.w	-.009	.003	-3.26	-.015	-.003

R= .57, R2 = .32,4; p< .01, .05, S.E.: Standard Error; b: unstandardized beta coefficient

According to the results in Table 2, all predictor values included in the regression analysis explain approximately 32% (R^2 =.32,4) of the variation in cyber bullying. Cyber victimization was found to have significant positive effects (b= .36, p<.01) on cyber bullying, while self-efficacy was determined to have significant negative effects (b= – .005, p<.01) on cyber bullying. The cyber victimization and self-efficacy variables were found to have significant interactional effect (moderating effect) on cyber bullying (b= .-009, p<.05).

The effects of the moderation variable as a result of the slope analysis are shown in Figure 1. Details of the moderation effect show that when self-efficacy is low, cyber victimization has a greater effect on cyber bullying. When self-efficacy is high, the effect of cyber victimization on cyber bullying increases, yet this effect is not as strong.

As a result, in the case of low self-efficacy, cyber victimization will have a greater effect on cyber bullying. This finding signifies that the relationship between cyber victimization and cyber bullying is moderated by self-efficacy.

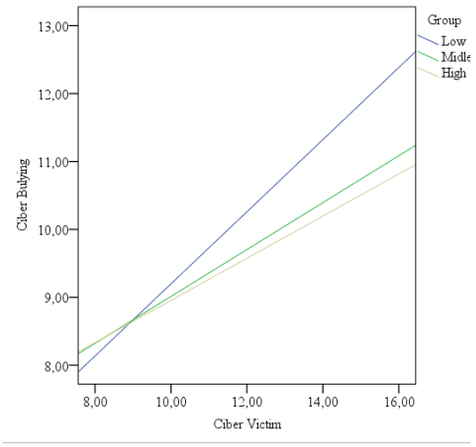

Figure 1. *Graphical representation of the moderation effect of self-efficacy*

4. DISCUSSION

This study examined the moderation role of self-efficacy between cyber victimization and cyber bullying. In the study, there was a linear relationship between cyber victimization and cyber bullying. It was found that there was a low-level, negative and significant relationship between self-efficacy with cyber victimization and cyber bullying. Another result of the study determined that self-efficacy has a moderation role in the relationship between cyber victimization and cyber bullying.

In the first finding of the study, it was determined that experiencing cyber victimization has a strong relationship in showing cyber bullying behaviors. Exposure to cyber bullying adversely affects social and emotional adaptation of adolescents (39). Individuals who experienced cyber bullying reported feelings of shyness, anger, sadness, disappointment, guilt and helplessness (40). Experiencing such negative emotions leads to an increased desire for revenge (41). The negative emotions experienced may drive the cyber victim to resort to bullying in order to take revenge (42). Patching and Hinduja (34) stated that cyber victims turn into cyber bullies due to their failure to effectively solve social problems and because they process social information in a hostile manner as a result of experiencing negative emotions such as anger. Therefore, it can be thought that experiencing cyber victimization can lead to cyberbullying behaviors.

This study revealed that self-efficacy plays a protective role in preventing cyber victims from becoming cyber bullies. This study found the interaction of cyber victimization with self-efficacy is a significant predictor of cyber bullying. More specifically, individuals with low self-efficacy display more cyber bullying behaviour in comparison to those with high self-efficacy. In other words, adolescents with low self-efficacy who have experienced cyber bullying, are more likely to display cyber bullying behaviour.

These results are compatible with Bandura's (26) social cognitive theory. Social cognitive theory states that self-efficacy beliefs are the basis of individuals' actions. The self-efficacy beliefs affect the behaviour pattern they choose and skills they develop. Self-efficacy levels can lead to healthier behaviour (27). As a result, individuals with high levels of self-efficacy about coping with cyber bullying effectively choose professional behaviour to cope with cyber bullying (43).

In this context, development of self-efficacy among cyber victims protects them from the adverse effects of victimization, and thus, reduces their aggression and prevents them from experiencing feelings of revenge. Bingöl (44) emphasized that having high self-efficacy will decrease cyber bullying. In a study conducted by Nikel (45), individuals with high self-efficacy reported they were able to solve social conflicts without feeling the need to resort to aggression, which supports this conviction. Individuals with high self-efficacy are able to deal with stressful social situations and prefer trusting behaviour as a social strategy (46, 47). Self-efficacy prevents negative peer relationships (29), and provides the opportunity for the individual to follow their personal norms in order to regulate their behaviour when faced with peer pressure (48). Furthermore, the finding that individuals with high social self-efficacy receive greater support from their friends when they experience cyber bullying reveals the protective role of self-efficacy (28, 10).

5. CONCLUSIONS

The results of this study reveal that when their self-efficacy levels are low, adolescents exhibit more cyber bullying behaviour. When the self-efficacy levels of adolescents are high, the effect of cyber victimization on cyber bullying was observed to be a little less strong. This result means that the relationship between cyber victimization and cyber bullying is moderated by self-efficacy.

Limitations and Recommendations

Despite revealing crucial results, this study has various limitations. First of all, the findings obtained from this study were collected using self-report measuring tools reliant on the participants' perceptions. Therefore, it is recommended that new studies employing different data collection methods be completed. Another limitation of the study is the use of the relational model. Therefore, causal inferences cannot be drawn from this study. Conducting new studies using experimental or longitudinal design will ensure the elimination of this limitation.

Various suggestions can be made in the context of this study. First of all, it was observed that self-efficacy plays a protective role against cyber victims becoming cyber bullies. In this context, psycho-education programs to be applied to cyber victims need to focus on boosting self-efficacy. Secondly, self-efficacy was measured integrally in this study. Later research focusing on the sub-dimensions of self-efficacy, and revealing their role in the relationship between cyber victimization and cyber bullying will be beneficial.

The findings of this research include vital results for school psychological counsellors. This research shows that adolescents exposed to cyber bullying are at risk of becoming cyber bullies. Therefore, school counsellors should take various precautions to prevent them from responding with cyber bullying after they are exposed to cyber bullying.

One of these precautions is to increase the self-efficacy of students. This research shows that individuals with high levels of self-efficacy can easily cope with the negative results of being exposed to cyber bullying. Therefore, school counsellors should develop programmes to increase the self-efficacy of students exposed to cyber bullying and should play a role during the implementation period.

Conflict of interest

The authors do not have any conflict of interest to disclose

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Clinical and Experimental Health Sciences

Effectiveness of Balance Exercises on Postural Control and Quality of Life in Patients with Lumbar Discopathy

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ABSTRACT

Objective: Lumbar discopathy is a common problem that affects musculoskeletal system. Many treatment modalities are in use for patients with lumbar discopathy. Balance exercises could be important for the lumbar discopathy due to the affected musculoskeletal system. This study was planned to investigate the effect of balance exercises on postural control and quality of life for lumbar discopathy.

Method: A total of 81 patients were divided into two as the intervention and the control groups. Therapeutic ultrasound, transcutaneous electrical nerve stimulation (TENS), hotpack and therapeutic exercises were applied to all patients 5 times a week during thirty sessions. Additionally, the intervention group received balance exercises. Pain (Visual Analog Scale), disability (Oswestry Disability Index), quality of life (Short Form-36) and postural control (Libra®) have been evaluated by same physiotherapist before and after the treatment.

Results: Statistically significant differences were found between the groups comparing pain, disability and postural control parameters ($p<0.05$). In the between group comparisons of the mean changes of the 'Quality of Life' assessment parameters, no significant differences were obtained between the intervention and control groups ($p>0.05$), except the 'physical role' parameter in favor of the control group ($p=0.04$).

Conclusion: This study demonstrated that physiotherapy modalities improve pain, disabiliy, quality of life and postural control parameters in patients with lumbar discopathy but adding classic balance exercises in physiotherapy treatment, do not have a remarkable effect in patients with lumbar discopathy.

Keywords: Lumbar discopathy, balance exercises, postural control, quality of life, physiotherapy

1. INTRODUCTION

Lumbar discopathy (LD) is a common health problem that causes low back pain (LBP) in most of the population. Most of adult population (80-85%) experience LBP at least once in their life. LD's constitute 64% of chronic LBP that mostly affects daily life activities negatively (1,2)

Many non-pharmacological therapy options such as manual therapy, exercise approaches, electrotherapy, massage, acupuncture and cognitive behavioral therapy are in use. Recent review study recommends most of these therapies when provided as a part of multimodal package (3). Appropriate exercise approaches have benefit in patients with LD. After assessment of the patient, suitable exercises programs could be prescribed such as stretching and strengthening exercises, core exercises or stabilization exercises (4-6). Besides that; patients should be informed about correct posture in order to prevent their mistakes and recurrence problems and to ensure correct body mechanics in daily life activities on the basis of ergonomic principles (7,8).

Balance and postural control disorders have explained widely in literature for patients with LBP. When the postures of the patients with LD were examined, the existence of flexed vertebral column, decreased lumbar lordosis, flexion position of the affected lower extremity and antalgic gait could be most seen (9). Balance problems in patients with LD are caused by pain, uneven distribution of load in the lower extremities, and muscle weakness. This asymmetry in the load distribution and accompanying pain, affects the proprioceptive input and forms the basis of balance problems and leads to loss of postural control. These problems negatively affect the individual's independence and mobility in daily life activities (10,11).

Most of the studies only evaluate balance or postural control in patients with chronic LBP or LD. Our study aims to investigate the effect of balance exercises on postural control and quality of life in addition to physiotherapy approaches in patients with LD.

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2. METHODS

This experimental study was designed as non-randomized controlled and carried out in a physiotherapy department after the approval of institute ethics committee (Ethics Committee of Marmara University, Institute of Health Sciences, Reference No: 16.01.2014-4).

The patients enrolled with LD aged between 18-70 years old who were admitted Physical therapy and rehabilitation department of 'Median Tıp Merkezi' in Istanbul, in the 6-month period in 2014. Patients who agreed to participate in the study filled an informed consent form. The including criteria were; diagnosis of LD by MRI, existing low back pain at least 3 months, existence of bulging or protruding discs, being able to understand and participate in program. The patients were excluded if they have extruded or sequestered disc, scoliosis, neurological sequelae, cardiac pacemaker or pregnancy. Patients who had spine or lower extremity surgery, rheumatologic, congenital, vestibular, neurologic diseases that could cause balance problems, were also excluded.

At first, the study enrolled 102 patients. Fifteen patients didn't meet the including criteria and one patient refused to participate. Rest of 86 patients were allocated into intervention group (IG) and control group (CG) by 1:1 allocation ratio. The allocation was made by sequence of incoming patients.

2.1. Procedures

Patients in both groups received the conventional physiotherapy procedure for 30 sessions (5 sessions per week, 6 weeks). Additionally, IG received balance exercises. The conventional physiotherapy procedure consists of *Ultrasound (US)* with frequency of 1 MHz and intensity of 1 W/cm² for 10 min; *Transcutaneous Electrical Nerve Stimulation (TENS)* with a frequency of 60-80 Hz and duration of 50-100 µs in conventional mode for 20 min; *Hotpack* with TENS for 20 min; and *exercises* including abdominal strengthening, lumbal traction and squat exercises that the patients waited 5 seconds at the end of each exercise and repeat each exercise 10 times.

IG received two classical balance exercises in addition to classical physiotherapy procedure. These are 'single leg stance exercise' determined as static balance exercise and 'tandem gait' determined as dynamic balance exercise. In the single leg stance exercise, subjects were asked to stand on one foot as long as they could, with their eyes open. In the tandem gait, subjects were asked to walk a total of 5 laps on a 2-meter-long line with the heel of the front foot touching the fingertip of the back foot. Patients were evaluated baseline and after six weeks of treatment. Evaluations were performed by a consider researcher who knew group which the cases were within the study. It was the same physiotherapist who did the evaluation and treatment.

2.2. Outcome measures

Visual Analog Scale (VAS) assesses pain subjectively using a 0-10 centimeters (cm) scale, where 0 indicates "no pain" and 10 indicate "worst pain" (12).

Oswestry Disability Index (ODI) is reliable and valid tool assesses the permanent functional disability. ODI contains 10 items related to limitations in daily life activities, with 0 (no disability) – 5 (severe disability) point scale (13). Turkish version of the tool is in use (14).

Short form 36 (SF-36) consists 36 items and assesses the quality of life (QoL) level. It has 8 sub-parameters as physical function, physical role, pain, general health, vitality, social function, emotional role difficulty and mental health. Each sub-parameter is scored on a scale of 0 to 100, where 0 is the lowest and 100 is the highest score (15). Reliable and valid Turkish version were used (16).

Libra (Easytech, Italy, 2013) was used to assess the postural balance. The subjects were asked to try to stay on the device in the form of a balance board, on both feet and without any support. Displacement of the body center of gravity on the virtual screen was controlled by the patient without slipping to the right or left. The evaluation consisted of 3 repetitions, each last 1 minute. The average score of the 3 repetition was recorded (17,18).

2.3. Statistical analysis

SPSS (Statistical Package for Social Sciences Inc. Chicago, IL, USA) Version 22.0 was used for data analysis. The sample size was calculated by using a previous study, which mentioned the impact of trunk balance exercises in chronic low back pain (19). The mean and standard deviation data of the VAS parameter were used with a power (1-type II error) of 0.90 and a Type I error of 0.05. Therefore, it was aimed to recruit at least 37 participants per group. In total, with a dropout rate of 20%, it was estimated that 44 patients would be recruited in each group. Kolmogorov-Smirnov test was used for the compatibility of the data with normal distribution. Mean values, standard deviations and the percentage of total for each variable of interest were calculated. Qualitative demographic data in groups was examined by Chi-square test. Wilcoxon T Test was used to determine the changes after the treatment and Mann-Whitney U Test was used to compare the difference between groups. Significance level was set at $p \leq 0.05$.

3. RESULTS

Total of 86 patients were recruited to the study. Two patients in IG and three patients in CG were excluded because they could not complete all sessions. Therefore 81 patients (IG:41 and CG:40) completed the sessions (Figure 1). The demographic characteristics of groups was similar at baseline (Table 1).

There was no significant difference in the severity of pain in between group comparisons (p=0.05) while it was significantly

better in each group before and after the treatment (p<0.01) (Table 2).

Significant improvements were obtained in ODI results in each group between before and after the treatment (p<0.01). But there was no significant difference in between group comparisons for the ODI results (p=0.99) (Table 2).

SF-36 with the sub-parameters of physical function (p<0.01), physical role (p<0.01), pain (p<0.01), vitality (p=0.05), social function (p=0.01) and mental health (p=0.04) showed significant difference in CG; physical function (p<0.01), pain (p<0.01) and general health (p=0.03) showed significant difference in IG, before and after treatment. In between group comparisons of the sub-parameters of the SF-36, no significant differences were obtained between the IG and CG (p>0.05), except 'physical role'(p=0.04) parameter in favor of the CG (Table 2).

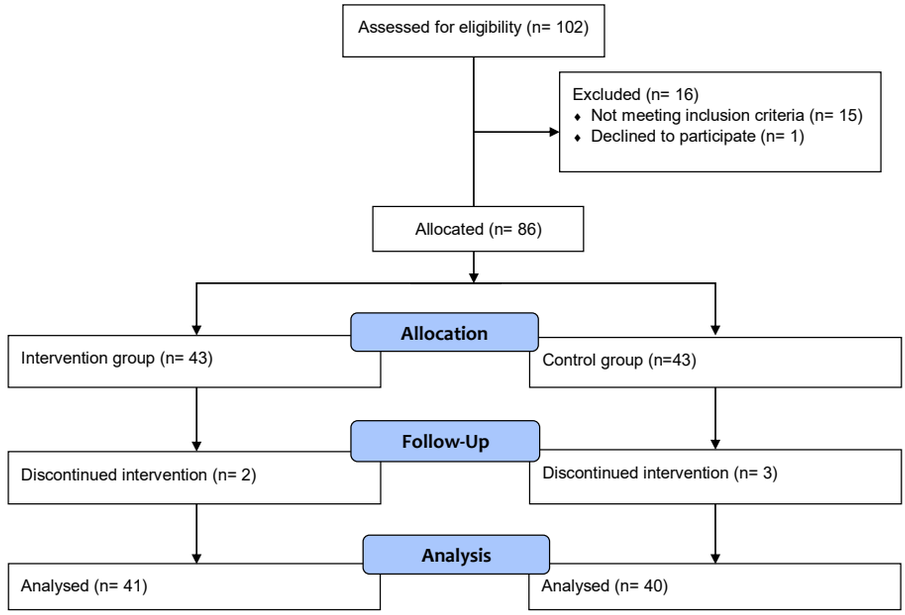

Figure 1. *Flow chart*

Table 1. *Baseline and demographic characteristics*

		Control Group (n=41)	Interventional Group (n=40)	Total (n=81)	P value
Age (year) mean±SD		48.59±10.49	47.5±12.29	48.05±11.36	0.91
Height (cm) mean±SD		163.6±8.95	163.55±7.85	163.58±8.37	0.81
Weight (kg) mean±SD		78.22±12.73	76.13±11.89	77.19±12.29	0.35
BMI (kg/m^2) mean±SD		29.24±5.02	28.53±4.55	28.89±4.78	0.42
Daily sitting time (hour) mean±SD		4.56±3.08	4.26±3.29	4.41±3.17	0.46
Gender n(%)	**Male**	14 (34.15)	10 (25)	24 (29.63)	0.26
	Female	27 (65.85)	30 (75)	57 (70.37)	
Occupational status n(%)	**Working**	16 (39.02)	14 (35)	30 (37.04)	0.44
	Non working	25 (60.98)	26 (65)	51 (62.96)	
Educational status n(%)	**No education**	4 (9.75)	3 (7.5)	7 (8.64)	0.47
	Primary	29 (70.73)	24 (60)	53 (65.43)	
	Secondary	6 (14.63)	7 (17.5)	13 (16.04)	
	University	2 (4.87)	6 (15)	8 (9.87)	
Stage of discopathy n(%)	**Bulging**	18 (43.9)	16 (40)	34 (41.98)	0.45
	Protrusion	23 (56.1)	24 (60)	47 (58.02)	

BMI: Body Mass Index, SD: Standard Deviation

Table 2. *Study outcomes*

		Control Group N=41			Intervention group N=40			P value
		Before Treatment X±SD	After Treatment X±SD	P value	Before Treatment X±SD	After Treatment X±SD	P value	(Between Group Comparison)
VAS (Pain, 0 to 10 cm)		5.46±2.42	4.41±2.39	**<0.01***	6.28±2.03	3.9±2.27	**<0.01***	0.05
ODI (Disability)		22.61±8.43	14.17±6.84	**<0.01***	23.15±6.32	14.23±7.26	**<0.01***	0.99
Libra (Postural Control)		8.47±3.24	6.7±2.31	**<0.01***	9.04±3.33	6.46±2.7	**<0.01***	0.17
SF-36 Quality of Life	Physical Function	65.12±21.58	76.46±16.93	**<0.01***	61.13±18.76	72.63±15.81	**<0.01***	0.6
	Physical Role	25.61±32.35	45.73±36.62	**<0.01***	21.25±27.47	28.75±35.15	0.1	**0.04****
	Pain	37.56±21.42	55.12±15.99	**<0.01***	41.75±18.1	52.75±20.38	**<0.01***	0.26
	General Health	59.02±20.5	62.8±19.5	0.15	50±17.9	55.5±18.46	**0.03****	0.44
	Vitality	52.07±20.49	58.88±20.16	**0.05****	41.63±18.72	44.88±22.69	0.42	0.31
	Social Function	54.57±23.01	65.55±23.01	**0.01****	57.19±24.98	59.69±24.1	0.54	0.25
	Emotional Role	40.65±39.1	51.22±39.52	0.07	38.5±35.04	39.17±41.27	0.92	0.15
	Mental Health	69.17±18.27	74.34±15.85	**0.04****	61.4±15.44	63.4±20.39	0.49	0.32

*VAS: Visual Analog Scale, ODI: Oswestry Disability Index, SD: Standard Deviation, cm:centimeter, *p<0.01, **p≤0.05.*

4. DISCUSSION

In this study, we aim to evaluate the effects of balance exercises on postural control and quality of life in patients with lumbar discopathy. The results of our study showed that the balance exercises that were selected with conventional therapy did not increase the positive effects of treatment on pain, disability, quality of life and postural control in patients with lumbar discopathy. There are many studies in the literature that is about investigating balance status or postural control in patients with lumbar discopathy or chronic low back pain (19-21). The difference of our study from these studies is to investigate the effectiveness of balance exercises added to the conventional treatment that is included electrotherapy modalities and therapeutic exercise program.

