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Original Article / Özgün Araştırma

Comparative Analyses of the Computer Aided Presentation and Brochure Based Information on the Knowledge of Mothers Regarding the Oral Health Status of Their 0-3 Years Old Children

Bilgisayar Destekli Sunum Şeklindeki Ağız Sağlığı Eğitimi ve Yüz Yüze Broşür Bazlı Bilgilendirmenin 0-3 Yaş Arası Çocuğu Bulunan Annelerin Çocuklarının Ağız ve Diş Sağlığı Hakkında Bilgi Düzeyine Etkisinin Değerlendirilmesi

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Keywords
Brochure-based information, computer aided presentation, dental health education, preventive dentistry, early childhood caries

Anahtar Kelimeler
Broşür bazlı bilgilendirme, bilgisayar destekli sunum, dental sağlık eğitimi, koruyucu diş hekimliği, erken çocukluk çağı çürüğü

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Abstract

Objective: The healthy habits that are created by parents during infancy play an important role in preventing oral and dental health problems, which cause decreased quality of life in children. In this study, the purpose was to evaluate the effectiveness of two different educational methods by providing oral and dental health training with computer-aided presentation (CAP) and Brochure-Based Information (BBI) methods for mothers who have 0-3 age group of children.

Materials and Methods: A total of 90 mothers who had children between the ages of 0-3 participated in this study. Before the training, the participants were asked to fill in the questionnaire forms that contained information on their demographic characteristics and oral hygiene practice knowledge, and the Pre-test (PreT) questionnaire forms that consisted of 12 questions to measure the information levels on oral and dental health of the participants. The participants were asked the same questions again immediately after the training (PostT1), and 3 months later (PostT2). Comparisons were made between the two types of training in terms of PreT, Post, T1 and Post-T2 follow-up periods. Additionally, time-dependent changes were calculated for each group. The chi-square, Mann-Whitney U, and McNemar test's were used during the statistical analyses. The $p<0.05$ level was considered significant in all analyses.

Results: In the PreT, no differences were found between the groups ($p>0.05$). The CAP group participants were found to be more successful in Q3 and Q4 in the PostT2 ($p<0.05$). Moreover, BBI group participants were found to be more successful in the PostT1 for Q12 ($p<0.05$) and for Q2, Q5, Q11, and Q12 in the PostT2. In some questions, the time dependent responses also significantly altered the CAP and BBI results.

Conclusion: It can be predicted that mothers have a lack of knowledge about the topics of the first dentist visit, early childhood caries and the use of fluoride toothpaste in children. The usage of the BBI method could have more successful outcomes in increasing the knowledge and awareness status of mothers compared to the CAP.

Öz

Amaç: Çocuklarda yaşam kalitesinin düşmesine neden olan ağız ve diş sağlığı problemlerinin önlenmesinde ebeveynlerin bebeklik döneminde oluşturdukları sağlıklı alışkanlıkların önemli rolleri vardır. Bu araştırmada, 0-3 yaş grubu çocuğu

olan annelere bilgisayar destekli sunum (BDS) ve broşür bazlı bilgilendirme (BBB) yöntemleriyle ağız ve diş sağlığı eğitimi verilerek iki farklı eğitim yönteminin etkinliğinin karşılaştırmalı olarak değerlendirilmesi amaçlandı.
Gereç ve Yöntemler: Araştırmaya 0-3 yaş arası çocuğu bulunan 90 anne katıldı. Eğitim öncesinde katılımcılardan içerisinde demografik özellikler ile oral hijyen uygulama bilgilerinin yer aldığı anket formları ve katılımcıların ağız ve diş sağlığı ile ilgili bilgi düzeylerini ölçmeyi amaçlayan 12 sorudan oluşan ön test (ÖT) anket formlarını doldurmaları istendi. Katılımcılara eğitimden hemen sonra (ST1) ve 3 ay sonra (ST2) aynı sorular tekrar soruldu. Her iki eğitim tipi arasında ön test, son test 1 ve son test 2 takip dönemleri açısından gruplar arasında karşılaştırmalar yapıldı. Ayrıca her bir grupta zamana bağlı değişimler hesaplandı. İstatistiksel analizler sırasında ki-kare, Mann-Whitney U ve McNemar testleri kullanıldı. Tüm analizlerde $p<0,05$ düzeyi anlamlı olarak kabul edildi.
Bulgular: ÖT'de gruplar arasında fark bulunmadı ($p>0,05$). BDS grubu katılımcılarının ST2 anketindeki 3. ve 4. sorularda daha başarılı oldukları görüldü ($p<0,05$). Ayrıca BBB grubu katılımcılarının ise ST1'de 12. soruda ($p<0,05$) ve ST2'de 2, 5, 11 ve 12. sorularda daha başarılı oldukları görüldü.
Sonuç: Annelerin ilk diş hekimi ziyareti, erken çocukluk çürükleri ve çocuklarda florlu diş macunu kullanımı konularında bilgi eksikliğine sahip oldukları tahmin edilebilir. BDS'ye göre BBB yönteminin kullanılması, annelerin bilgi ve farkındalık durumlarını artırmak için daha başarılı sonuçlara sahip olabilir.

Introduction

The dental caries during the primary teething period is a preventable and reversible process. However, if left untreated, pain, bacteremia risk, growth and developmental disorders, speech disorders, lack of self-confidence and damage to permanent teeth may occur (1).

Researchers reported that the main reason of the poor oral health of children might be inadequate knowledge and attitudes about oral and dental health of parents (especially mothers) and caregivers (2). Programs that promote health in early childhood are needed to be implemented to prevent the formation of dental caries (3). It has been shown in previous studies that there have been significant decreases in the incidence of dental caries among children groups who benefit from preventive programs (4-6). Although it is known that mothers have important roles in the care of her children, and that the main risk factors for many diseases are related with the lifestyle habits, it is considered that the trainings that will be provided especially for parents or caregivers with children between the ages of 0-3 may also reduce the risk of future dental problems (7-10). For this reason, parents and caregivers must have the right knowledge and attitude about oral health from as early periods as possible to give their children healthy oral habits.

When evaluated in terms of educational strategies, it was reported in previous studies that computer or web-assisted implementations (11), brochure-based trainings, face-to-face training and motivational interviews are necessary especially for pediatric age groups to protect and sustain oral health (12). Researchers evaluate and analyze various training techniques in a comparative manner (12-14). In general, the trainings that are given and repeated may increase oral health, the level of knowledge and the motivation in a much more comfortable way especially in non-hospital settings (13-15).

The hypotheses to be tested in this study are;

1- There may be differences between computer-aided presentation (CAP) and brochure-based information (BBI) training methods in terms of each question,

2- The educational methods can be different for each question in the tests to be applied in the pretests and post-tests (PostT) that will be applied in the CAP and BBI groups.

Materials and Methods

Study Design

The present study was conducted in a comparative manner with "Pre-test (PreT) - PostT model". CAP and BBI training types were evaluated comparatively.

In the present study, the CAP and face-to-face brochure-based oral health trainings were provided to the mothers with children between the ages of 0-3 in an isolated non-clinical/non-hospital setting. The trainings were carried out from August to November 2018. The approval of the Ethics Committee that was required for the study was obtained from the Directorate of the Ethics Committee of Clinical Research of the Faculty of Medicine of the Black Sea Technical University (protocol no: 2018/22; date: 02.03.2018).

Oral Health Training

Before the mothers were provided with oral dental health training, informed consent forms was read and signed by the participants in the scope of the study. The questions were compiled from previous studies on this subject in the questionnaire (7,16,17). The form including demographic variables, oral hygiene habits were recorded. Also, the questionnaires consisted of 12 questions was applied on the oral dental health of children (Attachment 1). The timeline training events were given in Figure 1. The oral-dental health training in the form of CAP was carried out with 7-8 people in groups in 6 sessions in the form of a collective presentation with the help of projector, the BBI about oral and dental health was completed with sessions held in 5 different days by interviewing the participants face-to-face with the brochures. After the questionnaires were completed, the mothers were given toothbrushes and fluoride-free toothpastes for their children.

Sample size calculation and randomization

The sampling size was calculated by using G Power 3.1.9.2 (Universitate Kiel, Germany) programme. The confidence interval =95% and the beta error =0.01 were used for the calculation based on the study of Hallas et al. (7) and the total number of sampling was determined as 80. However by considering the potential data losses, the averaged final number was set as 100. Each group was divided into groups with computer-assisted randomization programme for 50 mothers.

Attachment 1. Oral and dental health survey questions	
Q1	The results of oral health problems in children
Q2	Whether the cause of tooth caries bacterial origin
Q3	Primary teeth eruption time
Q4	The first dentist examination time
Q5	Dentist examination frequency
Q6	How healthy milk teeth look
Q7	Early childhood caries risk factors related to nutrition
Q8	Baby's tooth cleaning
Q9	Time of the children alone tooth brushing time
Q10	Fluoride toothpaste use age
Q11	Choosing snacks for kids
Q12	Relationship between nutrition and caries in children

Statistical Analysis

The SPSS (Statistical Package for Social Sciences, SPSS Inc. Chicago, IL, USA) 17.0 Statistical Package Program was used to analyze the data that were obtained in the study. The Kolmogorov-Smirnov, Mann-Whitney U, chi-square and McNemar tests were used for data analysis. The $p<0.05$ was considered to be significant in all analyses.

Results

A total of 100 participants who were identified as the targets were reached by telephone and informed about the study in the scope of our study. Ninety of those invited to the study participated in the study and 47 mothers were included in the CAP Group and 43 mothers in the BBI Group. The flowing diagram of the study was given in Figure 2.

The distributions of the socio demographic data of the mothers who participated in the study are shown in Table 1. In this context, there was only a statistically significant difference between the educational and occupational status of the mothers between CAP and BBI group ($p<0.001$), and no significant differences were detected in terms of the number of children between 0-3 years of age, the number of children in other age groups, the genders of the children between 0-3 years of age monthly income of the family, and the ages of the mothers ($p>0.05$). It was determined that 87.2% of the mothers in the CAP group were university graduates, 8.5% were high school graduates; and 44.2% of the mothers in the BBI group were university graduates and 25.6% were high school graduates. In the CAP group, 72.3% of the participants were employed as teachers, and housewives were the most common occupation group in the BBI group 60.5% (Table 1). When the oral hygiene habits of the mothers and their children were evaluated, no significant differences were detected between CAP and BBI groups ($p>0.05$) (Table 2).

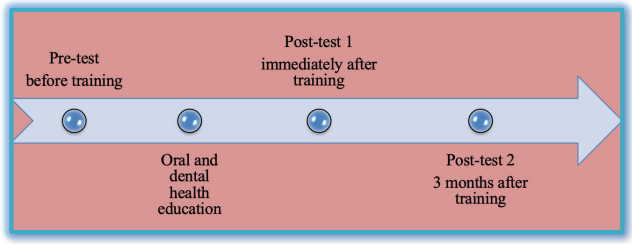

Figure 1. Timeline of study events

Table 1. Socio-demographic distribution of participants by groups

	CAP (n=47)	BBI (n=43)	Total (n=90)	[1]p,[2]p,[3]p
	n (%)	n (%)	n (%)	
Mother education				
Primary	2 (4.3%)	9 (20.9%)	11 (12.2%)	
High school	4 (8.5%)	11 (25.6%)	15 (16.7%)	[1]p<0,001
University	41 (87.2%)	19 (44.2%)	60 (66.7%)	
Master's degree	0 (0%)	4 (9.3%)	4 (4.4%)	
Number of children 0-3 years				
1 child	43 (91.5%)	38 (88.4%)	81 (90%)	[2]p=0.443; p>0.05
2 child	4 (8.5%)	5 (11.6%)	9 (10%)	
Number of children in other age				
0	23 (48.9%)	22 (51.2%)	45 (50%)	
1 child	15 (31.9%)	11 (25.6%)	26 (28.9%)	[2]p=0.790; p>0.05
2 child	7 (14.9%)	9 (20.9%)	16 (17.89%)	
3 child	2 (4.3%)	1 (2.3%)	3 (3.3%)	
Child gender				
1 girl	21 (44.7%)	18 (41.9%)	39 (43.3%)	
1 boy	22 (46.8%)	20 (46.5%)	42 (46.7%)	
2 girls	2 (4.3%)	1 (2.3%)	3 (3.3%)	[2]p=0.469; p>0.05
2 boys	0 (0%)	1 (2.3%)	1 (1.1%)	
1 boy ande 1 girl	0 (0%)	2 (4.7%)	2 (2.2%)	
Unknown answer	2 (4.3%)	1 (2.3%)	3 (3.3%)	
Average family monthly income				
0-2000 TL	3 (6.4%)	3 (7.0%)	6 (6.7%)	[2]p=0.060; p>0.05
2000-5000 TL	14 (29.8%)	23 (53.5%)	37 (41.1%)	
≥5000 TL	30 (63.8%)	17 (39.5%)	47 (52.2%)	
Mother occupation				
Teacher	34 (72.3%)	4 (9.3%)	38 (42.2%)	
Computer programmer	1 (2.1%)	0 (0%)	1 (1.1%)	
Doctor	1 (2.1%)	2 (4.7%)	3 (3.3%)	
Banker	1 (2.1%)	1 (2.3%)	2 (2.2%)	
Medical secretary	1 (2.1%)	1 (2.3%)	2 (2.2%)	
Lawyer	1 (2.1%)	0 (0%)	1 (1.1%)	
Teknician	0 (0%)	1 (2.3%)	1 (1.1%)	[1]p<0.001
Nurse	0 (0%)	2 (4.7%)	2 (2.2%)	
Housewife	8 (17%)	26 (60.5%)	34 (37.8%)	
Student	0 (0%)	1 (2.3%)	1 (1.1%)	
Architect	0 (0%)	1 (2.3%)	1 (1.1%)	
Business	0 (0%)	1 (2.3%)	1 (1.1%)	
Chemist	0 (0%)	1 (2.3%)	1 (1.1%)	
Operation responsible	0 (0%)	1 (2.3%)	1 (1.1%)	
Akademician	0 (0%)	1 (2.3%)	1 (1.1%)	
Mean age (minimum - maximum)	33.06±4.204 (25-46)	32.23±4.529 (25-43)		[3]p=0.153 p>0.05

[1]p: Pearson chi-square, [2]p: Fisher's Exact test, [3]p: Mann-Whitney U tests were performed. Values of p<0.05 were considered statistically significant. CAP: Computer aided presentation, BBI: Brochure based ınformation, TL: Turkish lira

The comparison between the groups based on the correct answers given by the participants in both groups to the questions in the questionnaire in the PreT, PostT1 and PostT2 is shown in Table 3. In this respect, no statistically significant differences were detected between the CAP and BBI groups in 12 questions answered in the PreT questionnaire (p>0.05), and it was determined that the BBI group was more successful in the Q12 (p=0.030) in the PostT1, and the BBI group was more successful in the in the Q2 (p=0.021), Q5 (p=0.044), Q11 (p=0.042) and Q12 (p=0.048) in the PostT2 questionnaire; and the CAP group was more successful in the Q3 (p=0.035) and in the Q4 (p=0.048) in the PostT2 questionnaire.

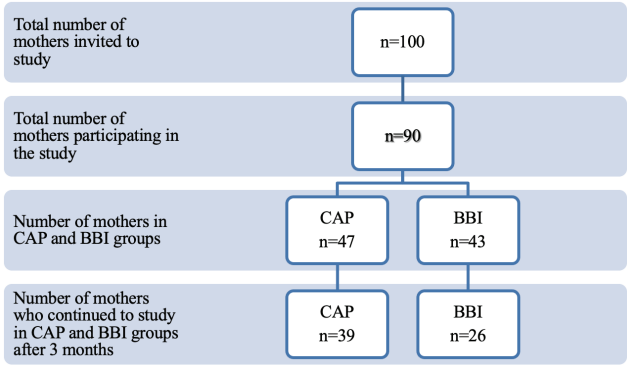

Figure 2. Distribution of the number of participants at baseline and follow-up periods
CAP: Computer aided presentation, BBI: Brochure based ınformatio

The correct answer numbers and rates of the mothers who participated in the study in CAP and BBI Groups are shown in Table 4. In this respect, in the CAP Group, "dental caries are bacterial (Q2)" and "the time when children started to brush their teeth (Q9)" was the questions that were most correctly answered (83%), and it was determined that the rate of the correct answers given to the question was the least with a rate of 17% in the question "the age of using fluoride toothpaste (Q10)"; however, it was also determined that the rate of this question increased at a significant level after the training. When the question-based intra-group changes were examined in the CAP Group, it was determined that there were statistically significant increases in the PreT and PostT1 in the Q1 (p<0.001), Q3 (p=0.008), Q4 (p<0.001), Q5 (p=0.027), Q6 (p=0.006), Q7 (p<0.001), Q10 (p<0.001) and Q11 (p=0.003); and between the PreT and PostT2 in the Q7 (p=0.012), Q10 (p=0.021) and Q12 (p=0.039); and there was a decrease at a statistically significant level in the Q1 (p=0.016) and Q4 (p=0.022) between the PostT1 and PostT2 questionnaires.

In the BBI group, "dental caries are bacterial (Q2)" was the most correctly answered question with a rate

Table 2. Oral hygiene habits

	CAP (n=47)	BBI (n=43)	Total (n=90)	2p
Oral hygiene habits of the mothers	**Yes (percent)**	**Yes (percent)**	**Yes (percent)**	
Would you go to the regular dentist twice a year?	16 (34%)	10 (23.3%)	26 (28.9%)	[2]p=0.186; p>0.05
Do you brush your teeth regularly twice a day?	41 (87.2%)	38 (88.4%)	79 (87.8%)	[2]p=0.564; p>0.05
Do you use additional products such as floss, mouthwash?	27 (57.4%)	23 (53.5%)	50 (55.6%)	[2]p=0.434; p>0.05
Do you consume more than one snack per day between the main meals?	34 (72.3%)	29 (67.4%)	63 (70%)	[2]p=0.391; p>0.05
Oral hygiene practices for your child				
Do you clean your child's teeth?	40 (85.1%)	30 (69.8%)	70 (77.8%)	[2]p=0.067; p>0.05
Do you add sugar-containing foods to the food you give to your child?	17 (36.2%)	16 (37.2%)	33 (36.7%)	[2]p=0.546; p>0.05
Have you ever taken your child to a dentist?	15 (31.9%)	9 (20.9%)	24 (26.7%)	[2]p=0.174; p>0.05

[2]p: Fisher's Exact test was performed. Values of p<0.05 were considered statistically significant. CAP: Computer aided presentation, BBI: Brochure based information

Table 3. Comparison of oral health information questionnaire between the groups in the beginning and follow-up periods

	PreT	PostT1	PostT2
Q1.'The results of oral health problems in children'	p>0.05	p>0.05	p>0.05
Q2.'Whether the cause of tooth caries bacterial origin'	p>0.05	p>0.05	[2]p=0.021; p<0.05
Q3.'Primary teeth eruption time'	p>0.05	p>0.05	[2]p=0.035; p<0.05
Q4.'The first dentist examination time'	p>0.05	p>0.05	[2]p=0.048; p<0.05
Q5.'Dentist examination frequency'	p>0.05	p>0.05	[2]p=0.044; p<0.05
Q6.'How healthy milk teeth look'	p>0.05	p>0.05	p>0.05
Q7. 'Early childhood caries risk factors related to nutrition'	p>0.05	p>0.05	p>0.05
Q8.'Baby's tooth cleaning'	p>0.05	p>0.05	p>0.05
Q9. 'Time of the children alone tooth brushing time'	p>0.05	p>0.05	p>0.05
Q10.'Fluoride toothpaste use age'	p>0.05	p>0.05	p>0.05
Q11.'Choosing snacks for kids'	p>0.05	p>0.05	[2]p=0.042; p<0.05
Q12.'Relationship between nutrition and caries in children'	p>0.05	[2]p=0.030; p<0.05	[2]p=0.048; p<0.05

[2]p: Fisher's Exact test was performed. Values of p<0.05 were considered statistically significant. PreT: Pre-test, PostT1: Post-test 1, PostT2: Post-test 2

of 88%. The least correctly answered question was the "early childhood caries (ECC) risk factors (Q7)" with a rate of 16%; however, it was also determined that it increased at a significant level right after the training. It was determined that the number of the correct answers given to all questions increased in the PostT1 questionnaire compared to the PreT questionnaire. When the PreT and PostT1 questionnaire answers were compared, it was determined that there were significant improvements in 8 questions. When the time-dependent variations were compared between the questionnaires in the BBI group, statistically significant increases were detected between the PreT and PostT1 in the Q1 (p<0.001), Q4 (p<0.001), Q5 (p<0.001), Q6 (p=0.013), Q7 (p<0.001), Q10 (p<0.001), Q11 (p<0.001) and Q12 (p=0.002); and between the PreT and PostT2 in the Q1 (p=0.016), Q6 (p=0.006), Q7 (p=0.012) and Q12 (p=0.006); and there were decreases at significant levels between the PostT1 and PostT2 in the Q4 (p=0.008), Q7 (p=0.039) and Q10 (p<0.001) (Table 4).

Sixty-five of the 90 mothers who participated in the study completed the study by also filling out the PostT2 questionnaire, 39 of these participants were CAP group and 26 were BBI group participants. To determine whether the attendance in our study had an effect on the outcomes of the study, intra-group and intergroup comparisons were made for the data of continuing (n=65) and non-continuing (n=25) mothers. No significant differences were detected between the participants who continued and who did not continue (missing) in any of the items in which the demographic data and oral hygiene habits were questioned in the intra-group comparisons. When the questions in the questionnaires were evaluated, it was determined that there were significant differences in terms of the continuing mothers only in the 5th question in the PreT questionnaire in the CAP group (p=0,028), and in the 6th question (p=0.029) in the PostT1 questionnaire in the BBI group.

As a result of the sensitivity analysis made by substituting the missing data in the responses of the participants who did not continue (missing) the study in the pre-training questionnaires with the answers given in the PostT2 questionnaire, which was carried out 3 months after the training, it was determined that there were no statistically significant in any of the 12 questions in the PostT2 questionnaire between the CAP and BBI groups (p>0.05)

Discussion

As a result of our study, it was determined in the inter-group and intra-group comparisons that there were no significant differences in all the questions; for this reason, the two hypotheses that were tested were not

Table 4. Intra-group comparison of oral health information questionnaire at baseline and two follow-up periods

	PreT		PostT1		PostT2		PreT-postT1		PreT-postT2		PostT1-PostT2	
	CAP	BBI	CAP	BBI	CAP	BBI	CAP	BBI	CAP	BBI	CAP	BBI
	n=47	n=43	n=47	n=43	n=39	n=26	[4]p	[4]p	[4]p	[4]p	[4]p	[4]p
Questions												
Q1. The results of oral health problems in children	31 (66%)	24 (56%)	46 (98%)	39 (91%)	31 (79%)	22 (85%)	[4]p<0.001	[4]p<0.001	[4]p=0.065 p>0.05	[4]p=0.016 p<0.05	[4]p=0.016 p<0.05	[4]p=0.625 p>0.05
Q2. Whether the cause of tooth caries bacterial origin	39 (83%)	38 (88%)	44 (94%)	40 (93%)	33 (85%)	25 (96%)	[4]p=0.18 p>0.05	[4]p=0.727 p>0.05	[4]p=0.754 p>0.05	[4]p=0.625 p>0.05	[4]p=0.219 p>0.05	[4]p=1.00 p>0.05
Q3. Primary teeth eruption time	38 (81%)	34 (79%)	46 (98%)	39 (91%)	35 (90%)	21 (81%)	[4]p=0.008 p<0.05	[4]p=0.180 p>0.05	[4]p=0.453 p>0.05	[4]p=1.00 p>0.05	[4]p=0.375 p>0.05	[4]p=0.375 p>0.05
Q4. The first dentist examination time	21 (45%)	17 (40%)	43 (91%)	39 (91%)	27 (69%)	16 (61%)	[4]p<0.001	[4]p<0.001	[4]p=0.134 p>0.05	[4]p=0.227 p>0.05	[4]p=0.022 p<0.05	[4]p=0.008 p<0.05
Q5. Dentist examination frequency	31 (66%)	24 (56%)	42 (89%)	41 (95%)	30 (77%)	22 (85%)	[4]p=0.027 p<0.05	[4]p <0.001	[4]p=1.00 p>0.05	[4]p=0.109 p>0.05	[4]p=0.227 p>0.05	[4]p=0.125 p>0.05
Q6. How healthy milk teeth look	31 (66%)	24 (56%)	41 (87%)	34 (81%) n=42	31 (79%)	22 (85%)	[4]p=0.006 p<0.05	[4]p=0.013 p<0.05	[4]p=0.146 p>0.05	[4]p =0.006 p<0.05	[4]p=0.375 p>0.05	[4]p=0.688 p>0.05
Q7. ECC risk factors related to nutrition'	13 (28%)	7 (16%)	36 (76%)	35 (81%)	23 (59%)	14 (54%)	[4]p<0.001	[4]p<0.001	[4]p=0.012 p<0.05	[4]p =0.012 p<0.05	[4]p=0.302 p>0.05	[4]p=0.039 p<0.05
Q8. Baby's tooth cleaning	31 (66%)	27 (63%)	29 (62%)	32 (74%)	28 (72%)	19 (73%)	[4]p=0.791 p>0.05	[4]p=0.302 p>0.05	[4]p=0.508 p>0.05	[4]p=0.388 p>0.05	[4]p=0.549 p>0.05	[4]p=0.688 p>0.05
Q9. Time of the children alone tooth brushing time	40 (83%)	32 (74%)	41 (87%)	34 (79%)	31 (79%)	22 (85%)	[4]p=0.727 p>0.05	[4]p=0.774 p>0.05	[4]p=1.00 p>0.05	[4]p=0.344 p>0.05	[4]p=0.688 p>0.05	[4]p=1.00 p>0.05
Q10. Fluoride toothpaste use age	8 (17%)	8 (19%)	29 (62%)	34 (79%)	16 (41%)	9 (35%)	[4]p<0.001	[4]p<0.001	[4]p=0.021 p<0.05	[4]p=0.388 p>0.05	[4]p=0.146 p>0.05	p<0.001
Q11. Choosing snacks for kids	30 (64%)	24 (56%)	41 (87%)	91 (39%)	28 (72%)	21 (81%)	[4]p=0.003 p<0.05	[4]p<0.001	[4]p=0.344 p>0.05	[4]p=0.039 p>0.05	[4]p=0.109 p>0.05	[4]p=0.375 p>0.05
Q12. Relationship between nutrition and caries in children	33 (68%)	28 (65%)	36 (76%)	40 (93%)	34 (87%)	24 (92%)	[4]p=0.481 p>0.05	[4]p=0.002 p<0.05	[4]p=0.039 p<0.05	[4]p=0.006 p<0.05	[4]p=0.267 p>0.05	[4]p=1.00 p>0.05

[4]p: McNemar test was performed. Values of p<0.05 were considered statistically significant. CAP: Computer aided presentation, BBI: Brochure based information, PreT: Pre-test, PostT1: Post-test 1, PostT2: Post-test 2, ECC: Early childhood caries

accepted for all questions, and were partially accepted.

Our study was conducted with a 72% participation rate in the 3-month follow-up period with a total of 90 mothers in two groups. Although the number of the participants was determined to be 100 in the sampling size calculation (7), 90 mothers were able to participate in the study, and in addition to the insufficient interest in the training through mass media, the fact that the society does not pay adequate attention for this issue may suggest that it is effective for mothers to engage in the caretaking of their children, and not be able to devote time to train them because of the criteria of having children between the ages of 0-3. A total of 65 of the 90 mothers who participated in

the study completed the study by filling in the PostT2 questionnaire in the 3rd month after the training.

In a similar study that was conducted in Iran, it was determined that the rate of participants who completed the study at the end of 3-month follow-up period was 80% (18), and in a study that was conducted by Hoeft et al. (19), they reached a follow-up rate of 75% at the end of 3 months. In the study that was conducted by Hallas et al. (7), only 10 of the 94 mothers participated in the continuation of the study. For this reason, it may be considered that the 72% participation rate in the study is acceptable. When the socio-demographic characteristics of the two groups were compared, it was determined that there is only a statistically significant difference between the mothers' educational status ($p<0.001$) and their professions ($p<0.001$); this and the lack of significant differences between the oral hygiene practices of the parents and their children and the answers given by them to initial questionnaires show that the participants of the two groups have very similar characteristics.

In the present study, the purpose was to compare only the effectiveness of the methods by using the same information and the same visuals in the trainings given to both groups. The participants in the BBI group in which the face-to-face training model was applied were trained with brochures during the training; however, these brochures were not given to the participants considering that the 3rd month check-ups would be carried out by e-mail and telephone, and not to create bias when comparing the sustainability of the information acquired in education. In a study that evaluated the oral health training conducted by Makvandi et al. (18), the mothers in the intervention group were informed by a booklet, and this booklet was given to the mothers to re-read them at an appropriate time and remember the messages given. In this study, different from the present study of ours, it was observed that there was only one intervention group. In the scope of our study, the values in the three periods were compared by conducting a questionnaire before, immediately after the training and 3 months after the training to determine whether the changes in the level of knowledge were sustainable. However, there are also researchers who recommend longer-term studies (7,20).

According to the answers given to the questionnaires by CAP and BBI group participants, no statistically significant differences were detected in any question in the PreT questionnaire ($p>0.05$), and both groups were similar in terms of the answers to the questions and in terms of the initial knowledge levels. It was determined that BBI group participants gave more accurate answers at significant levels to the in the Q12 ($p=0.030$) in the PostT1 questionnaire, and in the Q2 ($p=0.021$), Q5 ($p=0.044$), Q11 ($p=0.042$) and Q12 ($p=0.048$; $p<0.05$) in the PostT2 questionnaire; and CAP group participants gave more accurate answers at significant levels in the Q3 ($p=0.035$) and Q4 ($p=0.048$) in the PostT2 questionnaire. In the light of these results, it may be considered that there was a significant increase in the knowledge levels of the participants in the BBI group than of the participants of the CAP group; and that the training with BBI was more successful. Meanwhile, considering the higher educational status of the CAP group participants ($p<0.001$), it can be predicted that the participants' educational status was not effective as a result of our study.

In a study conducted by Hallas et al. (7), before the oral health training, the mothers were asked "do you think fluoride is safe and it helps prevent dental caries as your baby grows?", and 71.6% of the mothers said "yes"; and they were also asked "Do you think that dentist examination is important when your baby reached the age of 1, and 89.2% of the mothers said "yes" at a rate of 89.2%. When Weber and Gasparoni (21) questioned whether mothers who had children between 12-49 months used fluoride toothpaste for their children, it was observed that this rate increased after the training, and 30% of the participants initially answered "yes".

It was reported in another study that was conducted in Latin population that the correct answer rate was initially lower when the relationship between the development of caries and nutrition was questioned (21). In our study, the questions that were asked to the participants before the training were given a lower percentage about the question when the first dentist examination should be (Q4), ECC risk factors (Q7), and fluoride toothpaste use (Q10). It is observed that the correct answer rates given to these questions increased at significant levels compared to the initial values after the training in both the CAP and BBI groups.

The absence of a control group in the study might be considered as a limitation; however, the evaluation of the time-dependent changes in each group, in other words, the Quasi-experimental design of the study eliminated the need for a control group. Although the loss of participants in the follow-up periods appeared before us as another limitation, when we compared the continuing and non-continuing (missing) participants, no differences that could change the results of the study could be detected for each group. However, increasing the continuing participant rates in future studies will be useful.

Conclusion

It can be foreseen that both methods will increase the level of knowledge, but awareness may be higher due to the use of BBI in oral and dental health training that will be given to mothers. In addition, it was determined that missing knowledge was less in the time of the first dentist examination in children (Q4), the nutritional risk factors of the ECC (Q7), fluoride toothpaste use (Q10) compared to the other questions, and the long-term persistence of the knowledge learnt was less compared to the other questions. For this reason, it may be considered useful to allocate more time to these topics in the studies and social education programs that will be planned in the future.

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Ethics

Ethics Committee Approval: The approval of the Ethics Committee that was required for the study was obtained from the Directorate of the Ethics Committee of Clinical Research of the Faculty of Medicine of the Karadeniz Technical University (protocol no: 2018/22; date: 02.03.2018).

Informed Consent: Before the mothers were provided with oral dental health training, informed consent forms was read and signed by the participants in the scope of the study.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Ö.F.G., T.T., Concept: Ö.F.G., T.T., F.K., Design: Ö.F.G., T.T., Ö.B., F.K., Data Collection or Processing: Ö.F.G., A.K., Analysis or Interpretation: Ö.F.G., T.T., Ö.B., Literature Search: Ö.F.G., T.T., A.K., Writing: Ö.F.G., T.T.

Conflict of Interest: No conflict of interest was declared by the authors.

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Original Article / Özgün Araştırma

The Effect of Hypericum Perforatum on Alveolar Bone Healing After Tooth Extraction

Hypericum Perforatum'un Diş Çekimi Sonrası Alveolar Kemik İyileşmesine Etkisi

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Keywords
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Abstract

Objective: Hypericum perforatum L. (HP) (Hypericaceae) is used as a wound healing agent especially burns. Thus, the aim of this study was to evaluate the effect of an extract of HP on wound healing after tooth extraction with clinical, radiological and histopathological evaluations.

Materials and Methods: A unilateral upper central incisor was extracted from the rats. The rats were divided into four control and four test groups and were sacrificed 7, 14, 28, and 56 days after tooth extraction. HP was used in the test groups. Wound healing was evaluated clinically, radiologically and histopathologically.

Results: In clinical examinations, HP had a positive effect on wound healing. to the results of histopathological evaluations, with the use of HP, decrease in inflammation, better epithelial proliferation, connective tissue formation and cartilaginous tissue formation occurs. No effect was found on bone healing on radiological examination.

Conclusion: After tooth extraction the use HP extract result in better wound healing by clinical and histopathological evaluations. Further studies are required to evaluate the effects of oral surgery.

Öz

Amaç: Hypericum perforatum L. (HP) (Hypericaceae) özellikle yanıklarda yara iyileştirici ajan olarak kullanılmaktadır. Bu çalışmanın amacı, HP ekstraktının diş çekimi sonrası yara iyileşmesi üzerindeki etkisini klinik, radyolojik ve histopatolojik açıdan değerlendirmektir.

Gereç ve Yöntemler: Sıçanlarda tek taraflı üst orta kesici diş çekildi. Sıçanlar dört kontrol ve dört test grubuna ayrıldı ve diş çekildikten 7, 14, 28 ve 56 gün sonra sakrifiye edildi. Test gruplarında HP kullanıldı. Yara iyileşmesi klinik, radyolojik ve histopatolojik olarak değerlendirildi.

Bulgular: Klinik incelemelerde HP'nin yara iyileşmesi üzerinde olumlu etkisi vardır. Histopatolojik değerlendirme sonuçlarına göre HP kullanımı ile enflamasyonda azalma, daha iyi epitel proliferasyonu, bağ dokusu oluşumu ve kıkırdak dokusu oluşumu meydana gelir. Radyolojik incelemelerle kemik iyileşmesinde herhangi bir etki görülmedi.

Sonuç: Diş çekiminden sonra HP ekstraktının kullanılması klinik ve histopatolojik değerlendirmelere göre daha iyi yara iyileşmesi sağlamaktadır. Ağız diş ve çene cerrahisindeki etkilerin değerlendirilmesi için daha ileri çalışmalara ihtiyaç vardır.

Introduction

Successful wound healing involves initial inflammation, followed by cell proliferation, angiogenesis, epithelialization, and remodeling (1). In recent years, the use of herbal products to aid wound healing has increased; some of those products affect the various stages of healing and/or exert antimicrobial and antioxidant effects (2).

Hypericum perforatum L. (HP) has long been used as a folk medicine to accelerate the healing of wounds. Approximately 400 species of Hypericum from Europe, North America, Australia, New Zealand, Eastern Asia, and South America have been identified. Hypericum, a member of the Guttiferae family, grows in sunny areas and attains a height of 50-100 cm (3). The plant exhibits several pharmacological activities, including antimicrobial (antibacterial, antiviral, and antifungal), anti-inflammatory, and analgesic activities. Hypericum has traditionally been used to treat dermal injuries, to aid wound healing, to sterilize wounds prior to surgery (4-6), and to treat psychiatric problems like anxiety and depression due to its sedative effect (7). Recently, HP is also used to treat skin injuries and burns, especially injuries to soft tissue in modern times (8-11).

Beneficial effects of topical application of HP have been reported in both animal (12) and human (8) studies. However, research on HP in the field of dentistry is limited. Paterniti et al. (13) investigated the effect of HP on periodontitis in rats, Raak et al. (3) performed a systematic review on the use of HP for pain conditions, and Mendi et al. (14) evaluated the effect of HP on the differentiation of dental pulp *in vivo*. In this study, we evaluated the effect of HP on wound healing after tooth extraction by performing clinical, radiological, and histological analyses.

Materials and Methods

Preparation of HP Extract

Aerial parts of HP were collected around Kayseri/Turkey, washed, dried at room temperature, and stored in the Herbarium of the Faculty of Pharmacy, Erciyes University, Kayseri, Turkey. A total of 25 g of dried and crushed material was extracted into 500 mL methanol for 8 h using a Soxhlet extractor, evaporated to dryness at 40 °C in a rotary evaporator, and lyophilized.

Animals and Tooth Extraction

The experimental protocols were approved by the Erciyes University Animal Ethical Committee, Kayseri, Turkey (approval number: 11/124, date: 23.11.2011). We used 48 Wistar Albino rats (mean age, 1 year; weight, 250 g). The rats were housed in a temperature-controlled room (22±2 °C) under a 12/12 h light/dark cycle with free access to food and water, and were randomly divided into eight groups of six animals each (four test and four control groups).

Test Groups

Test group 1: The unilateral upper central incisor was extracted from rats. hp extract (0.2 cc) was inserted into the extraction sockets and the mucosae were sutured using 3.0 resorbable sutures. the rats were sacrificed 7 days after tooth extraction. the surgeries were performed under general and topical anesthesia. ketamine (ketalar; Pfizer, New York, NY, USA) (50 mg/kg) and xylazine (Rompun, Bayer, Leverkusen, Germany) (8 mg/kg) were used for general anesthesia, and articaine (Ultracain DS; Hoechst AG, Frankfurt, Germany) (0.2 mL) for local anesthesia and hemostasis.

Test group 2: Same as test group 1, but rats were sacrificed 14 days after tooth extraction.

Test group 3: Same as test group 1, but rats were sacrificed 28 days after tooth extraction.

Test group 4: Same as test group 1, but rats were sacrificed 56 days after tooth extraction.

Control groups

Control group 1: Same as test group 1, but HP extract was not used.

Control group 2: Same as test group 2, but HP extract was not used.

Control group 3: Same as test group 3, but HP extract was not used.

Control group 4: Same as test group 4, but HP extract was not used.

All surgeries were performed by the same operator. The surgical area was evaluated for any sign of infection. Animals with ≥15% weight loss or wound infection were excluded. There were animal losses in control groups 1 and 4 (one in each group).

Clinical Observation

Extraction socket healing was evaluated, and mucosal closure was graded as none (-), partial (+), or complete (++). Complete mucosal closure was graded as "complete", partial closure without complete

primer mucosal healing as "partial closure", and no closure of the socket or sign of alveolar osteitis as "none."

Radiological Examination

The maxillae were dissected out and fixed in 10% (v/v) formalin. All maxillae without decalcification were evaluated by micro-computed tomography (CT) (SkyScan 1172; SkyScan, Kontich, Belgium).

Sections were scanned using a 0.5 mm aluminum and copper filter (80 kV, 124 mA, rotation of 360° rotation step of 0.40°). The approximate scanning time per section was 70 minutes. Images with a resolution of 2,000×2,000 and pixel size of 13.68 µm were obtained. NRecon 1.6.9.4 (SkyScan) software was used to eliminate noise and artifacts. SkyScan DataViewer 1.5.0 (SkyScan) was used to eliminate positional errors in the sagittal, transverse, and vertical directions. The examination areas were limited by determining the first and last transversal section in which the extraction socket was seen using CTAn 1.13.5.1 software (SkyScan). Next, the extraction socket was isolated from the surrounding tissues and air spaces, and the optimal thresholding segmentation was determined.

Bone mineral density, tissue mineral density, formed bone volume (object volume; OV), formed bone percentage (percentage object volume; POV), formed bone intersection surface, formed bone structural thickness, and the "percentage of newly formed bone" (BVC) were evaluated.

Histopathological Evaluation

After micro-CT evaluation, tissue sections containing an extraction socket and surrounding mucosae were prepared for histopathological evaluation. The sections were fixed in 4% formalin solution. Decalcified bone sections were prepared using a cutting/grinding instrument (Exakt 300 CL; Exakt Apparatebau, Norderstedt, Germany) by cutting from the center of the extraction socket in the sagittal plane. Sections were thinned to 70 µm using a micro-abrasion instrument (Exakt 400 CS; Exakt Apparatebau) with 1,000-, 1,200-, and 2,500-mesh abrasion tools. Sections were stained with hematoxylin-eosin and examined under a light microscope (BX50; Olympus, Tokyo, Japan). Epithelial proliferation, inflammatory cell density (number of polymorphonuclear leukocytes), cartilage and bone formation, and collagen fiber density status was classified as none (-), moderate (+), or intense (++).

The clinical, radiological, and histological evaluations were performed independently by two blinded investigators. Any discrepancies were resolved by discussion and consensus.

Statistical Analysis

MINITAB 14 (Minitab Inc., State College, PA, USA) power analysis software was used for sample size determination. The required sample size was determined as five animals per group (α=0.05, 80% power). However, six animals were included in each group to offset any losses. SPSS 21.0 software (SPSS Inc., Chicago, IL, USA) was used for the statistical analysis. Fisher's Exact test, the chi-squared test, or the Mann-Whitney U test were used for between-group comparisons, as appropriate, and $p<0.05$ was taken to indicate statistical significance.

Results

Clinical Observations

At 7 days after extraction, mucosal closure was significantly higher in the test groups than the control groups ($p<0.05$), but not at 14, 28, or 56 days ($p>0.05$).

Radiological Examination

At 7 days after extraction, the OV, POV and BVC values were significantly higher in the control groups than the test groups ($p<0.05$, Figures 1, 2).

Histopathological Evaluation

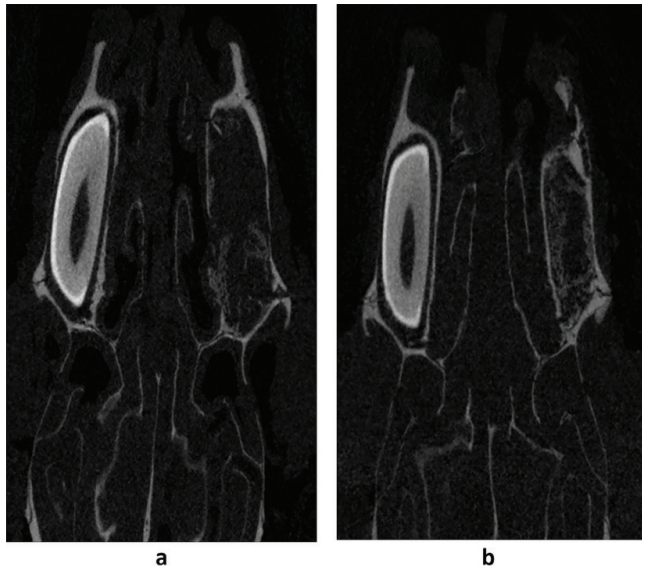

Figure 1. Horizontally section of micro-CT images. (a) 14 days control group. (b) 14 days test group
Micro-CT: Micro-computed tomography

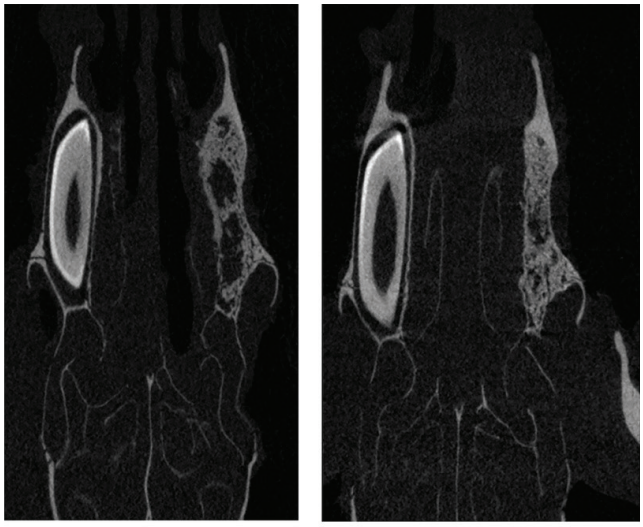

Figure 2. Horizontally section of Micro-CT images. (a) 56 days control group. (b) 56 days test group
Micro-CT: Micro-computed tomography

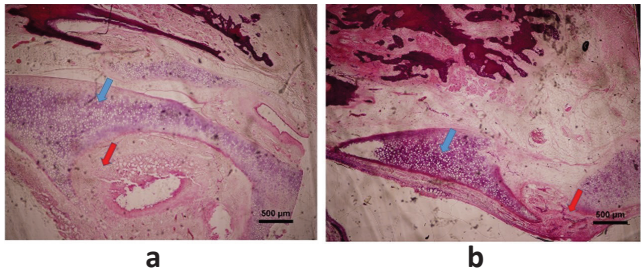

Figure 3. Connective tissue (red arrow) and bone (blue arrow) formation. H&E 500 µm. (a) 28 days control group. (b) 28 days test group
H&E: Hematoxylin and eosin

At 7 days after extraction, epithelial proliferation was significantly greater in the test groups and inflammatory cell infiltration was significantly greater in the control groups ($p<0.05$, Table 1). At 14 days, epithelial proliferation and connective tissue formation were significantly greater in the test groups and inflammatory cell infiltration was significantly greater in the control groups ($p<0.05$, Table 2). At 28 days, connective and cartilaginous tissue formation rates were significantly higher in the test groups ($p<0.05$, Figure 3). At 56 days, the cartilaginous tissue formation rate was significantly higher in the test groups ($p<0.05$).

Discussion

This study assessed the effect of HP extract on wound healing after tooth extraction based on radiological, and histopathological analyses. HP extract promoted wound healing according to the clinical and histopathologic evaluations; however, it did not significantly affect bone healing according to the radiologic evaluation. Therefore, HP may promote wound healing after tooth extraction.

HP increased mucosal closure, but the effect was significant only at 7 days after extraction. Previously, it was reported that HP significantly accelerates wound healing (8-12,15). However, those studies applied HP extract topically to the wound, whereas we delivered the extract directly to the extraction socket. This difference may explain the lower wound healing efficacy of HP in this study.

Histologically, wound healing involves an inflammatory phase, a proliferative phase, and a remodeling phase, all of which can be accelerated by treatment. The inflammatory phase is essential for wound healing, but should not be prolonged because this delays healing (16). In this study, HP extract reduced the severity and duration of the inflammatory phase. The test groups showed significantly lower inflammatory cell infiltration at 7 and 14 days, consistent with prior reports of the anti-inflammatory effect of HP, particularly during early phases of inflammation (10,12,15,17).

In this study, the HP extract promoted epithelial proliferation, and the formation of connective and cartilaginous tissue. Granulation tissue is primarily composed of fibroblasts, collagen, edema, and vessels (11). In the proliferative phase of wound healing, fibroblasts promote scar formation and collagen synthesis. The flavonoids in HP promoted epithelial cell proliferation and migration, and collagen synthesis, and increased the proportion of polygonal fibroblasts (9).

In this study, connective tissue formation peaked at day 14, and decreased at days 28 and 56, in both the test and control groups. During wound healing, collagen density peaks within the first 14 days and decreases gradually thereafter as bone tissue forms (18); this may explain our results. Also, HP reportedly accelerates epithelization in the early stages of primary and secondary wound healing (12,19,20).

Table 1. Analysis of histopathological evaluation results of control and test groups at 7 days time point

	Control group 1			Test group 1		
	-	+	++	-	+	++
Epithelial proliferation*	4 (80%)	1 (20%)	-	1 (16.7%)	4 (66.7%)	1 (16.7%)
Inflammatory cell infiltration†	-	1 (20%)	4 (80%)	-	3 (50%)	3 (50%)
Connective tissue formation	-	4 (80%)	1 (20%)	-	4 (66.7%)	2 (33.3%)
Cartilaginous tissue formation	-	3 (60%)	2 (40%)	-	4 (66.7%)	2 (33.3%)
Bone formation	-	-	-	-	-	-

Percentage of different histologic findings in the control and test groups. * and † indicates statistically significant difference between the control and test groups ($p<0.05$). (-): None, (+): Moderate, (++): Intense

Table 2. Analysis of histopathological evaluation results of control and test groups at 14 days time point

	Control group 2			Test group 2		
	-	+	++	-	+	++
Epithelial proliferation*	2 (33.3%)	3 (50%)	1 (16.7%)	-	3 (50%)	3 (50%)
Inflammatory cell infiltration†	-	2 (33.3%)	4 (66.7%)	-	4 (66.7%)	2 (33.3%)
Connective tissue formation‡	-	4 (66.7%)	2 (33.3%)	-	2 (33.3%)	4 (66.7%)
Cartilaginous tissue formation	-	4 (66.7%)	2 (33.3%)	-	3 (50%)	3 (50%)
Bone formation	-	6 (100%)	-	-	5 (83.3%)	1 (16.7%)

Percentage of different histologic findings in the control and test groups. *, † and ‡ indicates statistically significant difference between the control and test groups ($p<0.05$). (-): None, (+): Moderate, (++): Intense

The flavonoids in HP slow or prevent necrosis and improve vascularity, which reduces lipid peroxidation, increases wound circulation and collagen fiber strength, increases DNA synthesis, and prevents cell injury (11).

Collagen, which is the main structural protein of connective tissue, promotes healing by enhancing connective tissue formation and increasing tissue vascularization (21). Motta et al. (22) reported that collagen has a direct role in fibroblast metabolism and promotes the aggregation of fibroblasts, which in turn promotes collagen lysis and fibroblast proliferation. Furthermore, Öztürk et al. (9) and Hostanska et al. (23) reported that HP promotes wound healing by stimulating collagen synthesis and fibroblast migration, rather than cell proliferation. However, Süntar et al. (10) showed that the promotion of epithelialization and healing by HP is not related to fibroblast proliferation or angiogenesis.

HP did not significantly modulate bone formation in this study, in contrast to several previous studies; the difference may be due to use of differences in study methodologies. Halicioglu et al. (24) reported that systemic use of HP accelerated the formation of new bone in orthopedically expanded premaxillary sutures in rats. Damlar et al. (25) reported that, in rabbits, oil extracts of HP improved bone healing in defects filled with bovine-derived xenograft. Paterniti et al. (13) evaluated the effect of HP extracts on active inflammatory periodontal disease; HP significantly inhibited plasma extravasation and bone resorption during periodontitis.

The presence of an infection may interrupt or slow wound healing. Hyperforin shows antibacterial activity against Gram-positive bacteria (4). The wound healing effect of HP might be related to its antibacterial activity (20).

Conclusion

Within the limitations of this study, HP extract promoted wound healing after tooth extraction, according to clinical and histopathological evaluations. Because HP extract is infrequently used in dentistry and no study has assessed its utility for oral surgery wounds, including tooth extractions, our findings will guide future research on the utility of HP extract in oral surgery.

Ethics

Ethics Committee Approval: The experimental protocols were approved by the Erciyes University Animal Ethical Committee, Kayseri, Turkey (approval number: 11/124, date: 23.11.2011).

Informed Consent: Informed consent is not required.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: F.G.Ç., Concept: O.E., Design: O.E., Data Collection or Processing: F.G.Ç., Analysis or Interpretation: F.G.Ç., Literature Search: F.G.Ç., Writing: F.G.Ç.

Conflict of Interest: No conflict of interest was declared by the authors.

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Original Article / Özgün Araştırma

Comparison of Cameriere and Demirjian Methods for Estimating Adulthood in Turkish Population

Cameriere ve Demirjian Metodlarının Türk Popülasyonunda Erişkin Dönemi Tespit Etmesindeki Tanısal Etkinliklerinin Karşılaştırılması

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Keywords
Age estimation, Turkey, dental age, third molar index, forensic dentistry

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Abstract

Objective: The current study compares diagnostic performances of Demirjian's mineralization stage (DS) and Cameriere's third molar maturity index (I_{3M}) on estimating adulthood in Turkish individuals and to investigate a more suitable cut-off value in a sample Turkish population.

Materials and Methods: A retrospective study was conducted on a sample of digital panoramic images of 512 healthy individuals aged between 14 and 23 years old. The diagnostic capabilities were tested with receiver operating characteristic (ROC) curves, sensivities, specificity were evaluated and post-test probabilities were calculated with Bayes's theorem.

Results: The areas under the ROC were 0.88 for DS and 0.89 for I_{3M}. The sensitivity and the specifities of H stage were 41.4%, 97.9% for females and 64.3%, 93.4% for males, and I_{3M}<0.08 were 46.8%, 97.4% for females and 71.4%, 92.6% for males. The cut-off of the dataset I_{3M}<0.2 performed better for females, increasing sensitivity to 73.9%, post-test probability to 80.9% while decreasing specificity to 87.2%.

Conclusion: I_{3M}<0.2 performed better for females with higher sensitivity values while lowering the highest specificity values. Concerning legal rights and ethics, I_{3M}<0.08 or H stage provide the most accurate and consistent results in adulthood determination in males, whereas in females I_{3M}<0.08 and I_{3M}<0.2 provide sufficient results when a test with the highest specificity is sought.

Öz

Amaç: Çalışmanın amacı Demirjian diş gelişim basamakları (DS) ile Cameriere üçüncü molar indeksinin (I_{3M}), Türk bireylerin yetişkinlik ile çocukluk dönemlerinin ayırt edilmesindeki tanısal performansını değerlendirmek ve Türk popülasyonu örneklemine daha uygun bir indeks değeri varlığını araştırmaktır.

Gereç ve Yöntemler: Yapılan retrospektif çalışmaya 14-23 yaşları arasında 512 sağlıklı bireyin panoramik radyografileri dahil edildi. Her iki metodun tanısal etkinliği receiver operating characteristic (ROC) eğrisi ile test edildi, duyarlılıklar ve özgüllükleri hesaplandı, test sonrası olasılıklar Bayes testi ile değerlendirildi.

Bulgular: ROC eğrisi altındaki alanlar DS için 0,88 ve I_{3M}<0,08 için 0,89 olarak bulundu. Testlerin duyarlılık ve özgüllükleri H basamağı için kadınlarda sırasıyla

%41,4 ve %97,9, erkeklerde sırasıyla %64,3 ve %93,4; I_{3M}<0,08 için kadınlarda sırasıyla %46,8 ve %97,4, erkeklerde sırasıyla %71,4 ve %92,6 olarak gösterildi. Mevcut dataya en uygun indeks değeri olan I_{3M}<0,2 ise kadınlarda testin duyarlılığını %73,9'a, test sonrası olasılığı %80,9'a yükselterek daha etkin bir sonuç ortaya koydu, ancak testin özgüllüğü %87,2'ye düştü.
Sonuç: I_{3M}<0,2 indeks değeri kadınlarda daha yüksek duyarlılığı elde etmiş olsa da en yüksek özgüllük değerlerini düşürmüştür. Yasal haklar ve etik kurallar göz önüne alındığında, erkeklerde I_{3M}<0,08 indeks veya H basamağı, kadınlarda ise I_{3M}<0.08 veya I_{3M}<0,2 indeks değerleri yüksek özgüllükte doğru ve güvenilir sonuçları vermektedir.

Introduction

As worldwide migrations take place, forensic medicine is growing interest in the field of age diagnostics. Age estimation is based on anthropometric measures, signs of sexual maturity, radiological examination of the left hand, and dentition of individuals if the skeletal development is not yet completed (1). Because the skeletal development of the hand and wrist bones are completed around at the ages of 17-18 years, age estimation through radiological examination of the hand is not applicable for early adults. After 17-18 years, when the development of permanent dentition is almost completed, only third molars have the tendency to develop in late adolescence and early adulthood, thus contributing as a reliable method for legal age estimation (2-7).

According to the Turkish Institute of Statistics, there has been a 6.5% increase in the Turkish population in the last 5 years, with the population currently nearing 82 million, of which children make up 30% (8). As stated in Article 6 (1c) of the Turkish Criminal Code, any person who has not attained the age of 18 is a minor. Pursuant to Article 31 (3) of the Turkish Criminal Code, children who are between ages 15 and 18 at the time of the crime face imprisonment between 18-24 years if the crime requires heavy life imprisonment, and between 12-15 years if the crime requires life imprisonment. For other crimes, only two-thirds of the sentence is applicable, with imprisonment for each crime to not exceed 12 years (9). The provisions of the Turkish Criminal Code apply normally, without the abovementioned reductions, to persons over 18 years of age. Articles 103 and 104 of the Turkish Criminal Code states that sexually abuse of a child is punishable by 3 to 8 years imprisonment and sexual intercourse without the use of force or threat with a minor who has attained the age of 15 is punishable by 6 months to 2 years imprisonment (10).

It is crucial that an individual is accurately identified as a child or an adult when that person has committed or is suspected of committing a crime. As mentioned above, the type and length of any sanctions to be imposed for crimes committed may vary depending on whether a person is adult. Therefore, the uncertainty of age may result in a potential suspect facing higher or lower criminal sanctions than what would otherwise be applicable. Age determination based on teeth maturation is highly studied in forensic science due to the fact that the degree of dental development is less influenced by systemic factors rather than skeleton, and teeth are the most resistant part of the body in the decomposition process (11). To date, the correlation of third molar development and chronological age was studied in many studies and found effective (4,5,7,12-14); besides, a study by Thevissen et al. (15) conducted on 9 different populations with the same degree of third molar development, found that age was at most 14 months different. Consequently, the importance of population-based studies rises to estimate adulthood. For the determination of age based on the status of tooth formation, Demirjian's method among many other methods stands out to be the best method with its excellent interobserver agreement (3,4,12,13). One other proposed method which stands out for its ease of application is Cameriere's method. Previous studies have discussed the use of Cameriere's third molar maturation index (I_{3M}) which classifies patients with I_{3M} smaller than 0.08 as adults (2,5-7,14). Therefore, the first aim of the study is to compare both methods' sensitivity and specificity on distinguishing adults from minors, and whether there is a more suitable cut-off value for I_{3M} in Turkish population.

Materials and Methods

The retrospective study was approved by the Ethics Committee of İstanbul Okan University (decision number: 101/11, date: 26.12.2018). Power analysis indicated that 512 samples are required for the study (G Power 3.1.9.2). Nine hundred eight digital panoramic images of individuals aged between

14 and 24 years were collected at random from the digital archive of Faculty of Dentistry Department of Dentomaxillofacial Radiology between 2017-2018. The included radiographies belonged patients without any systemic disorders, dental anomalies, pathology present in alveolar bone, and whose left mandibular third molars were present. The included mandibular left third molars were non-decayed, free of periapical pathology and didn't consist of any dental restorations or root canal material. Panoramic radiographies of patients of unknown age or those lacking left mandibular third molars were excluded from the study. Digital panoramic images of 512 individuals were included, of which 306 were females and 206 were males. Images were exported in JPG format. The chronological age for each patient was calculated by subtracting the date of radiography taken from the date of birth (year/month).

Demirjian's mineralization stages (DS) of the tooth development were classified from enamel calcification till apices closure (A-H stages) (16). I_{3M} is calculated as the sum of the distances of open apices (a_1+a_2) divided by the length of the tooth (l) (Figure 1). When the apices of the tooth are completely closed, I_{3M}=0 is recorded. The measurements were done using ImageJ v1.52p and bundled with 64-bit Java for Windows (National Institutes of Health, Bethesda, USA; https://imagej.nih.gov/ij/download.html).

Statistical Analysis

Statistical analysis is done using IBM SPSS v21. Cohen's Kappa score (*κ*) and intra-class correlation coefficient (ICC) were used for inter and intra-observer reliability. Re-evaluation of randomly selected 100 radiographies were done by first and second observer after two weeks without the knowledge of age and sex of patients.

The diagnostic capabilities of the methods to estimate adulthood was tested with receiver operating characteristic (ROC) curves. The true positives, was defined as the proportion of correct classification of adults, hence sensitivity; and the true negatives, was defined as test's ability to classify minors, hence specificity of the methods were also evaluated. The sensitivity and specificity of the test were integrated for post-test probability (pp) of individuals attaining legal age (18 years of age or more). Pp is calculated by Bayes's theorem, where p=0 value equals to the present frequency of people who aged between 14-23 years old, and p1 and p2 represents the sensitivity and specificity of the relevant tests. The data was obtained from Turkish Statistical Institute, http://www.turkstat.gov.tr/UstMenu.do?metod=temelist (8).

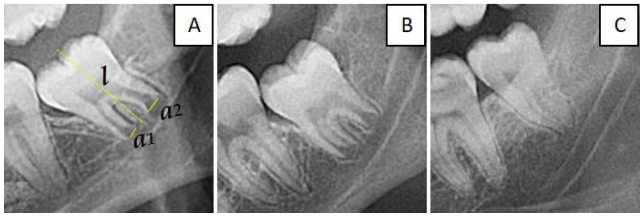

Figure 1. A) Representation for Cameriere's equation; l is the third molar length, a1 and a2 are the open apices distances, B) Third molar at G stage, C) Third molar at H stage

$$Pp = \frac{(p0*p1)}{(p0*p1)+(1-p2)(1-p0)}$$

Results

The results of κ and ICC for DS were highly consistent as 0.91 and 0.97, while κ and ICC values for I_{3M} were 0.89 and 0.94 respectively.

The ages of patients were not statistically different between groups according to sex. Mandibular third molars which are at B and C stages were too small in number to be taken into account, so the results include information obtained from D stage which corresponds to the time crown formation is completed. Only the ones grouped under H stage and I_{3M}<0.08 have statistically higher mean age for females than males (p<0.009) (Table 1).

To test the classification performance of methods to estimate adulthood, the patients who attained the age of 18 and older are classified as adults and the others as minors. The areas under ROC are 0.88 [95% confidence interval (CI), 0.85-0.91] for DS and 0.89 (95% CI, 0.86-0.91) for I_{3M} which indicated quite excellent and compatible performance of the methods (p<0.001).

The best cut-off value from the current data is calculated according to Youden Index J of 0.63 which corresponds to I_{3M}<0.2 with a range of 95% CI, ≤0,13-≤0.27. The I_{3M}<0.2 cut-off value for I_{3M} had 81.22% sensitivity and 81.87% specificity (p<0.001), positive likelihood ratios and negative likelihood ratios of the several cut-off values are also presented in Table 2. This means that a developed third molar with a maturity

index below 0.2 is more than 4.48 times more likely to be observed in an adult compared to a minor.

The stages of H, G and I_{3M}<0.08 and I_{3M}<0.2 are evaluated in terms of sensitivity and specificity and the results were summarized in Tables 3 and 4. In females both results presented high specificity values compared to males. In females the specificities of H stage and I_{3M}<0.08 were 97.8% and 94.4% whereas in males the results were 93.4% and 92.6% respectively. The sensitivities of H stage and I_{3M}<0.08 stayed quite low in females compared to males, being 41.4% and 46.8% respectively. In males the sensitivities of H stage and I_{3M}<0.08 were 64.3% and 71.4% respectively. The stages of H and I_{3M}<0.08 have high power to identify minors from adults for both sexes. The percentages of correct classification of adults and their post-test probabilities are summarized in Tables 3 and 4.

As the investigated new cut off value of I_{3M}<0.2 increased the sensitivity, the problem is that the probability of classifying minors incorrectly, hence the ratio true negatives is decreased from 97.4 to 87.2 for females and from 92.6 to 77.2 for males with respect to I_{3M}<0.08.

Discussion

Estimating adulthood is important for medicolegal reasons and the decision of methods on this matter should be consistent and reproducible. To minimize the possible error and have reliable outcomes, combination of methods is recommended since there is no perfect diagnostic test on the determination of legal age. Until now, both methods' reproducibility were studied and compared, and the idea of the present study is to apply both methods to a sample in the Turkish population.