Omokhodion and Sanya found that there is a relationship between sitting time exceeding 3 hours and low back pain in their study (22). In our study, the average daily sitting time of all patients was 4.41 ± 3.17 hours and 59.26% of the patients mentioned that did not walk every day. These results show that recommendations can be given to patients with LD to decrease the sitting time and increase the time of physically active.

Phadke and Yadav examined the effects of balance exercises in patients with chronic low back pain and compared to the pain, functional status, quality of life and balance status of patients between conventional and balance exercises group and conventional physiotherapy group. In this study, they were detected significant difference in all assessment area in both groups, but there was no significant difference between the groups in terms of all assessment results (20). Gatti et al. researched the efficacy of trunk balance exercises for individuals with chronic low back pain in the study. It has been reported that flexibility and balance exercises have no superiority over pain intensity compared to flexibility and strengthening exercises (19). In our study, pain intensity and functional disability of the patients was evaluated with VAS and ODI. Pain intensity and ODI scores of the two groups were similar at the baseline of the study. After the treatment program, pain and ODI scores decreased significantly in both the groups. When the mean of intergroup change in pain and ODI scores were compared, the groups did not have superiority over each other. These results show that balance exercises in addition to conventional treatment are not more effective on pain and functional disability.

Boskovic et al. investigated the effectiveness of conservative treatment in patients with lumbar radiculopathy and to evaluated the quality of life of patients with SF-36. This study reported that is achieved improvements in all parameters related to physical health but there is no significant difference in parameters related to mental health (23). In our study, we found only improvements in physical function and pain parameters of the quality of life in the control and intervention groups.

Tari et al. were used to Libra on patients' balance and postural control status in their studies (24). In our study, postural control was evaluated with Libra, and there were improvements on postural control in both groups. These results suggested that pain and trunk muscle weakness affecting postural control in patients with lumbar discopathy or chronic low back pain can be eliminated with therapeutic exercises in the physiotherapy program.

As a recommendation of this study demonstrated that physiotherapy modalities improve pain, disability, quality of life and postural control parameters in patients with lumbar discopathy. Whereas single leg stance and tandem stance exercises performed as classic balance exercises in physiotherapy treatment, do not have a remarkable effect in patients with lumbar discopathy.

Limitations of our study are that the effects of different balance exercises were not compared, and the effectiveness of conventional approaches and balance exercises could not be examined separately. In the future studies, we recommend

that different balance exercises be applied at different intensities and durations to examine their effectiveness in the long term.

5. CONCLUSION

The results of our study show that classical physiotherapy modalities and therapeutic exercise approaches are effective in patients with lumbar discopathy in terms of pain, disability, quality of life and postural control.

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Clinical and Experimental Health Sciences

Diabetes Risk Assessment with Blood Parameters of The First Degree Relatives of Patients with Type-2 Diabetes Mellitus

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ABSTRACT

Objective: This study was conducted to evaluate the diabetes risk in first degree relatives of Type-2 diabetic patients who were not diagnosed with diabetes and test the validity of the Finnish Diabetes Risk Score (FINDRISK) in determining the risk of type-2 diabetes.

Methods: First degree relatives of patients who were hospitalized in "İzmir Katip Çelebi University Atatürk Training and Research Hospital", who were not diagnosed with diabetes before, were included in this cross-sectional study. The sample of the research consisted of 200 people determined by power analysis. Data were collected by "a structured question form" and the FINRISK scale. Participants' height, weight, waist circumference were measured by the researcher, and venous fasting blood glucose (FBG) with Glycosylated Hemoglobin (HbA1c) were taken by the researcher.

Results: The average age of the participants was 46.93 ± 14.83 (18-86). It was determined that the vast majority of the individuals participating in the study were female (65%), 63% were married, 50.5% received high school or higher education, and 59% worked in an income-generating job. The findings was indicated that 41.5% of the participants were in the 'high-risk group' for type-2 diabetes according to the FINDRISK score scale. Sensitivity of FINDRISK score to FBG was 100% and specificity was 60%.

Conclusion: As a result of findings in this study indicate that It was determined that the risk of DM was high in the first degree relatives of the patients hospitalized with Type-2 DM and the FINDRISK score scale was a good scale in predicting new asymptomatic Type 2 diabetes.

Keywords: Risk for diabetes, FINDRISK, type 2 DM, FBG, HbA1c

1. INTRODUCTION

The prevalence of diabetes mellitus (DM) for adults worldwide is 8.3%. Although the prevalence of diabetes mellitus (DM) for adults is 7.9% in European countries, this rate is expected to reach 10.3% by 2035 (1). Almost half of all DMs in the world are concentrated in the 40-59 age group (184 million). In developing countries, diabetes is mostly seen between the ages of 35-64. Furthermore, DM is identified more frequently among individuals with medium and low incomes (77%), and the DM rate for this population is expected to increase to 86% by 2035 as well (1-3).

Such frequency of DM will cause serious complications and early deaths if its treatment and care are not adequately managed. According to the data issued by the International Diabetes Federation (IDF), nearly half of the deaths caused by diabetes worldwide occurred in individuals younger than 70 (1).

Approximately 10% of all diabetics patients have type-1 DM, while 90% have type-2 DM. By 2035, the number of type-2 diabetes patients is expected to increase by 55% worldwide (2,4). Adults with a history of DM among their first degree relatives and who are asymptomatic for diabetes are considered to be in the at-risk group for type-2 DM (5). The incidence rate of diabetes in children who have one parent with diabetes is 2-5% for type-1 DM and 15% for type-2 DM. However, the incidence rates for children with two diabetic parents is 10% in type-1 DM and 50% in type-2 DM (6).

Relieving the social and individual burdens of diabetes, preventing associated complications and minimizing the related death rate is possible only by early diagnosis of this disease and appropriate treatment (7).

Diagnosis, Treatment and Surveillance Guide for Diabetes Mellitus and Its Complications published by the Turkish Endocrinology and Metabolism Association (TEMD) in 2013, includes guidelines for determining which individuals are at risk for Type-2 diabetes. The guidelines suggest evaluating the answers to the 8 questions developed to determine the

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risk of Type-2 DM using the FINDRISK method. Individuals at risk for diabetes who score more than 20 are required to be included in protection programmes (8). A limited number of studies in Turkey used the FINDRISK Scale in screening studies for the type-2 diabetes, which is frequently observed in adults (9,10).

The present study was conducted to evaluate the diabetes risk in first degree relatives of Type-2 diabetic patients who were not diagnosed with diabetes, to test the validity of the FINDRISK score in determining the risk of type-2 diabetes, and to direct those with high risk to diagnosis.

Research Questions;

Question 1: According to the Fasting Blood Glucose (FBG) level, can the FINDRISK score be considered a valid scale with which to determine type-2 diabetes?

Question 2: Are there significant differences between the Glycated Haemoglobin (HbA1c) level and the venous FBG and the FINDRISK score according to gender?

2. METHODS

2.1. Objective

The research was conducted on first degree adult relatives of in-patients with type-2 DM at the İzmir Katip Çelebi University Atatürk Education and Research Hospital between August 2014 and May 2015.

2.2. Study Design

The present study is a complementary and methodological study.

2.3. Sample and Setting

The research population comprised type-2 DM inpatients' first degree relatives (mother, father, child, sister) who were not yet diagnosed with diabetes. The number of samples was determined 200 by using power analysis which the error amount was α=0.05 and 0.25 with a medium effect size with the power of the targeted test was 0.80 (80%).

2.4. Inclusion and Exclusion Criteria

The inclusion criteria were as follows: First-degree adult relatives of patients with type-2 DM who had not yet been diagnosed with diabetes, those who had no hearing, understanding and speech difficulties, those without cognitive impairment, who agreed to participate in the study, and who could have standing weight and height measurements were included in the study.

Second-degree relatives, underage relatives, unwilling to give blood samples, persons previously diagnosed with diabetes, and relatives who were unable to provide standing height and weight samples were not included.

2.5. Data Collection

The study data were collected using a "structured question form" and the "Fin Diabetes Risk Score Scale (FINRISK)", prepared based on the current literature (2,4,7,8,11,12).

The Structured Question Form comprises four sections:

The first section includes eight questions regarding information describing the respondents (age, gender, etc.). The second section includes open – and closed-ended questions to determine the respondents' status regarding type-2 diabetes risk factors [use of cortisone, cholesterol level, medications, etc.]. The third section records the FBG and HbA1c levels of respondents. Laboratory values are based on the ADA criteria (12) and evaluated as follows: FBG ≤ 99 mg/dL and HbA_1c ≤ 5.6% = No Risk, FBG 100 – 125 mg/dL and HbA1c 5.7 – 6.4% = Pre-diabetes, FBG≥126 mg/dL and HbA1c ≥ 6.5% = Diabetes. Blood samples were taken from respondents and delivered to the hospital laboratory by the researcher. All analysis costs were paid by the researcher. The FBG was evaluated by ARCHITECT Brand devices using a multi-constituent calibrator; The HbA1c was evaluated by ARCHITECT Brand devices calibrated by the Bio-Rad VARIANT-II TURBO haemoglobin test system. In the fourth section, the respondents' anthropometric measures [height, weight, waist size, body mass index (BKI)] were included. Participants' weight measurements were made in an empty room, inside without removing their clothes, and before breakfast, using a conventional scales calibrated by the hospital. The respondents' height (without shoes) and waist size (the narrowest diameter between the arcus costarum and the processus spina iliaca anterior superior) were measured using a non-elastic tape measure.

The FINRISK comprised eight questions (age, BKI, waist size, level of exercise, amount of fruits and vegetables consumed, hypertension status, previous status of blood glucose either at high or limited values, family history of diabetes). The FINRISK was developed by Jaakko Tuomilehto and Jaana Lindström (without laboratory tests to determine individuals with type-2 DM risk) in 1987 and its validity and reliability were analysed in 1992. The FINDRISK scores were evaluated according to Lindström (11), who conducted the reliability and validity study of the scale. The scores for a 10-year, type-2 diabetes risk were low = ≤7, moderate = 7-11, medium = 12-14, high = 15-20, and very high = ≥20. The minimum and maximum possible scores were 0 and 26, respectively.

In a number of studies, the breakpoint of the score was determined to be equal to or greater than 15, which determined the validity of the FINDRISK score or the risk of type-2 diabetes (9,10,13-19). In the present study, the breakpoint of the FINDRISK was also determined to be equal to or greater than 15; and such scores were determined to indicate a high or very high risk of type-2 diabetes.

2.6. Ethical Consideration

Before beginning the research, the necessary permission was obtained from the Adnan Menderes University, Faculty of Medicine, Board of Ethics for Non-Invasive Clinical Research (protocol no: 2014/387, serial no: 56989545/050-139) and from İzmir City Southern Public Hospitals Union General Secretariat for ethical considerations relevant to the study. In addition, following the necessary explanations, respondents' verbal consent was received. The question form and the FINDRISK scale were administered by the face-to-face interview method, and anthropometric measurements were completed by the researcher.

2.7. Statistical Analysis

Data analysis was conducted on the SPSS 18 package software. Categorical variables were evaluated using Pearson's Chi-Square, the multiple-span-four dimension chi-square test, and Fisher's Exact Test (α-Type error = 0.05).

3. RESULTS

This study was conducted on a total of 200 respondents to evaluate the risk of type-2 diabetes for adult 1st degree relatives of DM inpatients. Of the respondents, 51.5% had a mother or father with type-2 diabetes, and 18% had a sister or brother with type-2 diabetes.

Of the respondents, 65% were female, 63% were married, 50.5% had graduated from high school or a higher institution and 59% were employed. It was also determined that 90% of respondents were subject to the social security institution (SSI), and their income covered 65% of their expenditures (Table 1).

Table 1. *Distribution of demographical characteristics of respondents (N=200)*

		n	%
Gender	Female	130	65.0
	Male	70	35.0
Marital status	Married	126	63.0
	Single	74	37.0
Education level	Literate or illiterate	36	18.0
	Primary school	63	31.5
	High school and above	101	50.5
Employment status	Employed	118	59.0
	Unemployed	82	41.0
Social security	Have not	2	1.0
	SSI	180	90.0
	Private health insurance	12	6.0
	Green card	6	3.0
Income level	Income does not meet expenditures	64	32.0
	Income meets expenditure	130	65.0
	Income greater than expenditures	6	3.0

Age: 46.93 ± 14.83 (Min: 18 year, Max: 86 year)
Height: 166.5 ± 7.30 (Min: 150 cm, Max: 184 cm)
Weight: 71.47 ± 12.49 (Min: 43 kg, Max: 104 kg)
SSI: social security institution

The mean age of the females in the research group was 44.65 ± 13.67, the mean smoking duration was 10.57 ± 9.03, the mean FBG value was 96.34 ± 17.33 mg/dl, the mean HbA1c level was 5.55% ± 0.58%, the mean BKI value was 25.95 ± 3.11 kg/m^2, the mean waist size was 89.17 ± 12.57 cm, and the mean FINDRISK score was 13.43 ± 4.18.

The mean age of the males in the research group was 51.17 ± 16.05, the mean smoking duration was 17.14 ± 11.46, the mean FBG value was 97.09 ± 10.96 mg/dl, the mean HbA1c level was 5.53% ± 0.4%, the mean BKI value was 25.95 ± 3.11 kg/m^2, the mean waist size was 96.97 ± 8.64 cm, and the mean FINDRISK score was 13.6 ± 4.71.

The mean values of the female respondents with regard to their age, smoking duration and waist size variables were significantly lower than the corresponding values of the male respondents ($p<0.05$).

No statistically significant difference between the mean values of the FBG, HbA1c and BKI values of respondents were observed by gender ($p>0.05$) (Table 2).

Table 2. *Mean values of some diabetes risk factors and indicators of respondents according to gender (N=200)*

	Female		Male		p
	Mean±SD	Min.-Max.	Mean±SD	Min.-Max.	
Age	44.65±13.67	18-78	51.17±16.05	22-86	**0.005**
Smoking (year)	10.57±9.03	1-31	17.14±11.46	2-40	**0.018**
FBG	96.34±17.33	68-225	97.09±10.96	68-131	0.326
HbA1c	5.55±0.58	4.1-9.9	5.53±0.4	4,6-6,8	0.940
BKI	25.78±4.78	16.79-36.99	25.95±3.11	18,94-35,94	0.914
Waist size	89.17±12.57	62-125	96.97±8.64	72-120	**0.000***
FINRISK	13.43±4.18	6-24	13.60±4.71	5-24	0.863

p<0.001

According to the respondents' FINDRISK scores, when type-2 diabetes risk was evaluated, 5% were classified in the low-risk group, 28% were in the moderate-risk group, 25.5% were in the medium-risk group, 34.5% were in the high-risk group (diabetes development risk within ten years at 33%), and 7% were in the very-high-risk group (diabetes development risk within ten years at 50%). The respondents' mean FINDRISK scores were 13.49 ± 4.36 (Table 3).

Table 3. *Type-2 diabetes degree of risk and 10-year risk statuses according to the FINDRISK scores of respondents (N=200)*

Total score	n	%	Degree of risk	10-year risk
(<7)	10	5.0	Low	(1%) (1/100)
(7-11)	56	28.0	Moderate	(4%) (1/25)
(12-14)	51	25.5	Medium	(16%) (1/6)
(15-20)	69	34.5	High	(33%) (1/3)
(>20)	14	7	Very high	(50%) (1/2)
FINDRISK risk score: 13.49 ± 4.36 (Min: 5 score, Max: 24 score)				

The mean HbA1c level of the participants was 5.54 ± 0.52 (Min: 4.10, Max: 9.90) and the mean FBG values were 96.60 ± 15.37 (Min: 68, Max: 220). According to the diabetes diagnosis criteria published by the ADA (5); according to HbA1c results, 2% had diabetes, 38% had prediabetes; according to FBG results, 3% had diabetes and 30% had prediabetes (Table 4).

Table 4. *Distribution of the HbA1c and the FBG results according to the ADA values of the respondents with respect to the diabetes diagnosis criteria (N=200)*

HbA1c*	n	%
Normal (HbA1c = 5.6≤)	120	60
Prediabetes (HbA1c = 5.7 – 6.4)	76	38
Diabetes (HbA1c = 6.5≥)	4	2
FBG*		
Normal (FBG = 99 mg/dl ≤)	134	67
Prediabetes (FBG = 100-125 mg/dl)	60	30
Diabetes (FBG = 126 mg/dl ≥)	6	3
FBG: 96.60 ± 15.37 (Min: 68 mg/dl, Max: 220 mg/dl)		
HbA1c: 5.54 ± 0.52 (Min: 4.10%, Max: 9.90%)		

The HbA1c test must be conducted in laboratories using a method conforming to the International Glycohemoglobin Standardization Program (5). The TEMD does not suggest using the HbA1c as the sole diagnostic test in Turkey because deficiencies remain in terms of technical equipment, standardization and relevant high costs (4,8,12,20).

The sensitivity and specificity of the FINDRISK scale with regard to the FBG level were 100% and 60.3%, respectively; the positive predictive value and negative predictive value were 100% and 7.2%, respectively (Table 5 and Table 6).

Table 5. *Sensitivity and Specificity of the FINDRISK Results according to FBG Results*

FINDRISK	Fasting Blood Glucose (FBG)		Total
	Diseased	Healthy	
Diseased (≥15 score)	6 (100%)	77	83 (41.5%)
Healthy (≤14 score)	0	117 (60.3%)	117 (58.5%)
Total	6 (3.0%)	194 (97.0%)	200 (100%)

Table 6. *ROC analysis results for the prediction power of FINDRISK Scale Results according to FBG Results*

	Value	95% CI
Sensitivity	100	54,1 – 100,0
Specificity	60,3	53,1 – 67,2
+PV	7,2	2,7 – 15,1
-PV	100	96,9 – 100,0
AUC	0,802	0,699 – 0,904
p	<0,001	

In this study, the ROC diagram demonstrates the estimation strength of the FINDRISK score for the Type-2 diabetes status of recently diagnosed patients. These results are presented in Figure 1. The FINDRISK score was calculated at 0.802 (95% GA: 0.699-0.904), remaining below the ROC diagram plotted to determine the estimation strength of newly diagnosed type-2 diabetes (according to the FBG). The calculated AUC value was determined to be statistically significant (p<0.001). Thus, the FINDRISK score was successful in estimating newly diagnosed type-2 diabetes.