Several studies found sexual dimorphism for third molar development in H stage (3-5,7,17). As is the case

Table 1. Summary of DS and I_{3M} according to age and sex

		Male		Female		
		#	Mean age ± SD	#	Mean age ± SD	
DS	D	24	15.04±1.19	56	14.74±1.21	p=0.812
	E	50	15.5±1.15	70	15.07±1.58	p=0.925
	F	40	16.46±1.39	69	16.69±1.71	p=0.641
	G	36	17.66±1.61	55	18.32±1.95	p=0.072
	H	53	19.89±2.08	50	21±1.75	p=0.004*
I_{3M}	I_{3M}<0.08	60	19.71±2.05	57	20.67±1.87	p=0.009*
	0.08≤I_{3M}<0.2	92	18.95±2.21	107	19.44±2.34	p=0.096
	0.2≤I_{3M}<0.3	116	18.4±2.33	155	18.69±2.47	p=0.321
	0.3≤I_{3M}<0.5	161	17.74±2.33	216	17.96±2.55	p=0.445
	0.5≤I_{3M}	45	15.01±1.14	88	14.99±1.18	p=0.893

*Statistically significant differences, I_{3M}: Third molar maturity index, SD: Standard deviation

Table 2. Sensitivity, specificity, confidence intervals (CI), positive and negative LR of several cut-off points for I_{3M}

Cut-off value	Sensitivity	95% CI	Specificity	95% CI	LR+	LR-
<0.07	56.35	48.8-63.7	95.47	92.6-97.4	12.44	0.46
<0.08	58.56	51.0-65.8	94.86	91.9-97.0	11.4	0.44
<0.1	62.98	55.5-70	93.05	89.8-95.5	9.06	0.4
<0.15	72.93	65.8-79.3	88.52	84.6-91.7	6.35	0.31
<0.2	81.22	74.8-86.6	81.87	77.3-85.9	4.48	0.23
<0.25	87.29	81.5-91.8	74.32	69.3-78.9	3.4	0.17
<0.3	90.61	85.4-94.4	64.35	58.9-69.5	2.54	0.15

LR+: Positive likelihood ratios, LR-: Negative likelihood ratios, I_{3M}: Third molar maturity index, CI: Confidence interval

Table 3. Percentage values of sensitivity, specificity, correct classification, and posttest probabilities for females when G and H stages, I_{3M}<0.08 and I_{3M}<0.2 are used for legal age estimation

	G	H	I_{3M}<0.08	I_{3M}<0.2
Sensitivity	33.3	41.4	46.8	73.9
Specificity	90.8	97.9	97.4	87.2
Correct classification	64.1	83.5	85.4	82.3
Posttest probability	64.2	70.3	70.8	80.9

I_{3M}: Third molar maturity index

Table 4. Percentage values of sensitivity, specificity, correct classification, and posttest probabilities for males when G and H stages, I_{3M}<0.08 and I_{3M}<0.2 are used for legal age estimation

	G	H	I_{3M}<0.08	I_{3M}<0.2
Sensitivity	22.9	64.3	71.4	87.1
Specificity	85.3	93.4	92.6	77.2
Correct classification	69.9	77.4	79.1	80.5
Posttest probability	68.2	68.3	68.5	80.9

I_{3M}: Third molar maturity index

with previous studies, the results of the present study also had statistically significant higher mean ages for females with third molars corresponding H stage and for I_{3M}<0.08 than males, supporting earlier third molar maturation in males. Mincer et al. (18) found that 90% of males and 92% of females presenting third molars were at H stage in American children. H stage is stated as a cut-off value for estimation of legal age in Mexican and Colombian populations (12). Cameriere et al. (2) compared the sensitivity and specificities of G, H stages with 0.08 cut-off value for Italian population and concluded that the post-test probabilities of third molars at H stage and has I_{3M}<0.08 are both 98%. The sensitivity values of Galić et al.'s (7) study in Croatian individuals on 0.08 cut-off value was significantly high for both sexes, 84% and 91% for females and males respectively higher than the results of the current study. The specificity values of I_{3M}<0.08 is quite high being 97.4% for females and 92.6% for males and consistent with the previous studies (5-7,13,14,19,20). Even though the present study's sensitivity and specificity results are comparable to Cameriere et al.'s (2) study, post-test probabilities stayed low being at most 80.9% (2,6,7). The post-test probability of the present study when using H stage or cut-off value of 0.08 is 70% for females and 68% for males. Moreover, correct classification of females and males were low as 85.4% and 79.1% respectively for I_{3M}<0.08. The results of Cavrić et al.'s (5) study in black African population contradicted the results of the present study, their study showed only 10% incorrect classification with 94% pp. Another study on the French population tested I_{3M}<0.08 on the assessment of the adulthood and found lower specificities 88.8%, 88.4% with higher post-test probabilities 87.9%, 89.9% for females and males respectively when compared with the results of the present study (21).

The study of Gulsahi et al. (14) discussed the utility of 0.08 cut-off value in a sample of Turkish individuals. The results of their study had 94.6% and 85.9% sensitivities for females and males respectively with 100% specificity. From the ethical perspective, the results were consistent with the desired outcomes. In other words, the test's capability to distinguish minors and preserving their legal rights is 100%. The results of the current study revealed 46.8% sensitivity and 97.4% specificity for females and 71.4% sensitivity and 92.6% specificity for males. A recent study conducted on Turkish individuals had high specificity values being 96.2%, 94.9% with substantially lower sensitivity values being 55.4% and 75% for females and males, respectively (22). The variance of sensitivity values might be due to the influx of immigration, the increase of population, and the parallel increase of inaccurate civil registration in rural areas in recent years.

Another cut-off value was obtained from the dataset of the present study. When the new cut-off value, I_{3M}<0.2, was considered for legal age estimation, the sensitivity of the new value rises from 46.8% to 73.9% for females and 71.4% to 87.2% for males while specificity decreases from 97.4% to 87.2% for females and 92.6% to 77.2% for males. The post-test probabilities of I_{3M}<0.2 were increased for both sexes with respect to the results of I_{3M}<0.08. From the statistical perspective when ethics were not considered, a new cut-off value 0.2 may be used practically. Akkaya and Yilanci's (22) study also reported a better cut-off value for Turkish individuals being I_{3M}<0.19 for females which was very close to the cut-off value of the current study. The results suggest that newly identified cut-off value might produce more accurate discrimination. Here lies the dilemma of the current study that in expense of 27% decrease

in false negatives, could 10% decrease of specificity be ignored in females with the cut off I_{3M}<0.2 with respect to I_{3M}<0.08. Even though the desired values of sensitivity and specificity are obtained from the new cut-off value, ethical considerations of legal purposes determine its utility. Still, the aim is solely based on legal purposes and the 0.2 cut-off value has 12.8% and 22.8% chances of classifying minors as adults for females and males respectively. Such high chances of misclassification can easily violate minors' legal rights in the Turkish population.

Every year more and more migration occurs and Turkey with its strategic place being a bridge between middle-east and Europe becomes a suitable place for refugees and immigrants to settle. Therefore, the profile of the population changes slightly each year and up-to-date population-based studies are important for contributing new evidence and information for forensic science.

Conclusion

For age estimation, the methods should have high specificity to decrease the number of false classifications of minors as adults and protect their legal rights. The cut-off I_{3M}<0.2 seemed to increase the test performance with respect to sensitivity and post-test probability, while decreasing specificity values. Therefore, I_{3M}<0.08 and H stage might still be recommended for adulthood estimation for Turkish males, but for Turkish females, based on ethical and legal considerations I_{3M}<0.08 and I_{3M}<0.2 provide respectively the best and sufficient results.

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Ethics

Ethics Committee Approval: The retrospective study was approved by the Ethics Committee of İstanbul Okan University (decision number: 101/11, date: 26.12.2018).

Informed Consent: Retrospective study.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Concept: B.A., E.F., Design: B.A., C.B., Data Collection or Processing: B.A., M.Ş., G.K., Analysis or Interpretation: B.A., M.Ş., C.B., E.F., Literature Search: B.A., M.Ş., C.B., G.K., Writing: B.A., M.Ş., C.B., E.F.

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Original Article / Özgün Araştırma

Effects of Mechanical, Chemical and Combination Methods on Halitosis: A Systematic Review and Meta-analysis

Mekanik, Kimyasal ve Kombine Yöntemlerin Ağız Kokusuna Etkileri: Sistematik Bir İnceleme ve Meta-analiz

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Abstract

Objective: Halitosis or bad breath is one a problem that can have a profound effect on the quality of life associated with health. The purpose of this systematic review and meta-analysis was to investigate the treatments used to treat halitosis.

Materials and Methods: This study involved a systematic review and meta-analysis. It was used in conjunction with treatment, management, therapy, and therapeutics for the first-call combination of bad breath, bad breath, halitosis, pseudohalitosis, halitophobia. Finally, these articles were removed according to the text and 37 articles remained.

Results: The effect of chemical methods on the treatment of bad breath was significant; halitosis was reduced by 1.19 [95% confidence interval (CI), 1.57 to 0.78]. Additionally, there was heterogeneity between studies (Q=11.32). Mechanically, despite the presence of heterogeneity between studies (Q=5.41), the p-value was not statistically significant (p=0.15), with the effect of the combined methods, bad breath was reduced to 1.18 (with a 95% CI, 1.68 to -0.51). Additionally, there is heterogeneity (Q=12.14).

Conclusion: The results of this study show that chemical and compound methods are effective in reducing bad breath.

Keywords
Halitosis, bad breath, treatment, management

Anahtar Kelimeler
Halitozis, ağız kokusu, tedavi, yaklaşım

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Öz

Amaç: Halitozis veya ağız kokusu, sağlıkla ilişkili yaşam kalitesi üzerine önemli etkileri olabilecek sorunlardan biridir. Bu sistematik derleme ve meta-analizin amacı, halitozisin tedavisinde kullanılan yöntemleri incelemektir.

Gereç ve Yöntemler: Bu çalışma bir sistematik derleme ve meta analizdir. Veri tabanlarında ağız kokusu, halitozis, pseudohalitozis, halitofobi ile tedavi, yönetim, terapi ve terapötikler kelimelerinin kombinasyonları tarandı. Son olarak, bu makaleler metne göre değerlendirildi, 41 tanesi çıkarılarak 37 makale dahil edildi.

Bulgular: Kimyasal yöntemlerin ağız kokusunun tedavisi üzerindeki etkisi belirgindi; halitozis 1,19 düzeyinde azaltıldı [%95 güven aralığı (GA), 1,57 ila 0,78]. Ek olarak çalışmalar arasında heterojenite bulunmaktaydı (Q=11,32). Mekanik olarak, çalışmalar arasında heterojenite olmasına rağmen (Q=5,41), p değeri istatiksel olarak anlamlı değildi (p=0,15), kombine yöntemlerin etkisi ile ağız kokusu 1,18 düzeyine azaltıldı (%95 GA, 1,68 ila -0,51). Ek olarak heterojenite de bulundu (Q=12,14).

Sonuç: Bu çalışmanın sonuçları, kimyasal ve bileşik yöntemlerin ağız kokusunu azaltmada etkili olduğunu göstermiştir.

Introduction

Bad breath, also referred to as halitosis and oral malodor, is one of the grave problems that many people try to avoid. Under normal conditions, the human breath is odorless and has a distinctive odor called 'human odor' (1,2). Approximately, a quarter of the world population have halitosis, with the majority exhibiting this condition occasionally (3). Patients usually do not express the primary complaint; instead, the patients' family members and relatives become aware of the condition (3,4).

Dental practitioners estimate that only 25% of the population has halitosis, and the remaining 75% only has a medical condition or halitophobia (5). Several treatment modalities have been suggested for the treatment of halitosis; however, there is no consensus yet on a standard protocol for this problem.

Although many studies have been undertaken on halitosis, no systematic review or meta analysis has been published on techniques to evaluate the halitosis and the treatment modalities of halitosis. Therefore, the present systematic review and meta-analysis were undertaken to review the treatment modalities of halitosis during the past 20 years.

Materials and Methods

Combinations of the words halitosis, bad breath, oral odor, pseudohalitosis, and halitophobia with the words treatment, management, therapy, and therapeutics were used for the initial search. The search in databases brought up 1175 records, which were transferred to the Mendeley software. Then, a search based on 'author', 'year', and 'title' revealed 441 repeated records. The approval for the this study was approved by ethical committee of Kerman University of Medical Sciences (approval code: IR.KMU.REC.1397.472.).

Statistical Analysis

At the end of this stage, 737 records remained after the elimination of 441 repeated records. Finally, 37 articles (Table 1), were evaluated (Figure 1).

Results

The effect of chemical techniques on halitosis was statistically significant; i.e., halitosis decreased by 1.19% [at 95% confidence interval (CI): -0.78-1.57]. Also, there was a heterogeneity between the studies (Q=11.325) (Table 2).

Despite the heterogeneity between the studies (Q=5.41), the p-value did not indicate statistical significance (p=0.15) (Table 2).

The effects of combined techniques on the elimination of halitosis were significant; i.e., halitosis decreased by 1.18% (at a 95% CI: -0.51 to -1.68). In addition, there was heterogeneity between the studies (Q=12.142) (Table 3).

All work included initial search of the articles, selection of them, record and interpretation of the articles and meta-analysis was done by two authors of the article.

Discussion

The results showed that the pharmaceutical techniques are useful in this respect; however, mechanical techniques are useful, too. Also, a combination of chemical and pharmaceutical techniques was found to be effective.

Akkaoui and Enhibi (6) and Buunk-Werkhoven et al. (7) reported the results only based on questionnaires, with no tools for measuring the severity of halitosis, including halitometers and volatile sulfur compounds (VSC) analyses; therefore, they were eliminated. These reports are studies that have a very small sample size (1 to 6) with a specific medical condition or specific manifestations of a medical condition (8-10). These studies are suitable for introducing new diseases but cannot be generalized due to their very small sample size. Therefore, they are not included in systematic reviews. For example, Lopes et al. (9) evaluated the effect of photodynamic therapy with the use of methylene on the oral surfaces in five 14-16 year-old subjects.

Another study whose abstract was available was carried out by Yaegaki and Sanada (11) in 1992 on the effect of olive oil and essential oils on decreasing halitosis in comparison with repeated rinsing of the oral cavity. The researchers concluded that the use of these oils could decrease halitosisby 65% (11).

A study by Rassameemasmaung et al. (12) showed that the use of a herbal extract of G. mangostana might be useful as an adjunct in the treatment of halitosis.

An evaluation of the year of publication showed that 18 studies were published from 2015 on, and 19 articles were published from 2015 to 2018. Of all the studies published after 2015, almost half (nine

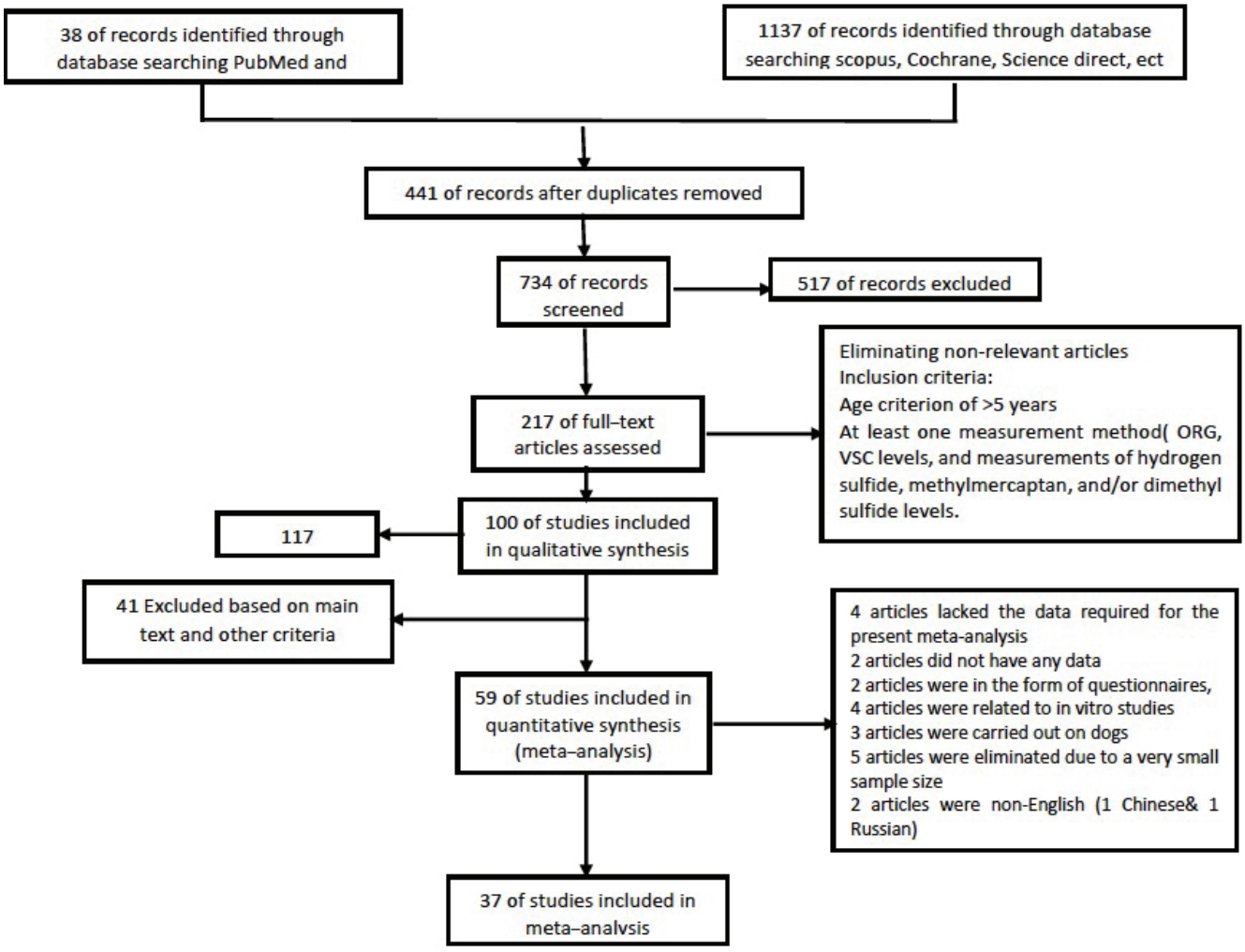

Figure 1. Flow diagram for study selection
ORG: Organoleptic scores, VSC: Volatile sulfur compounds

studies, 24.3% of all the studies) were published in 2016, indicating that the discrepancies in the results of studies on the treatment of halitosis have attracted the attention of researches more than ever.

A study by Porter and Scully (13) showed that the use of mechanical techniques, such as cleaning the surface of the tongue, brushing, use of dental floss, and periodontal treatments, could control the dental plaque and decrease halitosis.

In a study by Chen and Jin (14) 251 articles were included. These researchers showed that the mechanical cleaning of the tongue surface (brushing and scrubbing) significantly decreased halitosis (a decrease in VSC and Winkel tongue coating index, and an improvement in the periodontal status) in the case group. However, there was no significant difference between the case and control groups.

Several studies have shown a relationship between halitosis and periodontitis (15-17). At present, the majority of researchers believe that the two factors above affect each other (18).

Several studies have shown that chlorhexidine (CHX), essential oils, triclosan, cetylpyridinium chloride (CPC), chlorine dioxide, zinc salts, benzalkonium chloride, hydrogen peroxide, and sodium bicarbonate mouthwashes are successful when they are combined with mechanical techniques (19,20).

Studies by Thrane et al. (21), and Thaweboon and Thaweboon (22) showed that mouthwashes such as CHX, CPC, triclosan, essential oils, quaternary ammonium compounds, benzalkonium chloride, and hydrogen peroxide mouthwashes are effective in decreasing halitosis.

Table 1. Details of the information included in the systematic review and meta-analyses

No	Year - country	Author	QA score consort	Sample size and characteristics	Follow up period	Type of study and duration	Type of intervention	Methods of assessment halitosis
1	2002 Turkey	Ermis B	20	162 0 81 M, 71 F	--------	RCT	Mebendazole, placebo	Exitance of bad breath
2	2003 Netherland	Winkel EG		40 43.8±15.8 21 M, 19 F	At days 0 and 14	RCT	CHX (0.05%), CPC (0.05%), zinc-lactate (0.14%)	Organoleptic, VSC, WTCI, tooth staining
3	2004 Spain	Roldán S	21	19 40.4±19.1 9 M 10 F	1 and 3 months after baseline	RCT	Tongue scraper, CHX-0.05%, (CPC; 0.05%), zinc lactate (0.14%)	Organoleptic, VSC, tongue coating, periodontal, salivary flow, subgingival plaque
4	2006 Brazil	Faveri M	24	18 19.25±1.4 6 M, 12 F	--------	RCT	Tooth brushing; tooth brushing and inter-dental flossing; tooth brushing and tongue scraping; tooth brushing, inter-dental flossing and tongue scraping	VSC, organoleptically and by volatile sulphur
5	2006 Brazil	Dal Rio AC	25	38 0 13 M, 25 F	--------	Case control	CO_2 laser, shar plan lasers	VSC
6	2007 Greece	Katsinelos P	22	18 40.78±10.9 10 M, 8 F	6 weeks	Case control	20 mg omeprazole, 500 mg clarithromycin and 1,000 mg amoxicillin	Urea concentration H. pylori in the gastric mucosa
7	2009 Korea	Lee JS	25	88 47.7±8.4 39 M, 49 F	--------	RCT	Korea red ginseng	VSC, urea breath test, H.pylori culture, cell culture lyase, cystathionineγ
8	2009 Nigeria	Al-Abbasi AM	21	44	1 week	RCT	Tonsillectomy	Subjective and objective postoperative assessment
9	2010 Germany	Schaefer I	24	31 0 7 M, 24 F	3 months	RCT	0.06% Chlorhexamed, gum protection mouthrinse (plus 250 ppm sodium fluoride)	Stimulated saliva flow rate organoleptic, tongue coating, VSC, modified gingival index, plaque index, bleeding on probing index
10	2011 Turkey	Tanyeri HM	26	58 35.8 30 M, 28 F	------	RCT	Temperature-controlled RF tonsil ablation	Otolaryngologic examination
11	2011 India	Malhotra S	25	15 0 9 M, 6 F	7 days	RCT	CHX, essential oil mouth rinse Placebo	Organoleptic red complex organisms
12	2012 Korea	Song JA	24	N=66 Mean age =24.2±9.1 34 M, 32 F	14 day	Case control	A-solution, blended essential oils and diluted with distilled water	VSC, salivary pH, oral status

13	2012 Sweden	Erovic Ademovski S	23	21 45.7±13.3 11 M, 10 F	Day 1: 30 min after interand Day 14: 8-12 h after the last inter	RCT	Zinc acetate (0.3%) and CHX (0.025%) with and without adjunct tongue scraping	Measurements of H_2S, MM, and dimethyl sulfide levels VSC, bacterial samples from the tongue
14	2013 Sewden	Erovic Ademovski S	25	70 48.6±14.8 36 M, 34 F	3 months after treatment	RCT	Non-surgical periodontal therapy	Organoleptic scores, VSC, gas chromatograph, tongue coating
15	2013 Brazil	Oliveira-Neto JM	23	20 35.9 30 M, 14 F	--------	RCT	Tongue scraper. 2 mouthrinses (0.05% CPC and 0.12% CHX, soft-bristled toothbrush and fluoride toothpaste	VSC
16	2013 Germany	Wilhelm D	24	54	5 and 60 min after treatment	RCT	Tongue cleaning with tooth and tongue gel applied to the tongue cleaner	VSC, organoleptic
17	2014 Turkey	Ata N	23	34 28.28±9.3 26 M, 8 F	12 months	RCT	RF cryptolysis	VSC
18	2015 Italy	Marchetti E	24	20	--------	RCT	Lactobacillus brevis (CD2) - containing lozenges, compared with placebo	Tongue coating, VSC organoleptic, breath print constructed by BIONOTE®
19	2015 Iran	Sayedi SJ	23	77 case: 5.23±2.61 control: 5.78±2.29 40 M, 37 F	--------	RCT	Balsam fir, cinnamon, coriander, labrador tea, myrrh, peppermint, sage, sweet marjoram, thyme, winter savory	Biofilm formation, biofilm killing
20	2015 Japan	Aung EE	24	30 19.80±2.9 14 M, 16 F	14 Day	RCT	Tooth brushing, mouth washing with chlorine dioxide, tongue cleaning	VSC
21	2015 Turkey	Ileri Keceli T	23	69 NR 32 M, 37 F	14 Day	RCT	Scaling-polishing + tooth brushing + tongue brushing and scaling-polishing + tooth brushing	VSC, organoleptic assessment
22	2015 Brazil	Feres M	22	70 24.3±8.5 30 M, 40 F	12 Hours	RCT	Brushing with regular fluoride toothpaste alone, brushing with regular fluoride toothpaste followed by rinsing with a 0.075% CPC	Organoleptic examination, VSC
23	2016 India	Penala S	27	32 45.3 0	--------	RCT	Probiotics (Lactobacillus salivarius and Lactobacillus reuteri, scaling and root planning)	Microbial assessment, halitosis assessment, red complex organisms
24	2016 Turkey	Dereci O	23	60 43.7±3.1 31 M, 29 F	1st, 3rd and 6th months	RCT	Er, Cr:YSGG laser supported periodontal therapy	VSC, plaque index, probing depth, clinical attachment level, bleeding on probing
25	2016 Brazil	Costa da Mota AC	25	46 14.80±2.50 22 M, 24 F	--------	RCT	Antimicrobial photodynamic therapy	Microbiological analysis, VSC

26	2016 Germany	Seemann R	22	34 44.2 17 M, 17 F	12 h after the evening rinse and 12 h after the daytime rinse	RCT	Mouthwash Cb12 contains 0.3% zinc acetate dihydrate, 0.025% CHX, aqua, glycerin, hydrogenated starch hydrolysate, alcohol, sodium fluoride, PEG-40 hydrogenated castor oil, potassium acesulfame, citric acid and aroma Cb12 (a mixture of 0.3% zinc acetate and 0.025% CHX)	VSC, organoleptic score
27	2016 Turkey	Sökücü O	24	13 0 5 M, 8 F	1, 3, 5, 7, 9, 11, and 13 months after bonding	RCT	Orthodontic therapy consisted of molar bands with edgewise triple buccal tubes with vertical hooks	VSC, plaque index, gingival index, probing pocket depth
28	2016 Sweden	Erovic Ademovski S	24	21 45.7±13.3 11 M, 10 F	12 h after rinsing with placebo and five mouth rinse	RCT	Zinc acetate and CHX diacetate; zinc lactate, CHX, CPC; zinc acetate and CHX diacetate with reduced amounts of mint and menthol; zinc chloride and essential oil and chlorine dioxide	Gas chromatograph organoleptic
29	2016 Brazil	Lopes RG	22	45	--------	RCT	Antimicrobial photodynamic therapy, tongue scrabing	VSC
30	2017 Brazil	Silveira JO	21	30 FMD: 48.13 (±7.78) QS: 47.0 8 M, 22 F	90 days after treatment	RCT	Scaling and root planing	Organoleptic, VSC
31	2017 Turkey	Caygur A	21	60	Days 7, 14, 30	RCT	Glycine powder air-polishing combined with scaling and root planing	VSC
32	2017 Sweden	Erovic Ademovski S	22	46 48.89 0	3 Month	RCT	Zinc acetate and CHX diacetate containing mouth rinse, mouth rinse contained the same ingredients except for the active substances (0.3% Zn and 0.025% CHX)	Probing pocket depth, bleeding on probing, plaque index, organoleptic, VSC, H_2S and tongue coating
33	2017 India	Mamgain P	23	60 0 0	14th day, and the 21st day	RCT	Triphala and Ela decoction, CHX 0.2%	Gingival inflammation plaque, organoleptic
34	2018 Iran	Hashemian F	25	52 0 18 M, 34 F	7 days,1 month, and 6 months after the procedure	RCT	Temperature controlled RF, tonsil ablation, CO_2 laser cryptolysis	Tonsil smelling, pain bleeding
35	2018 Brazil	Gonçalves MLL	24	60 control: 37 case: 39 0	--------	Case control	Antimicrobial photodynamic therapy	VSC
36	2018 Turkey	Sezen Erhamza T	26	30		Case control	Rapid maxillary expansion	VSC, organoleptic
37	2018 Brazil	Gonçalves MLL	21	39	--------	RCT	Tongue scraping, Photodynamic therapy	Microbiological analysis halimetry

RCT: Randomized controlled trial, M: Male, F: Female, QA score: Quality score, VSC: Volatile sulfur compounds, CHX: Chlorhexidine, WTCI: Winkel tongue coating index, H. pylori: Helicobacter pylori, H_2S: Hydrogen sulfide, MM: Methyl mercaptan, CPC: Cetylpyridinium chloride, RF: Radiofrequency, FMD: Full-mouth debridement

Table 2. Overall estimate of the difference between the mean standard for reducing bad breath with pharmacological methods, mechanical methods and chi-square heterogeneity test

Pharmacological methods					
1st Author	**Year**	**SEM**	**95% CI**		**% weight**
			Lower	**Upper**	
Song JA	2012	-1.32	-1.95	-0.90	8.21
Ermis B	2002	-0.75	-1.35	-0.25	7.14
Winkel EG	2002	-1.56	-2.09	-1.20	7.29
Mamgain P	2016	-2.65	-2.69	-1.49	8.03
Seemann R	2016	-0.35	-0.078	0.80	7.02
Sayedi SJ	2015	-0.93	-0.25	-0.65	7.56
Costa da Mota AC	2016	-1.42	-2.15	1.17	7.23
Gonçalves MLL	2017	-1.71	-2.16	-1.18	8.19
Katsinelos P	2007	-2.52	-2.91	-1.81	7.95
Malhotra S	2011	-2.59	-2.94	-0.91	7.98
Schaefer I	2010	-2.19	-2.65	-1.65	7.15
Lee JS	2009	-0.64	-1.07	-0.20	8.02
Marchetti E	2015	-1.09	-0.22	-1.19	7.21
ErovicAdemovski S	2016	-1.31	-2.06	-0.34	7.78
ErovicAdemovski S	2013	-1.57	-1.59	-0.54	9.23
Pooled SMD		-1.19	-1.57	-0.78	100
Heterogeneity chi-squared = (P=0.001)					
I-squared (variation in OR attributable to heterogeneity) =82.1%					
Mechanical methods					
1st Author	**Year**	**SEM**	**95% CI**		**% weight**
			Lower	**Upper**	
Dal Rio AC	2006	-2.23	-2.89	-0.85	7.25
Hashemian F	2018	-2.23	-2.91	-0.65	7.45
Silveira JO	2016	-1.85	-1.89	-0.48	7.31
Ata N	2014	1.22	0.07	1.98	7.12
Faveri M	2014	-0.21	-0.54	0.97	6.90
Gonçalves MLL	2018	0.98	-0.09	-0.44	7.45
Tanyeri HM	2010	-1.44	-2.09	-0.44	7.51
Sökücü O	2016	-1.52	-2.21	-0.51	6.15
ErovicAdemovski S	2016	-1.31	-2.06	-0.34	7.78
Dereci O	2016	-1.57	-1.59	-0.54	7.56
Al-Abbasi AM	2009	-1.37	-2.01	-0.54	7.97
IleriKeceli T	2015	-.25	-1.45	0.88	6.21
SezenErhamza T	2018	-0.98	-0.46	-0.89	7.25
Pooled SMD		-1.12	-1.27	-0.62	100
Heterogeneity chi-squared = (P=0.015)					
I-squared (variation in OR attributable to heterogeneity) =71.2%					
SEM: Standard error of the mean, CI: Confidence interval, OR: Odds ratio					

Table 3. Overall estimate of the difference between the mean standard for reducing bad breath with combination methods and chi-square heterogeneity test

1st Author	Year	SEM	95% CI		% weight
			Lower	Upper	
ErovicAdemovski S	2012	-1.48	-2.09	-0.89	9.21
Feres M	2015	-1.45	-1.89	-0.71	9.65
Wilhelm D	2013	-2.47	-2.87	-0.95	10.25
Oliveira-Neto JM	2013	0.12	0.75	-0.62	8.65
Lopes RG	2015	-0.089	-1.18	-0.25	11.20
Caygur A	2017	1.32	0.21	2.01	9.54
Penala S	2016	-0.21	-1.75	0.62	9.41
Silvia Roldán	2005	-1.61	-2.42	-1.20	9.52
Aung EE	2015	-1.05	-0.45	1.22	6.54
Pooled SMD		-1.18	-1.68	-0.68	100
Heterogeneity chi-squared = (p=0.001)					
I-squared (variation in OR attributable to heterogeneity) =85.4%					
SEM: Standard error of the mean, CI: Confidence interval, OR: Odds ratio					

CHX has been considered the gold standard for the treatment of halitosis. CHX, in combination with CPC, decreased VSC levels to a great extent and decreased the counts of both aerobic and anaerobic bacteria in three hours. However, patients might not be interested in using CHX for a long time, because it has an unpleasant taste and stains the teeth reversibly (23).

These research works have shown that brushing the tongue with toothpaste could reduce the levels of VSCs for at least 1 h, and this was more effective than only brushing the teeth (24,25).

Conclusion

The results of the present study showed that the chemical and combined techniques are effective in decreasing halitosis.

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Ethics

Ethics Committee Approval: The approval for the this study was approved by ethical committee of Kerman University of Medical Sciences (approval code: IR.KMU.REC.1397.472.).

Informed Consent: This study is a systematic review and meta-analysis.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: M.A.H., Design: M.A.H., Data Collection or Processing: E.I., Analysis or Interpretation: E.I., Literature Search: E.I., Writing: M.A.H.

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Original Article / Özgün Araştırma

Evaluation of Saliva Melatonin Levels in Head-neck Radiotherapy Patients

Baş-boyun Radyoterapi Hastalarında Tükürük Melatonin Düzeylerinin Değerlendirilmesi

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Abstract

Objective: Melatonin (MT), which performs many missions such as the activation of the immune system, regulation of the body's circadian rhythm and body temperature, is a powerful antioxidant. This study investigated the impact of radiation on the saliva MT level in individuals with head and neck cancer.

Materials and Methods: Sixteen patients receiving head and neck radiotherapy were included in the study. The MT levels were measured in salivary samples taken from these patients before and after radiotherapy and in salivary samples taken from thirty healthy individuals who had no radiotherapy. Paired t-test and Student's t-test were used in the analysis of the data obtained.

Results: When the saliva MT levels were compared before and after radiotherapy in the patient group, although there was a decrease after radiotherapy, there was not found a statistically significant difference ($p>0.05$). Additionally, although the saliva MT levels before and after radiotherapy were determined to be lower than the control group, there was no significant ($p>0.05$).

Conclusion: Although there was no significant difference, the important decrease in saliva MT levels after radiotherapy in the patient group will enable us to understand more clearly the effect of ionizing radiation on saliva MT level.

Keywords
Head and neck cancer, melatonin, radiotherapy, saliva

Anahtar Kelimeler
Baş ve boyun kanserleri, melatonin, radyoterapi, tükürük

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Öz

Amaç: Bağışıklık sisteminin aktivasyonu, vücudun sirkadiyen ritminin ve vücut ısısının düzenlenmesi gibi birçok görevi yerine getiren melatonin güçlü bir antioksidandır. Bu araştırma baş ve boyun kanserli bireylerde radyasyonun tükürük melatonin seviyesine etkisini araştırmayı amaçladı.

Gereç ve Yöntemler: Çalışmaya baş ve boyun radyoterapisi uygulanan on altı hasta dahil edildi. Bu hastalardan radyoterapi öncesi ve sonrası alınan tükürük örneklerinde ve radyoterapi almamış otuz sağlıklı bireyin tükürük örneklerinde melatonin seviyeleri ölçüldü. Elde edilen verilerin analizinde paired t-testi ve Student's t-testi kullanıldı.

Bulgular: Hasta grubunda radyoterapi öncesi ve sonrası tükürük melatonin seviyeleri karşılaştırıldığında, radyoterapi sonrası azalma görülse de istatistiksel olarak anlamlı bir fark bulunmadı ($p>0,05$). Ayrıca, radyoterapi öncesi ve sonrası tükürük melatonin seviyeleri kontrol grubundan daha düşük bulunmasına rağmen anlamlı değildi ($p>0,05$).

Sonuç: Anlamlı bir fark olmamasına rağmen, hasta grubunda radyoterapi sonrası tükürük melatonin seviyesinde görülen belirgin azalma, iyonize radyasyonun tükürük melatonin seviyesine etkisini daha net anlamamızı sağlayacaktır.

Introduction

Head and neck cancers constitute approximately 3 to 5% of all cancer, and radiotherapy and/or chemotherapy are extensively used in the treatment (1,2). Radiotherapy relies on the ability of radiation to destroy tumor cells and has a considerable place in the treatment of head and neck cancers (3).

Ionizing radiation, with the toxic effect of reactive oxygen species (ROS) that it causes to increase, can cause tissue damage through multiple mechanisms such as cell membrane, DNA, RNA and protein damage, lipid peroxidation and enzyme oxidation (4-7). ROS are free molecules that occur during the conversion of nutrients to energy using oxygen, and it has been reported that these molecules have an important place in carcinogenesis (8). Antioxidant defense systems evolved to control ROS production and to get ahead of the harmful effects of ROS in the body. Antioxidants protect healthy cells against the harmful effects of radiation by various enzymatic (such as glutathione peroxidase, glutathione reductase, and superoxide dismutase) and non-enzymatic [such as glutathione, melatonin (MT), vitamins, selenium, uric acid, beta carotene, and alpha-tocopherol] systems (5,8-11).

In particular, MT is a powerful ROS cleansing antioxidant, which is secreted from foremost the pineal gland (epiphysis), lens, bone marrow, gallbladder, and gastrointestinal tract (12,13). It stimulates the circadian rhythm of the body, arrangement of body temperature, activation of the immune system, cell proliferation of osteoblasts, type I collagen synthesis and bone formation (12-14). It has anti-inflammatory and anti-tumoral effects due to its immunomodulating effect (12). It also prevents oxidative stress by stimulating some antioxidative enzymes or detoxifying free radicals (12,13).

In patients receiving head and neck radiotherapy, it is predicted that may occur changes in the salivary glands as well as changes in the quality and biochemical content of saliva. These biochemical changes are related to the mechanism of radiation's effect in the body, and it is very important in terms of patients' quality of life. The objective of our study was to investigate the essence of the saliva MT level by detecting biochemical changes in saliva before and after radiotherapy in individuals with head and neck cancer.

Materials and Methods

Study Design

In the present study, it was planned to investigate the level of MT in saliva samples that will be collected from head and neck radiotherapy patients admitted to Atatürk University Faculty of Medicine and healthy individuals. After all planning, by taking the informed consent form obeying the Declaration of Helsinki with the decision of Atatürk University Faculty of Dentistry Ethics Committee (decision number: 54, date: 21.06.2017), the saliva samples were taken from the patients who will receive radiotherapy before and after treatment and the healthy individuals. The data obtained as a result of biochemical analysis of saliva samples were compared statistically.

Patients

The patients who will receive head and neck radiotherapy in the Department of Radiation Oncology were referred to the Atatürk University Faculty of Dentistry to eliminate infection foci and to improve oral hygiene before radiotherapy. Sixteen head and neck cancer patients who had not been treated for cancer before and who did not have any periodontal and systemic problems that could lead to changes in saliva content were included in this study. Eight (50%) of patients diagnosed the head-neck cancer were laryngeal cancer, 3 (18.75%) lymphoma, 1 (6.25%) non-Hodgkin lymphoma, 1 (6.25%) nasopharyngeal cancer, 1 (6.25%) lower lip cancer, 1 (6.25%) maxillary sinus tumor, 1 (6.25%) mucoepidermoid carcinoma. The total fraction number of radiotherapy applied to the patients was between 13-36 days, the daily radiation dose was between 1.8-3 Gray (Gy), the total radiation dose was between 30-70 Gy, and no individuals received chemotherapy.

Also, thirty healthy individuals who consulted at Atatürk University Faculty of Dentistry and did not receive radiotherapy were included in the control group.

Saliva Samples

After all individuals in the study rinse their mouths with water in a quiet room, unstimulated saliva samples of these individuals were taken into Eppendorf tubes. In the patient group, saliva samples were taken before radiotherapy and in the second month after the start of radiotherapy. All samples were stored in a refrigerator at -80 °C.

Laboratory Analysis

The MT level of samples were analyzed by Human Enzyme-Linked Immunosorbent Assay Kit for MT, (Catalog No. CEA908Ge, Wuhan USCN Business Co., Ltd., USA) in Atatürk University Faculty of Medicine Biochemistry Laboratories.

Statistical Analysis

The data obtained after biochemical analysis were analyzed by SPSS (SPSS 25.0, SPSS Inc., IBM Corp., Chicago, IL) software program. While paired t-test was used to compare the measurements before and after radiotherapy, Student's t-test was used to compare the measurements before and after radiotherapy with the control group.

Results

The distribution of the control and patient groups by gender is shown in Table 1. While the mean age of the control group was 41.66±16.44, it was 50.68±13.51 in the patient group.

When the saliva MT levels (1252.83±437.8 pg/mL, 1003.04±543.94 pg/mL, respectively) were compared before and after radiotherapy in the patient group, although there was a decrease after radiotherapy, there was not found a statistically significant difference as shown in Table 2. When the saliva MT levels of the patient group were compared with the control group, although mean saliva MT levels before and after radiotherapy (1252.83±437.8 pg/mL, 1003.04±543.94 pg/mL, respectively) were determined to be lower than the control group (1314.5±504.2 pg/mL), these differences were no statistically significant as shown in Table 3 and Table 4.

Table 1. Distribution of the control and patient groups by gender

	Control group		Patient group	
	n	%	n	%
Male	18	60	14	87.5
Female	12	40	2	12.5

Discussion

Head and neck radiotherapy causes not only loss of function in major salivary glands but therewithal changes in the saliva flow ratio, quantity, viscosity, pH, buffering capacity, and biochemical parameters such as some enzyme levels, oxidant-antioxidant balance (15,16). It is believed that these changes in the salivary glands are mediated by radiation-induced free radicals and ROS (17).

The effects of radiotherapy on oxidant and antioxidant mechanisms are still controversial today. Khalil Arjmandi et al. (5), in their study on serum antioxidant/oxidant balance before and after radiotherapy, observed a statistically significant decrease in serum Selenium concentration, total antioxidant status (TAS), Superoxide Dismutase levels and Glutathione Peroxidase activity ($p<0.05$). Shariff et al. (7) also determined lower serum TAS levels before treatment in patients with head and neck malignancy than the control group and found this difference statistically significant. In the same study, they also observed a decrease in serum TAS levels after radiotherapy in the patient group compared to before treatment and associated this with increased oxidative stress. In contrast, Babiuch et al. (18) reported that TAS levels were higher in the patients with premalignant/malignant lesions. It has been suggested that this situation may be related to the

Table 2. Statistical comparisons of salivary melatonin levels before and after radiotherapy in the patient group

	n	Before radiotherapy	After radiotherapy	t	p-value
		Mean ± SD	Mean ± SD		
Melatonin (pg/mL)	16	1252.83±437.8	1003.04±543.94	1.540	0.144*

*Paired t-test, $p>0.05$, SD: Standard deviation

Table 3. Statistical comparison of salivary melatonin levels before radiotherapy and the control group

	Control group		Before radiotherapy		t	p-value
	n	Mean ± SD	n	Mean ± SD		
Melatonin (pg/mL)	30	1314.5±504.2	16	1252.83±437.8	0.413	0.682*

*Student's t-test, $p>0.05$, SD: Standard deviation

Table 4. Statistical comparison of salivary melatonin levels after radiotherapy and the control group

	Control group		After radiotherapy		t	p-value
	n	Mean ± SD	n	Mean ± SD		
Melatonin (pg/mL)	30	1314.5±504.2	16	1003.04±543.94	1.942	0.059*

*Student's t-test, p>0.05, SD: Standard deviation

increased activity of antioxidant defense systems in response to oxidative stress.

Although there are many studies on total antioxidant capacity in the literature, there is a limited number of studies on MT, one of the most important antioxidants. These studies are related either to the relation of MT with type II diabetes (12) and periodontal diseases (13,19,20) or to its therapeutic effect. Whereas, MT is today considered not only as a hormone but also as an important cell protector (17). It is important for oral defense because of its anti-inflammatory effects in the oral cavity and more effective than conventional antioxidants in reducing oxidative stress (21,22). In this context, our study will have an important place in contributing to the determination of the relationship between MT and radiotherapy.

In studies, it is shown that approximately 24-33% of active plasma MT is found in saliva (14,23). Therefore, in the evaluation of MT levels, it is recommended that the saliva samples, which are one of the low-cost and non-invasive methods, have recently been preferred to blood samples (14,24). Our study was planned on saliva samples because of to be more easily accessible and to be considered sufficient in the evaluation of the MT level.

In the present study, although there is no statistically significant difference, the decrease in saliva MT levels after radiotherapy may be attributed to ROS toxicity and oxidative stress resulting from ionizing radiation. Also, mitochondrial and metabolic dysfunctions and mutations in damaged cancer cells significantly increase ROS formation (25). In our study, the low levels of saliva MT before and after radiotherapy compared to the control group may also be related to the cancer development mechanism and tissue damage. In this context, saliva MT measurement can be considered as an assistant parameter in the evaluation of head-neck malignancies.

The limitation of the present study is the inability of patient follow-up for a long time because of time constraints. Patient follow-up is recommended to identify changes and complications that may occur in a long time after radiotherapy. Additionally, the study population remained small due to both the capacity of the oncology unit and the time constraints.

Conclusion

In conclusion, although there is no statistically significant difference in the present study, we can say that the decrease in the saliva MT level in head and neck malignancy patients is exacerbated by the effect of radiation, based on the marginal difference between groups. This too will enable us to understand clearly the effect of ionizing radiation on the saliva MT level. Although it is estimated that some biochemical changes occur in saliva with the effect of radiation, there are not many studies in the literature on MT levels in head and neck cancer patients. The present study will contribute to the data obtained in this respect. Also, a choice of saliva for examining the MT level will be an advantage in terms of both cost and sample collection compared to the commonly used blood tissue.

Ethics

Ethics Committee Approval: After all planning, by taking the informed consent form obeying the Declaration of Helsinki with the decision of Atatürk University Faculty of Dentistry Ethics Committee (decision number: 54, date: 21.06.2017).

Informed Consent: Informed consent was obtained.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: G.A., N.A., Concept: G.A., N.A., Design: G.A., N.A., Data Collection or Processing: G.A., Analysis or Interpretation: G.A., N.A., Literature Search: G.A., Writing: G.A., N.A.

Conflict of Interest: No conflict of interest was declared by the authors.

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Original Article / Özgün Araştırma

Knowledge Levels and Attitudes of Type 2 Diabetic Patients on Periodontal Health: A Cross-sectional Study

Tip 2 Diyabet Hastalarının Periodontal Sağlık Hakkındaki Bilgi Düzey ve Tutumları: Kesitsel Bir Çalışma

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Abstract

Objective: In our research, we determined the oral health behaviors and knowledge levels of the Turkish population with diabetes.

Materials and Methods: In this cross-sectional study, a self-reported questionnaire was used to assess the oral health practices, knowledge, and awareness items of the patients with a confirmed diagnosis of type 2 diabetes.

Results: A total of 439 respondents completed the questionnaire in a 6-month period. A majority of the participants (67%) were unaware about diabetes-periodontal disease relationship. However, only 9.6% of patients with diabetes were referred to a dentist for oral health. The rate of participants who received information about diabetes related increased periodontal risks by dentists was 19.4%.

Conclusion: Our findings showed that type 2 patients with diabetes had poor attitudes and knowledge about oral health.

Keywords
Oral hygiene, diabetes, periodontal disease

Öz

Amaç: Araştırmamızda, diyabetli Türk popülasyonunda ağız sağlığına ilişkin davranışlar ve bilgi düzeyleri değerlendirilmiştir.

Gereç ve Yöntemler: Kesitsel çalışmamızda, tip 2 diyabet hastalarında ağız sağlığına ilişkin tutumlar, bilgi düzeyleri ve farkındalık öğeleri bir anket ile değerlendirildi.

Bulgular: Altı aylık bir süreçte, 439 katılımcı anketi tamamladı. Katılımcıların büyük çoğunluğunun (%67) diyabet-periodontal hastalık ilişkisi hakkında yeterli farkındalığa sahip olmadığı belirlendi. Diyabetli bireylerin ağız sağlığına ilişkin diş hekimine yönlendirilme oranı çok düşük bulundu. Ayrıca diş hekimlerinin diyabete ilişkin artmış periodontal riskler hakkında hastalarını uyarma oranı %19,4 idi.

Sonuç: Bulgularımız tip 2 diyabetik bireylerin ağız sağlığına ilişkin zayıf tutum sergiledikleri ve yetersiz bilgi düzeyine sahip olduklarını göstermiştir.

Anahtar Kelimeler
Oral hijyen, diyabet, periodontal hastalık

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Introduction

Diabetes mellitus is a chronic metabolic disorder characterized by hyperglycaemia resulting from damage to insulin secretion or insulin activity or both (1). It is estimated that the prevalence of diabetes among adults in developing countries will have increased by

69% between 2010 and 2030, affecting 439 million individuals worldwide (2).

Periodontal diseases are inflammatory disorders of the periodontal supporting tissues that causes attachment loss and alveolar bone destruction (3). That the risk of periodontal disease occurrence in diabetic patients is two to three times higher (4). The severity of periodontal disease also increases, especially in long-term diabetic individuals with poor metabolic control (5).

Periodontal diseases can have significant effects on glycemic control in diabetics by inducing increased systemic chronic inflammatory condition (6). There is an increased risk of diabetes related complications such as cardiovascular events in diabetic patients with severe periodontitis (7). Several studies have shown that the treatment of periodontal diseases improves metabolic control of diabetes (8,9).

As evidence supporting the bi-directional relationship between diabetes and periodontal diseases has increased, prevention and control of periodontal diseases has become important for diabetic patients. However, several studies have shown that there is insufficient knowledge and awareness about oral and periodontal health among patients with diabetes (10,11).

The aim of this study is to evaluate the oral hygiene habits of diabetics in a small Turkish population and determine patients' knowledge of the link between diabetes and periodontal health.

Materials and Methods

Participants for the study were recruited from those patients attending to the outpatient clinic at the Department of Endocrinology in Pamukkale University Hospital. All patients had a confirmed diagnosis of type 2 diabetes mellitus. Patients who agreed to participate in the study signed consent forms before filling out the questionnaire. In a 6-month period, a total of 439 diabetic patients completed the survey.

The first part of the questionnaire recorded the demographic characteristics of participants metabolic control of diabetic patients was evaluated with glycated haemoglobin (HbA1c) levels. They were obtained from patient's medical records. Glycemic control was classified as three degrees. HbA1c <7% was considered as good controlled, HbA1c ≥7 to <8% as fair controlled and HbA1c <8 as poor controlled (12).

In the second part, gingival health data and the knowledge of the relationship between periodontal disease and diabetes were evaluated. Participants were asked if their dentists informed them about the risk of diabetes-related periodontal diseases and if their physicians referred to a dentist for periodontal health.

The study protocol was approved by the Ethics Committee of Pamukkale University with 60116787-020/44409 protocol number (date: 26.06.2018).

Statistical Analysis

Data were analysed by using Statistical Package for the Social Sciences (SPSS) version 22. Categorical variables were given as numbers and percentages. Pearson chi-square analysis was used for the differences between categorical variables. Statistical significance was considered as $p \leq 0.05$.

Results

A total of 439 (276 females and 163 males) patients completed the questionnaire. Demographic characteristics of participants are as shown in Table 1. The length of the diagnosis of diabetes was more than 10 years for 46% of the participants. The rate of diabetic patients with poor glycemic control was 36.2%. About 39% of the participants had one or more systemic diseases accompanying diabetes.

Periodontal Health Awareness and Attitudes to Dental Care

Dental care and oral hygiene behaviours of the respondents were shown in Table 2. The proportion of participants who had a dentist visit within last one year was 43.1% (189/439). The main reason for not visiting a dentist was finding unnecessary (68%, 170/250). Majority of the participants (62%) stated that they did not have any knowledge about gingival diseases. The rate of respondents who noticed bleeding in their gums was 31%. About 66% of the participants reported that they brush their teeth regularly.

Awareness of Diabetic Patients Related to Periodontal Disease

Participants were asked whether there is a relationship between diabetes and periodontal disease (Table 2). Nearly 67% of the respondents stated that they did not have any knowledge about the possible association between periodontal disease and diabetes. Majority of diabetic patients (90.4%)

Table 1. Demographic data of the participants

Characteristic		n	Percent
Gender	Female/male	276/163	37.1/62.9%
Age	20-29 years	12	2.7%
	30-39 years	32	7.3%
	40-49 years	82	18.7%
	>50 years	313	71.3%
Education	Elementary	295	67.2%
	High school	70	15.9%
	Collage	57	13%
	PhD	17	3.9%
Monthly income (TL)	<2000	262	59.7%
	2000-4000	146	33.3%
	4000-6000	28	6.4%
	>6000	3	0.7%
Smoking status	Smoker	78	17.8%
	Non-smoker	361	82.2%
Waist circumference	Male		
	Over-weight/ obese	67/85	41.1/58.9%
	Female		
	Over-weight/ obese	49/223	17.7/82.3%
Length of diagnosis of diabetes (years)	<1	32	7.3%
	1-5	102	23.2%
	5-10	103	23.5%
	>10	202	46%
HbA1c levels (mmol/ mol)	<7	90	20.5%
	≥7 to 8<	190	43.3%
	≥8	159	36.2%
Presence of diabetic co-morbidities	Yes	168	38.3%
	No	271	61.7%
Diabetes treatment	Oral medication	255	58.1%
	Insulin	174	39.6%
	Only diet	10	2.3%
Diet control	Yes	259	59%
	No	180	41%
Exercise	Yes	164	37.4%
	No	275	62.6%

TL: Turkish Lira, HbA1c: Hemoglobin A1c

were not referred to a dentist for their periodontal health. Similarly, 81% reported that their dentist did not inform them about the importance of periodontal health relating to their diabetes.

Table 2. Oral health awareness and dental care habits of participants

Characteristic		n	Percent
Dentist visit (within last year)	Yes	189	43.1%
	No	250	56.9%
Reasons for not visiting a dentist in the past year	Lack of necessity	170/250	68%
	Anxiety	37/250	14.8%
	Lack of time	31/250	12.4%
	Afraid of dental treatments	12/250	4.8%
Knowledge of gingival disease	Yes	167	38%
	No	272	62%
Gingival bleeding	Yes	136	31%
	No	303	69%
Frequency of tooth brushing	1/Day	159	36.2%
	2/Day	113	25.7%
	>2/Day	17	3.9%
	1/2-3 Day	72	16.4%
	Less than 1/2-3 Day	78	17.8%
Change of toothbrush	3 months	176	40.1%
	6 months	139	31.7%
	1 year	61	13.9%
	Less than 1 year	63	14.4%
Interdental cleaning	Yes	206	46.9%
	No	233	53.1%
Do you think there is an association between diabetes and periodontal health?	Yes	148	33.7%
	No	291	66.3%
Referral to a dentist by physician	Yes	42	9.6%
	No	397	90.4%
Information by a dentist on oral health	Yes	85	19.4%
	No	354	80.6%

Distribution of the questions determining knowledge and awareness according to gingival health attitudes are shown in Table 3. 80.2% of those who have knowledge about gingival diseases and 84.4% of the participants who are aware of diabetes is a risk factor for periodontal diseases reported brushing their teeth at least once a

Table 3. Distribution of the attitudes to gingival health by knowledge and awareness items

	Knowledge of gingival disease n (%)			Awareness of association between diabetes and periodontal health n (%)		
	Yes	No	P-value	Yes	No	p-value
Dentist visit (within last year)						
Yes	79 (47.3)	116 (42.6)	NS	74 (50)	121 (41.6)	NS
No	88 (52.7)	156 (57.4)		74 (50)	170 (58.4)	
Frequency of tooth brushing						
1/day	67 (40.1)	92 (33.8)	0.000	58 (39.2)	101 (34.7)	0.000
2/day	59 (35.3)	54 (19.9)		56 (37.8)	57 (19.6)	
>2/day	8 (4.8)	9 (3.3)		4 (7.4)	13 (4.5)	
1/2-3 day	16 (9.6)	56 (20.6)		19 (12.8)	53 (18.2)	
Less than 1/2-3 day	17 (10.2)	61 (22.4)		11 (7.4)	67 (23)	
Interdental cleaning						
Yes	90 (53.9)	116 (42.6)	0.022	86 (58.1)	120 (41.2)	0.001
No	77 (46.1)	156 (57.4)		62 (41.9)	171 (58.8)	
Information by a dentist on oral health						
Yes	83 (49.7)	2 (0.7)	0.000	61 (58.8)	24 (8.2)	0.000
No	84 (50.3)	270 (99.3)		87 (41.2)	267 (91.8)	
Knowledge of gingival disease						
Yes				92 (62.2)	75 (25.8)	0.000
No				56 (37.8)	216 (74.2)	

NS: Not significant

day. Participants who were informed by a dentist on periodontal health had significantly higher percentages of the knowledge of gingival diseases and diabetes-periodontal disease relationship (p=0.000). Awareness of the relationship in diabetes and periodontal diseases was significantly higher in respondents who have knowledge about gingival disease (p=0.000).

The attitudes to gingival health of diabetic patients who are at high risk for periodontal diseases were also evaluated separately and presented in Table 4. Those participants were less likely to report regular dentist visit, gingival bleeding, use an interdental cleaning tool, referral to dentist by physician and being informed in possible periodontal disease risk. About two thirds of these participants were unaware of diabetes is a risk factor for periodontal diseases.

Discussion

The key finding of our survey is that type 2 diabetic patients have insufficient knowledge and awareness about importance of periodontal health. These results are consistent with studies in the literature especially conducted in developing countries (10,13,14).

The rate of those who reported brushing their teeth more than once daily (29.6%) was lower than the proportions in the studies performed by Bowyer et al. (13) (67.2%). It has been reported that as the educational level and financial income increases, dental care and periodontal health status improves (15). Thirty-four percent of the participants remarked that they do not have regular brushing habits in present study. The fact that this rate is higher than the findings of Sandberg et al. (16) (8.7%) can be explained by the low education and economic status of our study group.

Regular dental visits are important for the maintenance of gingival health and prevention of periodontal complications, especially in diabetics. In a review, it was emphasised that the proportions of utilization dental services are very low, especially in

Table 4. Distribution of oral health variables by diabetes related factors with a high risk for periodontal diseases

	HbA1c level (≥8 mmol/mol) n (%)	Length of diagnosis (>10 years) n (%)	Presence of systemic diseases accompanying diabetes n (%)
Dentist visit (within last year)			
Yes	68 (42.8)	90 (44.6)	81 (48.2)
No	91 (57.2)	112 (55.4)	87 (51.8)
Knowledge of gingival disease			
Yes	52 (37.7)	71 (35.1)	59 (35.1)
No	107 (67.3)	131 (64.9)	109 (64.9)
Gingival bleeding			
Yes	48 (30.2)	54 (26.7)	51 (30.4)
No	111 (69.8)	148 (73.3)	117 (69.6)
Frequency of tooth brushing			
1/day	56 (35.2)	69 (34.2)	71 (42.3)
2/day	26 (16.4)	52 (25.7)	36 (21.4)
>2/day	7 (4.4)	9 (4.5)	5 (3)
1/2-3 day	36 (22.6)	36 (17.8)	23 (13.7)
Less than 1/2-3 day	34 (21.4)	36 (17.8)	33 (19.6)
Interdental cleaning			
Yes	76 (47.8)	92 (45.5)	82 (48.8)
No	83 (52.2)	110 (54.5)	86 (51.2)
A**wareness of association between diabetes and periodontal health**			
Yes	48 (30.2)	72 (35.6)	55 (32.7)
No	111 (69.8)	130 (64.4)	113 (67.3)
Referred to a dentist by physician			
Yes	13 (8.2)	20 (9.9)	16 (9.6)
No	146 (91.8)	182 (90.1)	152 (90.5)
Information by a dentist on oral health			
Yes	29 (18.2)	40 (19.8)	33 (19.6)
No	130 (81.8)	162 (80.2)	135 (80.4)

HbA1c: Hemoglobin A1c

low- and middle-income countries (17). Similarly, in present study, 43.1% of patients reported a dental visit within past 12 months which was consistent with studies conducted in low income countries (18), but lower than in high-income countries (13,16). Poudel et al. (17) stated that in high-income countries, dental care cost was the leading reason that discourage participants from using dental services, while the main underlying reasons are lack of necessity and dental anxiety in low-income countries. In our study, 68% of the participants who did not attend regular dental visits stated that they did not need it which can be due to not having sufficient knowledge about gingival health. Bahammam (19), have reported that diabetic patients who have regular dentist visits know that they are more vulnerable to gum problems than non-diabetics, also the rate of taking advice from their dentists about oral health care is 75%. While the rate of dental visit within last year was 43%, the proportion of patients who had been informed about periodontal disease risk related to diabetes by the dentist was below 20% in present study. This data may indicate that dental professionals do not take the medical history of patients on diabetes or not to take on responsibility of educating on diabetes related periodontal health. Those who are taken advice from healthcare professionals for dental check-up at least twice a year are more likely to visit a dentist in the last 12 months (14). In our study, the low rate of referral of the participants to a dentist by physicians for oral examination may have decreased dental attendance rates.

Nearly 67% of our study population were unaware that diabetes is a risk factor for periodontal diseases in line with most of the studies which reported more than half of diabetic patients have a lower knowledge about diabetes-periodontal disease relationship (19). On the internet-based survey of Amassi and Dakheel (20), 76% of the diabetic patients were aware of the adverse effects of diabetes on periodontal health, which is much higher than our results (33%). Furthermore, they emphasised that almost half of the participants in their study have under-graduate or postgraduate. This difference may be due to the fact that our population consist of only the patients attending to diabetes outpatient clinic and have lower education level.

The severity of periodontal disease is higher in individuals with poor metabolic control and long-term diabetes (5). As well as there is some evidence that periodontal infections influence the incidence of diabetic complications adversely (21), it has been reported that the number of microvascular complications is a risk factor for severe periodontal disease (22). These findings are important because at

least one co-morbidity was detected in 98.5% of Turkish patients diagnosed with type 2 diabetes for minimum 5 years (23). Therefore, in present study, we also evaluated the distribution of gingival health variables in respondents with poor metabolic control (HbA1c ≥8 mmol/mol), diagnosed with diabetes for more than 10 years and with co-morbidities accompanying diabetes. We found that gingival health practices were insufficient in these participants. However, the percentages of self-reported gingival bleeding, knowledge with gingival diseases and awareness of diabetes-periodontal disease relationship were low. Long-term periodontal care with regular periodontal maintenance visits ensured reductions in HbA1c levels of type 2 diabetic patients (24). As well as in a great majority of our study population, the percentages of referral and information provided by health and dental professionals about diabetes related periodontal diseases in patients with diabetic risk factors for periodontitis, were very low. It has been known that good oral health is strongly associated with regular dental visits.

The results of our study cannot be inferred from the general diabetic population in the country, due to the small study population and the low socio-economic status and education level of the respondents in our study. This is a limitation of our survey.

Conclusion

Our findings support that there is an essential requirement for increasing the awareness of association between periodontal disease and diabetes for maintaining periodontal health in diabetic patients. Therefore, it will be beneficial to enhance the knowledge of physicians and dentists on the bi-directional relationship between diabetes and periodontal diseases and to organize education programs to increase awareness about the importance and improvement of periodontal health in diabetic population. It will bring great benefit to include detailed periodontal examination and periodontal maintenance therapy to the program that planned for the management of diabetes in our society.

Ethics

Ethics Committee Approval: The study protocol was approved by the Ethics Committee of Pamukkale University with 60116787-020/44409 protocol number (date: 26.06.2018).

Informed Consent: Patients who agreed to participate in the study signed consent forms before filling out the questionnaire.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Concept: G.T.C., S.M.F., Design: G.T.C., S.M.F., Data Collection or Processing: S.M.F., Analysis or Interpretation: G.T.C., Literature Search: G.T.C., Writing: G.T.C.

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Original Article / Özgün Araştırma

Evaluation of Trabecular Structure Using Fractal Analysis in Patients Taking Proton Pump Inhibitors

Proton Pompa İnhibitörü Kullanan Hastalarda Trabeküler Yapının Fraktal Analiz ile Değerlendirilmesi

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Abstract

Objective: The aim of this study was to evaluate the changes in the trabecular bone structure in patients using proton pump inhibitors (PPI) by performing measurements with fractal analysis, mandibular cortical width (MCW) and panoramic mandibular index (PMI) on panoramic radiographs.

Materials and Methods: This study consists of 46 patients (20 males and 26 females) using PPI drugs regularly for at least 1 year and systemically healthy 46 persons (20 males, 26 females) as the control group. Fractal analysis was performed in five regions: the mandibular angle (ROI1), posterior mandible (ROI2), interdental area between apical of the second premolar and the first molar (ROI3), maxillary tuber region (ROI4), anterior mandible (ROI5). PMI and MCW as an indicative of osteoporosis, were measured on the panoramic radiographs.

Results: For re-evaluated measurements, interobserver and intraobserver agreements were found to be 0.985 and 0.987, respectively. ROI2-4 (1.732, 1.334, 1.333; respectively) were significantly lower in PPI users, whereas, there was no significant in ROI1 (1.605) and ROI5 (1.694). The values MCW and PMI were not statistically significant in PPI users.

Conclusion: Posterior region of the mandible, maxillary tuberosity and interdental region had lower trabecular microstructures in PPI users. The results of this study show that patients using PPI at least one year should be considered as regarding osteoporotic changes during dental treatment.

Keywords
Osteoporosis, proton pump inhibitors, fractal analysis, radiography, panoramic

Anahtar Kelimeler
Osteoporoz, proton pompa inhibitörü, fraktal analiz, radyografi, panoramik

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Öz

Amaç: Bu çalışmanın amacı, panoramik radyografilerde fraktal analiz, mandibular kortikal genişlik (MCW) ve panoramik mandibular indeks (PMI) ölçümleri ile proton pompa inhibitörü (PPI) kullanan hastalarda trabeküler kemik yapısındaki değişiklikleri değerlendirmektir.

Gereç ve Yöntemler: Bu çalışma, en az 1 yıldır düzenli olarak PPI ilacı kullanan 46 hasta (20 erkek ve 26 kadın) ve kontrol grubu olarak sistemik olarak sağlıklı 46 hastadan (20 erkek, 26 kadın) oluşmaktadır. Fraktal analiz için 5 bölge değerlendirildi: mandibular açı (ROI1), posterior mandibula (ROI2), ikinci premolar ve birinci molar dişlerin apikali arasındaki interdental alan (ROI3), maksiller tüber bölgesi (ROI4), anterior mandibula (ROI5). Osteoporozun bir göstergesi olarak PMI ve MCW indeksleri panoramik radyografiler üzerinde ölçüldü.

Bulgular: Gözlemciler arası ve gözlemci içi güvenilirlik sırasıyla 0,985 ve 0,987 olarak bulundu. ROI2-4 (sırasıyla; 1,732, 1,334, 1,333), PPI kullanıcılarında kontrol

grubuna göre önemli ölçüde daha düşükken, ROI1 (1,605) ve ROI5'te (1,694) gruplar arasında anlamlı bir farklılık bulunmadı. PPI kullanan hastalarda MCW ve PMI değerleri istatistiksel olarak anlamlı bulunmadı.
Sonuç: PPI kullanan hastalarda posterior mandibula, maksiller tüber ve interdental bölgenin trabeküler mikro yapısında azalma olduğu görülmüştür. Bu çalışmanın sonuçları en az bir yıl düzenli olarak PPI kullanan hastalarda çene kemiklerinde osteoporotik değişiklikler görülebileceğini göstermiştir.