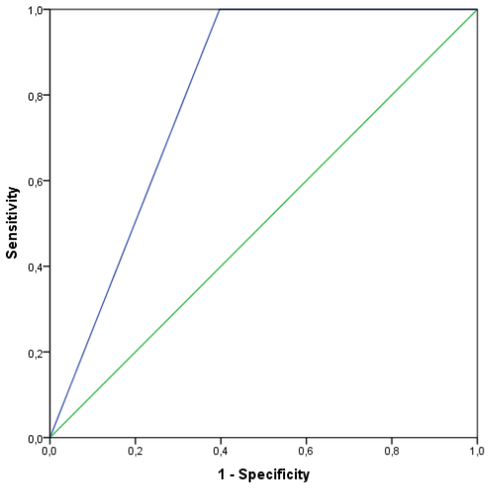

Figure 1. *ROC Curve Illustrating the Accuracy of FINDRISC Scores With Respect To FBG Results*

4. DISCUSSION

The present study was conducted to evaluate the diabetes risk of 1st degree relatives of inpatients previously diagnosed with the type-2 DM. The FINDRISK score was used to identify people at risk for Type 2 diabetes. This tool was developed by Tuomilehto and Lindström in 1992 and adapted into Turkish by the IDF. This scale was determined to be valid for predicting type-2 diabetes in a number of studies conducted in Finland and numerous other countries. In this study, the FBG was used as the standard, and corresponding results were compared.

The average age of the participants in the study was 46.93 ± 14.83 (18-86). The average age of women (44.65 ± 13.67) was found to be statistically significantly lower than men (51.17 ± 16.05) (Tables 2 and 5). According to data published by the ADA (12), individuals aged 45 and older are at risk for type-2 DM. According to data published by the WHO (21), type-2 DM is most frequently observed in individuals in the age group of 35-64 in developing countries. Age is reported to be an important risk factor for Type-2 DM and is usually seen in individuals over the age of 40. However, it has also been reported that the prevalent age of diabetes depends on different life styles; therefore, diabetes can occur in youth or even childhood (2,7,12,22).

In the present study, no significant difference was found between the mean BMI values of women (25.78 ± 4.78) and men (25.95 ± 3.11) (Table 2). In the study of Tarı Selçuk (19), it was reported that the average BMI was 27.09 ± 3.79 kg / m2 in men and 28.7 ± 4.48 kg / m2 in women. Costa et

al. (17) reported that there was no statistically significant difference between men (28.7 ± 4.0 kg / m2) and women (28.9 ± 4.9) in terms of average BMI. Makrilakis et al. (13), the mean BKI values were reported as 29.8 ± 5.7 kg / m2 in women and 29.4 ± 4.0 kg / m2 in men. Bayındır and Çevik et al. (23) reported mean BKI values for females and males at 28.44 ± 5.94 kg/m^2 and 28.09 ± 4.11 kg/m^2, respectively. These authors concluded a statistically significant correlation between these values.

Waist circumference values seen as a risk for Type-2 DM are > 88 cm in women and > 102 cm in men. In the present study, Females' mean waist size (89.17 ± 12.57 cm) was significantly lower than males' (96.97 ± 8.64 cm) (Table 2). In the study of Makrilakis et al. (13), it was reported that the average BMI was 29.6 ± 5.0 kg / m2, the average BMI for men was 29.4 ± 4.0 kg / m2, and the average BMI for women was 29.8 ± 5.7 kg / m2. The difference between them was significantly low. Bayındır – Çevik et al. (23) reported a mean waist size of 93.35 ± 15.72 cm and 99.38 ± 12.24 cm for females and males, respectively; the difference between the two measurements was determined to be significantly low. Costa et al. (17) reported the mean waist size as 93.4 ± 11.6 cm in women and 100.0 ± 10.0 cm in men. The difference between the two measurements was determined to be significantly low. In the study of Tarı-Selçuk (19), it was reported that the average waist circumference was 99.26 ± 8.51, the average waist circumference was 98.59 ± 8.20 cm for women, and 100.06 ± 8.83 cm for men. In study of Memiş, Gökçe, Gündoğmuş and Coşkunırmak (10), it was reported that the average waist circumference in women was 79.84 ± 10.00 cm, and the average waist circumference was 92.93 ± 12.58 cm in men.

Toktamış (24) reports that "Individuals are encouraged to quit smoking or at least dramatically reduce the number of cigarettes smoked daily in preventive programmes against type-2 DM". In this study, the mean duration of smoking in women (10.57 ± 9.03 years) was found to be statistically significantly lower than the average smoking period of men (17.14 ± 11.46 years) (Table 2).

The ADA (12) addressed Type-2 DM diagnosis criteria for venous FBG as <100 mg/dL = normal, 100-125 mg/dL = prediabetes and >125 mg/dl = diabetes. In this study, no significant difference was found between the mean "venous" FBG of women (96.34 ± 17.33 mg / dl) and the mean "venous" FPG of men (97.09 ± 10.96 mg / dl) (Table 2).

The ADA (12) assessed the HbA1c rate as "<5.7 normal", "5.7 – 6.4 prediabetes" and "≥6.5 diabetes". In this study, the mean HbA1c level was measured for females and males at 5.55% ± 0.58% and 5.53% ± 0.4%, respectively; the difference between them was not significant. The ADA (12) accepts the HbA1c ratio as <5.7 normal, 5.7-6.4 prediabetes, ≥6.5 diabetes. In this study, no significant difference was found between the mean HbA1c level of women (5.55% ± 0.58) and the mean HbA1c level of men (5.53 ± 0.4%) (Table 2). In the Tari-Selçuk (2013) study, was determined the average HbA1c level of participants was 5.70±0.81%, the average HbA1c level of men was 5.62±0.67%, and the average HbA1c level of women was 5.77±0.90%. In another study, Costa et al. (17) reported that there was no statistically significant difference between the average HbA1c levels by gender.

In this study, the difference between the mean FINDRISK of women (13.43 ± 4.18) and the mean FINDRISK of men (13.6 ± 4.71) was not statistically significant (Table 2). In Tarı Selçuk (19), the mean FINDRISK score was 12.22 ± 4.42; for females, the rate was 13.68 ± 3.96 and for males, 10.49 ± 4.32. In a study by Makrilakis et al. (13), it reported that the participants' mean FINDRISK score was 13.1 ± 4.9, and there was no statistically significant difference between the mean FINDRISK score of men (12.6 ± 4.9) and the mean FINDRISK score of women (13.6 ± 4.9). In the study of Costa et al. (17), the mean FINDRISK score was 11.8 ± 4.5. The rate for females was 12.0 ± 4.6 and 11.4 ± 44 for males; the mean FINDRISK score for females was significantly higher than for males. In the study of Taşdemir-Koçak, Öncel, Zincir and Seviğ (25) the mean FINDRISK score of the participants was reported 7.57 ± 0.13, and in the study of Coşansu, Çelik, Olgun, Özcan and Demir (9) the average FINDRISK score of the participants was reported 7.46 ± 4.62.

According to the ADA (5), individuals asymptomatic for diabetes and with first degree relatives diagnosed with DM are considered to be an at-risk group in terms of type-2 DM. Cheta et al. (26) reported that 33% of diabetic individuals have positive DM history in their families. In the CODIAB (27) study, 66% of diabetes cases had at least one diabetic relative. In current studies in the relevant literature, positive family history is particularly intensified with mothers as a first degree relative (28-30). In the present study, it was determined that the mother or father of half of the respondents (51.5%) was diagnosed with type-2 diabetes; brothers or sisters of one-fifth of (18%) respondents were diagnosed with type-2 diabetes as well. Contrary to the literature in the study of Bayrak, Koç and Suher (29), DM stories were reported in parents (23.9%) and siblings (34.3%) in the study of relatives of individuals with DM.

According to the respondents' FINDRISK scores, the risk degree for type-2 diabetes was assessed as low for 5% of respondents, moderate for 28%, medium for 25.5%, high for 34.5% (risk of developing diabetes within ten years is 33%) and very high for 7% (risk of developing diabetes within ten years is 50%) (Table 3). In the present study, according to the FINDRISK scores, respondents with scores ≥15 were considered to be "high risk" in terms of type-2 diabetes; respondents with scores ≤14 were considered "low risk". According to this classification, nearly half of the respondents (41.5%) were "high risk" in terms of type-2 DM; their risk of developing type-2 diabetes within the next 10 years is 33%.

Numerous studies have tested the validity of the FINDRISK score and used those scores to determine the risk of developing type-2 diabetes. Other studies similar to this study used ≥15 as the breaking point of the FINDRISK score (14-16). Hellgren et al. (14) conducted a study on FINDRISK scores (Swedish Version) and determined the type-2 diabetes risk to be 9.6%. Factors that may have influenced this result

included a mailing-based response method for the FINDRISK scale, a 56% response rate, the possibility of biased answers from respondents and the possibility of healthy persons participating in the study. The study of Costa et al. (17), conducted in Spain, reported that 27.0% of respondents were classified in the high-risk group in terms of type-2 diabetes. In that study, more than half of the respondents were female (66%), were obese (80%), and had abdominal obesity (55%). These findings are consistent with the results of the present study. In study of Coşansu et al (9) was determined low risk of Type-2 diabetes (8.7%) compared to this study. The reason for this is estimated to be related to the low average age of the participants (39.35 ± 10.40) and the high rate of men (74.8%). In a study by Bonaccorsi, Guarducci, Ruffoli and Lorini (18), conducted in Italy, the risk of type-2 diabetes was reported to be 22%. The type-2 diabetes risk was reported to be 28% in study of Tarı Selçuk (19). All of these study results reported lower rates than the present study observed. In this study, high type-2 diabetes risk (41.5%) was associated with the fact that the sample was selected from 1st degree relatives with type-2 DM. According to the results of the FINDRISK score (Greek version) in the study of Makrilakis et al. (13), The high risk of Type-2 diabetes (45%) supports this research result.

When the HbA1c and the FBG results of respondents were taken into consideration based on the diabetes-diagnosing criteria published by the ADA (12), according to HbA1c results, 2% had diabetes, 38% had prediabetes; according to FBG results, 3% had diabetes and 30% had prediabetes. In this study, the mean HbA1c and FBG scores of respondents were 5.54 ± 0.52 (4.10-9.90) and 96.60 ± 15.37 (68-220), respectively (Table 4). In national and international studies has been reported that individuals diagnosed based on their HbA1c scores experienced more metabolic problems than individuals diagnosed based on the FBG or the OGTT (8, 31, 32). In a national study, the metabolic risk profiles of the respondents classified in the “prediabetes” group based on the HbA1c (HbA1c 5.7%-6.4%) were observed to have deteriorated similar to the risk profiles of the individuals diagnosed with “Combined Glucose Tolerance Disorder” (IFG + IGT) (32). Clearly, using the HbA1c as diagnostic tool to diagnose and treat individuals at risk for complications would help prevent or retard the relevant complications. It is necessary to accelerate studies on the standardization of the HbA1c test by regulations enacted by the Ministry of Health (31). The HbA1c test is required in laboratories using methods conforming to the International Glycohemoglobin Standardization Program (NGSP) (5). The TEMD does not suggest employing the HbA1c test alone as a diagnostic tool in Turkey because deficiencies in terms of technical equipment and standardization have not yet been corrected and the associated costs are too high (4,8,12,20). Therefore, the FBG was used as the “golden test” in the present study.

In the present study, the validity of the FINDRISK score was investigated with respect to the venous FBG, one of the diagnostic diabetes tests. The ability of the FINDISK score to predict type-2 diabetes was assessed as an area in the ROC diagram. The AUC [0.802 (95% GA: 0.699-0.904)] value was determined to be statistically significant, and the FINDRISK score was deemed successful in predicting newly diagnosed type-2 diabetes ($p<0.001$) (Figure 1). Moreover, the sensitivity and specificity values of the FINDRISK scale with regard to the FBG level were 100% and 60.3%, respectively, and its positive and negative predictive values were 7.2% and 100%, respectively (Table 5 and Table 6). In the study of Tarı Selçuk (19), which investigated the reliability and validity of the FINDRISK scale in Turkey, the FINDRISK score was deemed the area remaining in the ROC diagram for estimating newly diagnosed type-2 diabetes and calculated as 0.84 (95% GA: 0.78-0.89). Makrilakis et al. (13) reported in their study on a Greek population that the FINDRISK score was valid for estimating type-2 diabetes and that estimation strength for newly diagnosed type-2 diabetes was the area remaining in the ROC diagram, calculated as 0.724 (CI: 0.68-0.76).

5. CONCLUSION

As a result of this study, indicates that the FINDRISK scale is successful in predicting newly diagnosed type-2 diabetes.

Suggestions;

-To determine the type-2 diabetes risk for individuals with relatives diagnosed with type-2 DM, the FINDRISK scale may be employed.

-All health employees in primary health care institutions in Turkey, internal medicine-endocrinology physicians and nurses and diabetes nurses could use the FINDRISK scale to determine individuals at-risk for type-2 DM. In addition, individuals diagnosed with type 2 DM in their first degree relatives should also be routinely screened for diabetes risk.

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Clinical and Experimental Health Sciences

Incidence of Cystic Echinococcosis in the East Azerbaijan, Iran, During 2011-2017: A Retrospective Epidemiological Study

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ABSTRACT

Objective: Cystic echinococcosis has been recognized as one of the most common zoonotic diseases in many parts of the world, which is caused by the larval stage of *Echinococcus granulosus.* The aim of this study was to investigate the incidence of Cystic echinococcosis cases in human in East Azerbaijan Province, Iran, during 2011-2017.

Methods: This cross-sectional descriptive epidemiologic study included seven-year period and investigated variables such as age, gender, involved organs, mortality, residential place types of patients and relations between these data. Data of the patients who had referred to the referral hospital in the East Azerbaijan, Iran with hydatid cysts were evaluated.

Results: The highest percentage of the cases were females (56.2%). The most common age range was 18-35 (33.5%). Liver was the highly involved organ, in 918 cases (60.2%). The highest case number was detected in the year 2015 (17.0%), while the year 2012 (11.4%) had the least rate of cases. The rural population with the highest rate of 62.2% had the most incidence. A survey of occupations showed that housewives (19.6%) had the highest rate in terms of variety among other occupations.

Conclusion: The results of this study confirmed the increasing incidence of this disease in the East Azerbaijan region. Because the disease in the region is endemic, public awareness and knowledge about the disease need to be increased in order to reduce related health problems and increase the level of prevention at the community level.

Keywords: Hydatid cyst, epidemiology, East Azerbaijan, *Echinococcus granulosus.*

1. INTRODUCTION

Cystic echinococcosis is a common parasitic disease which infects both human and animals and has global distribution. Echinococcosis is a recurrent disease and a serious public health challenge. This parasitic disease is present in most tropical and temperate regions around the world, and is particularly important in areas such as North Africa, South America, China, and the Middle East. (1). Cystic echinococcosis which is a larval stage, is mainly caused by accidental eating of the eggs spread by the stools of *Echinococcus granulosus* infected dogs. The disease can be widely spread in the regions where sheep breeding is considered as a major industry (2). This disease, which has several hosts (the final and intermediate hosts), is one of the most important infectious diseases affecting public health worldwide, especially in Iran (3). According to the World Health Organization, Iran is among the countries with moderate infection (4).

Most infections occur among ruminants and livestock breeders who directly contact the infected dogs (3). Cows do not play an important role in the transmission of the disease, because of the interaction between host and parasite in most cases, the cattle have the ability to calcify the parasite and do not play an important intermediate host role (1,2). Various studies have shown that the common strain in the Middle East and Arabic North Africa is the sheep strain (G1, G3), which causes disease in humans (1). The rate of infection in Iran was high among ruminants, and this rate was reported to be 11.1% among sheep in East Azerbaijan (4).

Studies in Turkey indicated that Cystic echinococcosis occurs throughout Turkey, the number of humans affected by the disease during the years 1987-1994 was 2663. The incidence of infection in the dogs found in Turkey was between 32%-40%. The rate and incidence of Cystic echinococcosis in domestic livestock in Turkey was 18.2% to 50.7% (5).

Cystic echinococcosis has no specific clinical symptoms. Therefore, it usually remains unknown to animals until slaughter, and it is randomly detected in humans. In

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order to diagnose the disease, it is advised to use several diagnostic methods, which include the use of radiography, ultrasonography, tomography, and immunological techniques (4,6).

Seroepidemiological study of the disease in different regions of Iran has shown different results (7,8). The rate of infection among people in Shemiranat region was 0.22% (9), while the rate of infection in Yasuj was reported to be 2%-18% (10). Human infection has been reported in Isfahan, Fars, Khorasan and Arak provinces more than other parts of Iran (3). In a study, conducted in Tabriz between 2011 and 2012, 206 cases of hydatid cysts were reported among people, indicating an increase in human cases in Tabriz (11).

In this study, we aimed to investigate the incidence of Cystic echinococcosis in the East Azerbaijan, Iran, during 2011-2017.

2. METHODS

This was a cross-sectional descriptive epidemiological study. For this study, all reports of the patients with hydatid cyst were collected from the central office of medical documents in the East Azerbaijan, over a period of 7 years (from winter 2011 to autumn 2017). After access to the patient records, information on the variables (age, gender, place of residence, occupation, involved organ, relapse, etc.) were collected and arranged. The sensitivity and specificity of each statistical method employed for evaluation of the results were investigated separately. Data were analyzed by SPSS software version 16.0, based on chi-square test followed by Fischer Advanced Test.

Figure 1. *Distribution of referral hospitals in cities of East Azerbaijan province*

Ethical Standards

An ethics committee approval was not provided in this study because the regulation on clinical research entered into force on 22 June 2016 (Official announcement NO:703/d/1152 of Deputy of Research and Technology – Ministry of Health and Medical Education) in Iran, and ethics committees within the scope of this regulation began to be formed thereafter. In this study archive data used for all patients.

3. RESULTS

In total, 857 female patients (56.2%) and 668 (43.8%) male patients with hydatid cysts with clinical findings and definite cases during a seven-year period were collected from referral hospitals in the central office of medical documents, East Azerbaijan (Table 1). Of 1525 patients studied, 918 cases (60.2%) had the liver form, 587 (38.5%) had the pulmonary form, and 20 cases (1.3%) had the brain form of the disease. In the liver form of the disease, females were more involved than males (561 females and 357 males). In the lung form of the disease, in contrast to the liver form, the incidence of disease in males was higher (301 males and 286 females). In the brain form, the number of cases in both sexes was equal (10 females and 10 males) (Table 2). According to the chi-square results, there was a significant difference in gender between the patients. In other words, more women than men had exposed to the disease (Tables 1).

Table 1. *Number and percentage of patients by gender (n=1525)*

	Frequency	%
Female	857	56.2 [a]
Male	668	43.8

P=.000; a. 0 cells (.0%) have expected frequencies less than 5.

Table 2. *Number and percentage of affected organs by gender*

	Female, n (%)	Male, n (%)	Total, n (%)
Brain	10 (%0/56)	10 (%0/56)	20 (1.3)
Liver	561(%36/7)	357(%23/5)	918 (60.2)
Lung	286(%18/7)	301(%19/8)	587 (38.5)
Total			1525

P=.000

The patients were in the age range of 0 to 65, and the patients were mostly in the age range of 18-35 (33.5%) (Figure 2). In addition, a significant correlation was observed between the involved organs and the gender. The results showed that the highest and the lowest incidence was related to 2015 (17.0%) and 2011 (11.4%) (Figure 3).

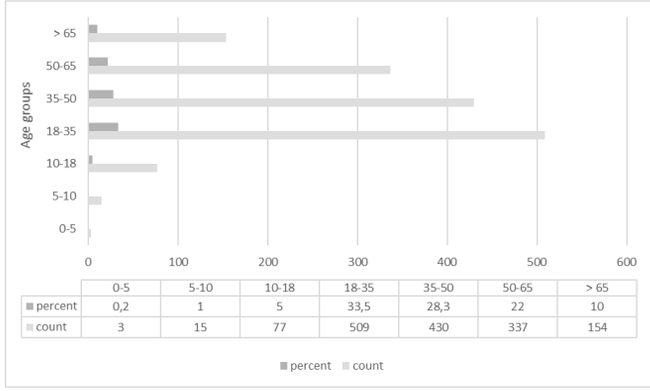

Figure 2. *Percentage and count of age groups in patients*

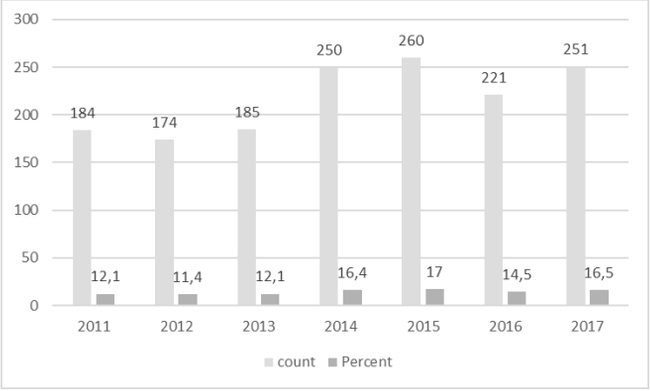

Figure 3. *Percentage and count of patients in different years*

The studied population were either urban or rural, with 62.2% of the population in the rural area and 37.8% in the urban area. The comparison between urban and rural population showed a significant difference, the number of rural patients referred to referral hospitals in this study was significantly more than the number of urban patients referred to these centers (Figure 4).

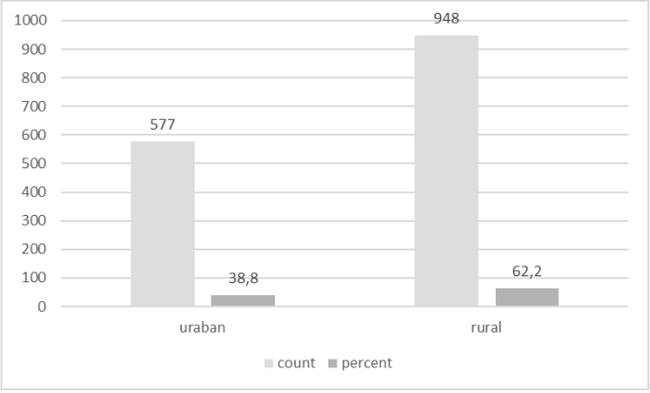

Figure 4. *Percentage of patients living in urban and rural areas*

Gender differences in the patients with brain disease and the patients with pulmonary lesions were not significant. However, there was a significant gender difference in the patients with hepatic impairment (Table 2. People with this disease had different scattering in occupations. But housewives, farmers and workers had a larger population than other occupations. (other occupations included more than 800 different jobs). Of all cases examined, 299 cases (19.6%) were housewives, 100 (6.5%) were farmers, 67 (4.4%) were workers, and 1059 had (69.5%) other businesses (Table 3).

The average referral of each patient to the hospital was 1.43. The number of the days when people were hospitalized varied from 3 to 95, with an estimated average number of 6.97 days per patient, based on the number of the patients (Table 4).

Only 8 cases (0.52%) of deaths were reported (4 cases of liver lesions, 4 cases of brain lesions). Recurrence of illness was seen in 63 cases, 4.13% of the patients (Table 5). 88 patients with liver form of disease, 114 patients with pulmonary form of disease, and 5 patients with brain form of disease were admitted to the intensive care unit in this study.

Table 3. *Occupations of patients (n=1525)*

Occupation	n	%
Housewife	299	19.6
Farmer	100	6.5
Worker	67	4.4
Others	1059	69.5

P=.000

Table 4. *Descriptive statistics (n=1525)*

	Min	Max	Sum	Mean
Admission times	1	7	2177	1.43
Hospitalized days	2	95	10623	6.97

P=.000

Table 5. *Descriptive statistics of mortality and recurrence of illness (n=1525)*

	n	%
Mortality	8	0.52
Recurrence of illness	63	4.13

P=.000

4. DISCUSSION

Hydatid cyst is one of the most common disease of humans and animals in Iran (3). Recently, the World Health Organization (WHO) has placed *Echinococcus granulosus* in the subgroup of neglected tropical diseases (NTDs) (12). Public health related, social and economic issues of this disease are of critical importance. Iran, especially rural areas, has been recognized as an endemic region for the disease, with high levels of infection. According to the WHO, Iran is among the countries with moderate rate of infected population. Echinococcosis is a recurrent disease and a serious public health challenge. *Echinococcus granulosus* has a high prevalence due to its large intermediate hosts in Iran and echinococcosis is one of the most important zoonotic parasitic diseases, the diagnosis of which has always been of great importance (3,13).

In regard to the results of the infective organs, several studies report that the liver in the first rank of involved organ, lung is in the second and brain in the third rank but there are reports about infection in different organs such as, spleen, bones, peritoneum and kidney. These reports support that *Echinococcus* larva has a primary tendency to infect the liver and secondarily to the lung. According to the results of the present study, 60.2% of the patients had liver, 38.5% had lung and only 1.3 % had brain involvement respectively. These localization rates of the disease in our study region has in conformity with other studies (6,9,10,11).

In correlation of the gender and involved organs, results showed that the most of the liver cases are in females and males have a high risk for lung form of the disease. The rate

of infection with hydatid cyst in each gender group depends on the local conditions within the area such as contact with vegetables contaminated with infected dog faces. It was reported in previous studies that the infection is higher in women as well (7,13). A 10 year-study conducted in Tehran reports that females (56.8%) had higher infection than males (43.2%) (14). According to the results of the present study females (56.2%) showed higher rate of infection than males (43.8%). These consequences showed that women have the highest chance of contact with sources of infection such as infected dogs, soil, vegetable etc. Furthermore, genetic differences between two genders may be responsible for a part of this difference (3,15).

Hydatid disease is generally considered to be a rural disease. Iranian women, especially in rural areas, have more contact with domestic animals and infected products. They are also in more contact with unwashed raw vegetables, which may be more contaminated with *Echinococcus* eggs. The results of present study showed that housewives were more prone to the disease, followed by workers and farmers, suggesting the major role of contaminated vegetables in spreading and increasing the disease. Results that were found in the other occupations showed that all people in the community were at risk of disease but because of variety of jobs (more than 800 different occupations) the results has not been made for all occupations that have been affected by the disease.

Epidemiologic studies conducted across Iran shows that the disease has an increasing trend (16).

According to the statistics, hydatid disease has been reported from 23 provinces of the country in several years (2002-2018) (7). Rokni integrated several reports of hydatidosis from 1981 to 2006 and reports showed that the highest risk in terms of age belong to 20-40 (3). A study in Sindh and Hyderabad, Pakistan that examined cystic echinococcosis in human, of the 43,656 cases inspected for 11 years, only 44 cases of hydatid cysts were recorded and age group of 21-40 years old, including 22 cases, had the highest rate of disease (17). Based on present study, most patients were in the age range of 18-35, which agreed with other studies in Iran. The prevalence of this disease in this age group could be due to the infections of children at an early age, which, given their limited and slow growth, are eventually marketed at this age (3).

Gholami in 2018 conducted a meta analyse beetwen 1995-2015 among 40 different study (totally 3090 cases). Results showed that spread of the disease in rural regions is more abundant than in urban areas(18). But in the other study in northern of Iran between 2005-2015 showed that the infection rates in urban population were higher than the rural people (19). High rate of infection in rural areas (62.2%) in present study can be related to the quality of life and social structure of rural areas, infected dog population in the region and culture of vegetables consumption; as a result, each region has different risk factors. The presence of infection in urban areas can also be an indicative of social health problem; therefore, preventive protocols in urban areas should be followed. Pet keeping, the presence of stray dogs in cities, and the lack of compliance between personal and public health can increase the risk of spread of the disease. In addition to the role of stray dogs in the spread of disease and their proximity to human societies, high survival of *Echinococcus* eggs in the environment (2.5 years), as well as high potential for the infection with parasite eggs are other factors involved in the spread of disease (20). The lack of a trend to reduce the incidence of disease over the years, has caused a lack in any preventive and controlling program in the region; therefore, effective preventive protocols should be followed. Patients usually notice their disorder when there is no choice except for surgical treatment. Hence, it seems that in addition to preventive protocols, screening tests must be designed in high-risk areas.

5. CONCLUSION

The hydatid cyst disease is endemic in our study region. One of the key ways to tackle this problem is to increase the level of awareness to prevent the disease. Control of the disease in different animals can play role as source of infection in human. The production of healthy vegetables can also be achieved through the control of disease in human. Considering all the above mentioned, the necessity of fundamental and applied studies simultaneously in endemic regions necessitates conducting this research.

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Clinical and Experimental Health Sciences

The Awareness and Knowledge of Dentists of Medication-Related Osteonecrosis of the Jaw

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ABSTRACT

Objective: Medication-related osteonecrosis of the jaw (MRONJ) is a serious condition affecting the quality of life of patients taking antiresorptive and anti-angiogenic drugs. The main purpose of this study was to evaluate dentists' awareness of MRONJ.

Methods: A questionnaire was administered to dentists. The questionnaire contained 20 questions on the demographic data of the participants and their awareness of MRONJ and the complications of antiresorptive or anti-angiogenic drugs. The responses were analyzed using Pearson's chi-square test and Fisher's exact test.

Results: In total, 141 dentists participated in the survey. Of these, 42.6% did not know about MRONJ. There was a statistically significant difference between specialist dentists and general dentists on the questions about the complications, effects, and usage of antiresorptive and anti-angiogenic drugs on necrosis formation ($p<0.05$). Dentists with less experience (seven years'<) were more knowledgeable about MRONJ (86.7%); compared to those with more than seven years' clinical experience.

Conclusions: This study showed that the knowledge level of dentists regarding the side effects of antiresorptive and anti-angiogenic medications is weak. Overall, MRONJ awareness among general dentists was poor.

Keywords: Antiresorptive, anti-angiogenic, medications, medication-related osteonecrosis of the jaw

1. INTRODUCTION

Bisphosphonates are antiresorptive agents and are widely used for the treatment of various pathologies, such as osteoporosis, Paget's disease, or hypercalcemia associated with some malignant tumors (as breast, prostate, and lung cancers, multiple myeloma) (1-3). Bisphosphonates and other antiresorptive drugs suppress osteoclastic bone resorption. However, these drugs may cause undesirable adverse effects, such as osteonecrosis (2).

Osteonecrosis of the jaw is a problem in patients taking bisphosphonates (3). Initially, avascular necrosis of the jaw was termed bisphosphonate-related osteonecrosis of the jaw (BRONJ). Recently, except for bisphosphonate drugs, other antiresorptive (denosumab) and anti-angiogenic drugs (bevacizumab and sunitinib) have been observed to cause the development of osteonecrosis in the jaw. The American Association of Oral and Maxillofacial Surgery (AAOMS) has suggested the use of the term medication-related osteonecrosis of the jaw (MRONJ) due to an increase in the number of osteonecrosis cases related to other antiresorptive and antiangiogenic drugs (4). The use of intravenous bisphosphonates represents an important part of osteonecrosis cases. The diagnosis of MRONJ in the current guidelines is defined as the presence of exposed bone in individuals with a history of treatment with antiresorptive or anti-angiogenic agents that occurs clinically with an intraoral or extraoral fistula lasting longer than eight weeks in the maxillofacial area, independent of radiotherapy or metastatic diseases (5-7).

MRONJ affects quality of life and causes serious morbidity. Patients with MRONJ may experience swelling, mucosal ulceration, pain, paresthesia, extra – and intra-oral fistula, suppuration, and deformity of the jaw. Although the pathophysiology of MRONJ has not yet been fully elucidated, several mechanisms are suggested: inhibition of angiogenesis, impaired remodeling, infection, trauma, suppression of immunity, and local toxicity (4,8-10). Dentoalveolar surgery, periodontal diseases, and dental prostheses are the most important risk factors for MRONJ (4,11). In addition, the duration of treatment and usage method (intravascular or oral) of antiresorptive and anti-angiogenic drugs are among the risk factors related to MRONJ (8, 10,12). Vescovi et al. found that 63.8% of 567 patients with BRONJ were related

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to preceding dentoalveolar procedures (13). Marx et al. reported that periodontitis was in 84% and dental caries was in 29% of patients with BRONJ (14).

It is important for dentists, clinicians, and patients to be aware of the potential adverse effects of antiresorptive and anti-angiogenic drugs (11). A few studies have evaluated the levels of knowledge and awareness of dentists regarding BRONJ (11, 15-17). The purpose of this study was to evaluate the awareness and knowledge of dentists regarding MRONJ.

2. METHODS

The cross-sectional study was approved by the Gazi University Ethics Committee (No: 2018-62) and was carried out by the Declaration of Helsinki. A questionnaire was administered to dentists working in oral and dental health centres. In this study, the principles of the AAOMS regarding MRONJ were followed (1,4). Validated questionnaires from previous studies were prepared and given to participants (11,16,18). Detailed information about the study was given to participants, and their consent was obtained. Participation in the survey was voluntary. The questionnaire was administered by two researchers (GA: an oral and maxillofacial radiologist with experience 6 years, AE: a maxillofacial surgeon with experience 6 years). Also, approval and permission for the study were obtained from the relevant institutions.

2.1.Survey

The survey contained 20 questions and two parts. In the first part, demographic information including age, gender, years of professional experience, and field of specialty was recorded. In the second part, we asked questions about experiences treating patients using antiresorptive and anti-angiogenic drugs, risk factors, indications of drugs, diagnostic criteria, awareness of MRONJ.

2.2.Statistical analysis

All statistical analyses were performed with IBM SPSS Statistics 23·0 (Armonk, New York). The number and percentage of dentists according to demographic factors (gender, age, years of professional experience, and field of specialty) were calculated and the descriptive statistics were reported. Pearson's chi-square test was used to determine if there is a significant relationship between the answers to questions and the properties of dentists. Fisher's exact test as an alternative to Pearson's chi-square test is used to examine the relation of two different binary properties. The level of significance was set at 0.05.

3. RESULTS

A total of 196 dentists was asked to participate in the survey. Twenty-three dentists have refused to participate, and thirty-two dentists were excluded from the study due to incomplete questions. Among participants, 90 (63.9%) were females, 51 (36.1%) were males. The distribution of the demographic characteristics of the participants is shown in Table 1. Hundred and five dentists were general dentists, thirty-six dentists were specialists.

Table 1. *Demographic features of dentists*

	Male	Female	Total
Age			
24-34 age	9(17.3%)	43(82.7%)	52 (36.8%)
35-44 age	31(49.2%)	32(50.8%)	63(44.7%)
45+ age	11(42.3%)	15(57.7%)	26(18.5%)
Total	**51 (36.1%)**	**90 (63.9%)**	**141 (100%)**
Clinical experience			
1-3 years	0(0.0%)	3(100.0%)	3(2.2%)
4-7 years	5(18.6%)	22(81.4%)	27(19.4%)
8-11 years	4(18.1%)	18(81.9%)	22(15.8%)
12-16 years	15(48.4%)	16(51.6%)	31(22.4%)
17-20 years	12(44.4%)	15(55.6%)	27(19.4%)
20 + years	13(44.8%)	16(55.2%)	29(20.8%)
Specialty education			
Yes	6(16.6%)	30(83.4%)	36(25.5%)
No	45(42.8%)	60(57.2%)	105(74.5%)
Field of specialty			
Oral and Max. Radiology	2(40.0%)	3(60.0%)	5(13.9%)
Oral and Max. Surgery	0(0.0%)	6(100.0%)	6(16.7%)
Restorative Dentistry	0(0.0%)	5(100.0%)	5(13.9%)
Endodontics	0(0.0%)	2(100.0%)	2(5.5%)
Orthodontics	1(100.0%)	0(0.0%)	1(2.8%)
Pediatric Dentistry	0(0.0%)	10(100.0%)	10(27.8%)
Periodontology	1(50.0%)	1(50.0%)	2(5.5%)
Prosthodontics	2(40.0%)	3(60.0%)	5(13.9%)
Total	**6 (16.7%)**	**30 (83.3%)**	**36 (100.0%)**

The sources of information about MRONJ were summarized in Table 2. This knowledge about MRONJ had been acquired primarily from a university education (n:27, 33.3%), postgraduate training (n:17, 20.1%), or journal/literature (n:24, 29.7%). Alendronate (n:45, 31.9%) and Zoledronate (n:45, 31.9%) were the most frequently known drugs among dentists. However, fifty-six (39.7%) dentists did not know of any of these drugs. Specialists were more knowledgeable about antiresorptive and anti-angiogenic drugs, compared to general dentists ($p<0.05$) (Table 3).

Sixty (42.6%) dentists did not knowledge about MRONJ. Most of the specialists knew MRONJ (91.7 %). Only 42% of participants reported taking a medication history from patients before dental treatment. Similarly, specialist dentists (88.9%) were more aware of complications of the antiresorptive and anti-angiogenic drugs than general dentists. According to the results of the analysis summarized

in Table 4, statistically, significant differences were found between specialists and general dentists in the questions about complications of drugs and the effect of usage method of drugs on necrosis formation (p<0.05). There were also statistically significant differences between specialists and general dentists in the questions about the diseases using antiresorptive and anti-angiogenic drugs (p <0.05). The most stated diseases were osteoporosis (27.6%) and bone metastases (30.4%). A total of 42.9% of general dentists did no comment about the diseases using antiresorptive and anti-angiogenic drugs. When comparing responses about treatment options in patients using these drugs, there were no statistically significant differences between specialists and general dentists (p>0.05). Bone sequestrate (n:58, 41.1%) and expose bone areas (n:65, 46.1%) were the most known symptoms in the MRONJ diagnosis, whereas thickening of lamina dura was the rare answer (n:10, 7.1%). Statistically significant differences were detected (p <0.05) for all of the findings, except halitosis and enlargement in periodontal space. The findings showed that specialist dentists were more aware, compared to general dentists (Table 4).

The clinical experiences influenced the answers to some of the questions such as knowledge about MRONJ and complications of drugs (Table 5) (p<0.05). Less experienced dentists had more knowledgeable about MRONJ (86.7%). Moreover, less experienced dentists (66.7%) had more knowledgeable about the effect of drugs on necrosis formation, compared to medium experienced and the most experienced dentists. Also, there were statistically significant differences among three experience levels on the questions about clinical and radiological findings of MRONJ (for changes in the structure of bone and expose bone regions) (p<0.05). Expose bone regions (46.1%) were the most known symptoms or signs for MRONJ among participants.