Introduction

Proton pump inhibitors (PPI) are commonly prescribed for peptic ulcer, gastroesophageal reflux disease, Helicobacter pylori infections. PPI irreversibly links the proton pump of the parietal cells in the stomach to hinder acid secretion (1). However, concern for the adverse effects of PPI due to being overprescribed is growing. Recent studies indicated that long-term PPI using increases the risk of osteoporosis and fractures, and causes kidney disease, hypomagnesemia and iron deficiency (2). Lastly, the Food and Drug Administration (FDA) issued a warning in 2010 that spine and hip fractures can be observed in patients who intake PPIs for one year or longer (3). Although the effect of PPI drugs on bone metabolism is not known exactly, hypochlorhydria and hypergastrinemia are reported two main mechanisms. A review of the literature showed that there are several studies indicating that long intake of PPI could negatively affect implant osseointegration and increase the failure rate of implant (4-6).

Fractal analysis is a widely used method in recent years to characterize the trabecular structure of the bone, because it is a non-invasive method, free of charge and allows examining conventional radiographs. It is also a widely used method in medical research for examining microstructure of calcaneus bone, especially in osteoporotic patients.

However, osteoporotic effect of long term PPI intake on maxilla and mandible is still unknown. To answer this question, this study was performed. The aim of this study is to evaluate the altering trabecular structure of maxilla and mandible in PPI users using fractal analysis and mandibular cortical width (MCW) and panoramic mandibular index (PMI) as an indicator for osteoporosis.

Materials and Methods

Study Sample

This retrospective study consisted of 46 patients using PPI (26 female and 20 male, age between 16 and 77 years) and sex, age matched control group who applied to the Department of Oral and Maxillofacial Radiology for various dental complaints. Patients who regularly used PPI at least 1 year included in this study. The number of samples for the fractal dimensions (FD) with a 95% confidence level and an 80% power was determined as minimum n=27 for each group. In study group, 34.7% of patients (n=16) used PPI less than 5 years, 30.4% of patients (n=14) used PPI between 5-10 years, 34.7% of patients (n=16) used PPI at least 10 years. Based on the medical history of patients, active pharmaceutical ingredients were lansoprazole (32.6%), esomeprazole (40%), pantoprazole (30.4%). Exclusion criteria for this study were; patients had bone related diseases or used drugs which affected bone formation, panoramic radiography with distortion, poor image quality, lesion, infection or cyst in selected areas, regions including dentition. Ethics committee approval was obtained from Altınbaş University Clinical Research Ethics Committee (approval number: 28, date: 05.11.2020).

Fractal Analysis

All panoramic images were performed with Newtom 2D Panoramic (Newtom, Verona, Italy) with the same exposure parameters (72 kVp, 7 mA, 13.6 s). FD of samples were calculated using an image processing program, imageJ bundled with 64 bit Java 1.8.0 which was developed by the National Institutes of Health.

In this study, 5 regions of interest (ROI) were identified on the panoramic radiography. Selected ROIs were as follows (Figure 1):

1. ROI1: 35x35 pixels in the mandibular angle
2. ROI2: 30x70 pixels in the posterior mandible
3. ROI3: 18x18 pixels in the area between apical of the second premolar and the first molar
4. ROI4: 18x18 pixels in the maxillary tuber region
5. ROI5: 30x70 pixels in the anterior mandible

FD was performed based on the method used by White and Rudolph (7) (Figure 2).

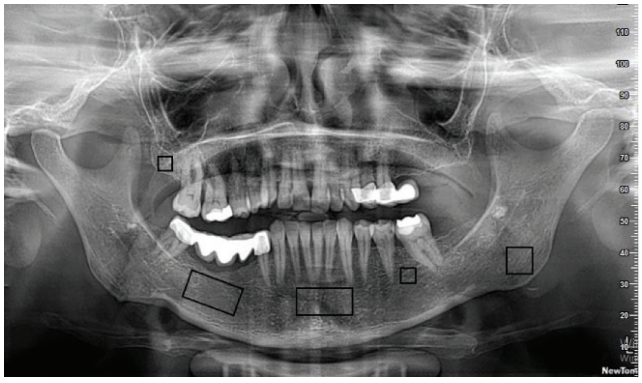

Figure 1. ROIs were selected for fractal analysis on panoramic radiography
ROI: Regions of interest

Initially, selected regions were identified and duplicated (Figure 2A). Then the Gaussian filter (sigma=35 pixels) was used for blurred image and extraction of detail and noise (Figure 2B). The blurred image was removed from the original image and the mean value of 128 was applied to show radiographically components of the bone (Figure 2C). The resulting image was made binary to separate bone marrow from trabeculae (Figure 2D). Median filter was used to remove noise from the image (Figure 2E). Then the binary image inverted to show trabeculae then converted to the skeletonize type (Figure 2F, 2G). This step allows to demonstrate skeletal structure of the bone. Skeletonized image may be superimposed on the original image to show the trabecular structure of the selected region (Figure 2H). Lastly, the image segmented into squares with dimensions of 2, 3, 4, 6, 8, 12, 16, 32, 64. This process is led to measure heterogeneity of the bone as well as. The slope of the logarithmic regression line shows the value of the FD.

Panoramic Mandibular Index

A ratio of MCW to distance between inferior border of mandible and mental foramen was calculated for PMI measurement based on the method of Benson et al. (8) (Figure 3A).

Mandibular Cortical Width

Two parallel lines were drawn across the inferior and superior border of the mandibular cortex at the level of mental foramen. Then a perpendicular line between two parallel lines was measured (Figure 3B).

Statistical Analysis

IBM SPSS 25.0 (SPSS, Chicago, IL, USA) was used for statistical analysis. Normality of the sample data was assessed using Shapiro-Wilk test. We used a student t-test as a parametric test for radiomorphometric indexes, and Mann-Whitnet U test as a non-parametric test for fractal analysis. FD calculations for 20% of all samples were applied twice by two oral and maxillofacial radiologists who had 4 years experience for both observers at 2 weeks intervals. Interobserver and intraobserver agreement were evaluated by intraclass correlation coefficient (ICC). The level of statistical significance is set as $p<0.05$.

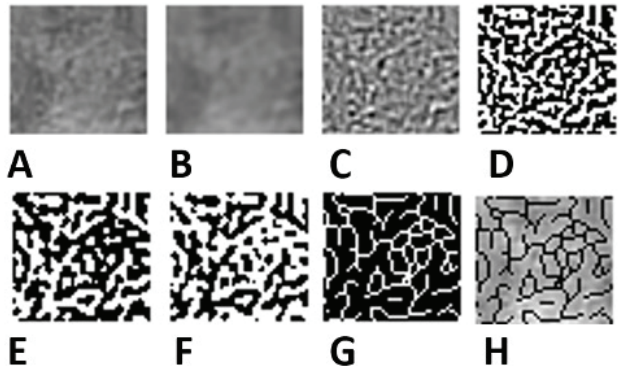

Figure 2. Fractal analysis procedures. Image was selected and duplicated (A). Gaussian filter was applied on the selected image (B). The blurred image was subtracted from the original image (C). The image made binary (D). Median filter was used to remove noise from original image (E). Invertion (F). Skleletonization (G). Demonstration of trabeculation on original image (H)

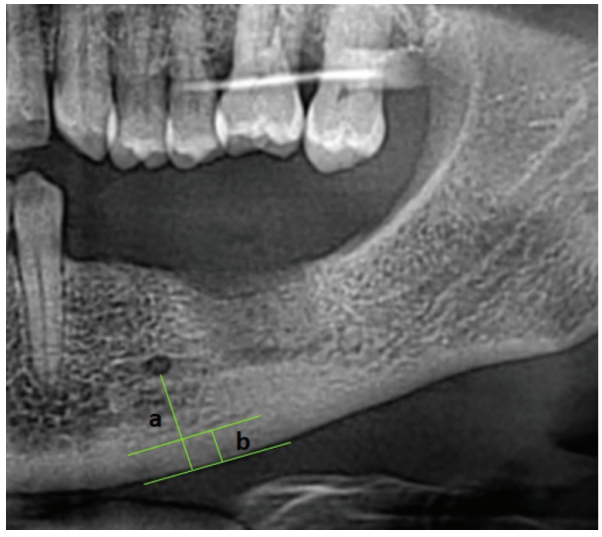

Figure 3. Measurements of PMI and MCW on panoramic radiography
PMI: Panoramic mandibular index, MCW: Mandibular cortical width

Results

For re-evaluated measurements, interobserver and intraobserver agreement was found to be 0.974 and 0.970, respectively which shows excellent reliability (Table 1). Descriptive analysis and results based on student t-test were shown in Table 2. FD values of posterior area of mandibula were higher than anterior for both groups. Posterior mandibula (ROI2), interdental bone (ROI3) and tuber maxilla (ROI4) were significantly lower in PPI users whereas, there was not significant in mandibular angle (ROI1) and anterior mandibula (ROI5). Whereas, tuber maxilla (ROI4) has the highest difference (p=0.01), difference of the interdental bone (ROI3) was the lowest rate (p=0.041) among selected interested regions. The values of MCW and PMI as a radio morphometric index were not statistically significant in PPI users.

Discussion

In recent years, researchers indicated some adverse effects of PPI including Vitamin B12 deficiency, hypomagnesaemia, increased risk of bone fracture, thrombocytopenia (2). Lastly, FDA warned that long-term using of PPI may increase the risk of bone fracture (3).

Table 1. Inter-rater and intra-rater reliability

	ICC	%95 confidence limits		p
Intra-observer	0.974	0.942	0.988	0.000*
Interobserver	0.970	0.934	0.987	0.000*

*Statistically significant in the study group *p<0.05, ICC: Intraclass correlation coefficient

Association between PPIs and increased fracture risk is still unknown. There are a lot of conflicting results between previous studies. Increasing of the histamine level, hyperparathroidism, hypochlorhydria, an adverse impact of PPI on bone turnover have been recently discussed by the researchers (5,9-11). Our results promote previous findings and reveal that long-term effects of PPIs may be deleterious on bone formation. In accordance with the FDA warnings, a majority of clinicians are still overprescribing this medicine. Strict controls on the administration of PPI should be applied by clinicians.

The recent literature linked some drugs may interact with PPIs (12,13). A cohort study with a large population indicated coadministration PPIs with bisphosphonates could be a high risk of hip fractures happening. Researchers denoted a growing concern about oral bisphosphonates and PPIs, which were commonly used by elderly patients. Authors remarked this result needed further studies to explain interaction between two drugs (12). Another study assessed the efficacy of risedronate and PPIs on vertebral structures. This randomized clinical trial revealed that using risedronate with PPIs was not relevant to vertebral fractures (13). Since patients had bone formation disorders and used drugs that affect bone formation were excluded in our study, the relationship between bisphosphonates and fracture risk was not evaluated. On the other hand, in our study, patients were often taking PPIs to impair the effect of non-steroidal anti-inflammatory drugs (NSAI) on gastrointestinal systems. PPI coadministration with NSAI could cause osteoporotic changes on bone.

Table 2. Descriptive analysis of sample data

	PPI users						Control group						p
	N	Mean	SD	Min	Max	Range	N	Mean	SD	Min	Max	Range	
ROI1	46	1.605	0.004	1.597	1.610	0.013	46	1.609	0.018	1.598	1.690	0.092	[1]0.394
ROI2	46	1.732	0.059	1.567	1.833	0.266	46	1.76	0.061	1.641	1.868	0.227	[1]0.019*
ROI3	46	1.337	0.021	1.29	1.365	0.075	46	1.346	0.023	1.318	1.4	0.082	[1]0.041*
ROI4	46	1.333	0.022	1.267	1.365	0.098	46	1.346	0.025	1.309	1.409	0.100	[1]0.010*
ROI5	46	1.694	0.020	1.61	1.753	0.143	46	1.704	0.038	1.642	1.834	0.192	[1]0.379
MCW	46	3.182	0.653	1.6	4.5	2.9	46	3.268	0.565	2	4.1	2.1	[2]0.582
PMI	46	0.230	0.078	0.12	0.532	0.413	46	0.3	0.066	0.171	0.456	0.284	[2]0.792

Min: Minimum, Max: Maximum, SD: Standard deviation, *Statistically significant in the study group *p<0.05, [1]Mann-Whitney U test, [2]Student's t-test, PPI: Proton pump inhibitors

Number of studies evaluating dental procedures affected by PPI-related bone formation disorder are limited to date. In recent years, the retrospective cohort study assessed the impact of PPI intake on dental implant osseointegration in 1,918 implants and the failure rate of osseointegration was found 4.30 times more likely among PPI users than nonusers (7). Similarly, another large cohort study investigated whether the negative effect of PPI on osseointegration and rate of implant failure was found 6.8% and 3.2% in PPI users and nonusers, respectively (6). However, these retrospective studies had some potential confounding factors such as duration of treatment, regular user and type of PPI. Regular PPI intake is crucial due to the fact that based on detailed anamnesis obtained from patients in this study, most of the patients only used PPI for 2 or 3 weeks when they had a gastrointestinal system related symptoms. In our study, patients used PPI regularly at least one year were included. Recently, Al Subaie et al. (4) observed the postoperative effect of PPI on osseointegration in rats tibia by using Micro-computed tomography. Results of this study indicated the serious effects of administration of omeprazole after implant surgery on bone healing. Younger rats were preferred for this study due to having a better bone healing process. However, outcomes of the study indicated that the effect of administration of PPI could be likely more harmful in elderly patients. Our concern is that long-term preoperative use of PPI may have more deleterious effects on bone healing than only postoperative use for 2 weeks.

To our knowledge, this is the first study to evaluate trabecular structure on panoramic radiographs using fractal analysis in PPI users. In the present study, we selected the ROIs as large as possible based on suggestions of the study by White and Rudolph (7). Alterations in anterior maxilla and posterior mandible were found statistically significant in a previous study evaluating morphological structure of maxilla and mandible among osteoporosis and control groups (8). Another study reported that the supracortical area above the angulus mandible and anterior to the mental foramen were significantly lower among patients using the aromatase inhibitors (14). In our study, FD values of posterior mandible (ROI2) and tuber maxilla (ROI4) were found significantly lower among PPI users than control group, whereas the difference in anterior region of mandible (ROI5) was not statistically significant. Alterations in the tuber maxilla is the highest difference among selected ROIs, and corpus mandible is the second region. A possible explanation of this result may be that because osteoporotic changes associated with cancellous bone and maxilla had more trabecular structure and less cortical bone than mandible (15). Bones with the high ratio of trabecular bone to cortical bone are eightfold more sensitive to blood circulation due to high turnover of trabecular bone (16). Additionally, explanation of being not different in the mandibular anterior region may be this reason. In the present study, the maxillary tuber region had the highest difference among selected ROIs. This result revealed that osseointegration of implant in the maxillary tuberosity could be difficult in the long term of PPI use due to low bone quality. We could not select maxillary anterior region because the image quality of maxilla anterior bone is low due to artefacts (16).

Our results revealed that values of MCW and PMI were not statistically significant. The possible explanation of this result could be that osteoporotic alterations developed more rapidly in trabecular bone than cortical bone and parameters of MCW and PMI depend on cortical bone of mandible (17).

In our study, changes in bone microstructure was assessed by using fractal analysis. Fractal analysis is non-invasive, free of charge and high reproducibility technique. There are numerous applications of fractal analysis in dentistry (17-20). Principle of fractal analysis depends on the box counting method and calculates the number of FDs. FD values inversely correlate with the bone mass. However, standardization of parameters on radiographs is a crucial factor for reliable outcomes. A recent study indicated that parameters of radiography such as exposure time can change the voxel size which result in different outcomes (21). Another study showed that differences in image resolution between panoramic radiography and cone beam computed tomography result in different FD values. Authors offered that using panoramic radiography in fractal analysis studies due to high image resolution of panoramic radiography (22).

As a limitation of the present study, patients have used drug for different durations so their effects may be different from each other in clinical practice.

Further studies are needed to evaluate the relationship between duration of use and osteoporosis risk. One other limitation is that the radiologic bone structure of the patient before using the drug is not known exactly. Therefore comparative radiological studies before and after PPI use are needed to obtain more meaningful clinical results.

Conclusion

Current study aims to evaluate the trabecular structure of mandible and maxilla in patients intake PPI at least one year. Posterior region of mandible, maxillary tuberosity and interdental region had lower trabecular microstructure in PPI users. PMI and MCI as an indicator of osteoporosis were not statistically significant. Patients using PPI at least one year should be considered as regard osteporotic changes while dental treatment.

Ethics

Ethics Committee Approval: Ethics committee approval was obtained from Altınbaş University Clinical Research Ethics Committee (approval number: 28, date: 05.11.2020).

Informed Consent: Retrospective study.

Peer-review: Internally peer-reviewed.

Authorship Contributions

Concept: Ş.Ö., Design: Ö.O., Z.Z.Y., Data Collection or Processing: Ş.Ö., Analysis or Interpretation: Ş.Ö., Ö.O., Literature Search: Ş.Ö., Z.Z.Y., Writing: Ş.Ö., Ö.O., Z.Z.Y.

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Original Article / Özgün Araştırma

Socio-demographic, Behavioral and Subjective Factors Affecting the Knowledge and Attitudes on Periodontal Health Among Turkish Pregnants

Türk Gebelerinin Periodontal Sağlığa İlişkin Bilgi ve Tutumlarını Etkileyen Sosyo-demografik, Davranışsal ve Öznel Faktörler

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Pregnancy, periodontal health, knowledge, attitude, socio-demographic, behavioral

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Abstract

Objective: This study aimed to assess the knowledge and attitudes toward periodontal health of Turkish women and determine their socio-demographic, behavioral, and subjective factors.

Materials and Methods: The sample of cross-sectional study consisted of 407 pregnant. Data on socio-demographic factors, behavioral patterns, pregnancy-related characteristics, self-reported periodontal health, willingness to initiate the periodontal treatment, and dental care referral and advice from a family doctor/gynecologist, the knowledge and attitudes toward periodontal health were collected using a structured questionnaire.

Results: Oral health knowledge was associated with education level and self-perception of tooth mobility, whereas oral health attitudes were associated with education level, dental flossing, dental check-up during pregnancy, the absence of self-reported gingival bleeding, gum swelling and redness, and tooth mobility among pregnant women. Having a higher education level, using daily flossing, having a check-up during pregnancy, the absence of self-reported gum bleeding and tooth mobility, having a history of premature birth, having longer periods of gestation, and having greater knowledge are predictors of more positive attitudes. Having a higher education level and having more positive attitudes are the predictors of better knowledge.

Conclusion: Most pregnant women have limited knowledge about the effect of periodontal health on birth outcomes; nevertheless, the level of knowledge about the effect of periodontal health on pregnancy is high.

Öz

Amaç: Bu çalışmanın amacı Türkiye'de yaşayan gebelerin, periodontal sağlığına yönelik bilgi ve tutumlarını değerlendirmek ve sosyo-demografik, davranışsal ve subjektif faktörleri belirlemektir.

Gereç ve Yöntemler: Bu kesitsel çalışmaya 407 gebe dahil edildi. Gebenin sosyo-demografik faktörleri, davranış kalıpları, gebelik özellikleri, kendisi tarafından bildirdiği periodontal sağlık durumu, periodontal tedaviyi kabul etme isteği, dental tedaviler için aile hekimi/kadın doğum uzmanı tavsiyesi ile sevk edilmesine ilişkin

verileri ve periodontal sağlığa yönelik bilgi ve tutumlarını içeren soruların bulunduğu anketler katılımcılara uygulandı.
Bulgular: Ağız sağlığı hakkındaki bilgi eğitim düzeyi ve gebenin kendisi tarafından bildirilen diş mobilitesi ile ilişkiyken; ağız sağlığı ile ilgili tutumlar ise eğitim düzeyi, diş ipi kullanımı, gebelikte diş hekimi muayenesi, gebenin kendisinin bildirdiği dişeti kanamasının, diş eti şişliğinin ve diş mobilitesinin olmaması ile ilişkilendirildi. Eğitim düzeyinin yüksek olması, günlük diş ipi kullanımı, gebelikte diş hekimi muayenesi, kendisi tarafından bildiren diş eti kanaması ve diş mobilitesinin olmaması, geçmişte yaşanılan erken doğum öyküsüne sahip olma, gebelik süresinin uzun olması ve daha fazla bilgiye sahip olma daha pozitif tutumların prediktörü olarak tespit edildi. Daha yüksek eğitim düzeyine ve daha olumlu bir tutuma sahip olmak da daha iyi bir bilgi seviyesinin prediktörü olarak bulundu.
Sonuç: Bu çalışmanın sonuçlarına göre gebelerin çoğunluğunun periodontal sağlığın doğum sonuçları üzerindeki etkisi hakkında sınırlı bilgileri varken, periodontal sağlığın gebelik üzerindeki etkisine ilişkin bilgi düzeyleri yine de yüksek bulundu.

Introduction

During pregnancy, a woman may be more prone to periodontal diseases due to the hormonal changes and the alteration in the immune system (1). Evidences reported the negative effects of pregnancy on periodontal health and the link between periodontal disease and adverse pregnancy outcomes, including low birth weight, preterm birth, preeclampsia, and miscarriages (2,3). In recent years, recognizing the importance of periodontal health in pregnancy has resulted in increased interest in studying the knowledge and attitudes of pregnant women. Previous studies showed that most pregnant women had limited knowledge about periodontal health and its possible effects on pregnancy and negative attitudes towards the safety of periodontal treatment (4-9).

To improve the oral health of pregnant women and their baby, collaborative oral health promotion strategies between medical and dental health professionals and the antenatal care policy are needed (8,10-13). In recent years, although the importance of dental care attendance and the safety and effectiveness of the preventive, diagnostic and restorative dental treatments during pregnancy has been emphasized in the routine antenatal examination (14,15), the prevalence of dental service usage and the percentage of pregnant women who received oral health counseling during their pregnancy are still low and periodontal health is neglected in oral health education programs (12,16,17).

In Turkey, there are a few studies which examined the awareness, attitudes and knowledge on periodontal diseases among pregnant women according to socio-demographic factors (18-21). Along with clinical factors, the wide range of socio-demographic, psychological, behavioral and subjective factors which may affect the knowledge and attitudes of pregnant women must be considered when developing culturally sensitive oral health promotion programs (8,11-13,22,23). There is scarcity of studies evaluating the relationship between these factors and the knowledge and attitudes of pregnant women. Therefore, the aims of the study are to assess the knowledge and attitudes towards periodontal health among Turkish pregnant women and to determine the socio-demographic, behavioral and subjective factors affecting their knowledge and attitudes. We hypothesized that significant differences would exist in the knowledge and attitudes of Turkish pregnant women in term of socio-demographic, behavioral, and subjective factors.

Materials and Methods

This study was conducted on a consecutive sample of 407 pregnant women attending antenatal clinic at the Faculty of Medicine of Bolu Abant İzzet Baysal University, Department of Obstetrics and Gynecology, between April 2019 and June 2019.

Based on the reported 39% (p) prevalence of pregnant women who visited a dentist in the last year (24), a desired 95% confidence level, desired precision of 5% (d), considering non response rate of 10%, a minimum sample size of 402 subjects was calculated using the formula $n=z^2\ p\ q/d^2$. All procedures performed in this study were approved by the Clinical Researches Ethics Committee of Bolu Abant İzzet Baysal University (decision no: 2018/221, date: 13.12.2018). Participation was voluntary, and informed written consent was also obtained from each pregnant woman before the start of the study. Inclusion criteria for participation were pregnant women who were healthy and aged 18 years or older. The exclusion criteria were inability or unwillingness to

give informed consent, not speaking/reading/writing Turkish, and a personal history of chronic diseases and psychiatric illness. Eligible pregnant women were recruited in the waiting room by a researcher (Ü.M.U.) and informed written consent was obtained from pregnant women after providing information about the study.

Data Collection

Data on socio-demographic and behavioral factors, pregnancy-related characteristics, self-reported periodontal measure, willingness to initiate the periodontal treatment, and having a dental care referral and advice from family doctor/gynecologist, the knowledge and attitudes towards periodontal health were collected using a structured questionnaires.

Instrument

The Knowledge Scale which consists of 10 items with a 3-point Likert scale (0= disagree, 1= neither agree nor disagree, 2= agree) was used to determine pregnant women's knowledge about dental health, nutrition, smoking, periodontal disease as well as its effect on pregnancy outcomes. This scale scores ranged from 0 to 20, with higher scores indicating a higher level of knowledge.

Pregnant women's attitudes towards healthy eating, periodontal treatment, oral hygiene and routine dental checkups during pregnancy were assessed using the attitude scale. This scale consisted of 4 items that are scored using a three-point scale ranged agree (2) to disagree (0). Scale scores ranged from 0 to 8, with higher scores indicating more positive attitudes.

Development and Validation of the Scale of Knowledge and Attitudes

The initial item pool of 31 items (knowledge 18 items and attitudes 10 items) was generated by two researchers (T.P.) and (G.U.) through comprehensive reviews of published literatures and existing instrument (4,6,18). Following the generation of the initial item pool, face and content validity of items developed in this study were evaluated by an expert panel consisting of comprising two periodontists, one dental public health specialist, one biostatistician, and one gynecologist. They independently rated each item using the item-level content validity index (I-CVI) for its relevancy ('not relevant -1' to 'very relevant-4') and clarity ('not clear-1' to 'very clear-4'). An I-CVI ≥0.78 was defined as having good content validity (25). Twelve items with an I-CVI value of <0.78 (knowledge 6 items and attitudes 6 items) that had I-CVIs of 0.78 were excluded from the questionnaire and the final scales were obtained.

According to the recommendations of the expert panel, two knowledge items were revised to improve their clarity and understanding and reassessed by this panel.

According to the opinion of the expert group, two questions were included in this survey: willingness to initiate the periodontal treatment (yes vs. no), and having a dental care referral and advice from family doctor/gynecologist (yes vs. no). Pregnancy-related characteristics, including the trimester of pregnancy, the number of pregnancies, and having a history of premature birth (yes vs. no) were obtained through the pregnant women's medical records. Socio-demographic variables included age (years), monthly family income (<Turkish Lira (TL) 1,000 (low), TL 1,000-2,999 (lower-middle), TL 3,000- 4,999 (upper-middle), ≥ TL 5,000 (high) (26), employment status (unemployed vs. employed), location (rural vs. urban), having national health insurance (yes vs no), and educational level (≤8 years vs. >8 years) (27).

Five self-reported measures of general and oral health behavior were included: tooth brushing frequency (≥ twice a day vs ≤ once a day) (28), use of dental floss (yes vs. no) (28); dental attendance patterns (regular vs. problem-oriented) (28); dental check-up during pregnancy (yes vs. no) (4, 6) and smoking during pregnancy (yes vs. no) (29).

Self-reported periodontal disease was assessed using the three items on gingival bleeding, gum swelling /readness, and tooth mobility. Response options for each item were "yes" and "no (30,31). Self rated oral health was assessed using a single-item question with ordinal response options, which were grouped into good (excellent, very good, good) and bad (fair and poor) (32).

Statistical Analysis

Descriptive statistics were used to summarize the study. Data normality was tested using the Kolmogorov-Smirnov test. Due to the non-normal distributions, the Mann-Whitney U test was used to test significant differences between two groups while Spearman's rank correlation coefficient was used to evaluate the relationship between two variables

measured on an ordinal or continuous scale. Two multiple linear regression analyses with backward stepwise selection method were conducted for knowledge and attitudes to identify its related factors.

All variables found to be significant ($p<0.10$) in univariate analysis were entered into the multivariate regression analysis. R^2 statistic was used to determine the proportion of variance explained by the predictors. The standardized β coefficients were calculated for all variables. Reliability of the scales was assessed using the Cronbach's alpha coefficient and the corrected item-total correlation. A Cronbach's alpha value of 0.5-0.69 is considered for a new scale as acceptable (33). For item-total correlation, we considered a value greater than 0.20 (34).

In addition, the test-retest reliability of the scales was assessed using the intra-class correlation coefficients (ICC) in a subsample of 46 pregnant women who were reinterviewed about 2 week after the first interview. According to Walter et al. (35), the required sample size for the test retest reliability was calculated was determined based on the following parameters: $\alpha=0.05$, $\beta=0.20$, acceptable ICC=0.80, and hypothesized ICC=0.90. Following these parameters, 46 women were required. We considered an ICC greater than 0.75 as excellent agreement (36).

Results

The sample consisted of 407 pregnant women, with mean age of 29.08 [standard deviation (SD)=5.67]. Of these, 67.3% were housewives, 47.9% lived rural, 39.3% had formal school education, 83.8% had health insurance, 40% were in lower-middle income groups, 43% were in the third trimester period, 89.9% did not smoke. The mean number of pregnancies in the sample was 2.33 (SD =1.10; range, 1-6). 75.9% had a problem-oriented dental visits pattern, 30.5% brushed their teeth ≥ twice a day, 57.7% didn't use dental floss, and 29.7% visited the dentist for dental check-ups during their pregnancy. More than half of women perceived signs of gingival bleeding (56.3%) and gum swelling and readness (56.5%) but only 18.4% reported the presence of tooth mobility. Of these, 7.1% had a dental care referral and advice from family doctor/gynecologist, 83.5% were willing to initiate the periodontal treatment during pregnancy, and 88% rated their oral health as good (data not shown).

Mean knowledge was 16.96 (SD =2.36), mean attitude was 7.55 (SD =1.13). The corrected item total correlations were all positive and more than 0.20, supporting the internal reliability of these scales. The Cronbach's alpha was 0.63 for knowledge and 0.79 for attitude. For test-retest reliability, ICC's for knowledge and attitude were 0.84 and 0.86, respectively (data not shown).

As seen in Table 1, the total score on the knowledge scale was positively correlated with income ($r=0.106$, $p<0.05$). Women with 8 years or less of schooling had lower knowledge ($p=0.001$) and attitude scores ($p=0.041$). The scores on the attitude ($p<0.001$) and knowledge ($p=0.04$) were found to be lower among pregnant women who reported "tooth mobility". Lower attitude scores were found among pregnant women who reported gingival bleeding ($p=0.008$), gum swelling and readness ($p=0.007$), not having dental check ups during pregnancy ($p=0.001$), and no flossing ($p=0.025$). Among pregnancy-related characteristics, increasing gestational stage ($r=0.155$, $p<0.05$) and having a history of premature birth ($p=0.002$) were associated with more positive attitudes.

As seen Table 2, the final model explained 21.8 and 8.9% of the variation in attitude and knowledge, respectively. Having a higher education level, the use of dental floss, having a check-up during pregnancy, having a history of premature birth, having longer periods of gestation, having greater knowledge, the absence of gum bleeding and tooth mobility were found to be significant predictors of more positive attitudes. Higher education level and positive attitudes were the predictors of better knowledge.