Table 2. *Information sources of dentists about MRONJ*

	Specialist dentists	General dentists	Total
University education	10(30.3%)	17(35.4%)	27(33.3%)
PhD/postgraduate training	17(51.5%)	0(0.0%)	17(20.1%)
Journals and literature	10(30.3%)	14(29.1%)	24(29.6%)
Congress/Seminar	4(12.1%)	2(4.1%)	6(7.4%)
Internet/Television	5(15.1%)	11(22.9%)	16(19.7%)
Patient anamnesis	3(9.1%)	0(0.0%)	3(3.7%)
Drug experiences	0(0.0%)	3(6.2%)	3(3.7%)

Table 3. *Table showing the knowledge about antiresorptive and anti-angiogenic drugs of specialist dentists and general dentists*

Drugs	Specialist dentists	General dentists	Total	P-value
Alendronate (Fosamax®)	20(55.6%)	25(23.8)	45(31.9%)	0.001*
Risedronate (Actonel®)	8(22.2%)	4(3.8%)	12(8.5%)	0.002*
Ibandronate (Boniva®)	4(11.1%)	1(1.0%)	5(3.5%)	0.015*
Pamidronate (Aredia®)	5(13.9%)	3(2.9%)	8(5.6%)	0.026*
Denosumab (Xgeva,Prolia®)	9(25.0%)	17(16.2%)	26(18.4%)	0.318
Zoledronate (Zometa, Reclast®)	21(58.3%)	24(22.9%)	45(31.9%)	0.000*
Sunitinib (Sutent®)	7(19.4%)	2(1.9%)	9(6.3%)	0.001*
Sorafenib (Nexavar®)	3(8.3%)	0(0.0%)	3(2.1%)	0.016*
Bevacizumab (Avastin®)	8(22.2%)	2(1.9%)	10(7.1%)	0.000*
Sirolimus (Rapamune®)	-	-	-	-
None	4(11.1%)	52(49.5%)	56(39.7%)	0.000*

** significant at 0.05*

Table 4. *Knowledge of specialist and general dentists regarding complications, risk factors, and the treatment options of patients taking antiresorptive and anti-angiogenic medication*

Variables	Specialist dentists	General dentists	Total	P-value
Knowledge about MRONJ? (Yes)	33(91.7%)	48 (45.7%)	81(57.4%)	0.000*
Do you take a medication history from patients before an invasive dental procedure? (Yes)	21(58.3%)	39(37.1%)	60(42.6%)	0.032*
Do you know the complications of these drugs? (Yes)	32(88.9%)	43(41.0%)	75(53.1%)	0.000*
Are there any effect duration and usage method (oral, intravenous, intramuscular) of drugs on necrosis formation? (Yes)	29(80.6%)	35(33.3%)	64(45.3%)	0.000*
Diseases using antiresorptive and anti-angiogenic drugs (Most)?				
Prostate CA	9(25.0%)	11(10.5%)	20(14.1%)	0.050*
Breast CA	17(47.2%)	13(12.4%)	30(21.2%)	0.000*
Multiple Myeloma	9(25.0%)	10(9.5%)	19(13.4%)	0.026*
Osteoporosis	16(44.4%)	23(21.9%)	39(27.6%)	0.016*
Bone Metastases	17(47.2%)	26(24.7%)	43(30.4%)	0.011*
Paget's Disease	8(22.2%)	12(11.4%)	20(14.1%)	0.163
Malign Hypercalcemia	10(27.8%)	7(6.7%)	17(12.1%)	0.002*
Lung CA	9(25.2%)	5(4.8%)	14(9.9%)	0.001*
None	8(22.2%)	45(42.9%)	53(37.5%)	0.030*
The treatment options in patients using these drugs?				
I make no treatment.	11(30.6%)	40(38.1%)	51(36.1%)	0.547
I perform all dental treatments without surgery.	4(11.1%)	13(12.4%)	17(12.1%)	1.000
I make simple surgical treatments (such as tooth extraction).	4(11.1%)	5(4.8%)	9(6.3%)	0.233
I perform all surgical treatments, including the implant.	-	-	-	-
I make all dental treatments after consulting the medical physician.	22(61.1%)	49(46.7%)	71(50.3%)	0.176
What are the clinical and radiological findings of the MRONJ?				
Halitosis	5(13.9%)	8(7.6%)	13(9.2%)	0.317
Ulceration of mucosa	12(33.3%)	14(13.3%)	26(18.4%)	0.010*
Drainage (inflammation)	13(36.1%)	13(12.4%)	26(18.4%)	0.003*
Mobility and sensitive teeth	13(36.1%)	13(12.4%)	26(18.4%)	0.003*
Expose bone regions	26(72.2%)	39(37.1%)	65(46.1%)	0.000*
Thickening of lamina dura	7(19.4%)	3(2.9%)	10(7.1%)	0.003*
Enlargement in periodontal space	4(11.1%)	7(6.7%)	11(7.8%)	0.472
Changes in the structure of bone trabecula	17(47.2%)	26(24.8%)	43(30.4%)	0.020*
Sequestration areas	21(58.3%)	37(35.6%)	58(41.1%)	0.020*

** significant at 0.05; MRONJ: Medication-related osteonecrosis of the jaw*

Table 5. *Correlation between clinical experience and responses*

Variables	Clinical experience			Total	P-value
	Less exp. (1-7 years)	Medium exp. (8-16 years)	Experienced (17 + years)		
Knowledge about MRONJ? (Yes)	26(86.7%)	26(49.1%)	28(50.0%)	81(57.4%)	0.001*
Do you take a medication history from patients before an invasive dental procedure? (Yes)	17(56.7%)	24(45.3%)	19(33.9%)	60(42.6%)	0.118
Do you know the complications of these drugs? (Yes)	24(80.0%)	23(43.4%)	28(48.2%)	75(53.1%)	0.004*
Are there any effect duration and usage method (oral, intravenous, intramuscular) of drugs on necrosis formation? (Yes)	20(66.7%)	17(32.1%)	27(46.4%)	64(45.3%)	0.010*
Diseases using antiresorptive and anti-angiogenic drugs (Most)					
Prostate CA	6(20.0%)	6(11.3%)	8(14.3%)	20(14.1%)	0.555
Breast CA	7(23.3%)	11(20.8%)	12(21.4%)	30(21.5%)	0.962
Multiple Myeloma	7(23.3%)	3(5.7%)	9(16.1%)	19(13.6%)	0.063
Osteoporosis	10(33.3%)	12(22.6%)	17(30.4%)	39(28.1%)	0.514
Bone Metastases	14(46.7%)	13(22.6%)	16(28.6%)	43(30.4%)	0.068
Paget's Disease	7(23.3%)	6(11.3%)	7(10.7%)	20(13.6%)	0.219
Malign Hypercalcemia	6(20.0%)	4(7.5%)	7(12.5%)	17(12.2%)	0.250
Lung CA	3(10.0%)	3(5.7%)	8(14.3%)	14(10.1%)	0.327
None	9(30.0%)	26(47.2%)	18(32.1%)	53(37.5%)	0.172
The treatment options in patients using these drugs?					
I make no treatment.	10(33.3%)	20(35.8%)	21(37.5%)	51(36.1%)	0.929
I perform all dental treatments without surgery.	2(6.7%)	5(9.4%)	10(17.9%)	17(12.2%)	0.234
I make simple surgical treatments (such as tooth extraction).	2(6.7%)	3(5.7%)	4(7.1%)	9(6.4%)	0.951
I perform all surgical treatments, including the implant.	-	-	-	-	-
I make all dental treatments after consulting the medical physician.	16(53.3%)	31(56.6%)	24(42.9%)	71(50.3%)	0.334
What are the clinical and radiological findings of the MRONJ?					
Halitosis	5(16.7%)	6(11.3%)	2(3.6%)	13(9.2%)	0.114
Ulceration of mucosa	6(20.0%)	10(17.0%)	10(17.9%)	26(18.4%)	0.942
Drainage (inflammation)	8(30.8%)	8(30.8%)	10(38.5%)	26(18.4%)	0.421
Mobility and sensitive teeth	6(20.0%)	10(18.9%)	10(17.9%)	26(18.4%)	0.970
Expose bone regions	20(66.7%)	21(39.6%)	24(42.9%)	65(46.1%)	0.045*
Thickening of lamina dura	3(10.0%)	3(5.7%)	4(7.1%)	10(7.1%)	0.763
Enlargement in periodontal space	3(10.0%)	5(7.5%)	3(5.4%)	11(7.8%)	0.724
Changes in the structure of bone trabecula	15(50.0%)	13(24.5%)	15(26.8%)	43(30.4%)	0.037*
Sequestration areas	16(21.7%)	22(42.3%)	20(35.7%)	58(41.1%)	0.288

** significant at 0.05; MRONJ: Medication-related osteonecrosis of the jaw*

4. DISCUSSION

Osteonecrosis of the jaw in patients using antiresorptive (bisphosphonate, denosumab) and anti-angiogenic drugs (Sunitinib, Sorafenib, Bevacizumab, Sirolimus) may develop after simple surgical procedures such as tooth extraction (4,18). In recent years, the incidence of this complication has increased due to the frequent prescription of these drugs by oncologists and physicians (4). To the best of our knowledge, there are a few limited numbers of studies the assess the knowledge, opinions, and awareness of dentists about MRONJ (7,18). In the study in the United Kingdom, 90% of dentists were uninformed of the effect of antiresorptive and antiangiogenic drugs excluding bisphosphonates on MRONJ (7). Escobedo et al. evaluated the capabilities in the decision of clinical problems in patients using different medications

among dentists. The question regarding implant surgery in patients using oral amino-bisphosphonates had the lowest percentage of correct responses among participants (18). De Lima et al. reported that 72.1% of dentists and 75% of dental students did not know the bisphosphonates (11). In Mexica, Vinitzky-Brener et al. revealed that 99.7% of dentists were not found enough knowledge about the prevention, diagnosis, and treatment of BRONJ (16). In this study, the awareness, opinion, and knowledge of dentists regarding MRONJ were evaluated. The results showed that only 57.4% of dentists were knowledgeable about MRONJ.

Distinct committees have improved various consensus documents to guide the clinical management of patients treated with antiresorptive medication. In the literature, there is consensus that the education of dentists on the prevention and management of MRONJ should be increased (4,19,20). In 2014, the AAOMS published a position paper that highlighted the low risk of developing MRONJ in patients taking oral bisphosphonate therapy for less than four years and confirmed that these patients may be treated successfully in primary care (4). Patients taking intravenous bisphosphonate or denosumab have an increased risk of developing MRONJ and surgical procedures should be avoided in these patients (4). Previous studies have reported that dentists must be aware of the type, dosage, and usage duration of bisphosphonates to minimize the risk in patients taking them (8,15,21). In the study of Tanna et al., only 2% of general dental practitioners were aware that denosumab could cause osteonecrosis of the jaw other than bisphosphonates (7). Escobedo et al. (18) reported that Alendronate was the most known drug among dentists (77.2%), while Zoledronic acid was the most recognized drug among dental students (97.3%). In a study by De Lima et al., commercial brand names of bisphosphonates were only recognized by around 15% of dentists and dental students (11). In the present study, Alendronate and Zoledronate were the most known medications among dentists (31.9%), but 39.7% of dentists did not recognize anyone of antiresorptive and anti-angiogenic drugs.

Antiresorptive drugs are used to control cancer-related situations, including hypercalcemia of malignancy and bone metastases of solid tumors such as breast, prostate, and lung cancers. Anti-angiogenic agents have displayed effectiveness in the treatment of renal cell carcinomas, gastrointestinal tumors, and neuroendocrine tumors (4,7,22). Lopez – Jornet et al. reported that 51.6% of dentists have the correct information for bisphosphonate indications (17). The study results of De Lima et al. showed that 65.4% of dentists did not know the medical indication of bisphosphonates (11). In the present study, osteoporosis (27.6%) and bone metastases (30.4%) were the most known diseases on the question regarding the medical indications of bisphosphonates and anti-angiogenic drugs. 37.6% of dentists did not know any of the diseases regarding the indication of antiresorptive and anti-angiogenic medication.

Patients taking antiresorptive and anti-angiogenic medications should be informed about oral hygiene to prevent osteonecrosis (23). Dentists should have sufficient knowledge of these novel medications and MRONJ. Routine dental care containing prophylaxis, non-operative periodontal treatments, restorative applications, and fixed and removable prosthodontics is not contraindicated in these patients. Because advanced oral surgery, apical surgery, and dental implants can create a risk in this group of patients, dentists should exercise caution against the possibility of necrosis (21,23). Yoo et al. reported that only 33.3% of Korean dentists taken the medication anamnesis of patients before oral surgery or treatment (15). The results of our study showed that 57.4% of the dentists reported taking medication anamneses from patients (antiresorptive and anti-angiogenic drug history) before dental treatment.

In our study, we asked participants regarding the treatment protocol of patients using antiresorptive or anti-angiogenic drugs and 31.6% of the dentists reported that they would not perform any dental treatment. In the study of Lopez – Jornet et al. (17), 73.3% of dentists correctly marked the question about treatment procedures, including surgical procedures, which can be performed in patients using oral bisphosphonates for less than three years. The early findings of MRONJ can be difficult to distinguish clinically and radiographically, this complicates the treatment process of the disease (21). In the current study, bone sequestrate (n:58, 41.1%) and expose bone areas (n:65, 46.1%) were the most known symptoms regarding MRONJ diagnosis.

In previous studies, while dentists with more professional experience (> 5 years) had greater clinical experience in the treatment of patients with osteonecrosis, dentists with more professional experience had a lower knowledge about possible complications (11,15). Escoba et al. showed that dentists with professional experience (20 years>) obtained significantly lower results in the interpretation of clinical cases (18). In Brazil, De Lima et al. stated that 59.6% of dentists and 58% of dental students did not know the oral adverse effect of bisphosphonates in BRONJ (11). In our study, statistically significant differences were observed between clinically experienced dentists and less experienced dentists about MRONJ. Less experienced dentists (< 7 years) knew more about MRONJ. We investigated the correlation between postgraduation training and knowledge levels about MRONJ. There were statistically significant differences between specialist and general dentists regarding MRONJ awareness, drug complications, and drug effects on necrosis formation. The results of our study showed that dentists with postgraduate training had more information about MRONJ than general dentists. Education about the risks and adverse effects of antiresorptive and anti-angiogenic medication usage needs to be improved in many areas. Knowledge of the risk of MRONJ for dentists and patients should be increased through explanatory brochures, guideline booklets, and, of course, communication between doctors and patients (23).

5. CONCLUSION

The results of this study showed that the knowledge level of dentists on the side effects of antiresorptive and anti-angiogenic medications is weak. However, specialist dentists had more information about MRONJ, compared to general dentists. Overall, MRONJ awareness among general dentists was poor.

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Clinical and Experimental Health Sciences

The Bond Strength of Universal Adhesives with Different Acidities to Calcium Silicate-Based Materials

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ABSTRACT

Objective: To compare the micro-shear bond strengths of 3 different universal adhesives to 2 different calcium silicate-based materials.

Methods: A hole was prepared in the center of the top surfaces of 60 cylindrical acrylic blocks, and the blocks were randomly divided into 2 groups (n=30) according to biomaterial filling; NeoMTA Plus (Avalon Biomed Inc. Bradenton, FL, USA) and ProRoot MTA (Dentsply Tulsa, Tulsa, OK, USA). Biomaterials were prepared according to the manufacturers' instructions. The sample surfaces were flattened after the initial setting, and the samples were incubated for 24h at 37°C. After placing the biomaterials in the prepared holes, the specimens were randomly divided into 3 subgroups (n=10) according to adhesive; G-Premio Bond (GC Corp., Tokyo, Japan), All-Bond Universal (Bisco, Inc., Schaumburg, IL, USA) and Single Bond Universal (3M ESPE, St Paul, MN, USA). Adhesives were applied to the specimens and polymerized. A micro-hybrid composite resin (Filtek Z250, 3M ESPE, MN, USA) was then placed on the specimen surfaces and polymerized. Micro-shear bond strengths were tested using a universal testing device (LRX, Lloyd Instruments, Farnham, UK). A Shapiro-Wilk test confirmed normal distribution, and 2-way ANOVA was used for statistical analysis.

Results: No significant differences were found in the shear bond strengths of any of the tested adhesives to either of the calcium silicate-based materials ($p>0.05$).

Conclusion: The results indicate that the acidity of a universal adhesive does not affect the bond strength of composite resin to calcium silicate-based materials.

Keywords: Calcium Silicate, NeoMTA, ProRoot MTA, Shear Strength, Universal Adhesive

1. INTRODUCTION

Vital pulp treatment (VPT) aims to maintain the vitality and health of dental pulp in cases where pulp exposure has occurred due to caries or traumatic injury (1). Several procedures have been developed to protect pulp vitality in permanent teeth. Indirect pulp capping involves the controlled excavation of deep caries to prevent pulpal exposure followed by the application of a biomaterial as a protective layer to maintain pulp vitality. With direct pulp capping, a protective layer of biomaterial is applied directly on exposed pulp tissue, and with pulpotomy, the same procedure is performed after partial amputation of the pulp (2). The biomaterial used in VPT must not only be biocompatible and capable of maintaining pulp vitality, it also needs to adhere to both dentin and restorative material and resist the forces generated during restoration placement and function (3). Calcium hydroxide (CH) has long been accepted as the gold standard; however, the materials has some limitations, such as degradation after acid-etching, insufficient adhesion, high solubility in oral fluids, tunnel defects inside the dentin bridge, and pulp-chamber obliteration caused by excessive dentin formation (4).

Several materials have been proposed as alternatives to CH, including calcium hydroxide liners, dentin bonding agents, mineral trioxide aggregate, glass ionomer cement, zinc oxide/eugenol, calcium silicate, and medical Portland cement (5). Mineral Trioxide Aggregate (MTA), comprised of bismuth oxide and modified Portland cement, was developed as a durable, biocompatible alternative for use in various endodontic applications (6). Over the years, studies have shown that MTA can be used successfully in pulpotomy procedures, pulp capping, apexification and root-canal obturation as well as for treating perforations and internal root resorption (6,7). In addition to biocompatibility, MTA possesses low solubility, is able to set in wet conditions and in the presence of blood, and can prevent bacterial leakage;

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however, the material is difficult to manipulate and has a long setting time (8).

The bond strength between the pulp-capping and restorative materials plays a very important role in restoration quality (9). Some studies have suggested that restoration with a resin composite and a bonding agent can be performed immediately following MTA placement. Moreover, acid-etching prior to composite application has been shown to produce surface changes that increase the bond strength of resin-based materials; however, it has also been shown to reduce the compressive strength and surface microhardness of MTA (10,11). Recently, universal adhesives (UAs) have been introduced that can be used in either etch&rinse, self-etching, or selective-etching modes, depending upon clinical conditions and the clinician's preferences. These new, single-bottle adhesives have become popular due to their simple application procedures and short application times (12). These adhesive systems differ from etch-and-rinse adhesives in several aspects, such as the initial pH, type of acidic monomer, the concentration of water and solvents, and the hydrophilicity of the bonding layer and they can be classified as mild, moderate and acidic systems depending on their initial pH (13). Previous studies have stated that the bond strength of MTA to resin can be affected by adhesive solvent type (acetone, ethanol, or water) and filler content (10); however, the effect of UA acidity on the bond strength of MTA to resin composite has not been investigated. Therefore, this study aimed to compare the micro-shear bond strength of three diferrent UAs with different acidity levels (G-Premio Bond-1,5 (14), Single Bond Universal-2,7 (15) and All-Bond Universal-3,2 (16) to ProRoot MTA and NeoMTA Plus. The null hypothesis was that there would be no difference in the micro-shear bond strength of UAs with different acidity levels to the calcium silicate-based biomaterials tested.

2. METHODS

Ethics committee approval was not taken due to in vitro design of the study. This study does not include human participants. Thus, no consent form was required. Sample size was calculated a priori using the effect size of a previous study (17) with analysis of variance (fixed effects, omnibus, 1-way) test from F test family and an alpha-type error of 0.05 and a power beta of 0.95 (G*Power 3.1 for Mac.; Heinrich Heine, Universitat Dusseldorf, Dusseldorf, Germany). Ten specimens per group were indicated as the minimum sample size to observe the same effect.

Material composition and manufacturer details are given in Table 1. A 5-mm dia. x 2-mm h. hole was prepared in the centre of the top surfaces of 60 cylindrical acrylic blocks, which were then randomly divided into 2 groups (n=30) according to biomaterial filling. NeoMTA Plus (Avalon Biomed Inc. Bradenton, FL, USA) and ProRoot MTA (Dentsply Tulsa, Tulsa, OK, USA) were prepared according to the manufacturers' instructions by mixing the ProRoot MTA powder with the liquid provided and the NeoMTA Plus powder with the anti-washout gel provided. The materials were transferred into the holes and compacted using a spatula. The samples were covered with wet cotton pellets, stored at 37°C and 100% humidity for 24 hours to allow the materials to set completely, and then polished with 600-grit SiC paper (#600, Tigre; Pinceis Tigre SA, Castro, Brazil) for 60 seconds to obtain uniform, flat surfaces.

Table 1. *The chemical compositions and manufacturer details of the tested materials*

Material	Main components	Manufacturer
ProRoot® Mineral Trioxide Aggregates	Tricalcium silicate, bismuth oxide, dicalcium silicate, tricalcium aluminate, calcium sulfate dehydrate or gypsum	Dentsply Tulsa Dental, OK, USA
NeoMTA Plus	**Powder:** Tricalcium silicate (Ca_3SiO_5), Dicalcium silicate (Ca_2SiO_4), and Tantalum oxide (Ta_2O_5). **Liquid:** Water (H_2O) and proprietary polymers.	Avalon Biomed, Bradenton, Florida
G-Premio Bond	MDP, 4-MET, MEPS, methacrylate monomer, acetone, water, initiator, silica filler,pH:1,5	GC, Tokyo, Japan
Single Bond Universal	MDP, Bis-GMA, HEMA, DMA, methacrylate functional copolymer, filler, ethanol, water, initiators, silane pH:2,7	3M ESPE, St Paul, MN, USA
All-Bond Universal	MDP, Bis-GMA, HEMA, ethanol, water, initiators pH:3,2	Bisco Inc, Schaumburg, IL, USA
Filtek Z250 Universal Restorative System	Zirconia/silica filler, UDMA, Bis-GMA and Bis-EMA resins.	3M ESPE,MN, USA

Bis-GMA: bisphenol-A-glycidyldimethacrylate; Bis-EMA: ethoxylated bisphenol-A-dimethacrylate; MDP: 10-methacryloyloxydecyl dihydrogen phosphate; HEMA: 2-hydroxyethyl methacrylate; 4-MET: 4-methacryloyloxyethyl trimellitate; MEPS: methacryloyloxyalkyl thiophosphate methylmethacrylate; UDMA: urethane dimethacrylate; DMA: N, N-dimethylacrylamide.

Specimens were then randomly divided into 3 subgroups according to universal adhesive (n=10). G-Premio Bond (GC Corp., Tokyo, Japan), All-Bond Universal (Bisco, Inc., Schaumburg, IL, USA) and Single Bond Universal (3M ESPE, St Paul, MN, USA) were applied according to the manufacturers' instructions and polymerized with a LED light-curing unit (Elipar S100, 3M ESPE, MN, USA) for 10 s. A silicone tube (0.8 mm internal dia. x 2 mm h.) was positioned on the centre of each sample, and composite resin (Filtek Z250, 3M ESPE,MN, USA) was placed inside the tubes and polymerized for 20

seconds. Specimens were stored at 37°C and 100% humidity for 24 hours.

Micro-shear bond strength was tested using a knife-edge blade mounted in a Universal Testing Device (LRX, Lloyd Instruments, Farnham, UK) (Figure 1). A load was applied with a crosshead speed of 1mm/min., and the load at failure was recorded in Newtons and converted into MPa. Failure modes were evaluated by a single operator under a stereomicroscope (Nikon SMZ 745T; Tokyo, Japan) at x40 magnification and categorized as either mainly adhesive, mainly cohesive within the resin cement, or mixed (Figure 2).

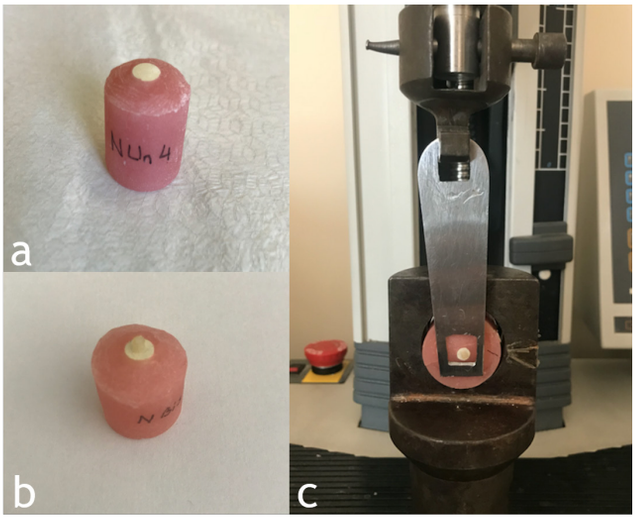

Figure 1. *a) Preparation of the samples, b) sample bonded with composite material, c)The universal testing machine*

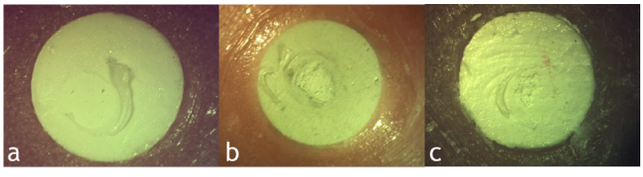

Figure 2. *Images of fractured samples showing: a) Adhesive failure in resin composite bonded to NeoMTA Plus b) Cohesive failure of resin composite bonded to ProRoot MTA c) Mixed failure in ProRoot MTA.*

2.1. Statistically analysis

A Shapiro-Wilk test confirmed a normal distribution of data. Micro-shear bond-strength data were statistically analyzed using two-way analysis of variance, and a chi-square test was used to analyse the distribution of failure modes. Statistical analysis was performed with the software SPSS v.21.0 (IBM, SPSS Inc., Chicago, IL, U.S.A.) with the level of significance set at 5%.

3. RESULTS

Mean micro-shear bond strength values and standard deviations of the groups are given in Table 2. No significant differences were observed in the micro-shear bond strengths of any of the adhesives tested to either NeoMTA Plus or ProRoot MTA ($p>0.05$). Moreover, all the tested adhesives showed similar bond-strengths to both of the calcium silicate-based biomaterials tested ($p>0.05$). Failure modes are given in Table 3. The majority of failures in the ProRoot MTA subgroups were cohesive failures, whereas both adhesive and cohesive failures were observed in the NeoMTA Plus subgroups.

Table 2. *Mean Shear Bond Strength Values (MPa) and Standard Deviations of Each Groups (n=10). Different superscript lower case letters in each row and capital letters in each column indicate indicate statistically significant differences (P<0,05)*

	NeoMTA Plus	ProRoot MTA	N
G-Premio	3.58 ± 0.64^{aA}	3.35 ± 0.53^{aA}	10
All-Bond Universal	2.71 ± 1.15^{aA}	3.09 ± 0.81^{aA}	10
Single Bond Universal	3.75 ± 1.12^{aA}	3.11 ± 0.41^{aA}	10

Table 3. *Distribution of Failure Modes within Groups (n=10)*

		Adhesive	Cohesive	Mixed
NeoMTA Plus	G-Premio	4	4	2
	All-Bond Universal	6	1	3
	Single Bond Universal	2	2	6
ProRoot MTA	G-Premio	-	6	4
	All-Bond Universal	2	2	6
	Single Bond Universal	2	7	1

4. DISCUSSION

The hypothesis of this study was accepted, because there was no difference in the micro-shear bond strength of UAs with different acidity levels to the calcium silicate-based biomaterials tested.

ProRoot MTA is the most commonly used and most studied brand of endodontic repair material and is used in surgical as well as non-surgical procedures. Composed mainly of tricalcium silicate (53.1%), dicalcium silicate (22.5%), bismuth oxide (21.6%), with small amounts of tricalcium aluminate and calcium sulfate (18), its thin, hydrophilic particles cure in a humid environment at 12.5 pH over a period of 3-4 hours (19). NeoMTA Plus is a new material composed of finely powdered tricalcium silicate that incorporates tantalum oxide (Ta_2O_5) rather than bismuth oxide as a radiopacifying agent to prevent discoloration and is mixed with a water-based gel to impart good processing properties (20). By changing the powder-gel ratio, NeoMTA Plus can be used in a variety of

applications, with a thin mixture used in orthograde filling and a thicker mixture used in retrograde filling. According to the manufacturer, NeoMTA Plus can be used in vital pulp treatment (for pulp capping, pulpotomy, and as a cavity liner/base), root apexification, root repair (resorption and perforation), root-end filling, and root-canal sealing (21).

The adhesive properties of restorative materials are most commonly evaluated according to bond-strength, assessment of which has become a well-recognized method of analyzing material performance *in vitro*. In this study, the adhesive properties of tricalcium silicate-based materials and universal adhesives were evaluated by measuring shear bond strengths (10).

Due to its hydrophilic properties, ProRoot MTA requires moisture to initiate setting (22). For this reason, the application of a damp cotton pellet for 3-4 hours is recommended in order to supplement tissue fluids and provide two-sided hydration during perforation repair and pulp capping (6). A previous study reported this method to provide optimal results with a 24 h application, whereas double-hydration of ProRoot MTA for 72 hours resulted in a significant reduction in bond strength that the authors attributed to the potential negative impact of excessive humidity and possible solubility of the material (23). Therefore, in the present study, MTA was stored in 100% humidity for 24 h before bonding.

In order to simplify application procedures and reduce technical errors, one-step self-etch adhesives have been developed. Studies have noted that the acidic monomers used in self-etch adhesives play a key role in their enamel and dentin bonding performance (24). Generally, self-etch adhesives are classified as either “strong” (pH<1), “intermediately strong” (pH≈1.5), “mild” (pH≈2) and “ultra-mild” (pH≥2.5) (25), with the pH value known to strongly affect the solubility of the smear layer and the depth of demineralization of underlying dentin (26,27). Considering the differences in pH of the adhesives used in this study (G-Premio Bond: 1.5; Single Bond Universal:2.7; All-Bond Universal:3.2), the degree of biomaterial dissolution was expected to vary. Previous studies have stated that micromechanical retention and therefore bond strength increases with increases in surface porosity of tricalcium silicate based cements (28,29). Phosphoric acid etching has been suggested to provide greater microretention and potentially increases the bonding effectiveness of resinous materials by significantly enhancing the surface energy of substrate material (10). Moreover, Yelamali et al. have reported two-step etch-and-rinse and two-step self-etching primer systems to perform significantly better than an all-in-one adhesive system in terms of bond strength when bonding composite resin to white MTA (30). In contrast to these findings, the present study indicated differences in acidity among universal adhesives to have no significant effect on shear bond strentgh values, suggesting that microretention is not noticeably improved by lowering the acidity of self-etch adhesives.

Although the adhesives tested vary according to pH, they all contain the monomer 10-MDP, whose binding mechanism to dentin has been explored in a previous study. According to the authors, when an adhesive containing MDP is rubbed onto dentin, the surface is partially demineralized up to a submicron depth, and the MDP reacts with the released Ca ions to create nano-layers of MDP-Ca salts within the hybrid layer (31). In light of this earlier finding, it may be suggested that the bonds between the adhesives tested in the present study and both ProRoot MTA and NeoMTA are strengthened by chemical chelation of the Ca ions contained in these tricalcium silicate-based biomaterials (1).

However, despite the interaction between the MDP monomer and the calcium ions, the bond strengths of the adhesives to the tested cements were considerably lower than the threshold values of 17-20 MPa that are reportedly required to sufficiently resist contraction forces and produce gap-free restoration margins (32,33). It is likely that the bond between the cement and adhesives could be increased by using the same UAs in etch&rinse mode to increase porosity of the resin cement, thereby improving microretention.

With regard to fracture modes, according to Tate et al. (34) a bond is considered to be acceptable when fracture occurs within a material rather than at the bonded interface, i.e. when the fracture is cohesive rather than adhesive. In the present study, cohesive fracture predominated in the ProRoot MTA group.

5. CONCLUSION

The findings of the present study showed that the acidity of universal adhesives has no effect on the bond strength of tricalcium silicate-based biomaterials to restorative materials.

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Clinical and Experimental Health Sciences

Examination Of The Relationship Between Depression and Body Mass Index (BMI) Among University Students

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ABSTRACT

Objective: The objective of this study is to examine the level of depression of university students and evaluating its relationship with Body Mass Index (BMI).

Methods: In this correlation study, a questionnaire with 18 questions (age, gender, department, meal consumption, cigarette-alcohol usage) questioning demographic information and eating habits. and "Beck Depression" inventory was conducted voluntarily to 800 undergraduate students studying at Istanbul Aydın University. Height, weight and waist circumstance were measured and calculated. SPSS 22.0 program was used for data analysis.

Results: The gender ratio of the students in this study was 42,9% male and 57,1% was female. Students who have the average age 21,22±1,801 years, %7,0 are underweight in terms of BMI, 66.6% are normal, 23.9% are overweight and 2.5% are obese. The BMI ratios of the students show a statistically significant difference ($p < 0.05$); male students are more overweight and more obese than female students. 53.1% of students have normal level of depression, 22.8% mild mood changes level, 10.5% have moderate level, 4.8% have severe depression and 1.7% extreme depression.

Conclusion: University students' depression level is normal and there is no relationship between the BMI and depression levels. There was relationship between students' depression levels and age-height length, body weight and waist circumference measurements; depression levels increase as the students decrease in height; as the weight, BMI and waist circumference increases, depression levels slightly increase.

Keywords: BMI, Body Mass Index, Depression, University Students, Beck Depression inventory.

1. INTRODUCTION

The university period is a special period. With the start of university education, changes in their eating habits are also observed due to reasons such as young people leaving their familiar family environment, being more affected by their friends, paying attention to their external appearance and making their free choices become more evident. The most distinctive feature of this period is the efforts to adapt to the new establishment to be established with both psychological and nutritional style (1). University period is a duration in which nutritional habits are shaped due to environmental factors, especially friends, and they carry a particularly high risk in terms of gaining wrong eating habits (2-3). Nutritional habits formed during the university period also continue in adulthood. Inadequate and unbalanced eating habits gained during this period may adversely affect the school success of students, and may lead to the formation of obesity, diabetes, cardiovascular diseases, and some cancers (4-5-6-7).

Depression is an increasingly common disease. It is estimated that approximately 5.0% of the population in our country (6.4% in women, 3.2% in men) has major depression. It is known that 10.0% of men and 20.0% of women experience short or long clinical depression at some point in their lives in our country (8). Depression is a disease accompany with sleep, sexual desire and appetite disorders that the person don't enjoy life, the person feels deeply in sorrow, pessimistic thoughts about the future, intense regret and guilt feelings about the past, sometimes can be occur suicidal thoughts and death can be seen (9).

There are many scales in the literature for the diagnosis of depression. One of them is the Beck depression scale. The Beck Depresson Scale is used to determine the risk of depression in individuals and to determine the severity of depression. There are a total of 21 titles and four articles per title. It is filled by asking the individuals to give their answers according to themselves, and a depression classification is

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made according to the score obtained (10-11). According to Beck's (2005) model, depression occurs with emotions, thoughts, motivation and symptoms in the physiological nature. Depression starts with disturbances in thought, emotional depression comes as a secondary condition (12).

The most obvious symptoms in depression; loss of interest, depressed mood (depression), inability to enjoy life, a general lack of energy and reluctance. The clinical picture in depression may vary according to the severity of the complaints, age, and other accompanying psychopathological or medical conditions. It is possible to group the symptoms and clinical appearance of depression as behavioral, emotional, cognitive, somatic and motivational symptoms (13).

The World Health Organization (WHO) is widely used to determine obesity. BMI is an indicator that evaluates body weight according to height. In adults, BMI were used: underweight (<18.5 kg/m^2), normal-weight (18.5 – 24.9 kg/m^2), overweight (25 – 29.9 kg/m^2), obese (>30 kg/m^2) (14). The World Health Organization has determined that over the age of 18, 1.9 million adults overweight (39.0%) and 650 million adults were pre-obese (13.0%) in 2016 (15).

The increase in obesity causes the formation of diseases that cause not only physical but also mental problems (16). Studies indicate that depressive symptoms in university youth are increasing day by day, and the most important psychological disorder that threatens this group is depression (17-18). The American Psychiatric Association (APA: American Psychological Association) defines depression as a common and serious medical disease that negatively affects how we feel, our way of thinking, and our behavior (19). Studies show that there is a two-way relationship between depression and BMI; has a high BMI; reported that obesity increases the risk of depression and depression is an important determinant in the formation of obesity (20-21). In another similar study, it is thought that depression is seen as a complication after obesity in adults and depression causes obesity in children (22). In another study, it was reported that psychosocial stress is associated with high energy intake and thus obesity, that is, high BMI (23-24).

This study was planned and conducted to determine the level of depression in university students and to evaluate its relationship with BMI.

2. METHODS

The universe of this study consists of 25.000 students studying at Istanbul Aydın University. In line with the purpose of the study, stratified random sampling method was used. 13 faculties were included in the research. These faculties; Faculties in the field of health (Faculty of Medicine, Dentistry, Health Sciences), Faculties in the field of Science and Social Sciences (Faculty of Arts and Sciences, School of Foreign Languages, Faculty of Fine Arts, Faculty of Law, Faculty of Economics and Administrative Sciences, Faculty of Communication and Public Relations, Architecture and Faculty of Design, Faculty of Engineering) and Faculty of Sport Sciences. The students are included in the sampling group considering the number of students studying in these faculties. It was concluded that the participation of 384 students would be sufficient in the calculation of the sample volume within the range of 0.05 sample error and 95% confidence. However, the study was completed with 800 students between February and March 2020 due to students who showed great interest to participate voluntarily. It was started to be implemented after the Ethics Committee Approval numbered 61351342 – / 2020-61 and dated 29.01.2020 was obtained from Üsküdar University Non-Interventional Research Ethics Committee. Criteria for inclusion in the study; having undergraduate education at Istanbul Aydın University, not having mental-brain retardation, not having a diagnosed psychological disorder, and not using psychological drugs and nutritional supplements. The research is voluntary and a questionnaire including the BECK depression inventory was applied to the students who agreed to participate in the study with 18 questions (age, gender, department, meal consumption, cigarette-alcohol usage) questioning demographic information and eating habits. weight, height, and waist circumference were measured.

Body weights of students; With the Tefal 1063 Premiss brand (sensitive to 100 g, 150 kg capacity) scale; the height was measured with a non-flexible tape measure without shoes and feet united and fixed on the wall in the Frankfurt plane (the eye and auricle are flush and the angle between the head and neck is 90 degrees).

The BMI values of the students were calculated by dividing their body weight (kg) by the square of their height (kg / m^2)] and classified according to the WHO's BMI classification. Measurement of waist circumference was measured while the student was standing and his abdomen was relaxed and the arms were on both sides, feet together, without applying pressure from the midpoint between the lower rib and the crystalline with a 0.1 cm sensitive non-flexible tape measure (25).

2.1. Beck Depression Inventory

The Beck Depression Inventory was developed by Beck in 1961. BDI is used to determine the risk of depression and to measure the level and severity of depressive symptoms (26). The validity and reliability study in our country was carried out by Hisli in 1989 (11). Cronbach's alpha value was found as 0.80. Each item of BDI determines a depression-specific behavioral pattern in the past week and includes 21 self-assessing sentences with four options, going from low to high (0-3). The total score that can be obtained from the scale varies between 0-63. Evaluation; 1-10: Normal, 11-16: Mild mood changes, 17-20: Clinical depression at the border, 21-30: Moderate depression, 31-40: Severe depression,> 40: Extreme depression. It takes about 15 minutes to complete the test.

2.2. Data Analysis

SPSS 22.0 program was used for data analysis. Number, percentage, average, standard deviation, min, max. values

are shown in a table. The data showed normal distribution according to the normality test results. Independent sample t test, Annova (one way variance analysis), Pearson correlation test analyzes were performed in the analysis of the data providing parametric test assumptions.

3. RESULTS

This study has 800 students who participated. The average age of the students is 21.22 ± 1.801, 76.8% of the students are between the ages of 19 and 22, 22.6% are between the ages of 23-25 and 0.6% are over or at the age of 26. 97.4% of students are single and 2.6% are married. 94% of students do not have chronic diseases, 6% of them have. 56.2% of the students do not smoke, 34.8% smoke and 9% state that they quit smoking. Alcohol usage among students; 41.2% drink alcohol, 54.6% do not drink alcohol, and 4.2% state that they have quit drinking alcohol (Table 1).