Table 3 showed that most of the pregnant women knew about the increased risk of caries due to frequent consumption of sugar containing foods and beverages (94.3%), the main cause of periodontal disease (90%), the relationship between eating foods high in protein, calcium, phosphorus and vitamins A, C and D and good oral health during pregnancy (89%), the first signs of gum disease (86%), the cause of tooth loss (85%), the control methods in the prevention of periodontal disease (75%), and the negative effect of smoking (72%). More than half of them didn't know about the increased risk for low birth weight, preterm birth, and pre-eclampsia due to periodontitis (60.7%), and the best time to perform non-surgical periodontal therapy during pregnancy (59.2%). In addition, 74%

Table 1. Differences in scores of oral health knowledge and attitude according to socio demographic, behavioural and subjective characteristics of study participants (n=407)

Characterictics		Knowledge Mean ± SD	Attitudes Mean ± SD
Age (years) (r)		0.011	-0.040
Income (r)		0.106*	-0.031
Employment status[a]	Unemployed (n=274)	16.87±2.31	7.55±1.15
	Employed (n=133)	17.12±2.47	7.54±1.09
p-value		0.103	0.679
Location[a]	Rural (n=195)	16.81±2.43	7.47±1.23
	Urban (n=212)	17.09±2.30	7.62±1.02
p-value		0.171	0.220
National health insurance[a]	Yes (n=341)	16.95±2.39	7.55±1.12
	No (n=66)	17.01±2.22	7.51±1.15
p-value		0.884	0.726
Educational level[a]	≤8 years (n=160)	16.44±2.68	7.41±1.28
	>8 years (n=247)	17.30±2.08	7.64±1.02
p-value		0.001	0.041
Toothbrushing[a]	≤once a day (n=283)	16.90±2.26	7.55±1.10
	≥twice a day (n=124)	17.08±2.59	7.53±1.20
p-value		0.108	0.933
Dental flossing[a]	Yes (n=172)	17.08±2.33	7.65±1.01
	No (n=235)	16.86±2.39	7.41±1.26
p-value		0.326	0.025
Smooking[a]	Yes (41)	16.68±3.10	7.34±1.29
	No (366)	16.99±2.27	7.57±1.11
p-value		0.905	0.161
General dental attendance pattern[a]	Regular (n=98)	16.87±2.19	7.46±1.21
	Problem-oriented (n=309)	16.98±2.42	7.57±1.10
p-value		0.377	0.283
Dental check-up during pregnancy[a]	No (n=286)	16.75±2.68	7.16±1.56
	Yes (n=121)	17.04±2.22	7.71±0.84
p-value		0.775	0.001
Self-rated oral health[a]	Good (n=358)	17.03±2.33	7.55±1.12
	Bad (n=49)	16.40±2.55	7.51±1.19
p-value		0.089	0.990
Self-reported gingival bleeding[a]	Yes (n=229)	16.82± 2.51	7.37±1.36
	No (n=178)	17.13±2.16	7.78±0.67
p-value		0.402	0.008
Self reported gum swelling and readness[a]	Yes (n=230)	16.92±2.47	7.40±1.29
	No (n=177)	17.00±2.22	7.73±0.84
p-value		0.806	0.007
Self-reported tooth mobility[a]	Yes (n=75)	16.09±3.13	6.74±1.79

	No (n=332)	17.15±2.11	7.73±0.82
p-value		0.043*	<0.001
Self-reported willingness to initiate the periodontal treatment[a]	Yes (n=340)	16.91±2.45	7.52±1.15
	No (n=67)	17.20±1.83	7.65±1.02
p-value		0.920	0.412
Receipt of oral health referral and advice from family doctor/gynecologist[a]	Yes (n=29)	16.75±2.21	7.65±0.89
	No (n=378)	16.97±2.38	7.54±1.14
p-value		0.414	0.961
Number of pregnancies (r)		0.020	-0.031
Gestational stage (r)		0.078	0.155*
History of premature birth[a]	Yes (70)	17.02±2.37	7.80±0.80
	No (337)	16.67±2.33	7.49±1.18
p-value		0.161	0.002

*Correlation is significant at the 0.05 level (2-tailed); [a]Statistical evaluation by Mann-Whitney U test, SD: Standard deviation, r: Spearman's rank correlation coefficient

Table 2. Socio-demographic, behavioral and subjective predictors of the knowledge and attitudes

Model	The predictors					
Attitudes	**(R^2=0.235; adjusted R^2=0.218)**	**B**	**Std. error**	**β**	**t**	**Sig.**
	Having a higher education level	0.105	0.051	0.095	2.064	0.040
	Using dental flossing	0.206	0.103	0.090	2.005	0.046
	Having a check-up during pregnancy	-0.392	0.116	-0.158	-3.382	0.001
	Self reported absence of gum bleeding	0.355	0.104	0.156	3.401	0.001
	Self-reported absence of tooth mobility	0.679	0.135	0.233	5.016	0.000
	Having a history of premature birth	-0.354	0.136	-0.118	-2.616	0.009
	Having longer periods of gestation	0.190	0.063	0.137	3.022	0.003
	Having greater knowledge	0.078	0.022	0.164	3.590	0.000
Knowledge	(R^2=0.10; adjusted R^2=0.089)					
	Having a higher education level	0.319	0.110	0.138	2.887	0.004
	Having more positive attitudes	0.402	0.107	0.192	3.766	0.000

B: Unstandardized coefficients, β: Standardized coefficients, Std. Error: Standard error

of them had false knowledge about the relationship between hormonal change during pregnancy and periodontal health.

Most women had a positive attitude towards eating a balanced diet for their own health and that of their unborn child (92.6%), giving more attention their oral care for preventing periodontal diseases (92.4%), visiting the dentist for a check-up before pregnancy (91.1%), the effects of periodontal treatment on her pregnancy and the health of her baby (86%) (Table 4).

Discussion

In Turkey, oral health as one of consultancy issues was included in the recent Prenatal Care Management Guide of Ministry of Health, 2014 (37). Family medicine and obstetric practitioners have a responsibility for giving advise and information on oral and dental health (37). Hovewer, earlier studies (18,19) reported that the inter-professional collaboration among healthcare providers and dental professionals is required to improve the oral health care and dental care-seeking behaviour of Turkish

Table 3. The distribution of item responses of the knowledge scale

	Yes n (%)	Don't know n (%)	No n (%)
1. During pregnancy, frequent consumption of sugar containing foods and beverages increase caries risk	384 (94.3)	18 (4.4)	5 (1.2)
2. Hormonal change during pregnancy can affect periodontal tissue	46 (11.3)	60 (14.7)	301 (74)
3. Untreated periodontal diseases may lead to loss of teeth	344 (84.5)	53 (13)	10 (2.5)
4. The gingival health is controlled by effective oral hygiene measures (e.g, brushing twice daily and daily flossing)	307 (75.4)	78 (19.2)	22 (5.4)
5. The main cause of periodontal disease is bacterial plaque	365 (89.7)	35 (8.6)	7 (1.7)
6. Periodontitis is associated with a higher risk of low birth weight, preterm birth, and pre-eclampsia	129 (31.7)	247 (60.7)	31 (7.6)
7. Non-surgical periodontal therapy (scaling and root planing) during the second trimester is safe	158 (38.8)	241 (59.2)	8 (2)
8. Bleeding gums are one of the first signs of gum disease	349 (85.7)	53 (13)	5 (1.2)
9. Smoking during pregnancy increases the risk of health problems for baby and mother	292 (71.7)	105 (25.6)	10 (2.5)
10. Eating foods high in protein, calcium, phosphorus and vitamins A, C and D is necessray for good oral health during pregnancy	362 (88.9)	31 (7.6)	14 (3.4)

Table 4. The distribution of item responses of the attitude scale

	Agree n (%)	No opinion n (%)	Disagree n (%)
1.Pregnant womens must eat a balanced diet for for their own health and that of their unborn child	377 (92.6)	27 (6.6)	3 (0.7)
2.Pregnant womens must pay attention their oral care for preventing periodontal diseases	376 (92.4)	27 (6.6)	4 (1.0)
3.Women must go to the dentist for dental check-ups before pregnancy	375 (91.1)	28 (6.9)	4 (1.0)
4.Periodontal treatment has a positive effect in my pregnancy and the health of my baby	350 (86)	35 (8.6)	22 (5.4)

pregnant women as well as to increase the awareness towards these topics of medical providers. In addition, Turkish pregnant women had limited knowledge about periodontal disease and its possible effects on pregnancy. To develop the collaborative efforts, the factors affecting the knowledge and attitudes towards periodontal health should be determined culturally sensitive. In this study, we aimed to assess the knowledge and attitudes towards periodontal health among Turkish pregnant women and determine its socio-demographic, behavioral and subjective predictors.

We found that the majority of pregnant women had the proper knowledge about periodontal diseases (i.e. developing due to the bacteria, gingival bleeding, and tooth loss when untreated), caries risk and nutrition which are consistent with previous studies (10,38). This may be explained by the fact that more attention has been paid to these topics in television programs and advertisements in recent years. The knowledge level of Turkish pregnant women on the link hormonal change and periodontal tissue and the association between periodontal disease and adverse pregnancy outcomes were better when compared with previous studies (5,7,38). Hovewer, there is a need for improving Turkish pregnant women's knowledge on the periodontal health, symptoms and necessary treatments during pregnancy. Compared to the previous studies (10,39). This study showed that Turkish pregnant women were more aware of the main topics of oral health and the importance of a balanced diet for their own health and unborn child. The knowledge level and positive attitudes of Turkish pregnant women were similar to those pregnant in developed countries (11,12,29). We found that most of Turkish pregnant women had positive

attitudes about the paying attention their oral care for preventing periodontal diseases, the necessity of for dental check-ups before pregnancy, the positive effect of periodontal treatment on their pregnancy and baby's health.

In line with previous study of Asa'ad et al. (5), most Turkish pregnant women stated that the gingival health is controlled by effective oral hygiene measures (e.g, brushing twice daily and daily flossing). Contradictory to these findings the Jordanian and Malaysian studies reported that most pregnant women did not believe that frequency of teeth brushing should be increased during pregnancy (38,40). Although most of Turkish pregnant had positive attitudes towards the importance of proper oral hygiene behaviours in maintaining gingival health, only less than half brushed their teeth more than twice a day and used dental floss daily. In contrast to our findings, most pregnant women in some countries reported tooth brushing twice daily (10,17,30). Decreasing in oral hygiene behaviours may be attributable to the complaints experienced during pregnancy such as nausea, sensitivity to smell and vomiting (19). In line with our findings, recent studies showed that use of dental floss were less preferred by pregnant women than tooth brushing due to a lack of awareness (15,16,25,39).

The limited number of studies (17,23) reported that poorer rating of oral health was significantly associated with having dental caries and dental pain which were the main reason for visiting dentist among pregnants. Minority of pregnants were not aware of having some periodontal problems. The most striking result of this study, that although more than half of Turkish pregnants had self-reported symptoms such as gingival bleeding, swelling, readness, most of them perceived their oral health as good. This may be associated with the beliefs that bleedings gums in pregnancy is normal (30) and gingival changes is due only to hormonal changes (5) that go away on their own after the baby's birth (7,9).

Only 30% of Turkish pregnant women reported visiting a dentist during pregnancy. This finding similar to previous studies (9,12). We found that only 39% had limited knowledge on the safety of non-surgical periodontal therapy during the second trimester, whereas most pregnants (83%) had willingness to start periodontal treatment if their doctor recommends. This limited knowledge on this topic may be related to the fact that pregnants were not generally advised for a dental check up by healthcare professionals (7,17). As reported in a study of Honkala and Al-Ansari (17) the majority of Turkish pregnants reported that they were not referred by family doctor/gynecologist to a dentist and didn't take advice from them for oral health. In line with previous studies (7,9,12,18,19), we suggest that the effective oral health training programs are needed for gynecologist or obstetrician to increase their knowledge and attitudes towards the importance of oral health in pregnancy as well as to encourage dental visits of pregnant women.

Previous studies have reported lower rates of dental care use when pregnant women have dental problems, even in countries that provide free access to public dental services (8,18,19,29). This may be related negative beliefs about the safety of dental treatment during pregnancy and the attitudes of medical as well as dental health providers (9,12,16,26). Both pregnant women and medical providers should be educated about the importance of receiving dental care for the maintenance of optimal oral health by a multidisciplinary team (7,9,12,18,19).

The knowledge level of pregnant women about the harmful effects of smoking during pregnancy and the prevalence of non-smoking were found to be higher, which are consistent with findings in other published studies (5,38).

Confirming the study hypothesis, the results of bivariate analyses showed that knowledge was positively correlated with income and education level. Similar findings were reported by previous studies that higher levels of income (11,24) and education (11,13) were associated with better knowledge. Turkish pregnant women with higher knowledge level had lower perception of tooth mobility. The results of multivariate analyses showed that higher education level and positive attitudes were the predictors of better knowledge. This may be explained by the fact that having higher levels of education and income may increase women's access to correct and relevant oral health informations (5). These findings support previous studies that suggests that the socio-demographic factors should be taken into account when planning oral health promotion strategies and programmes for pregnant women (11,24).

Attitudes of Turkish pregnant women were found to be correlated with the level of education which is in line with previous studies (13,23). We found that self perceptions of tooth mobility and gingival bleeding were important predictors of lower attitudes. In addition, Turkish pregnant women with lower attitudes reported no flossing and not visiting the dentist for dental check ups during pregnancy. This is not surprising considering the fact that positive oral health behaviors and practice are influenced by pregnant women's attitudes (11).

Increasing gestational stage and having a history of premature birth were associated with more positive attitudes. Negative prior experiences during pregnancy may lead to increase the awareness on periodontal health and the attitudes towards gaining oral care.

To the best of our knowledge, this was the first study evaluating the socio-demographic, behavioral, and subjective predictors of oral health knowledge and attitudes among Turkish pregnant women. However, this study has some limitations. This study performed was based on a sample of pregnant women attending a maternal health center in the western Black Sea region of Turkey which limits the generalizability of these results. Future large population and clinical based studies are needed to confirm the results obtained from this study. Memory-recall errors and social desirability bias may threaten the validity of self report data used in this study. Oral health assessments were not performed in this study because there was not a separate room for oral examination and diagnosis. Further studies are required to examine the agreement between the normative and subjective perceptions of periodontal health and the validity and reliability of subjective measures among Turkish pregnant women. Despite these limitations, this study may provide valuable information to the oral health and antenatal providers in the designing the effective oral health education programs and specifying culturally specific messages for pregnant women receiving antenatal care.

Conclusion

A majority of the pregnant women has adequate knowledge about general oral health, positive attitudes, and the effect of periodontal health on pregnancy; though, their knowledge regarding periodontal health on birth outcome is limited. Having a higher education level was found to be a predictor for both Turkish pregnant women's knowledge and attitudes. A significant positive association was found between the knowledge and attitudes. Pregnancy related characteristics, subjective evaluation of periodontal health, and behavioural factors were associated with attitudes. These findings may provide an insight into the identification of pregnant with low level of dental awareness and attitudes and the development of effective health education strategies through the collaboration between medical and dental care providers. Both obstetrician and dentists should work together to improve the awareness and attitudes of pregnants and to optimize preventive dental and general health strategies. It is obvious that efforts to educate pregnants of the benefits of continued dental care before and during pregnancy is needed. Nevertheless, this requires educating dentists and obstetricians of the guidelines and recommendations for dental care for pregnants.

Ethics

Ethics Committee Approval: All procedures performed in this study were approved by the Clinical Researches Ethics Committee of Bolu Abant İzzet Baysal University (decision no: 2018/221, date: 13.12.2018).

Informed Consent: Participation was voluntary, and informed written consent was also obtained from each pregnant woman before the start of the study.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: T.P., K.P., G.U., Ü.M.U., Concept: T.P., K.P., G.U., Ü.M.U., Design: T.P., K.P., G.U., Ü.M.U., Data Collection or Processing: T.P., K.P., G.U., Ü.M.U., Analysis or Interpretation: T.P., K.P., G.U., Ü.M.U., Literature Search: T.P., K.P., G.U., Ü.M.U., Writing: T.P., K.P., G.U., Ü.M.U.

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Original Article / Özgün Araştırma

Elimination of Calcium Hydroxide from Simulated Internal Resorption Cavities Using EDDY

Kalsiyum Hidroksitin EDDY Kullanılarak Yapay İç Rezorbsiyon Kavitelerinden Uzaklaştırılması

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Abstract

Objective: Calcium hydroxide (CH) is a preferred interappointment medicament to increase antimicrobial activity in intracanal irregularities such as internal root resorption (IRR). This study aimed to compare the effectiveness of EDDY, passive ultrasonic irrigation (PUI), sonic irrigation, CanalBrush (CB), manual dynamic irrigation (MDI) and syringe irrigation (SI) in the elimination of CH from IRR cavities.

Materials and Methods: One hundred and twenty-eight single-rooted teeth with straight root canals were prepared using rotary instruments and split longitudinally. A standardized artificial IRR cavity was prepared in the middle third of each half of the specimens. For the negative control group, four teeth were selected which did not receive any further treatment. The remaining roots were filled with CH and distributed into a positive control (n=4) group and 6 experimental groups (n=20): SI; MDI; PUI; EDDY; CB, and Rispisonic (RS). Five-mL 5% sodium hypochlorite and 5 mL 17% ethylenediaminetetraacetic acid were used as irrigants. The quantity of CH remnants was scored. Results of the scoring were analyzed using statistical analyses.

Results: None of the tested methods could create cavities free of CH. PUI and EDDY were found to be significantly more effective than the other investigated methods ($p<0.05$). SI was significantly less efficient compared to the other tested groups ($p<0.05$).

Conclusion: The activation of irrigants increased the elimination of CH from the IRR cavities. Among the investigated activation regimens, PUI and EDDY was more effective than CB, MDI, RS.

Öz

Amaç: Kalsiyum hidroksit (KH), internal kök rezorpsiyonu (İKR) gibi kanal içi düzensizliklerdeki antimikrobiyal aktiviteyi artırmak için seanslar arasında tercih edilen bir medikamandır. Bu çalışmanın amacı, yapay İKR boşluklarından KH'nin uzaklaştırılmasında EDDY, pasif ultrasonik irrigasyon (PUİ), sonik irrigasyon, CanalBrush (CB), manuel dinamik irrigasyon (MDİ) ve geleneksel iğne irrigasyonunun (İİ) etkinliğini karşılaştırmaktı.

Gereç ve Yöntemler: Düz kök kanallı 128 tek köklü diş, döner aletler ile genişletildi ve uzunlamasına ortadan ayrıldı. Numunelerin her bir yarısının orta üçte birlik kısmında standart yapay bir İKR kavitesi hazırlandı. Negatif kontrol grubu için dört

diş seçildi ve herhangi bir tedavi uygulanmadı. Geri kalan kökler KH ile dolduruldu ve bir pozitif kontrol (n=4) ve 6 deney grubuna (n=20) dağıtıldı; İİ, MDİ, PUİ, EDDY, CB ve Rispisonic (RS). İrrigant olarak 5 mL %5 sodyum hipoklorit ve 5 mL %17 etilen diamin tetra asetik asit kullanıldı. KH kalıntıları skorlandı. Skorlama sonuçları istatistiksel testler kullanılarak analiz edildi.
Bulgular: Araştırılan protokollerin hiçbiri, İKR kavitelerinden KH'yi tamamen ortadan kaldıramadı. PUİ ve EDDY diğer tekniklerden önemli ölçüde daha fazla etkiliydi (p<0,05). İİ, diğer test edilen gruplardan anlamlı derecede daha az verimliydi (p<0,05).
Sonuç: İrrigantların aktivasyonu, simüle edilmiş İKR kavitelerinden KH'nin eliminasyonunu artırmıştır. İncelenen aktivasyon tekniklerinden PUİ ve EDDY, KH'nin uzaklaştırılmasında CB, MDİ ve RS'den daha etkiliydi.

Introduction

Internal root resorption (IRR) is an inflammatory process that starts within the pulp space presenting destruction of dentin and possible invasion of the cementum in teeth with pulpitis and pulp necrosis (1). Due to the limitations of chemomechanical debridement of IRR cavities, irrigation and medication becomes a critical step. Sodium hypochlorite (NaOCl) and different chelators are used to eliminate bacteria, biofilm, necrotic and granulation tissues from IRR cavities.

To increase the effect of disinfection, calcium hydroxide (CH) has been used as an interappointment dressing (2). However, CH medicament applied to the root canal should be eliminated from the canal walls before the permanent obturation procedures are performed (3).

EDDY (VDW, Munich, Germany) features a size-25, 4% taper, sterile, non-cutting flexible polyamide tip driven with a sonic-powered irrigation activation device at lower frequencies. According to the manufacturer, it is claimed that it creates a three-dimensional movement that triggers "cavitation" and "acoustic streaming". Till date, there is limited information with regard to its CH removal capacity (4).

Rispisonic (RS, Micro-Mega, Besançon, France) files which are used within the sonic handpiece (SONIC AIR® MM 1500, Micro-Mega, France) have a non-uniform taper that increases with file size. Because they are barbed, it is necessary to perform continuous small repositioning movements to prevent the file from being inadvertently bound to the canal walls and damage the completed canal preparation during agitation. The operating frequencies of sonic instruments are lower than ultrasonic instruments and produce smaller shear stress while they have higher amplitude and tip movement (5,6).

There is limited research investigating the competency of EDDY and RS tips on the elimination of CH from artificial IRR cavities. The purpose of this *in vitro* study is to assess how effective EDDY and RS are in the elimination of CH from artificial IRR cavities compared with syringe irrigation (SI), manual dynamic irrigation (MDI), passive ultrasonic irrigation (PUI) and CanalBrush (CB) protocols. The null hypothesis was that the choice of the irrigation technique has no effect on the amount of CH eliminated from IRR cavities.

Materials and Methods

The present study was approved by the Ethics Committee of the Bolu Abant İzzet Baysal University (decision number: 2019/204, date: 07.11.2019) and signed informed consent/assent was obtained from all patients.

A priori power analysis was performed to calculate the adequate number of samples to be included in the study. An effect size of 0.40 was added to a power b=93% and a=5% input into an F-test family for analysis of variance. The power analysis showed that a total of 120 specimens (20 for 6 test groups) were required. Eight specimens were selected per positive and negative groups. A total size of 128 samples was required for this study.

One hundred and twenty-eight extracted-intact permanent maxillary incisor teeth were selected. The teeth have a single root and a similar size/shape measured from the cemento-enamel junction to the root apex. Two radiographs of each root were taken from mesio-distal and bucco-lingual directions to verify the presence of a single-rooted canal without calcifications, resorption or previous root canal treatment with the canal curvature limitation taken as <10° (7). The criteria for exclusion were fractures/cracks/caries on the tooth surface and immature root apices. All teeth were decoronated with the help of a high-speed bur under copious water spray to standardize root lengths as 15 mm. Conventional

endodontic access cavities were prepared using diamond fissure burs with a high-speed hand piece under water cooling. The working length of canals was measured by placing a size-15 K-file (Dentsply Maillefer, Ballaigues, Switzerland) into the root canal until the tip of the file was visible at the apical terminus under stereomicroscope and shortening 1.0 mm from this point. To obtain teeth with similar canal size, only teeth whose working length could be reached passively with a size 20 K-file without exceeding the apical foramen were used. All canals were enlarged with rotary nickel-titanium instruments up to X4 (size 40) (ProTaper Next, Dentsply Maillefer, Switzerland) with a crown down sequence at working length. During root canal preparation, 2 mL 5% NaOCl, delivered with a 30-gauge needle, was used between each file change. After the chemomechanical preparation was completed, the roots were inserted in Eppendorf vials filled with impression material. Afterwards, the roots were extracted from the completely set impression material and two longitudinal grooves were created on lingual and buccal surfaces of the root using a water-cooled diamond disc. The roots were longitudinally split in a bucco-lingual direction using a small chisel and hammer. Both root halves were examined under a stereomicroscope to confirm that the halves could be reassembled without gaps.

A simulated resorption cavity (1.6 mm diameter and 0.8 mm deep) was prepared in the middle third of each half of the specimens at a distance of 5 mm above the anatomic apex, using a round diamond bur (Diatech, Coltène/Whaledent AG, Altstätten, Switzerland). The photographs were taken using a stereomicroscope (Olympus SZX10, Tokyo, Japan) equipped with a digital camera (Olympus DP73, Tokyo, Japan) at 20× magnification to confirm the cavity dimensions.

The cavities and root canal walls were flushed with 5 mL 17% EDTA for 1 minute (min) followed by 5 mL 5% NaOCl with a duration of 1 min duration and agitated with a toothbrush to eliminate debris. Five mL of distilled water and paper points were used for the final irrigation and to dry canal, respectively. The 120 roots were randomly allocated into six experimental groups (n=20). Eight specimens were used as negative (n=4) and positive (n=4) controls. The CH paste was not inserted in the samples of the negative control group.

CH powder (Promida, Eskişehir, Turkey) was prepared with distilled water at a liquid/powder ratio of 1.5:1. The prepared CH paste was placed into the main root canal and the cavities using paper points under observation through a stereomicroscope. The root halves were reassembled with wax, avoiding applying the wax to the main canal and cavities throughout the process. Nonetheless, each of the apices were sealed with wax in order to guarantee a closed system. Two radiographs were taken to verify that the cavities were fully filled with CH. The orifices of the root canals were restored with temporary filling material. The specimens were remounted into the Eppendorf vials and kept in 37 °C for 14 days at 100% humidity.

Four of the remaining 124 roots were used as positive controls and no irrigation procedures were performed on them.

After the coronal access was opened, an X4 file (Dentsply Maillefer, Switzerland) and 1 mL 5% NaOCl were used to create a pathway up to the working length for instruments and irrigation needles.

SI; The root canals were flushed with 5 mL 5% NaOCl for 1 min, followed by 5 mL of 17% EDTA for 1 min using a 30-gauge endodontic needle (Canal Clean, South Korea). The needle was placed 1 mm short of the working length and the flow rate of irrigants was set as 0.08 mL/s.

MDI; canals were irrigated with 2.5 mL 5% NaOCl followed by manually performed in-and-out motions up to 7-8 mm of the working length using a well-fitted ProTaper Next X4 (Dentsply Maillefer) gutta-percha cone at an approximate rate of 100 strokes per min for 30 seconds. This procedure was repeated 2 times followed by irrigation with 5 mL 17% EDTA activated by a new ProTaper Next X4 gutta-percha cone for 1 min at the same rate.

PUI; Canals were irrigated with 2.5 mL 5% NaOCl thereafter an ultrasonic tip with a size-25, %2 taper (Irrisafe, Satelec Acteongroup, France) was inserted up to 1 mm of the working length. The tip inserted to the handpiece of a Satelec P5 Newtron XS (Acteon Group, Merignac, France) ultrasonic system was activated at a power setting of 9 for 30 seconds without contacting or locking the wall. This procedure was repeated 2 times, followed by irrigation with 5 mL 17% EDTA for 1 min at the same rate, employing the same activation method. Each tip was used for 4 roots and then replaced with a new one.

EDDY; The irrigation cycles were executed in the same manner as the PUI group with the exception that an EDDY (VDW, Munich, Germany) with a size-25, %4 taper tip, 28 mm length, mounted on a sonic handpiece (SonicMax, Endo-Perio Sonic Handpiece, Maximum Dental Inc, Japan) set at maximum speed was used.

CB; Irrigation was performed under the same technique as the PUI group with the exception that a medium size CB (Coltene/Whaledent GmbHCo. KG, Langenau, Germany) set at 600 rpm was used adopting a gentle in-and-out motion.

RS; Irrigation was performed under the same technique as the PUI group with the exception of a size 3 RS file tip (Micro-Mega, Besançon, France), 22 mm length with a size-25, %2 taper, mounted on a SONIC AIR® MM 1500 sonic handpiece (Micro-Mega, France) set at maximum speed.

During the activation process, in cases where the level of irrigants in the canal decreased, a drop solution was added. A total volume of 5 mL 5% NaOCl and 5 mL 17% EDTA was used for each sample. Finally, canals were flushed with 5 mL of distilled water to eliminate further solution action. A total of 15 mL irrigant was used for each specimen.

Digital images of simulated IRR cavities were photographed at 20× magnification using a stereomicroscope. Two calibrated endodontists independently scored the coded images.

The following scores were used: 0: empty cavity, 1: less than 50% of the cavity is covered with CH, 2: CH covered more than 50% of the cavity, and 3: the cavity is fully covered with CH (Figure 1).

Calibration was conducted by twice scoring a total of 100 reference images at an interval of 48 h by two observers. The results were discussed by both investigators. If any disagreement occurred, a consensus was reached through discussion.

Statistical Analysis

All photographs were evaluated by the calibrated examiners 1 week later and intraobserver and interobserver reproducibility were measured using the weighted coefficient kappa. According to the Shapiro-Wilks test, the data was not distributed normally; therefore non-parametric tests were performed. The scoring results of CH remnants were analyzed by means of Kruskal-Wallis and the Mann-Whitney U tests. The significance level was set at 0.05.

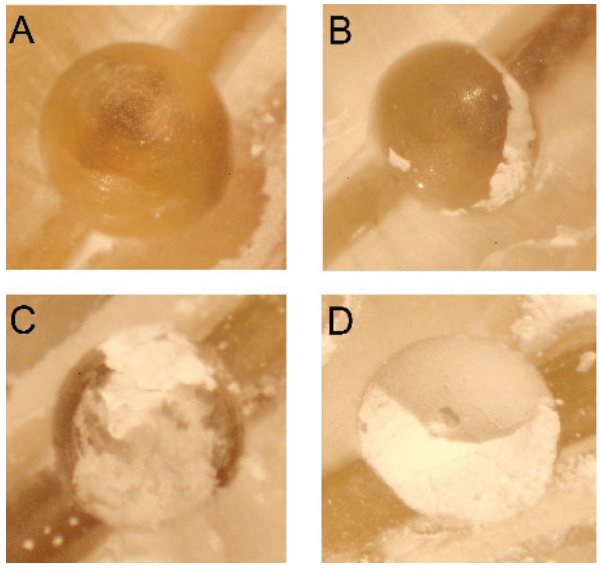

Figure 1. Score system for evaluation of remnants of CH in the artificial cavity. (A) Score 0; empty cavity (B) Score 1; less than 50% of the cavity is covered with CH (C) Score 2; CH covered more than 50% of the cavity (D) Score 3; the cavity is fully covered with CH
CH: Calcium hydroxide

All analyses were performed using SPSS 20.0 software (SPSS Inc., Chicago, IL, USA).

Results

The Cohen Kappa value was 0.889 for two examiners, indicating good agreement and intraindividual reproducibility was found to be 94% and 92%, respectively. Table 1 shows the median and interquartile ranges of all tested groups. Each of the negative control specimens had a result score of 0 while all the positive control specimens showed a result score of 3. There was a significant difference among the positive and negative control groups and all the investigated groups ($p<0.05$). None of the tested methods were able to create cavities free of CH. PUI left significantly less CH remnants in comparison to all tested groups ($p<0.05$) but was found to be similar to the EDDY ($p>0.05$). SI removed significantly less CH than the other activation protocols ($p<0.05$). RS and CB eliminated significantly more CH than MDI ($p<0.05$); however, no significant differences were found between these two groups ($p>0.05$). MDI was significantly more efficient than SI but less successful compared with the other tested groups ($p<0.05$).

Discussion

The purpose of this study was to investigate the efficacy of different irrigation devices and compare them with the irrigation of ethylenediaminetetraacetic acid (EDTA) and NaOCl in the elimination of CH from artificial IRR cavities. The main outcome of this study was that PUI and EDDY were more effective than RS, CB and MDI in the elimination of CH. The null hypothesis that the choice of the irrigation technique would have no effect on the amount of CH eliminated from IRR cavities was rejected.

In previous studies, it was reported that removal of medicaments before the completion of the root canal filling, strengthens the bond between dentin and sealer by creating sealer tags in the dentinal tubules and it also results in a good seal (3,8). CH provides a physical barrier that prevents the development of microorganisms and reinfection. However, in agreement with the findings of this study, it is seen in the literature that CH could not completely be eliminated from the root canal recesses with any of the methods and irrigation solutions being used currently (4,9-13).

EDDY tips have been recently introduced as sonic irrigation activation tips which are made of polyamide driven at a frequency of 6000 Hz by an air-driven handpiece. The EDDY was tested in the present study for its superior ability to remove CH from artificial IRR cavities, as reported in a recent study (14). In another study, it was reported that PUI and EDDY have similar CH medicament removal capacity form artificial IRR cavities when the cavities were created by bur (4). In this study, EDDY eliminated CH fully in 47.5% of the IRR cavities, whereas PUI eliminated CH fully in 60% of the cavities. The CH removal percentages of the other investigated irrigants were 32.5%, 17.5%, 0% and 0% for RS, CB, MDI, and SI, respectively. However, no significant difference was found between EDDY and PUI (Table 1). This may be due to the fact that although EDDY is used with frequencies below 30,000 Hz, it has a flexible tip made of polyamide which can freely swing inside the root canal and create a cavitation effect.

PUI is the most popular method in activating the irrigant for the removal of debris and bacteria (15-17). Cavitation and acoustic streaming of the irrigant by means of ultrasonic wave have a critical role in the effectiveness of PUI. This feature has enabled PUI to be superior to sonic activation as stated in many studies (9,10,18). In the present study, PUI was found to be superior to other tested methods, which was consistent with previous studies (11,13), while it performed similarly to EDDY (Table 1). In a previous study, it has been shown that PUI with EDTA and NaOCl was superior to CB and SI in the elimination of CH from simulated IRR cavities (10). Although these results are in line with those of Topçuoglu et al. (18), the results showed that PUI was not able to completely remove CH from artificial IRR cavities.

Table 1. The scoring results of the simulated IRR cavities

Groups	Median	IQR	Minimum	Maximum
SI	2.0[A]	1.0	1.0	3.0
MDI	2.0[B]	1.0	1.0	3.0
PUI	0.0[C]	1.0	0.0	1.0
EDDY	1.0[C]	1.0	0.0	2.0
CB	1.0[D]	1.0	0.0	3.0
RS	1.0[D]	2.0	0.0	2.0

SI: Syringe irrigation, MDI: Manual dynamic irrigation, PUI: Passive ultrasonic irrigation, CB: CanalBrush, RS: Rispisonic, IQR: Interquartile range, IRR: Internal root resorption. Values with different superscript letters were statistically different at $p<0.05$ (Kruskal-Wallis and Mann-Whitney U tests)

CB features a polypropylene brush with high flexibility for endodontic use. The manufacturer suggests that it helps cleaning by activating the irrigation solutions and directing the irrigation solution to the canal irregularities that cannot be touched by instruments. Contrary to our results, in the limited number of studies (10,18), CB removed a similar amount of CH from resorption cavities compared to SI. This discrepancy may be attributable to the differences in the irrigation protocol in which irrigants were refreshed every 30 seconds. However, CB was less effective than PUI in clearing resorption cavities. This is in accordance with the findings of previous studies (10,18). The researchers found CB to be effective in cleaning the main canal however, further examinations showed that it tends to fill the canal irregularities with CH remnants (10,18,19). In a recent study, it has been shown that CB removed less CH from the main canal compared to EDDY and RS (20).

The SONIC AIR® MM 1,500 handpiece which is hooked directly to the dental unit with a RS file was

originally developed for the purpose of shaping the root canals (5). However, in many studies, it was used to activate the irrigants. The investigators found that the efficacy of RS in the removal of debris was superior to SI while it was comparable to PUI (21). However, to date, no data are available in the literature with regard to its effectiveness on the removal of CH. In this study, RS removed significantly less CH compared with PUI and EDDY.

The effect of NaOCl alone or the combined use of EDTA and NaOCl in the elimination of medicaments from root canals was investigated in previous studies. Some previous studies reported that the addition of chelators to NaOCl did not enhance the removal of CH compared with NaOCl irrigation (22). However, other studies reported that NaOCl showed the least favorable results of CH removal when used without a combination containing a chelating agent (8,23). In another study, PUI with NaOCl was found more effective on the elimination of CH than SI with NaOCl and PUI with water (24). However, EDTA is considered to be superior to NaOCl in the elimination of CH and smear layer (23). In the present study, the canals were flushed with 2.5 mL EDTA followed by NaOCl to resemble an actual clinical application cycle.

In previous studies, artificial standardized grooves or cavities were used to investigate the effectiveness of different irrigation protocols or solutions in the elimination of intracanal medicaments (9-11,13-15,18,22,24). The use of standardized cavities provides a standardization in the size and location of cavities as well as the amount of medicament used. This allows a good agreement between the intraobserver and interobserver. However, the artificial cavities created by a bur do not represent the complexity and irregularities of resorption cavities or isthmuses. In addition to this, evaluation of only visible CH remnants overlooking the residues in the dentinal tubules are the limitations of the presented study (4,22).

Conclusion

The activation of irrigants has improved the elimination of CH from simulated IRR cavities. Among the investigated activation regimens, PUI and EDDY were more effective than CB, MDI, RS in CH removal. However, none of the tested methods were able to create cavities free of CH.

Ethics

Ethics Committee Approval: The present study was approved by the Ethics Committee of the Bolu Abant İzzet Baysal University (decision number: 2019/204, date: 07.11.2019).

Informed Consent: Informed consent/assent was obtained from all patients.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Concept: H.G., İ.Ö., Design: H.G., İ.Ö., Data Collection or Processing: H.G., İ.Ö., S.T.İ., Analysis or Interpretation: H.G., İ.Ö., S.T.İ., Literature Search: H.G., İ.Ö., S.T.İ., Writing: H.G., İ.Ö.

Conflict of Interest: No conflict of interest was declared by the authors.

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Original Article / Özgün Araştırma

Differences in Obstetric Outcomes and Antenatal Follow-up Between Syrian Refugees and Resident Women: A Retrospective Comparative Study in a Maternity Hospital Aydın, Turkey

Türkiye'de Yaşayan Suriyeli Mülteciler ile Yerel Gebeler Arasındaki Obstetrik Sonuçlar ve Antenatal İzlemedeki Farklılıklar: Retrospektif Karşılaştırmalı Bir Çalışma, Aydın Kadın Doğum ve Çocuk Hastanesi, Türkiye

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Obstetric outcome, antenatal care, refugees

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Abstract

Objective: This study compared Syrian refugees' antenatal follow-up characteristics and pregnancy outcomes with Turkish residents.

Materials and Methods: We performed a retrospective observational study of the births in a Gynecology and Pediatrics Hospital in Aydın-Turkey, between 01.10.2009 and 01.06.2019. The data were obtained from the birth data archives and the medical and laboratory records of the hospital. The demographic characteristics and obstetric-neonatal outcomes were compared between 634 Syrian refugees and 21,092 Turkish residents. The laboratory results and antenatal screening data were compared between 634 Syrian refugees and randomly selected 715 Turkish residents.

Results: Maternal age was significantly lower, adolescent pregnancy rate (age <19), and the parity was significantly higher in the refugee group (p<0.001). Although the cesarean-section (C/S) delivery rate was higher in Turkish residents, the primary C/S delivery rate was similar. C/S delivery due to previous repetitive C/S delivery was significantly higher in the refugee group (p=0.041). Preterm-birth rate (<37 gestational weeks), low-newborn birth weight (<2,500 g), and stillbirth rate was not significantly different between the groups. Mean hemoglobin levels and the rate of maternal anemia was not significantly different between the groups. The attendance to antenatal screening tests (first-trimester combined test, triple test, glucose tolerance tests) was significantly lower in the refugee group (p<0.001).

Conclusion: Compared to Turkish residents, Syrian refugee women had some significant differences in terms of demographic specifications, the application of antenatal screening tests, and laboratory test results. However, the obstetric and neonatal outcomes were not statistically different.

Öz

Amaç: Bu çalışmada, Suriyeli mültecilerin antenatal izlem özelliklerini ve sonuçlarını Türk vatandaşları ile karşılaştırmayı amaçladık.

Gereç ve Yöntemler: Çalışma, 01.10.2009 ve 01.06.2019 tarihleri arasında Aydın Kadın Doğum ve Çocuk Hastanesi'nde gerçekleşen doğumların incelendiği retrospektif gözlemsel bir çalışmadır. Her iki grubun verileri hastanenin doğum veri arşivlerinden ve hastanenin tıbbi ve laboratuvar kayıtlarından elde edildi. Altı

yüz otuz dört Suriyeli mülteci ile 21.092 Türkiye'de ikamet eden kişinin demografik özellikleri ile obstetrik ve neonatal sonuçları karşılaştırıldı. Altı yüz otuz dört Suriyeli mülteci ile rastgele seçilmiş 715 Türk vatandaşının laboratuvar sonuçları ve antenatal tarama kullanımına ilişkin verileri karşılaştırıldı.

Bulgular: Suriyeli mülteci gebeler anlamlı olarak daha gençti, adölesan gebelik sayısı (anne yaşı <19) ve parite mülteci grubunda anlamlı olarak daha yüksekti (p<0,001). Sezaryen (C/S) doğum oranları Türk vatandaşlarında daha yüksek olmasına rağmen, primer C/S doğum oranları gruplar arasında anlamlı farklılık göstermedi. C/S doğum endikasyonları arasında, sadece mükerrer C/S doğum öyküsü nedeniyle C/S doğum mülteci grubunda anlamlı olarak daha yüksekti (p=0,041). Preterm doğum oranları (<37 gebelik haftası), düşük yenidoğan doğum ağırlığı (<2.500 gr) ve ölü doğum oranları gruplar arasında anlamlı bir farklılık göstermedi. Ortalama hemoglobin düzeyleri ve maternal anemi oranları açısından gruplar arasında anlamlı bir farklılık bulunmadı. Mülteci grubunda antenatal tarama testlerine (birinci trimester serum kombine testi, üçlü test, glukoz tolerans testleri) katılım anlamlı derecede düşüktü (p<0,001).

Sonuç: Türk vatandaşları ile karşılaştırıldığında, Suriyeli mülteci kadınların demografik özellikleri, antenatal tarama testlerinin uygulanması ve laboratuvar test sonuçları açısından bazı anlamlı farklılıkları gözlenmiştir, ancak obstetrik ve neonatal sonuçlar gruplar arasında istatistiksel olarak farklı bulunmamıştır.

Introduction

Over 7 million Syrian citizens were forced to leave their country to escape the civil war in March 2011. Currently, about 3,6 million registered Syrian refugees live in Turkey (1). Though refugees, especially pregnant women, are considered a vulnerable group, the Republic of Turkey Ministry of Health provides unrestricted access to health care services for the Syrian refugees (2).

Despite unrestricted access to health care services, pregnant refugee women may encounter numerous barriers to reaching antenatal care services due to low socioeconomic status, language, and cultural barriers (3,4). As shown in previous studies, this vulnerable population is more prone to perinatal complications such as low birth weight, preterm birth, increased cesarean-section (C/S) delivery rate, intrapartum bleeding, and puerperal infections (5-7).

The current study aimed to compare the pregnancy characteristics, perinatal risk factors, attendance to antenatal screening tests, and obstetric-neonatal outcomes of Syrian refugees with Turkish residents, both delivered in a public hospital (Aydın Gynecology and Pediatrics Hospital) located on the western coast of Turkey between 2009 and 2019.

Materials and Methods

A retrospective study was conducted using the data retrieved from the births in Aydın Gynecology and Pediatrics Hospital between 01.10.2009 and 01.06.2019. The hospital's birth data archives and laboratory results system were scanned to obtain information about the antenatal follow-up data and the obstetric-neonatal outcomes. The data of the Syrian refugee women were compared with Turkish residents. The ethical approval for the study was received from the İzmir Katip Çelebi University Non-Interventional Clinical Studies Institutionel Review Board (decision number: 289, date: 03.10.2018), and permission was obtained from the hospital's management to scan the archives.

The birth data, including maternal age, number of pregnancies and parity, the gestational week at delivery, number of multiple gestations, mode of delivery, C/S delivery indication, primary C/S delivery rate, newborn gender, birth weight, preterm delivery (<37 gestational weeks) rate, post-term pregnancy rate (>42 gestational weeks), low newborn birth weight (<2,500 g), macrosomic newborn (>4,500 g), and number of stillbirths, were compared between 634 Syrian refugees and 20,438 Turkish residents. Additionally, the laboratory data including hemoglobin (Hb) level, mean corpuscular volume (MCV), platelet count, hepatitis B surface antigen (HBsAg) and hepatitis B surface antibody (anti-HBs) status, glucose tolerance test (GTT) results, the number of antenatal screening tests (first-trimester combined test, triple test, and GTTs) performed, and blood type status were compared between 634 Syrian refugees and randomly selected 715 Turkish residents.

Statistical Analysis

Statistical analyses were performed by SPSS version 20.0 (IBM. Armonk, NY, USA). Kolmogorov-Smirnov test was used to assess the normal distributions of continuous variables. The descriptive variables were expressed as mean ± standard deviation or median (range). Independent samples t-test or Mann-Whitney U test were used to compare numerical variables. The categorical data were analyzed by the

χ^2 test or Fisher's Exact test. P<0.05 was considered to be statistically significant.

Results

Compared to Turkish residents, maternal age was significantly lower, adolescent pregnancy (maternal age <19) rate and the parity were significantly higher in the refugee group (all p<0.0001) (Table 1). Gestational age at delivery, preterm birth (<37 gestational weeks) rate, low newborn birth weight (<2,500 g) rate, and stillbirth rate were not statistically different between the groups. Although newborn birth weight was significantly higher in the Turkish residents, the amount of macrosomic newborns (>4,500 g) was not significantly different between the groups (p<0.010, p=0.3293 respectively). C/S delivery rate was significantly higher in Turkish residents (p<0.0001), but the primary C/S delivery rate was similar in both groups (Table 2). Among C/S delivery indications, only C/S delivery for previous repetitive C/S delivery was significantly higher in the Syrian refugee group (p=0.0416) (Table 3). Mean Hb value, platelet count, the number of anemic (Hb <11 mg/dL), and thrombocytopenic patients (platelet <150,000/mcL) at submission for delivery were not significantly different between the groups. However, MCV was significantly lower and women who have an MCV <80 fL were significantly higher in the Syrian refugee group (p=0.0016 and p=0.0081 respectively). Serum fasting glucose levels were not statistically different between the groups. Among patients who have performed an oral GTT for gestational diabetes mellitus (GDM) screening, the number of patients diagnosed with GDM was not statistically different between the groups. The number of patients accepted receiving a GTT, first-trimester combined test, and triple test was significantly lower in the Syrian refugee group (all p<0.0001). HBsAg positivity was not significantly different between the groups. However, patients who are positive for Anti-HBs were significantly higher in the Turkish resident group (p<0.0001) (Table 4). We analyzed the blood types of both ethnic groups. In Turkish pregnant women, A Rh-positive was the most common blood type (38.24%). 0 Rh-positive was the most common blood type in the Syrian refugee group (31.54%). Rh (-) prevelance was comparable between the groups, 10.9% in Turkish women, and 11.67% in Syrian refugees (p=0.667).

Table 1. Demographic characteristics of the study groups

Variable	Turkish residents n (mean ± SD) or n (%)	Syrian refugees n (mean ± SD) or n (%)	p-value
Maternal age	20,430 (27.26±5.84)	634 (23.65±5.45)	<0.0001*
Adolescent pregnancy (<19 years)	848 (4.15%)	90 (14.19%)	<0.0001*
Parity	710 (1.07±1.04)	610 (1.34±1.55)	0.0001*

*Statistically significant p-value, SD: Standard deviation

Discussion

In the present study, although some significant differences in demographic specifications, application of antenatal screening tests, and laboratory test results between Turkish resident and Syrian refugee pregnant women were observed, the perinatal outcomes were not statistically different between the groups.

Our study showed that maternal age was significantly lower, and the adolescent pregnancy rate and the parity were significantly higher in the refugee group. Our findings were in line with the results of the previous studies conducted in Turkey (8,9). In our study, the C/S delivery rate in both groups was higher than 10 to 15%, as stated by the World Health Organization (WHO) (10). The C/S delivery rate in our study was significantly higher in Turkish pregnant women. This finding was in line with the previous studies conducted in Turkey (11) and other countries (5,9). Unlike our study, Huster et al. (11) reported that refugees had increased complications diagnosed during the delivery period that often required emergent C/S delivery leading to higher C/S rates compared to Lebanese women. In our study, the primary C/S delivery rate was not different between the groups. Unlike our study, in the previous two studies, the authors reported lower C/S delivery rates in nulliparous Syrian patients (8,12). We thought that the lower C/S delivery rate among Syrian refugees might be due to the higher parity rate among the refugees, and may be due to the beliefs and motivation of Syrian women regarding vaginal delivery. Turkish residents' higher C/S delivery rate may be due to older maternal age and increasing chronic diseases (13).

Table 2. Neonatal and Obstetric outcomes of the study groups

Variable	Turkish residents n (mean ± SD) or n (%)	Syrian refugees n (mean ± SD) or n (%)	p-value
Newborn sex			
Female Male Ambiguous	9,944 (48.65%) 10,492 (51.33%) 2 (0.01%)	324 (51.10%) 310 (48.90%)	0.2410
Stillbirth	118 (0.57%)	4 (0.63%)	0.8610
Multiple pregnancy	420 (2.5%)	12 (1.89%)	0.8874
Type of delivery			
Vaginal delivery	12,674 (62.01%)	470 (74.13%)	<0.0001*
C/S delivery	7,664 (37.49%)	159 (25.07%)	<0.0001*
Vacuum delivery	100 (0.50%)	5 (0.79%)	0.4425
Primary C/S delivery	3,572 (46.60%)	75 (47.17%)	0.9519
Newborn weight (g)	20,436 (3208.55±521.7)	634 (3074±492.08)	<0.0001*
Gestational age at delivery (weeks)	20,438 (38.73±1.69)	634 (38.81±1.89)	0.4252
Preterm birth (<37 weeks)	1,937 (9.48%)	55 (8.67%)	0.5396
LBW (<2,500 g)	1,534 (7.50%)	61 (9.62%)	0.0566
Macrosomic newborn (>4,500 g)	106 (0.52%)	1 (0.16%)	0.3293
Post-term pregnancy (>42 gestational weeks)	190 (0.93%)	9 (1.42%)	0.2952

*Statistically significant p-value. LBW: Low birth weight, C/S: Cesarean-section, SD: Standard deviation

Table 3. C/S delivery indications of the study groups

C/S delivery indication	Turkish residents n (%)	Syrian refugees n (%)	p-value
A single history of C/S delivery	2,935 (38.29)	51 (32.07)	0.1174
History of two or more C/S deliveries	1,127 (14.70)	33 (20.75)	0.0416*
Cephalopelvic discrepancy	667 (8.7)	13 (8.17)	1
Arrested labor	721 (9.4)	14 (8.8)	0.8913
Macrosomic fetus	178 (2.32)	2 (1.25)	0.5895
Fetal distress	977 (12.74)	20 (12.57)	1
Multiple pregnancies	277 (3.61)	6 (3.77)	0.8296
Breech presentation	495 (6.46)	13 (8.17)	0.4132
Eclampsia	2 (0.02)	0	1
Hypertensive disorders	61 (0.79)	0	1
Transverse lie	29 (0.37)	2 (1.25)	0.1303
Placental abnormalities	68 (0.88)	1 (0.62)	1
Fetal cord prolapsus	12 (0.15)	0	1
Others	115 (1.5)	4 (2.51)	0.3082

*Statistically significant p-value, C/S: Cesarean-section

In our study, the indications for C/S delivery were broadly similar in both groups. The most common indication for performing a C/S delivery was the history of previous C/S delivery in both groups. Among the C/S delivery indications, only the C/S delivery rate due to repetitive C/S history was significantly higher in the Syrian refugee group. Similar to our study, it was reported that the history of a repeated C/S delivery

was the most common reason for C/S deliveries among the Syrian refugees in Lebanon and Jordan (5,11).

Regarding the obstetric outcomes, we found that the mean gestational week at delivery, the preterm birth rate (<37 gestational weeks), and the stillbirth rate were not significantly different between the groups. Although the mean newborn birth weight of the refugee group was significantly lower than residents, the rate of low newborn birth weight and fetal macrosomia was not significantly different between the groups. Unlike our study, in several studies conducted either in Turkey or other countries, the preterm birth rate and unfavorable obstetric outcomes were significantly higher among the refugee group (6,12). In their study, Thomas et al. (14) evaluated the effect of cultural and linguistic diversity on pregnancy outcomes, and they did not find a significant relationship between refugee status and adverse outcomes. They showed that attendance to interpretation services reduced the likelihood of adverse outcomes (14). In a systematic review where the pregnancy outcomes among immigrant women in the United States and Europe were evaluated, the authors showed that the prevalence of low birth weight among migrants varied by the host country and the composition of migrants to different regions (15). According to the authors, the primary determinant of migrant health was the migrant "regime" in different countries at specific periods. In our study, we thought that the lack of statistical difference, in terms of adverse obstetric outcomes between the two groups, might be related to refugee policies implemented by the Republic of Turkey and the fact that most of the refugees included in our study are residing in houses rather than camps which have unfavorable conditions. We thought that the lower mean newborn weight among Syrian refugees might be due to ethnic differences.

We also found that the mean Hb levels and the rate of prenatal anemia were not significantly different between the groups. In their study, Erenel et al. (8) reported that, although Syrian patients had significantly lower Hb levels compared to Turkish patients, the mean Hb level of the Syrian patients was not less than 11 g/dL. This finding was similar to the mean Hb level of the Syrian refugees in our study, which was 11.47 g/dL. They said that, compared to residents, refugees had poor antenatal care, but adverse perinatal outcomes were not observed among them. In our study, although the rate of GDM was not statistically different between the groups, we would not be able to report the exact prevalence of GDM in both groups because few either Turkish residents or Syrian refugee pregnant women performed a GDM screening test (11.34% and 4.60% respectively). Similar to our study, Ozel et al. (12) could not state and compare the prevalence of GDM in the refugee group

Table 4. Laboratory characteristics of the study groups

Variables	Turkish residents n (mean ± SD) or n (%)	Syrian refugees n (mean ± SD) or n (%)	p-value
Hb (g/dL)	715 (11.56±1.44)	634 (11.47±1.47)	0.2547
Hb <11 g/dL	233 (32.59%)	211 (33.28%)	0.8164
MCV (fL)	715 (80.91±6.92)	634 (79.68±7.34)	0.0016*
MCV <80 fL	277 (38.74%)	291 (45.89%)	0.0081*
Platelet* 1,000/mcL	715 (206.6±58.64)	634 (217.1±63.91)	0.0016*
Platelet <15,0000/mcL	117 (16.36%)	84 (13.25%)	0.1254
Fasting blood glucose level (mg/dL)	99 (84.11±9.551)	26 (84.50±12.12)	0.8619
Accept GTT	81 (11.34%)	29 (4.60%)	<0.0001*
GDM (+)	29 (35.8%)	9 (31.03%)	0.8203
Accept genetic screening tests	423 (59.16%)	190 (29.97%)	<0.0001*
HBsAg (+)	9 (1.26%)	2 (0.32%)	0.0696
Anti-HBs (+)	232 (51.79%)	25 (19.23%)	<0.0001*

*Statistically significant p-value. SD: Standard deviation, Hb: Hemoglobin, MCV: Mean corpuscular volume, GTT: Glucose tolerance test, GDM: Gestational diabetes mellitus, HBsAg: Hepatitis B surface antigen, Anti-HBs: Hepatitis B surface antigen

due to the low attendance rates of Syrian pregnant women in GDM screening tests.

In the present study, we found that the rate of patients who performed the first-trimester combined test or triple test was significantly lower in the refugee group. Furthermore, the rate of patients who had performed GTT for GDM screening was significantly lower in the refugee group. In another study conducted in Turkey, Ozel et al. (12) reported that rates of antenatal follow-up, first-trimester serum combined test, triple testing, and GDM screening were significantly lower in the refugee group. In their study, Abbasi-Kangevari et al. (16) reported that Syrian refugee pregnant women did not comply with both Iran national guidelines or WHO antenatal care recommendations, and they thought that Syrian women kept their cultural behavior in terms of antenatal care utilization. Similarly, we thought that the difference might be due to low socioeconomic status, language barrier, social isolation of the refugees, or differences in religious and cultural perspectives on events of both groups.

In our study, the difference in the seroprevalence of Hepatitis B infection between Turkish and Syrian pregnant women was not statistically significant. Our results were in line with the study conducted by İnci et al. (17) in Turkey. However, in our study, the rate of pregnant women who have antibodies against HBsAg was significantly higher in Turkish residents. The low anti-HBs rates among Syrian refugees were in line with the previous two studies conducted in Syria (18,19).

The study had a retrospective design, and the data were obtained from a single hospital. For that reason, we could not scan the data, including nutrition habits, weight gain during pregnancy, education levels, number of members in the family, and monthly financial incomes of the family for each patient.

Despite its retrospective design, our study has a large data set that compares the obstetric-neonatal outcomes and antenatal monitoring parameters of Turkish residents with Syrian refugees through the data obtained from over 21,000 pregnant women.

Conclusion

Compared to Turkish residents, Syrian refugee women had some significant differences in terms of demographic specifications, application of antenatal screening tests, and laboratory test results, but the obstetric and neonatal outcomes were not statistically different between the groups. We thought that the results of our study can provide informative data to the governments of countries hosting refugees for the organization of health care policies. Multicenter studies with more extensive series and investigating different lines about this subject are needed.

Ethics

Ethics Committee Approval: The ethical approval for the study was received from the İzmir Katip Çelebi University Non-Interventional Clinical Studies Institutionel Review Board (decision number: 289, date: 03.10.2018).

Informed Consent: Retrospective study.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Concept: E.B.G., Design: E.B.G., Data Collection or Processing: S.K.K., Analysis or Interpretation: E.Ş.G., Literature Search: E.Ş.G., Writing: S.K.K.

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Original Article / Özgün Araştırma

Effect of Different Surface Treatments on the Surface Roughness and Orthodontic Bond Strength of Partially-stabilized Zirconia

Farklı Yüzey İşlemlerinin Parsiyel Stabilize Zirkonyanın Yüzey Pürüzlülüğü ve Ortodontik Bağlanma Dayanımına Etkisi

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Anahtar Kelimeler
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Abstract

Objective: To investigate the effect of different surface treatments on the surface roughness (R_a) and shear bond strength (SBS) of partially stabilized zirconia (Y-PSZ) with different yttrium content.

Materials and Methods: Zirconia samples were milled from 5Y-PSZ and 4Y-PSZ disks and divided into 5 groups: Control (C), sandblasting (APA), single-step self-etch primer etching (MEP), Er:YAG laser (ER), and femtosecond laser (FS) irradiation (n=11). Surface R_a was measured and metallic mandibular incisor brackets were bonded. SBS test was performed after thermocycling. Data were analyzed using two-way ANOVA and Tamhane's T2 tests (α=0.05).

Results: Only surface treatment affected R_a and SBS ($p<0.001$). FS groups had the highest ($p<0.001$), and C groups had the lowest values R_a ($p\leq0.001$). MEP groups had lower R_a than ER and APA ($p\leq0.002$). FS and APA groups resulted in the highest SBS ($p<0.001$), while MEP groups achieved significantly higher SBS than ER groups ($p<0.001$). The C group showed the lowest SBS ($p<0.001$).

Conclusion: Within the limitations of this *in vitro* study, sandblasting and FS irradiation was found to be the most effective surface treatments for metallic bracket bonding to Y-PSZ.

Öz

Amaç: Bu çalışmanın amacı, farklı yüzey işlemlerinin parsiyel stabilize zirkonyanın (Y-PSZ) yüzey pürüzlülüğü (R_a) ve makaslama bağlanma dayanımına (SBS) olan etkisini değerlendirmektir.

Gereç ve Yöntemler: Zirkonya örnekler, 5Y-PSZ ve 4Y-PSZ disklerinden frezeleme ile hazırlandı ve beş gruba ayrıldı: Kontrol (C), kumlama (APA), tek aşamalı kendiliğinden pürüzlendiricili primer ile pürüzlendirme (MEP), Er:YAG lazer (ER) ve femtosaniye lazer (FS) ışınlama (n=11). R_a ölçüldü ve metalik mandibular kesici braketler yapıştırıldı. SBS testi, ısıl döngüden sonra gerçekleştirildi. Veriler, iki-yönlü ANOVA ve Tamhane T2 testleri ile analiz edildi (α=0,05).

Bulgular: R_a ve SBS sadece yüzey işlemlerinden etkilenmiştir ($p<0,001$). FS grupları en yüksek R_a'yı ($p<0,001$), C grupları ise en düşük R_a'yı göstermiştir ($p\leq0,001$). MEP grupları, ER ve APA'dan daha düşük Ra sonuçları vermiştir ($p\leq0,002$). FS ve APA

grupları en yüksek SBS ile sonuçlanırken (p<0,001), MEP grupları ER gruplarından önemli ölçüde daha yüksek SBS elde etmiştir (p<0,001). C grupları ise en düşük SBS'yi göstermiştir (p<0,001).
Sonuç: Bu *in vitro* çalışmanın sınırlamaları dahilinde, Y-PSZ metalik braket bağlanması için APA ve FS ışınlamasının en etkili yüzey işlemleri olduğu bulunmuştur.

Introduction

Monoclinic, tetragonal, and cubic are 3 different crystallographic structures of zirconia (1). Dental zirconia usually contains 3 mol% of yttrium oxide, which stabilizes the tetragonal phase (2,3). Even though zirconia has exceptional physical properties, it is opaque (3,4) and technical complications have been reported (1,5). Monolithic zirconia, which has reduced alumina content was introduced to overcome these problems (6,7). Monolithic zirconia has better translucency (3), yet it still lacks the translucency that glass-ceramics present (6). Recently introduced partially stabilized zirconia (Y-PSZ) (3) has increased yttrium oxide that introduced the cubic phase along with the tetragonal phase (2,6,8). Cubic phase reduces the light scattering at the borders of zirconium dioxide crystals resulting in a more translucent material (6).

Orthodontic treatment and esthetic dental restorations are increasing their popularity among adult patients (9-12). However, traditional adhesives are unsatisfactory to maintain adequate bonding between the porcelain surface and the orthodontic bracket (13). Various lasers have been suggested for bracket bonding (14,15). Among them, Ti:sapphire femtosecond laser (FSL), which emits ultrashort pulses (1 fs =10^{-15} s) not only produces a clean surface, but also reduces phase transformations (15) and have a slight heating effect (9). Several studies investigated the effect of FSL on the shear bond strength (SBS) of orthodontic brackets (9,16,17). However, to authors' knowledge, no study has examined the impact of lasers on the SBS of metal brackets to Y-PSZ. Moreover, a single-step self-etching ceramic primer [Monobond Etch & Prime (MEP); Ivoclar Vivadent, Schaan, Liechtenstein] has been launched recently and the number of the studies on the effect of this material on zirconia are limited (11,13,18). Therefore, the purpose of this study was to investigate the effect of different surface treatments on the surface roughness (R_a) and SBS of Y-PSZ with different yttrium content. The null hypotheses were that surface treatments and material type would not affect R_a or SBS.

Materials and Methods

One hundred and ten specimens were milled (CEREC inLab MC X5; Dentsply Sirona, Bensheim, Germany) from 5 mol% and 4 mol% (Ceramill Zolid FX and Zolid HT+; Amann Girrbach, Pforzheim, Germany) zirconia discs and sintered in a furnace (1450 °C, 8 h, Ceramill Therm; Ivoclar Vivadent, Schaan, Liechtenstein). Specimens were then polished with silicone-carbide papers (#600, 800, and 1000) to final dimensions (12x12x1.5 mm), embedded in auto-polymerizing acrylic resin (SC; Imicryl, Konya, Turkey), and divided into 5 groups (n=11):

Group C: No treatment

Group APA: Sandblasting with 50 µm Al_2O_3 (Korox; BEGO, Bremen, Germany) particles at 2 bar pressures from 10 mm distance for 10 s (2).

Group MEP: MEP etching for 60 s. A micro-brush was used to apply the product for the first 20 s. After 60 s, specimens were rinsed and air-dried.

Group Er:YAG laser (ER): ER laser beams (Fotona; AT Fidelis, Ljubljana, Slovenia) delivered perpendicularly approximately from 2 mm were used to irradiate specimens with a non-contact hand piece (R02) that has an integrated spray nozzle (Wavelength: 2940 nm, frequency: 10 Hz, pulse duration: 10 s, pulse width: 100 µs, energy level: 400 mJ, power: 4 W).

Group FS: FSL consists of two basic units, one of which is oscillator seed laser (Quantronix, Ti-Light, NY, USA) that produces 3 nJ per laser pulse energy with 85 MHz repetition rate at the wavelength of 800 nm. Another unit amplifier laser operates at 1-3 kHz repetition rate with 3.5 mJ per pulse with the fundamental laser wavelength at 800 nm (Quantronix, Integra-C-3.5, NY, USA). The micromachining unit (Quantronix, Q-Mark, NY, USA) capable of operating in accordance with the FSL and the system that can be controlled by computer. F-theta lens was focused on the specimen from 11 cm. In the present study, a marking speed with 10 mm/s, skip speed with 125 mm/s and the repetition with five times were carried out in 10 mm x 10 mm square for each zirconia specimen. All specimens were ablated using 200 mW

laser power and 9.819 10^{13} W/cm^2 laser intensity to create a checkered pattern.

Specimens were cleaned ultrasonically for 10 min (Whaledent Biosonic; Whaledent Inc., New York, USA). R_a of the specimens were measured from 5 different regions with a 2-dimensional contact profilometer (MarSurf PS1; Mahr GmbH, Göttingen, Germany) and these values (μm) were averaged. One additional specimen from each group was prepared as described and observed with scanning electron microscope (SEM) (EVO LS-10; Zeiss, Cambridge, UK) at 25 kV (500× and 1000× magnifications).

A light-polymerized adhesive primer (Tranbond XT Primer; 3M Unitek, Monrovia, California, USA) was applied to pretreated zirconia surfaces and mandibular incisor orthodontic metal brackets (Mini Master; American Orthodontics, Sheboygan, Wisconsin, USA), which had an average surface area of 10.37 mm^2 were bonded by using an adhesive resin (Transbond XT; 3M Unitek, Monrovia, California, USA). Excess cement was removed with an explorer and the specimens were irradiated with an LED curing unit (Bluephase; Ivoclar Vivadent, Schaan, Liechtenstein) from the occlusal and the gingival bracket edges for 20 s at 1200 mW/cm^2 intensity. Bonding was performed by a single experienced clinician (B.B.D.). Specimens were then subjected to thermocycling (5,000 cycles at 5-55 °C, dwell time of 15 s) to replicate an intraoral period of relatively six months (19).

A universal testing machine (Mod Dental Micro Shear Tester; Esetron Smart Robotechnologies, Ankara, Turkey) with a crosshead speed of 0.5 mm/min was used for SBS tests. The knife-edge rod was positioned perpendicular to the edge of the brackets' base. SBS (MPa) was calculated by using the equation:

SBS (MPa) = Maximum load (N) / Surface area (mm^2)

Adhesive remnant index (ARI) was determined by using a stereomicroscope (Olympus SZ61; Olympus Corp, Tokyo, Japan) at 45× magnification to assess the failure modes. One sample from each group exhibiting the dominant ARI score was analyzed by using SEM (64×). The scores for ARI were as follows (20):

Score 0: No adhesive on the specimen,

Score 1: Less than 50% of adhesive on the specimen,

Score 2: More than 50% of adhesive on the specimen,

Score 3: All adhesive on the specimen, with distinct impression of the bracket mesh.

Statistical Analysis

Data were analyzed (SPSS 23, SPSS Inc; Chicago, IL, USA) by using 2-way analysis of variance (ANOVA) and Tamhane's T2 tests. In addition, the correlation between these parameters was evaluated with Pearson's correlation analysis (α=0.05). Number of specimens was decided based on a power analysis (power: 0.80, α: 0.05, and effect size: 0.4).