Table 1. *Demographic information of students*

Variables	Male (n: 343)		Female (n:457)		Total (n:800)	
Age (years)	**n**	**%**	**n**	**%**	**n**	**%**
19-22	263	76,7	351	76,8	614	76,8
23-25	79	23,0	102	22,3	181	22,6
26 ≤	1	0,3	4	0,9	5	0,6
Mean ± SD / Min. Max	21,22±1,801 / (19-39)					
Marital Status						
Married	14	4,1	7	1,5	21	2,6
Single	329	95,9	450	98,5	779	97,4
Total	343	100,0	457	100,0	800	100,0
Chronic Disease Status						
Yes	13	3,8	35	7,3	48	6,0
No	330	96,2	422	92,7	752	94,0
Smoking status						
Yes	149	43,4	129	28,2	278	34,8
No	146	42,6	304	66,5	450	56,2
Quit	48	14,0	24	5,3	72	9,0
Drinking Alcohol						
Yes	154	44,9	176	38,5	330	41,2
No	169	49,3	268	58,6	437	54,6
Quit	20	5,8	13	2,9	33	4,2

The mean body weight, height, BMI, waist circumference measurements of the students participating in the study showed a significant difference according to gender ($p < 0.05$); body weight, height, BMI, waist circumference averages were higher in male students than female students. The mean BMI and waist circumference measurements were 24.16 ± 2.96kg / m^2, 85.64 ± 11.35 cm for male, respectively; in flushing 21,89 ± 3,17 kg / m^2, 71,20 ± 8,71 cm. The min and max values of BMI and waist circumference measurements for male, respectively; 16.90-34.20 kg /m^2 52.0-134.0 cm. for female; it was determined as 16,0-35,80 kg / m^2, 50,00-110,00 cm. (Table 2).

When the distribution of BMI according to the gender of the students is examined; 34.7% of male are slightly overweight and 3.2% are obese; 15.8% of female are slightly overweight and 2.0% are obese students and BMI (underweight, normal, overweight, obese) rates of female and male statistically differ significantly ($p < 0.05$) (Table 3).

Depression scores of students by gender; the average depression score of males is 10.88, the average depression score of female is 12.85 (Table 4).

When the depression levels of students according to their age distribution are examined; according to the age distribution, there was no statistically significant difference ($p > 0.05$) between them, the ones with the extreme depression levels were between 2.1% and 19-22 years old. When the distribution of depression levels according to the gender of the students is examined; BMI (underweight, normal, overweight, obese) rates of female and male students differ significantly. ($p < 0.05$); Depression level of female students was found higher than male. Of the normal level, 57.7% are male and 49.7% are female; 23.0% of the mild mood changes ones are males and 22.5% are female; 5.5% of those at the border are male and 8.1% are female; 7.9% of the moderate level are male and 12.5% are female; of those who are severe, 5.0% are male, 4.8% are female and 0.9% of those who are extreme level are male and 2.4% are female. When the distribution of depression levels according to the BMI values of the students is examined; no statistically significant difference was found between BMI values and depression levels. ($p > 0.05$) Depression rates of students who are underweight, normal, overweight, and obese are close to each other. 6.8% of those at normal depression level are underweight , 65.6% are normal, 24.5% are overweight, and 3.1% are obese. 7.7% of the mild mood changes depression level ones are underweight, 67.0% are normal, 23.6% are overweight and 1.6% are obese. 3.6% of those at the border are underweight, 71.4% are normal, 23.2% are overweight and 1.8% are obese. 6.0% of moderate depression level people are underweight, 67.9% are normal, 23.8% are overweight and 2.4% are obese; 7.7% of those who are severe depression level are underweight, 61.5% are normal, 28.2% are overweight and 2.6% are obese; 21.4% of those who are extreme depression level have underweight and 78.6% have normal BMI values (Table 5).

When the depression levels of the students, age, height, body weight and waist circumference measurements were examined; A negative, weak but significant relationship was found between students' depression levels and height ($r = -,085$, $p < 0.05$), A weak but significant correlation was found with the level of depression and body weight. ($r = -,099$, $p < 0.05$). A weak but significant relationship was found positively with the level of depression and BMI. ($r = -,072$, $p < 0.05$). A weak but significant relationship was determined with the level of depression and waist circumference. ($r = -,082$, $p < 0.05$). In other words, as the height of the students decreases, their depression levels increase, as their weight, BMI and waist circumference increase, their depression levels slightly increase (Table 6).

Table 2. *Anthropometric Measurements of Students According to Gender and Average and Min-Max Values of Body Composition*

Anthropometric Measures	Male (n:343)		Female (n:457)		Total (n:800)		P Value
	Mean + SD	Min.-Max.	Mean + SD	Min.-Max.	Mean + SD	Min. – Max.	
Body weight (kg)	78,61±11,48	49,0-130,0	59,74±9,20	41,60-100,0	67,83± 13,86	41,60-130,0	,000
Height (m)	179,65±7,28	155,0-195,0	165,38±6,23	148,0-185,0	171,50 ± 9,74	155-195	,000
BMI (kg/m^2)	24,16±2,96	16,90-34,20	21,89±3,17	16,0-35,80	22,86±3,28	16,0-35,80	,000
Waist circumference (cm)	85,64±11,35	52,0-134,0	71,20±8,71	50,00-110,00	77,39±12,23	50,0 – 134,0	,000

Table 3. *BMI Values of Students by Gender*

	Male (n:343)		Female (n:457)		Total (n:800)	
BMI classification	**n**	**%**	**n**	**%**	**n**	**%**
Underweight (<18,50kg/m^2)	8	2,3	48	10,4	56	7,0
Normal (18,50-24,99kg/m^2)	205	59,8	328	71,8	533	66,6
Overweight (25,00-29,99kg/m^2)	119	34,7	72	15,8	191	23,9
Obese (>30kg/m^2)	11	3,2	9	2,0	20	2,5
	Chi-Square Value 70,783, p:0.000					

Table 4. *Descriptive values of students' Depression scores by Gender*

Gender	Depression scores	
	Mean±SD.	Min. – Max.
Male (n:343)	10,88±10.01	1-62
Female (n:457)	12,85±10.81	1-63
Total (n:800)	12,01±10.51	1-63

Table 5*. Investigation of students' depression levels according to different variables*

Variables Ages (Years)		Depression Levels						Total
		Normal	Mild mood changes	At the border	Moderate	Severe	Extreme	
19-22	n	328	142	39	66	26	13	614
	%	53,4	23,1	6,4	10,7	4,2	2,1	100,0
23-25	n	93	39	17	18	13	1	181
	%	51,4	21,5	9,4	9,9	7,2	0,6	100,0
≥26	n	4	1	0	0	0	0	5
	%	80,0	20,0	-	-	-	-	100,0
Gender	Chi-Square Value: 8.557; p:0.575							
Male	n	198	79	19	27	17	3	343
	%	57,7	23,0	5,5	7,9	5,0	0,9	100,0
Female	n	227	103	37	57	22	11	457
	%	49,7	22,5	8,1	12,5	4,8	2,4	100,0
Total	n	425	182	56	84	39	14	800
	%	53,1	22,8	7,0	10,5	4,9	1,7	100,0
	Chi-Square Value: 10.831; p: ,055							
BMI								
Underweight	n	29	14	2	5	3	3	56
	%	6,8	7,7	3,6	6,0	7,7	21,4	7,0
Normal	n	279	122	40	57	24	11	533
	%	65,6	67,0	71,4	67,9	61,5	78,6	66,6
Overweight	n	104	43	13	20	11	0	191
	%	24,5	23,6	23,2	23,8	28,2	0,0	23,9
Obese	n	13	3	1	2	1	0	20
	%	3,1	1,6	1,8	2,4	2,6	0,0	2,5
Total	n	425	182	56	84	39	14	800
	%	53,1	22,8	7,0	10,5	4,9	1,8	100,0
	Chi-Square Value: 11,392 ; p: ,724							

Table 6. *Correlation of students' depression level and age, height, weight, waist circumference*

		Beck Total	Age	Height	Body weight	BMI	Waist circumstance
Beck Total	r	1					
	p						
Age	r	-,026	1				
	p	,469					
Height	r	-,085*	,022	1			
	p	,016	,526				
Body weight	r	-,099**	,020	,717**	1		
	p	,005	,576	,000			
BMI	r	-,072*	,018	,240**	,805**	1	
	p	,041	,609	,000	,000		
Waist circumstance	r	-,082*	,047	,497**	,818**	,738**	1
	p	,020	,185	,000	,000	,000	
*. Correlation is significant at the 0.05 level (2-tailed).							
**. Correlation is significant at the 0.01 level (2-tailed).							

4. DISCUSSION

The university period is a special period in parallel with the separation of the students from the family, the transition to a new friend environment and the changing external factors, eating habits and lifestyle. The decisive feature of this period is the efforts to adapt to the new conditions that is established with both the psychological and the eating habits (1). The gender ratio of the students in this study was 42,9% male and 57,1% was female. Students who have the average age 21,22±1,801 years, %7,0 are underweight in terms of BMI, 66.6% are normal, 23.9% are overweight and 2.5% are obese. The BMI (underweight, normal, overweight, obese) ratios of the students show a statistically significant difference ($p < 0.05$); male students are more overweight and more obese than female students. 53.1% of students have normal level of depression, 22.8% minimal level, 10.5% have moderate level, 4.8% have severe depression and 1.7% extreme depression. No statistically significant difference was found between BMI values and depression levels of students ($p > 0.05$); depression rates of students who are underweight, normal, overweight, and obese are close to each other. There was no statistically significant difference ($p > 0.05$) between students' age groups and depression levels; depression was observed at a higher level in the 19-22 age group. A statistically significant difference was found between students' depression levels and age-height length, body weight and waist circumference measurements ($p < 0.05$); depression levels increase as the students decrease in height; as the weight, BMI and waist circumference increases, depression levels slightly increase.

In this study, majority of students are normal in terms of BMI and male students are more overweight and obese than female students. Similarly, in their studies on university students, majority of students were found to be neutral in terms of BMI, and female students were more overweight and obese than male students (27-28-29-30). This situation can be explained by the fact that female students have more normal BMI than male students and have normal BMI, as a result of their efforts to be appreciated they pay attention to their nutrition, in parallel.

In this study, when the level of students' depression is examined; depression level for majority of students was found to be normal. Similarly, in the study of Güzel on university students, the depression level of the majority of students was found to be normal (31). In Abdullayev's study on university students in 2019, the depression level of the majority of students was found to be normal (32). In the study of Arkoç on university students in 2019, the depression level of the majority of students was found to be normal (33). This can be explained by the fact that students' mental reactions are at a level that can manage negative processes, depending on the system and events they are in, as well as individual differences. And this can be explained by the fact that depression levels can be normal.

In this study, students in the low age group were found to be more depressed than students in the high age group. This may be due to the higher number of students with low age groups, or because lower age groups have difficulty adapting to the new university environment through the process of leaving the family environment.

When the BMI and depression levels of the students were examined, the depression levels of the underweight, normal, overweight, and obese students were found to be close to one another. In a different way, in the study of Güzel on university students in 2016, the depression level of students who were normal and 1st degree overweight was found to be lower than the depression level of individuals with high BMI (31). This can be explained by the fact that the differences in perceptions of students towards their weight may have different effects on their psychology.

5. CONCLUSION

University students' depression level is normal and there is no relationship observed between the BMI and depression levels. There was relationship between students' depression levels and age-height length, body weight and waist circumference measurements; depression levels increase as the students decrease in height; as the weight, BMI and waist circumference increases, depression levels slightly increase. This study will enlighten on future studies due to the insufficient studies on this subject in our country. In addition, by contributing to the formation of a psychologically healthy, happy young population, in determining the factors that will affect the depression levels of young people, in order to assist the countries' health policies under the heading of preventive mental health and the fight against obesity, and the health of individuals in this direction. On the other hand, it is important in terms of contributing to reducing the social burden by decreasing the health expenditures that individuals make in this direction.

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Clinical and Experimental Health Sciences

Additive Manufacturing (3D Printing) Methods and Applications in Dentistry

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ABSTRACT

Computer Aided Design-Computer Aided Manufacturing technologies (CAD-CAM) are often used in dentistry. Along with technological developments, techniques of additive manufacturing (3D) which has a lot of advantages have been improved and found a field of practice. Today, metals and metal alloys, polymer and composite, ceramic materials are produced and used through additive manufacturing techniques. With additive manufacturing in dentistry, dental implants, prosthetic restorations, maxillofacial implants and prostheses, dental models, custom trays, occlusal splints, orthodontic models and devices can be produced and used in tissue engineering. The aim of this study is to profile and evaluate the additive manufacturing methods, materials, and application fields in dentistry.

Keywords: 3D printing, dentistry, computer aided design-computer aided manufacturing, stereolithography

1. INTRODUCTION

During recent years, the tendency towards digital systems in material production has been increasing (1). After the first use of Computer Aided Design and Computer Aided Manufacturing (CAD-CAM) technologies in dentistry in 1980's by Mormann, CAD-CAM systems have become widespread with precocity (2, 3). All CAD-CAM systems are made up of 3 components. The geometry of the products turned into digital data that will be processed by the computer through numerous scanning methods. These digital data are designed by the CAD method to acquire the desired layout. Afterwards, the production of the desired material is carried out by the CAM method accordingly to the data previously created (1,4).

CAD-CAM systems have three different production concepts in dentistry, such as chairside production, laboratory production and centralised fabrication in a production centre (4). CAD-CAM systems in dentistry has been in widespread use for years with inlay, onlay, crown and bridge restorations, laminate veneer, removable partial denture frameworks, surgical guides, abutments and maxillofacial prosthesis (5).

CAD-CAM systems that is widely used in the material production in dentistry is called Substractive Manufacturing. In this method, the designed object obtained by the milling method in which additional material is abstracted from the block used for production. CAM software automatically sends the design created with CAD to the CNC (Computer Numeric Controlled) machine and locates it for the milling system of the machine (6). The accuracy of the positioning specified as 10µm. In this process, 3 axis milling system is used and the aimed design is acquired with the milling cuttlers moving over the x,y,z plain (7). This technology allows the utilizing of unmanipulable materials. It is not affected by the working conditions and has the advantage of using homogeneous materials. However, since the production is made by the removing of materials out of the solid block, the amount of waste material is a lot (1). The materials used in this system for production are metals, resin materials, silica-based ceramics, infiltrated ceramics and metal oxide ceramics (4).

The other CAD-CAM system that has been developed and used in recent years is Additive Manufacturing system. It is also called the solid freeform processing, rapid prototyping and 3D printing (1). This system has been recognised by International Standards Organisation (ISO) and American Society for Testing and Materials (ASTM) as "the process of merging the materials layer by layer, unlike the reduction method for the production of the desired material from 3D model data" (8).

Similar to the milling technique, the data is acquired by intraoral scanning. It is followed by 3D modelling with the help of CAD software; the materials are created layer by layer based on the computer generated design (Standard

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Tessellation Language) (STL). The design is usually divided into two-dimensional layers (9). Afterwards, the additional manufacturing machine carries out the production throughout x,y directions. Every layer is added after another and the material is made in three dimensions (1,10). For the production of the final restoration, procedures such as the removal of the support structures created during the production, washing and heat treatment are required (9). The thickness of the produced layers is between 15-500 microns. If the thickness of the layers is under 50 microns, the layers cannot be distinguished with the naked eye (11).

Additive manufacturing systems have advantages such as a more sensitive production in complex geometry, flexibility in design and custom designs, saving materials as opposed to conventional methods (12). The disadvantage is that with the additive manufacturing method, materials can be produced in different sizes between micro size to macro size; however, the sensitivity of the production can vary according to the method in use. The resolution, surface quality and interlaminar bonding problems may occur when post-production methods such as micro size 3D manufacturing and sintering are required. The limited materials existing for 3D manufacturing cause hardship in the usage of this technology in various industries. Thus, the development of suitable materials for 3D manufacturing and the development of the mechanical qualities of these materials are necessary (12).

The materials used in the system for production are metals and metal alloys (titanium, stainless steel, aluminium, chrome cobalt, molybdenum), polymer and composite materials (thermoplastic polymers, polylactide, polycaprolactone, polyglycolide, acrylic resin) and ceramics (alumina, zirconia) (12,13).

1.1. Methods of Additive Manufacturing Technologies

1.1.1. Vat Polymerization

A. Stereolithography (SLA): Stereolithography has been developed to produce solid objects by adding a polymerisable material (i.e. UV) over each other. This system is known as the solid-form screening process and 3D printing technology. The UV light beam pre-programmed to meet the design is used to create the cross-section of the object in a surface or the layer of the UV-polymerised liquid. The object is then moved away from the liquid surface as programmed and then the next cut is created and the layer that completes the object is added. This procedure is continued until the whole object is created (14,15). This system has advantages such as having the speed to produce in a single day, anatomical diagnostic cast, prosthetic restorations, injection model and creating a master model for various metal castings, better surface quality and less use of raw materials (16). The disadvantage of this system is that the material production needs support structures. Thus, additional material is consumed, and the removal of support structures increases the post-production time (15,17). Since this technology uses light-sensitive polymer materials, the field of use is limited. It is not used for mass production. The cost of the machine is high (16).

B. Digital Light Processing (DLP): DLP system was developed by Larry Hornbeck in 1987 (15,18). DLP was accepted to the same additive manufacturing category as SLA by ASTM since they are similar (19). The main difference between SLA and DLP is the light source that is created by the arc lamp of the image or the little mirrors that are microscopically placed in the matrix over the semiconductor digital micro signal device (DMD). The liquid photopolymer is exposed to the light coming from the projector. DLP projector shows the image of the 3D model to the liquid photopolymer. Radiation passes through a UV transparent window. The process is repeated until a three-dimensional object is produced (15).

1.1.2. Powder Bed Fusion

A. Selective Laser Sintering (SLS): In this production technology, the production is carried out by sintering method by applying a laser beam (Nd-YAG) (20). The utilised materials are in powder form and laser carries out the production by sintering the powder. The advantages of this system are that the usage of support materials is not needed during production and the material produces has high durability and stiffness. There are various finishing options. The disadvantages are that the material surfaces are porous. Usage of adhesives such as cyanoacrylate may be needed to provide the connection between layers (21,22).

B. Direct Metal Laser Sintering (DMLS): This technology is used to create materials with high accuracy and better mechanical resistance. In this technology, laser beams are used to melt the metal powder and the metals are produced layer by layer. The advantages of the system are that it can produce high resistance materials has a high accuracy rate and it can produce complex morphologies productively. The disadvantage is that porosity and deterioration can be observed depending on the material (23,24).

C. Selective Laser Melting (SLM): In this production technique, contrary to the partial melting observed in SLS and DMLS productions techniques, the metal powder is completely melted. This way, the creation of porous internal structures and granular surfaces are prevented. In SLM, the materials are better bonded. Materials with advanced mechanical properties and higher densities are produced (25). The most widespread fibre laser used in SLM to process the metal powder is the CO_2 laser (26). The disadvantage of this production technique is that there are fluctuations in temperatures during production; due to this high internal tension occurs in the material produced. The materials need heat treatment post-processing (27).

D. Electron Beam Melting (EBM): A strong electron beam produces the product as a layer by using metal powder. The raw material which is under the vacuum is stored and combined by an electron beam. The advantages of this system are that it can obtain high energy levels with narrow beams, it can efface foreign materials in a vacuumed environment,

it produces low energy and needs low maintenance. The disadvantages are the vacuum cost which is too expensive, and it needs maintenance. At the same time, the EBM transmit x-ray during the production (16,28).

Fused Deposition Modelling (FDM)

Thermoplastic material is heated and produced by adding layer by layer. In this process, the printing end can use various materials simultaneously by jetting. The advantages of this system are that it can produce high resistance materials that are reasonably priced and resistant to moisture. There is more than one material colour. The disadvantages are that the mechanical properties and the surface quality are weak and the number of thermoplastics that can be used is limited. Usually, it causes bulges showing lines in every layer post production. To rid of these lines, additional processes such as polishing and sanding may be needed. Supporting materials may be needed during production and temperatures may experience fluctuations (12,29).

1.1.4. Material and Binder Jetting

A. Inkjet 3D Printing (IJP): It is one of the main methods of ceramic production in 3D. In this method, the ceramic suspension is collected over the sublayer as droplets. Then the droplets are thickened by being added layer by layer and adequate level of resistance is achieved. This method is swift and productive. Two main types of ceramics, wax essential inks and liquid suspensions are used. Wax essential inks are melted and thickened by collecting over cold raw material. Liquid suspensions are thickened by liquid vaporisation. The particle size distribution, viscosity and solid material context, extrusion speed, nozzle size and production speed of the ceramics are the determinant features of the quality of the produced material. The advantages of this system are that it can produce complex materials in less time and expense. The disadvantages are that the production process is hard, the resolution is low and the adhesion between layers is hard to protect. The sized of the materials are limited and the expense is high (12,16).

B. Polyjet 3D Printing: It is a technique in which the advantages of stereolithography (high resolution and good surface quality) and material jetting methods (high production speed and large production capacity) are combined (11). With this technique, two materials are created during production: production and support materials (30). The advantage of this technique is that it can produce multiple materials simultaneously. It is possible to produce 3D coloured materials that are hard to produce with SLA and DLP. Objects made from poly materials with various optical and mechanical properties can now be produced without the need for additional stages. The disadvantages are that contrary to other production methods, the support material has a denser structure and due to this, it requires more material use (31). The narrow production window of the utilised materials impairs the viscosity and surface tension (11).

1.1.5. Laminated Object Manufacturing (LOM)

3D models are added layer by layer through laser use. Adhesives are used to connect the layers and the production is completed by the repetition of the processes. The advantages of this system are that it can produce large-size materials, and produces fast, accurate and in high resistance. The disadvantages are that the production requires a lot of experience and time and the produced materials' surface quality and dimensional stability are low. Also, the removal of excess material post-production is time-consuming as opposed to powder bed fusion methods. Thus, it is not recommended for complex morphologies (12,16).

1.2. The Application Fields of Additive Manufacturing in Dentistry

Production of dental implants (32), maxillofacial prostheses (33), prosthetic restorations (16), occlusal splints (34), dental models (16), surgical guides (16) , custom trays (35), orthodontic models and devices (36) and usage in tissue engineering (37) are carried out by additive manufacturing methods in dentistry.

1.2.1. Dental Implants

The implants on the market offer a limited variety of design by means of length, diameter and thread parameters. Considering the personal oral and clinical conditions, custom dental implants eliminate the difference between existing standardised designs and the oral conditions of the patient (32). Implants with desired designs and features can be produced with three-dimensional production techniques (38). The 3D templates are created during implant surgery. And the implant replaces the missing tooth with a more reliable and economical method than conventional methods. The production of implants with personalised and complex geometry can be carried out in a short time with different material types. Better surface quality is obtained in dental implants with this method (16).

Osman et al. (39) evaluated the dimensional stability, surface and mechanical properties of zirconia implants and zirconia discs produced with DLP method. It is detected that the DLP method is efficient in the production of custom zirconia implants with adequate dimensional stability. At the same time, it has been reported that the mechanical properties of the produced materials show flexural strength close to the ceramics produced by conventional methods.

In the 3-year prospective study Tunchel et al. (40) carried out, the survival and success rates of additive manufacturing and the titanium dental implants produces were evaluated. It was found that in the titanium dental implants manufactured with the additive method, the general implant survival rate for single-tooth spaces in both jaws is 94.5% and the clinical results were successfully received up to three years.

1.2.2. Prosthetic Restorations

After the intraoral scanning is done with this method, metals and metal alloys, polymers and composites and ceramic materials can be used to produce inlay-onlay (41), temporary and permanent crown-bridge restorations (16,42), crown-bridge substructures and partial prosthesis frameworks (43), complete dentures (44) and save on time. In the study conducted by Ahlholm et al. (41) the inlay and onlay restorations were produced with milling and 3D manufacturing techniques and compared by their accuracies. The accuracy of the restorations produced with the 3D manufacturing technique was found to be almost as same as the restorations produced with the milling technique.

In a study, the accuracy of complete dentures produces by additive manufacturing was compared to the complete dentures produced by CAD-CAM milling. It was reported that the complete prosthesis produced by the milling method has higher accuracy than those produced by 3D manufacturing (45). Lin et al. (44) reported that they produced temporary removable complete denture with DLP method using a photopolymeric resin and Wilkes et al. (46) reported that they produced bridge restoration substructure that is 80% zirconia and %20 alumina with SLM method. Tahayeri et al. (42) compared the mechanical properties of temporary crown and bridge restorations produced by stereolithography technique and conventional methods. It was stated that temporary crown and bridge restorations produced by the additive technique have adequate mechanical properties of intraoral use.

1.2.3. Maxillofacial Implant and Prostheses

Titanium and polymer (Polyetheretherketone) materials were used to produce maxillofacial implants and protheses with additive manufacturing methods. The production is faster than the milling method. It has the advantage of using homogeneous and uniform materials (43,47,48).

Scollozi et al. (49) produced maxillofacial prosthesis using PEEK material in their study and provided defect reconstruction. They emphasized that this technique not only achieve a predictable correction for congenital or acquired deformities but also aesthetic expectations. Unkovkskiy et al. (33) reported that they reconstructed the maxillofacial defect by producing a nasal prosthesis with the additive manufacturing technique by using silicone material.

1.2.4. Occlusal Splints

Occlusal splints can be produced with additive manufacturing methods and used for diagnosis and treatment (34). Venezia et al. (34) produce occlusal splint with 3D manufacturing technique using acrylic resin. They stated that this production technique saves time for the physician and the patient since it is chairside, the accuracy and precision of the splint produced is high and can be reproduced when necessary.

1.2.5. Dental Models

Additive manufacturing methods have the potential to produce custom models. This model also can be used as a guide model (16). At the same time, education models in the field of medicine and dentistry can be produced with additive manufacturing techniques. Since the produced models demonstrated the anatomy well and are colourful, they can be used in education and research (50). Alshawaf et al. (51) compared in their study the dental models they produced with the SLA method and conventional stone casts. The reported that the accuracy of the models produced with the SLA method is lower than the conventional methods. In their study, Choi et al. (52) compared the fracture toughness and flexural bond strength of the artificial teeth they produced with heat cured, milling and 3D manufacturing methods after thermal ageing. It was concluded that the fracture toughness and bond strength of the artificial teeth produced by heat cured decreased significantly with ageing and the bond strength of the artificial teeth produced by milling and 3D manufacturing technique was low and not affected by aging. It has been reported that unlike milling and 3D manufacturing methods, the heat cured artificial teeth have the highest bond strength to various prostheses.

1.2.6. Surgical Guides

This technology produces high precision surgical guides. It improves the reliability of the applied surgical method and improves patient outcomes (16). Turbush et al. (53) produced bone-supported, tooth-supported, and mucosa-supported surgical guides using CBCT data for implant planning and placement protocols with the SLA technique and compared their accuracy. It has been reported that the accuracy of the mucosa-supported guides is lower than the other two techniques. In their study, Kim et al. (54) produced surgical guides with 3 different 3D printers (stereolithography, polyjet, multijet) and compared their accuracy. It has been reported that the highest accuracy is in the surgical guides produced with PolyJet technique and the least accuracy is in the multijet technique.

1.2.7. Custom Trays

Custom trays can also be produced with additive manufacturing technologies. The CAD design of the custom tray allows the control of a homogeneous area for the impression material and reduces manual procedures (35). Using the FDM technique, Chen et al. (35) produced a custom tray for a fully edentulous mandible. They found out that the custom tray produced by this method has higher accuracy than conventional production methods.

1.2.8. Tissue Engineering

Tissue engineering is the process of tissue production using a combination of cells and materials. Cells can accumulate on a 3D tissue scaffold or can be allowed to proliferate without

a tissue scaffold (13). The tissue scaffold must direct and stimulate tissue regeneration and should be biodegradable (37). In scaffold-free tissue engineering, polymeric support structures are required for the movement of blood and nutrients. This application can be used in dental tissue regeneration where the pulp cavity can be filled with cells and microstructured biomaterials (55). Shuai et al. (56) reported that they produced bone tissue scaffolds using nano-hydroxyapatite with the SLS method.

1.2.9. Orthodontic Models and Devices

With the additive method, the orthodontic model, devices used in the treatment of irregularities in teeth and jaws can produce. The dentist can scan with the intraoral method and design an orthodontic model and device, and finally produce this device with additive manufacturing technologies (57). Jindal et al. (57) compared the accuracy of thermoplastic-based aligners produced by the traditional method and resin-based aligners produced by the 3D method. They reported that the resin-based aligners produced with the 3D method have high geometric accuracy, better mechanical properties and they shorten the processing time.

1.3. Materials Used with Additive Manufacturing Methods

1.3.1. Metals and Metal Alloys

Metal and metal alloys can be produced by traditional casting, milling technique and additive manufacturing. 3D manufacturing of metals consists of melting metallic raw material (powder or wire) using an energy source such as a laser or electron beam. The molten material is solidified by adding layer by layer. With additive manufacturing, high accuracy and fast production can be achieved (12).

Although well-designed studies have been conducted on the properties of titanium alloy produced using SLS (especially Ti_6Al_4V), little has been produced on other materials that can be produced using the same technology. Additive manufacturing techniques such as direct metal laser sintering (DMLS) have been used to overcome the difficulties encountered during the production of high hardness materials such as CoCr (cobalt-chrome) with traditional casting and milling techniques. The shrinkage during casting and the high hardness of CoCr making it difficult to produce by milling technique were eliminated by DMLS technology (58). In their study, using the titanium alloy with SLM method, Kanazawa et al. (59) produced a maxillary complete denture infrastructure, evaluated their hardness and microstructures, and concluded that they were suitable for clinical use.

Uçar et al. (60) compared the internal fit of CoCr alloy crowns produced by laser sintering technique and traditionally produced CoCr alloy and Ni-Cr alloy crowns. As a result, no significant difference between the 3 crown materials and internal fit between the crowns has been found.

In a study evaluating the metal-ceramic bond characteristics of Co-Cr alloys produced by casting, milling and selective laser melting methods, the oxidation surface and interfacial characterization and composition before porcelain application were evaluated and the ceramic bonding strength was assessed by 3-point bending test. It was concluded that the oxidation surface and thickness of CoCr alloys depend on the different manufacturing techniques used. The bond strength was found to be 37.7 ± 6.5 MPa for casting restorations, 43.3 ± 9.2 MPa for milling restorations and 46.8 ± 5.1 MPa for SLM restorations. Statistically significant differences were reported between the 3 groups that were tested (61).

Revillia-Leon et al. (62) produced and compared titanium frameworks for complete arch implant-supported prostheses using SLM and EBM additive manufacturing technologies. The implant-prosthesis discrepancy did not show a significant difference between SLM and EBM additive manufacturing technologies. Titanium frameworks produced by additive manufacturing have been reported to be a clinically acceptable implant-prosthetic discrepancy.

1.3.2. Polymer and Composite Materials

Polymers are considered to be the most widely used materials in 3D manufacturing due to the variety of materials and ease of adaptation to different methods. Polymers are available in the form of thermoplastic filaments, reactive monomers, resin or powder for 3D manufacturing. The advantages of producing composite materials with 3D manufacturing are that they identify the geometry with high accuracy and are more cost-effective than other traditional production methods. Since the durability of polymer materials produced by additive manufacturing is low, their usage areas are limited. Researches aimed at improving the low mechanical properties of 3D-produced polymers and leading to the development of various methods and materials for the production of improved polymer composites with better performance are being conducted (12,63). It has been reported that adding fibre to polymer materials can increase the mechanical properties of polymers (64).

Polymer material production with additive manufacturing technique is slower compared to traditional methods such as milling technique and injection moulding. However, with this method, CAD-guided production of many material systems with complex morphology and properties is provided (11).

Additive manufacturing techniques used in the production of polymer and composite materials are SLA, SLS, FDM and IJP. The most used method to produce polymer composites and thermoplastics with low melting points is FDM (63). However, the materials used must have good physical properties and must be environmentally friendly. Polymers commonly used in additive manufacturing are polymers such as Acrylonitrile butadiene styrene (ABS) and polylactic acid (PLA). ABS has good mechanical properties but gives an unpleasant smell during production, PLA is environmentally friendly, but its mechanical properties are poor (65). Photo polymeric

resins can polymerize when activated by UV light in the SLA method (11). Also, the thermomechanical properties of photopolymers still need improving. The molecular structure and sequence of the polymers produced depend on the thickness of the layers due to UV exposure and variable density (12).

Selective laser sintering is the second most used method in polymer production. Polymers used in production by selective laser sintering include polystyrene, polyamides, and thermoplastic elastomers (12).

PLA-based composite materials are used in the production of tissue scaffolding in additive manufacturing (12). Senatov et al. (66) have created PLA-based hydroxylapatite scaffolds with a porosity of 30% for bone implants. A combination of PLA and bioactive CaP has been produced by additive manufacturing to create a 3-dimensional biocompatible scaffold for various tissue engineering applications (32).

With the latest technological developments, the usage of silicone material in the production of facial prostheses has begun. Jindal et al. (67) reported that the mechanical properties of the produced prosthesis depend on the composition of the silicone material.

1.3.3. Ceramic Materials

The ceramic material is widely used in dentistry due to its positive properties such as biocompatibility, good mechanical and optical properties, chemical stability, and thermal conductivity (1, 68). In addition to its positive features, it has disadvantages such as fragile structure and difficulty of production processes. The ceramic material can be produced in dentistry using traditional methods (69), substractive manufacturing and additive manufacturing (1) techniques.

Ceramic components are traditionally produced by manufacturing methods such as injection moulding, die pressing, tape casting, gel casting, etc. The desired morphologies are created from a powder mixture with or without binders (69). After production, the components must be sintered at high temperatures to densify. However, these manufacturing techniques cause limitations in terms of long processing times and high costs (70). The high melting points of ceramics make it difficult to melt under normal heating methods. Although it is possible to melt some ceramics, this process may cause a new phase formation. During cooling, thermal shock can occur, leading to cracks. On the other hand, various factors (sintering temperature and duration, particle size and distribution, the content of binders) related to the production stages of ceramic materials and the properties of the raw materials used affect the porosity. The increasing porosity negatively affects the mechanical properties of the final product (1).

The production of ceramic components by milling technique is extremely difficult due to their extreme hardness and brittleness. It is difficult to obtain good surface quality and dimensional stability with this method. Milling tools are subject to severe wear. Failures such as cracking and breaking can be seen in ceramic materials (70).

The production of ceramics with 3D printing techniques was first performed by Marcus et al. (71) and Sachs et al. (72) in the 1990s. To date, a wide variety of 3D manufacturing techniques have been developed for ceramic production, with material science and technological developments (70). In the production of ceramics with 3D manufacturing methods, Inkjet 3D manufacturing technique, powder bed fusion and SLA methods are frequently used (12). Inkjet 3D manufacturing method is considered as the main method of producing dense ceramic that will not need post-production processes. Inkjet 3D manufacturing requires a stable suspension with controlled rheology that flows easily, does not clog, and has an effective drying process (73).

With the SLA method, the moving beam polymerizes the ceramic suspension containing light-sensitive substances. Thus, stratified production of a three-dimensional object is possible. As a result, a material consisting of an organic matrix containing ceramic powder particles is produced. This part is also called the green body. The additive manufacturing of ceramics is a three-step process. Once the green body has been produced, a two-step thermal process (debinding and sintering) is required. In the debinding process, the organic matrix is burned at temperatures up to 550 ° C. In the first step, the diluent evaporates. Thus, porosity are formed in the green body. The porosities then facilitate diffusion and evaporation of the pyrolyzed polymer components. The burning of the organic part and the sintering procedure leads to weight loss and volume shrinkage (74).

Another additive manufacturing technique used in ceramic material production is selective laser sintering (SLS). However, heating during fusion and cooling down to room temperature after production can cause thermal shock and cause cracks in the ceramic (73).

Ceramics are often used in dentistry in crown and bridge restorations, endodontic posts, orthodontic brackets, dental implants, and abutments (1, 75). Materials such as zirconia and alumina are used in the additive production of ceramics.

1.3.4. Zirconia Ceramics

Zirconia, a polycrystalline ceramic is generally stabilized by 3 mol% yttria (3Y-TZP) for dental applications. Zirconia ceramics can be stabilized in tetragonal or cubic phases depending on the additive used (Y_2O_3, MgO, CaO), its concentration and temperature during heat treatment (1). Its components and additives have positive properties such as hardness, abrasion resistance, high texture compatibility due to post-production sintering procedures and heat treatments (76,77). 3Y-TZP ceramics, which are frequently used today, are used in the construction of dental crowns and especially long bridge restorations in the anterior and posterior region (76,78). It has been reported that zirconia is biocompatible with oral tissues and osteoconductive. Bone formation becomes easier as a result of the contact between the zirconia ceramic

and the bone. Studies also emphasize that zirconia does not cause allergic reactions or taste changes (79-81).

Ebert et al. (82) reported that using zirconia ceramic suspensions, they created crowns-sized dense three-dimensional components with inkjet 3D printing technique (Inkjet 3D printing). It has been observed that the failure rate of the produced and sintered samples is low. With this study, it was possible to obtain samples with a density of 96.9%, comparable to the 3Y-TZP traditionally produced by cold isostatic pressing.

Lian et al. (83) reported that they successfully produced zirconia ceramic, while Cheng et al. (84) reported that they successfully produced the yttria-containing zirconia ceramic using the SLA method. Moin et al. (85) reported that they successfully produced the root analogue implant with zirconia ceramic using the DLP manufacturing method.

1.3.5. Alumina Ceramics

Alumina ceramics, also called aluminium oxide (Al_2O_3), are used in endodontic posts, orthodontic brackets, dental implants, crown and bridge substructure production and ceramic abutments (75). According to the US Food and Drug Association (FDA), high purity alumina should be used. High purity alumina generally has 99.99% purity and has been developed as an alternative to metal alloys for dental applications (1).

Maleksaeedi et al. (86) produced the alumina material with high density and improved mechanical properties using vacuum infiltration inkjet 3D manufacturing method. It has been reported that the properties of the materials produced by this technique are highly dependent on the appropriate suspension concentration, the thickness and size of the produced material, and the complexity of the morphology.

Uçar et al. (87) compared the fracture mechanics, microstructure, and elemental composition of alumina-based on stereolithography with the alumina ceramics produced by pressing and CAD-CAM methods. They compared the flexural strength of these materials using In-Ceram alumina and alumina-based on stereolithography. They concluded that SLA-based alumina is a promising technique for ceramic production in dental applications.

2. CONCLUSION

Additive manufacturing can be made thanks to the materials and techniques developed with the use of CAD-CAM applications in dentistry. Production carried out using metals and metal alloys, polymer and composite materials and ceramics with additive manufacturing. With additive manufacturing in dentistry, dental implants, prosthetic restorations, maxillofacial implants and prostheses, dental models, custom trays, surgical guides, occlusal splints, orthodontic models, and appliances can be produced and used in tissue engineering. Since additive manufacturing techniques have new and many advantages, research is being done on them. The mechanical and biological properties of the restorations produced with this technique should be examined and evaluated in detail before oral use in the clinic.

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