Results

Surface treatments had a significant effect on R_a and SBS ($p<0.001$), whereas the effect of material type ($p\geq0.192$) and the interaction between the main factors ($p\geq0.312$) were nonsignificant for both parameters. For 4Y-PSZ, FS group (4.87±0.41) had the highest R_a ($p<0.001$), whereas C group (0.24±0.04) showed the lowest ($p\leq0.001$). APA (0.49±0.05) and ER (0.47±0.06) groups had similar values ($p=0.997$) that were higher than that of MEP group (0.36±0.04) ($p\leq0.002$). For 5Y-PSZ, FS group (5.13±0.62) had the highest ($p<0.001$) and C group (0.26±0.07) had the lowest R_a ($p\leq0.001$). APA (0.48±0.04) and ER (0.47±0.05) groups had similar values ($p>0.05$) that were higher than that of MEP (0.39±0.04) group ($p\leq0.006$).

For 4Y-PSZ, FS (19.88±2.69) and APA (18.74±2.38) groups had the highest ($p<0.001$) and C group (3.46±0.47) had the lowest SBS ($p<0.001$). MEP group (13.27±2.39) had higher SBS than ER group (5.42±0.59) ($p<0.001$). For 5Y-PSZ, FS (20.58±2.47) and APA (19.47±2.63) groups had the highest SBS values ($p<0.001$). MEP group (12.83±2.46) had higher SBS than ER group (5.15±0.82) ($p<0.001$). C group (2.82±0.38) had the lowest values ($p<0.001$). Pearson correlation analysis showed that there was no significant correlation between R_a and SBS for any of the groups ($p\geq0.154$).

Figures 1 and 2 depict surface alterations after surface treatments. In general, surface treatments resulted in similar surface modifications for both materials. C groups showed little to no irregularities, while MEP etching led to somewhat similar surfaces with more pronounced grooves caused by the etchant. APA generated an irregular topography and roughness of the treated surfaces was apparent. ER treatment was characterized with concave and convex

areas, and evident microcrack formation. FS groups presented the most precise surface change with clear, square shaped depressions and no microcracks.

Representative SEM images of the bond failures are presented in Figures 3 and 4, while bond failure types (n and %) are shown in Table 1. In both C groups, 100% of the specimens demonstrated score 0. While the failure types of ER and MEP treated specimens were scores 0 and 1, APA and FS groups showed scores 2 and 3.

Discussion

Even though material type was not effective, surface treatments resulted in significant differences

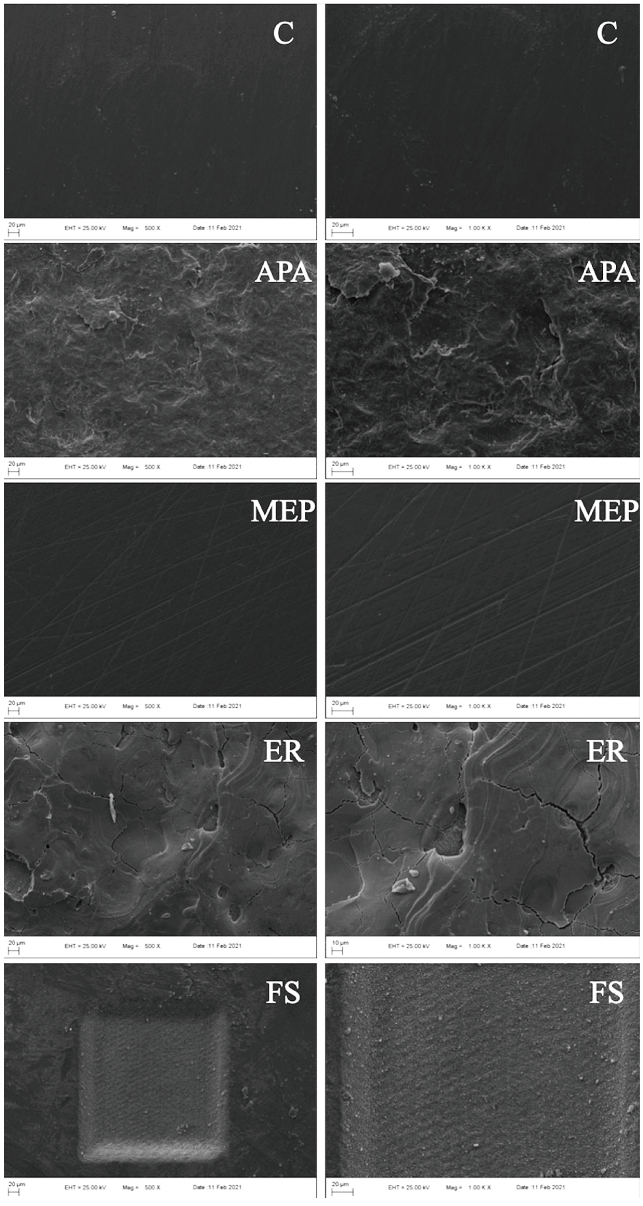

Figure 1. SEM images (500× and 1000×) of 4Y-PSZ specimens after surface treatments
C: Control, APA: Sandblasting, MEP: Monobond Etch & Prime etching, ER: Er:YAG laser irradiation, FS: Femtosecond laser irradiation, SEM: Scanning electron microscope, Y-PSZ: Partially stabilized zirconia

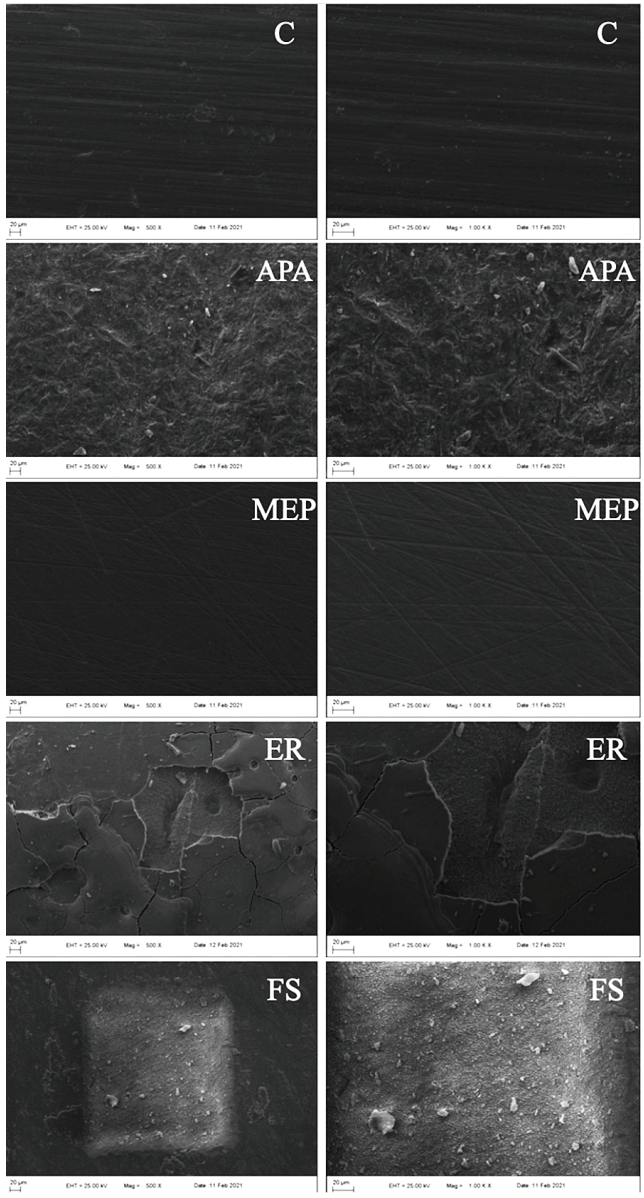

Figure 2. SEM images (500× and 1000×) of 5Y-PSZ specimens after surface treatments
C: Control, APA: Sandblasting, MEP: Monobond Etch & Prime etching, ER: Er:YAG laser irradiation, FS: Femtosecond laser irradiation, SEM: Scanning electron microscope, Y-PSZ: Partially stabilized zirconia

in SBS and R_a. Therefore, the null hypotheses were rejected.

Previous studies have shown that zirconia had higher R_a after FSL irradiation when compared with APA (1,9). The result of this study supports this finding as the specimens of FS groups had the highest R_a. SEM images of FS groups revealed the conspicuous surface alteration with precise square shaped depressions, which were also evident to naked eye. These depressions contributed to a greater surface area that orthodontic resin cement penetrated, which may have led to greater SBS values. R_a results of MEP treated specimens were inferior to those treated with APA, ER, and FS. As seen in SEM images, MEP treated

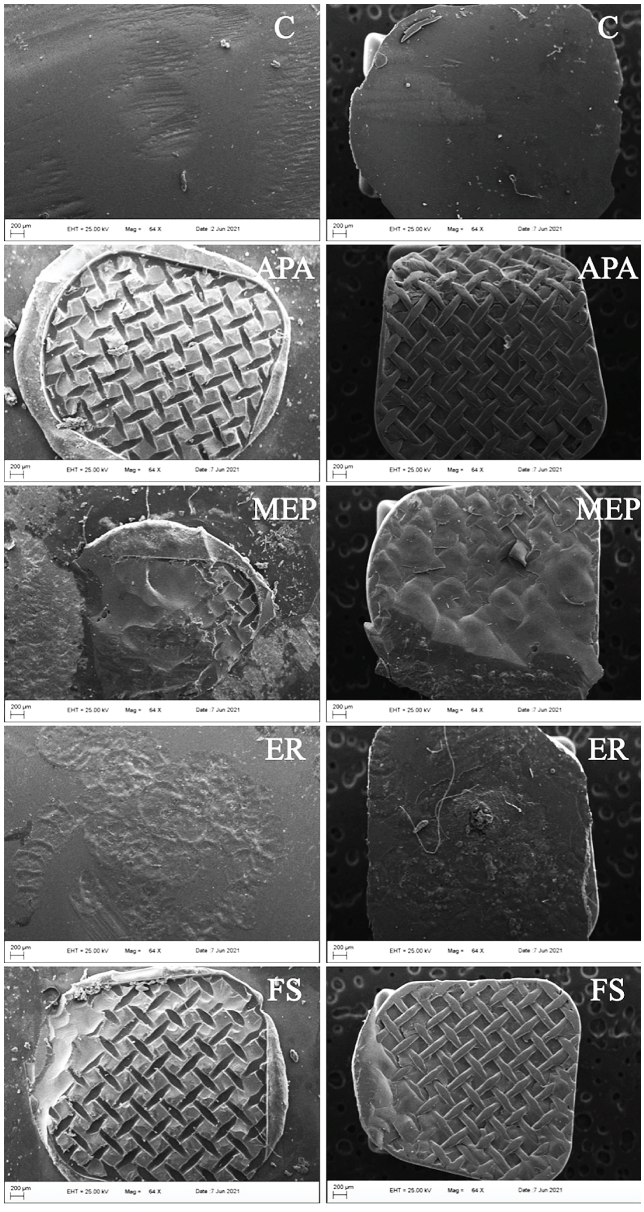

Figure 3. SEM images (64×) of the debonded 4Y-PSZ specimens and metallic bracket surfaces after SBS test
C: Control, APA: Sandblasting, MEP: Monobond Etch & Prime etching, ER: Er:YAG laser irradiation, FS: Femtosecond laser irradiation, SEM: Scanning electron microscope, Y-PSZ: Partially stabilized zirconia, SBS: Shear bond strength

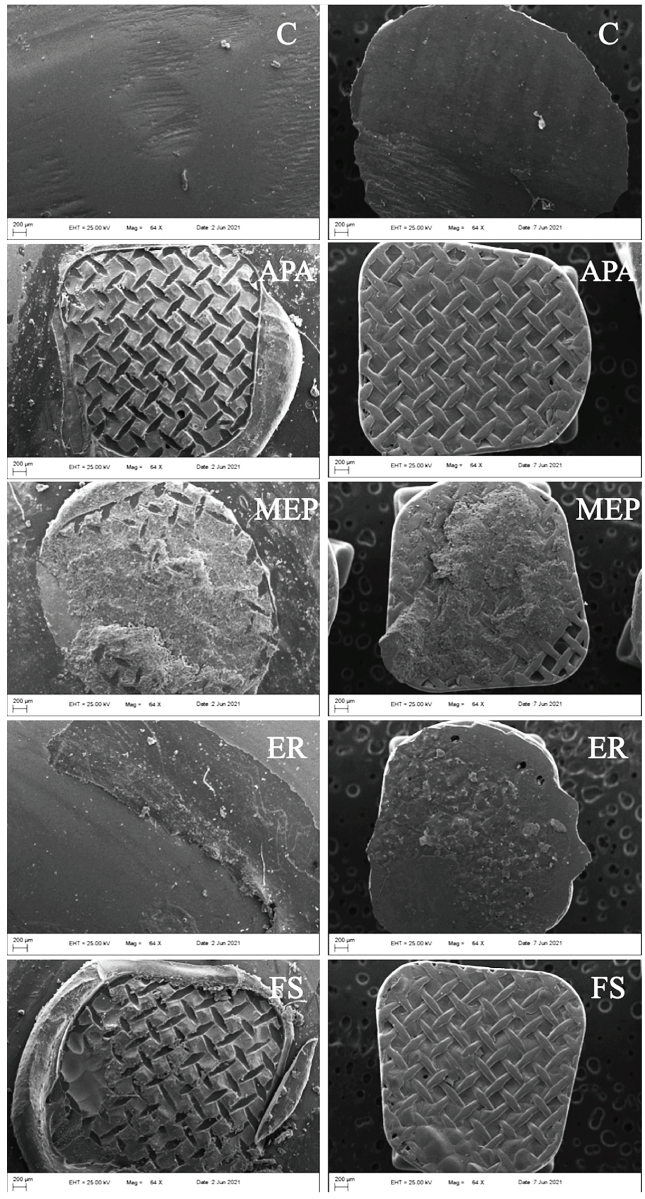

Figure 4. SEM images (64×) of the debonded 5Y-PSZ specimens and metallic bracket surfaces after SBS test
C: Control, APA: Sandblasting, MEP: Monobond Etch & Prime etching, ER: Er:YAG laser irradiation, FS: Femtosecond laser irradiation, SEM: Scanning electron microscope, Y-PSZ: Partially stabilized zirconia, SBS: Shear bond strength

Table 1. Bond failure mode scores (ARI) (n and %)

	C		APA		MEP		ER		FS	
	4 Y-PSZ	5 Y-PSZ	4 Y-PSZ	5 Y-PSZ	4 Y-PSZ	5 Y-PSZ	4 Y-PSZ	5 Y-PSZ	4 Y-PSZ	5 Y-PSZ
Score 0	11 (100%)	11 (100%)	0 (0%)	0 (0%)	4 (36%)	3 (27%)	5 (45%)	3 (27%)	0 (0%)	0 (0%)
Score 1	0 (0%)	0 (0%)	0 (0%)	0 (0%)	6 (55%)	6 (55%)	6 (55%)	8 (73%)	0 (0%)	0 (0%)
Score 2	0 (0%)	0 (0%)	3 (27%)	3 (27%)	1 (9%)	2 (18%)	0 (0%)	0 (0%)	2 (18%)	3 (27%)
Score 3	0 (0%)	0 (0%)	8 (73%)	8 (73%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	9 (82%)	8 (73%)

ARI: Adhesive remnant index, C: Control, APA: Sandblasting, MEP: Monobond Etch & Prime etching, ER: Er:YAG laser irradiation, FS: Femtosecond laser irradiation, Y-PSZ: Partially stabilized zirconia

specimens displayed more consistent and flat surfaces than these groups, which justifies the R_a results.

Different surface treatments have been suggested to enhance the SBS between ceramic surfaces and orthodontic brackets (2,9,12-14,16,17), as debonding is frequently encountered (19). Nonetheless, the ideal surface treatment for bracket bonding is still unclear (9). Reynolds (21) reported SBS values higher than 6 MPa as clinically acceptable. However, SBS should also be at a reasonable level so that no cohesive damage occurs during debonding (10) and the least amount of adhesive remains on the zirconia surface (5). In this study, SBS values ranged between 2,5 MPa to 24,85 MPa for 4Y-PSZ, and from 2,42 MPa to 26,19 MPa for 5Y-PSZ. Furthermore, specimens of APA, MEP, and FS groups presented SBS values higher than 6 MPa. Therefore, these treatments may be considered clinically satisfactory.

Sandblasting produces a rough ceramic surface depending on the pressure and particle size (1,19). In this study, zirconia surfaces were treated with 50 µm Al_2O_3 particles at 0.2 MPa pressure. Several studies have evaluated the effects of sandblasting on the SBS of Y-PSZ and concluded that sandblasting with 0.2 MPa resulted in higher SBS values (2-4). However, a recent study reported similar values while comparing the SBS of metallic brackets to Er:YAG laser treated and sandblasted 5Y-PSZ. In addition, ARI scores of both groups were predominantly 2 (5). Contrarily, APA-5 had higher SBS values than ER-5 in the present study and this difference may be attributed to the parameters of the surface treatments. Furthermore, SBS of APA groups were higher than those of other groups, except for FS.

In a previous study, MEP treated zirconia was shown to have SBS higher than 6 MPa even after 10,000 cycles of thermocycling (13). This finding was further supported by another study, in which MEP provided SBS as high as 32.3 MPa after similar thermocycling (11). Similarly, SBS values of MEP groups exceeded 6 MPa, which may be associated with the significant increase in surface energy even with a minimal change in R_a (Figure 1, 2) (11). However, the knowledge on the effect of MEP on Y-PSZ is scarce and these results should be interpreted carefully. Furthermore, MEP has acidic components and possible toxic effects should be considered during intraoral application (18).

FSL irradiation of ceramics for orthodontic purposes has been scarcely studied (9,16,17). García-Sanz et al. (9) reported 200 mW output power and 60 µm inter-groove distance as the ideal parameters for treating 3Y-TZP. The same study also showed that these parameters led to higher SBS values than sandblasting (25 µm Al_2O_3 at a pressure of 2.5 bar for 20 s), which coincides with another study (16). In this study, FS resulted in nonsignificantly higher SBS values than APA, which may be associated with different parameters used. Although adequate SBS was achieved, clinical utilization of FSL is questionable considering the system costs and dimensions (16).

Specimens of APA and FS groups mainly had ARI score 3, which is an indicator of bond failure between orthodontic cement and metallic bracket. Considering that the greater amount of cement retained on the restoration surface means less chance of ceramic damage, FSL irradiation and sandblasting may be considered as the most zirconia-friendly treatments. However, this study did not evaluate the possible effects of these treatments on the mechanical behavior or phase transformation of Y-PSZ. Therefore, future studies investigating these parameters are needed to support this interpretation.

Even though the present study aimed to compare new-generation zirconias, absence of 3Y-TZP, which

can be used monolithically in the posterior region is a limitation. In addition, the present study did not involve a zirconia primer, which may increase SBS (7). Another limitation was that a checkered depression pattern was created for FS groups. However, it is possible to engrave other geometrical designs (9,16) and distinct patterns might affect R_a and SBS. Since the esthetic expectations of patients are rising, preference of metallic bracket might also be a limitation.

Conclusion

Within the limitations of this study, the type of Y-PSZ did not affect R_a or SBS values. FSL irradiation and sandblasting emerged as the most effective and zirconia-friendly treatments. Single-step self-etching primer may be a valid surface treatment for Y-PSZ.

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Ethics

Ethics Committee Approval: This article does not contain any studies with human participants or animals performed by any of the authors.

Informed Consent: Informed consent is not required.

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Authorship Contributions

Surgical and Medical Practices: M.D., Y.G., Concept: M.B.D., B.B.D., Design: M.B.D., B.B.D., Data Collection or Processing: B.B.D., M.D., Analysis or Interpretation: M.B.D., Y.G., H.Ş.K., Literature Search: B.B.D., M.D., Writing: M.B.D., Y.G., H.Ş.K.

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Original Article / Özgün Araştırma

A CBCT-assisted Evaluation of Single Reciprocating File Instrumentation in Curved Root Canals with a Prior Glide Path Preparation

Eğimli Kök Kanallarında Rehber Yol Oluşturularak Tek Eğeli Resiprokal Eğe ile Gerçekleştirilen Enstrümantasyonun CBCT ile Değerlendirilmesi

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Keywords
Glide path, reciprocating motion, transportation, centering ability

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Rehber yol, resiprok eğe, transportasyon, merkezleme kabiliyeti

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Abstract

Objective: The primary aim of the current study was to compare the centering ability and transportation of the Reciproc Blue system in curved root canals with and without prior glide path preparation, using cone beam computed tomography (CBCT). Additionally, the amount of time required for root canal instrumentation was also compared.

Materials and Methods: Forty root canals of maxillary molar teeth with curvature angles 30°-40° were included in this *in vitro* study. All root canals were divided randomly into 2 experimental groups (n=20) as follows: group 1: the root canals were instrumented with a Reciproc Blue R25 instrument, and group 2: glide path was prepared with path-file and the root canals were instrumented with a Reciproc Blue R25 instrument. Working times with Reciproc Blue files were recorded for each group. All teeth were scanned with CBCT before and after instrumentation to evaluate the centering ability and transportation. The data were statistically analyzed using the Shapiro-Wilk test and t-test ($p<0.05$).

Results: No significant difference was observed between the tested groups regarding the centering ability and transportation ($p>0.05$). The time required for instrumentation using Reciproc Blue after glide path preparation was statistically less than that without the glide path preparation group ($p<0.001$).

Conclusion: In curved root canals, glide path preparation before instrumentation with the Reciproc Blue file had no effect on the centering ability and transportation. However, the amount of time required for root canal instrumentation with the Reciproc Blue files was significantly decreased when the files were used after the preparation of the glide path by path-files.

Öz

Amaç: Bu çalışmanın birincil amacı, şekillendirme öncesi rehber yol hazırlanan ve hazırlanmayan eğimli kök kanallarında Reciproc Blue sisteminin merkezleme kabiliyetini ve meydana gelen transportasyon miktarını konik ışınlı bilgisayarlı tomografi (KIBT) kullanarak karşılaştırmaktır. Ayrıca her iki grupta kök kanal genişletme süreleri kıyaslanmıştır.

Gereç ve Yöntemler: Bu *in vitro* çalışmaya kırk adet 30°-40° eğrilik açısına sahip kök kanalı dahil edildi. Tüm kök kanalları 2 deney grubuna ayrıldı: 1. grup: path-file ile rehber yol oluşturulduktan sonra Reciproc Blue kanal eğesi ile kök kanal şekillendirmesi gerçekleştirildi, 2. grup: Rehber yol oluşturmaksızın Reciproc Blue kanal eğesi ile kök kanal şekillendirmesi gerçekleştirildi. Her grup için Reciproc Blue eğeleri ile çalışma süreleri kaydedildi. Merkezleme kabiliyetini ve transportasyonu

değerlendirmek için tüm dişler şekillendirmeden önce ve sonra KIBT ile tarandı. Veriler Shapiro-Wilk ve t-testi ile istatistiksel olarak analiz edildi.
Bulgular: Merkezleme kabiliyeti ve transportasyon açısından test edilen gruplar arasında anlamlı bir fark tespit edilmemiştir (p>0,05). Rehber yol oluşturulan grupta Reciproc Blue kanal eğesi ile şekillendirme için gereken sürenin, rehber yol oluşturulmayan gruptan istatistiksel olarak daha az olduğu gözlenmiştir (p<0,001).
Sonuç: Eğimli kök kanallarında, Reciproc Blue eğesi ile şekillendirme öncesinde rehber yol oluşturmanın merkezleme kabiliyeti ve transportasyon üzerinde hiçbir etkisi olmamıştır. Ancak, path-file ile rehber yol oluşturulduğunda Reciproc Blue eğesi ile kök kanalı içindeki çalışma süresi önemli ölçüde azalmıştır.

Introduction

Cleaning and shaping of the root canals are the most important stages that affect the success of the root canal treatment. However, the shaping process may not always be completed in ideal conditions. Although preserving the original root canal anatomy is substantial, deviations from the root canal anatomy such as root canal straightening, transportation and ledging may occur depending on the severity of the root canal curvature and characteristic features of the instruments (1).

The glide path is defined as an access route to the root canal up to the apical foramen (2,3). Glide path creating provides an understanding of the root canal anatomy, permits safer and faster action during root canal instrumentation (4). Glide path creation can be performed with stainless steel hand files as well as using nickel titanium (NiTi) files designed for this purpose. There are several NiTi glide path files (PF) produced with different alloy and cross-section properties. PF; Dentsply Maillefer is a rotary glide path system and manufactured from a conventional NiTi alloy. The system includes three files with .02 taper and ISO 13, 16, 19 tip sizes (1). PF are claimed to better preserve the original root canal anatomy by creating less irregularity in the root canal while preparing the glide path (5).

Reciproc Blue (VDW, Munich, Germany) is a reciprocating NiTi instrument and have S-shaped cross-section. Reciproc Blue files (R25, R40 and R50) are produced with a heat treatment that changes the molecular structure of the alloy and gives the tool a blue color (6). The heat treatment process improves the flexibility and strength of the Ni Ti files.

The aim of this study was to compare centring ability and transportation of Reciproc Blue files with and without glide path by using cone beam computed tomography (CBCT) imaging and to evaluate time required for root canal instrumentation with Reciproc Blue files. The null hypothesis of the study was that preparation of glide path prior to root canal instrumentation would not effect the centring ability and canal transportation of Reciproc Blue system.

Materials and Methods

The current study was approved by the Ege University Faculty of Medicine Clinical Research Ethics Committee (approval number: 18-10.2/42, date: 30.10.2018) and was funded by University Scientific Research Project Fund. An initial power analysis was performed according to a previous study (7) and it was confirmed that a minimum sample size of 16 teeth per group is required (80% power, α=5%).

Selection of Teeth

Mature extracted human maxillary molars without calcified root canals, internal or external resorption, or root fractures were included.

Access cavities were prepared using diamond burs, and the apical patency was confirmed using 10# K-file. In order to measure the root canal curvature, K-type files #10 was placed in the root canals and digital radiographs were taken from all teeth and root canal curvature was measured following Schneider's (8) method using AutoCAD 2007 program (Autodesk Inc., San Rafael, CA, USA). As a result, a total of 40 mesial or distal root with single canal (with canal curvature of 30°-40°) were selected. In order to provide standardization of the position of the teeth before and after root canal preparation, the teeth were placed in a custom-made specimen holder and all radiological and clinical procedures were performed without removing the teeth from this holder.

The teeth were divided into 2 groups as follows:

Group 1. Reciproc Blue: Reciproc Blue files #25 were directly used in root canals without glide path preparation.

Group 2. PF - Reciproc Blue: Reciproc Blue files #25 were used in root canals after creating a glide path by PFs.

CBCT Before Root Canal Instrumentation

The teeth were positioned with the custom-made specimen holder in which each root could be aligned perpendicularly to the beam and placed in the same position on the CBCT device before and after instrumentation, so that reproducible images could be obtained. In order to determine the scanning direction, a small notch was prepared on the crown of each tooth. The roots were scanned before and after instrumentation by using the CBCT [Kodak 9000 three-dimensional (3D)] operating at 64 kV and 6.3 mA; volumes were obtained with 5x3.8 cm field of view (FOV) and with a spatial resolution of 76 mm was selected.

Root Canal Instrumentation

Group 1 (Reciproc Blue): The root canals were prepared directly by using Reciproc Blue #25 files in the Reciproc all mode with an X-Smart plus motor (Dentsply, Maillefer Ballaigues, Switzerland). No glide path was created in this group. A 10 K-file was used with the purpose of determining full working length (WL) according to the manufacturer instructions. Then, root canal instrumentation was performed using the Reciproc Blue R25 instrument with a slow in-and-out pecking motion. The amplitude limit of the file was approximately 3-4 mm with a very light pressure. After every third pecking, the file was removed from the canal and the root canal was rinsed with 2 mL 2.5% sodium hypochlorite. This procedure was followed until WL was reached.

Group 2 (PF - Reciproc Blue): path-files #1-3 were used for glide path creation with copious 2.5% sodium hypochlorite irrigation. Next, the root canals were prepared using Reciproc Blue #25 files as same as group 1.

One experienced operator completed all root canal instrumentation (S.M.K.). During the instrumentation procedure, each root canal was irrigated at 2 mm shorter than the WL using a closed ended side-vented needle (30-G) (Kerr Hawe Sa, Bioggio, Switzerland). Time required for root canal instrumentation with Reciproc Blue files were also recorded and compared.

CBCT After Root Canal Instrumentation

The teeth with the custom-made specimen holder were placed at the same position on the CBCT device and were scanned under the same conditions used before instrumentation. For both groups, the degree of transportations and centring ability that were created during instrumentation were measured using the CS 3D imaging software program (Figure 1).

Transportation and Centring Ability Evaluation

Canal transportation was calculated at 3 mm and 5 mm distance from the apex according to the following formula;

Degree of canal transportation = (a1-a2) - (b1-b2)
= (c1-c2) - (d1-d2)

a1, b1, c1, d1: The shortest distance between the mesial, distal, buccal and palatal wall of the canal and the mesial outer surface of the root, respectively, before instrumentation.

a2, b2, c2, d2: The shortest distance between the mesial, distal, buccal and palatal wall of the canal and the mesial outer surface of the root, respectively, after instrumentation.

A result of 0 indicates that no transportation has occurred; all other results indicate that transportation has occurred.

Centring ability was calculated with the following formula:

(a1-a2)/(b1-b2) or (b1-b2)/(a1-a2) and (c1-c2)/(d1-d2) or (d1-d2)/(c1-c2)

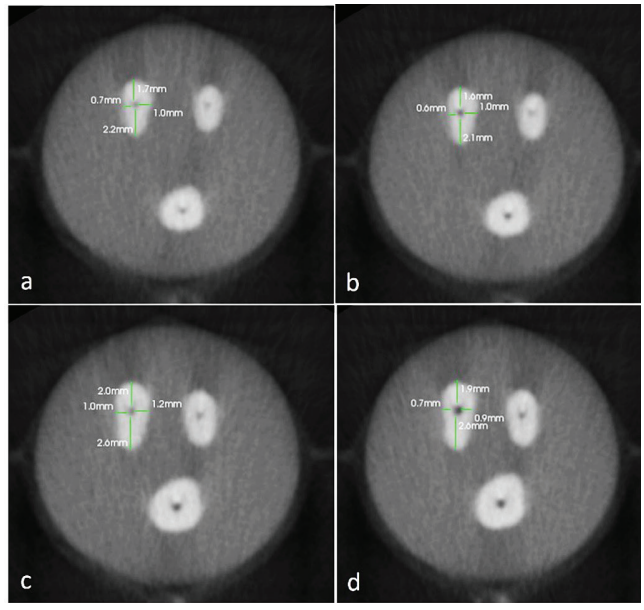

Figure 1. Representative CBCT images of 3 mm (a and b) and 5 mm (c and d) cross sections before (a and c) and after (b and d) preparation
CBCT: Cone beam computed tomography

According to this formula, a value of '1' indicates that the root canal remains completely in the centre, while other values indicate that the direction of the root canal has changed.

Statistical Analysis

The statistical analysis was performed with the GraphPad prism version 6.0 for Windows package program (GraphPad Software, LA Jolla California, USA). The Shapiro-Wilk test was used to determine the normal distribution of continuous variables. Centring ability, transportation and working time comparisons were analysed using the t-test. All analyses were performed at $p<0.05$ statistical significance level.

Results

Amount of time required for canal preparation: Time required for root canal instrumentation using Reciproc Blue after creating a glide path (66.2±11.8 seconds) was found to be statistically significantly lower than in the group without a glide path preparation (89.4±11.6 seconds) ($p<0.001$).

Transportation results: Mean, standard deviation and p-values of transportation for both 3 and 5 mm sections are shown in Table 1. No statistically significant difference was observed between the groups for both directions in transportation findings in both 3 mm and 5 mm sections ($p>0.05$).

Centring ability results: Mean, standard deviation and p-values of centring ratio for both 3 and 5 mm sections are shown in Table 2. There was no statistically significant difference between the groups in terms of centring ratio in each section ($p>0.05$).

Table 1. Mean canal transportation (mm ± standard deviation) at 3 and 5 mm after preparation with Reciproc Blue and path-file + Reciproc blue

	Direction	Reciproc Blue	Path file + Reciproc Blue	p-value
3 mm	Mesio-distal Bucco-palatal	0.030±0.172 0.040±0.169	0.035±0.159 0.005±0.198	0.924 0.446
5 mm	Mesio-distal Bucco-palatal	0.015±0.153 0.010±0.177	0.005±0.153 0.060±0.153	0.682 0.346

Table 2. Mean centring ability (mm ± standard deviation) at 3 and 5 mm after preparation with Reciproc blue and path file + Reciproc Blue

	Direction	Reciproc Blue	Path file + Reciproc Blue	p-value
3 mm	Mesio-distal Bucco-palatal	0.45±0.406 0.52±0.422	0.35±0.378 0.43±0.431	0.415 0.498
5 mm	Mesio-distal Bucco-palatal	0.54±0.391 0.47±0.45	0.40±0.377 0.44±0.46	0.227 0.851

Discussion

This *in vitro* study was carried out to examine whether the creating a glide path before instrumentation influences the level of transportation and centring ability of the Reciproc Blue file in curved root canals. According to the results, the null hypothesis was rejected in terms of time required for root canal instrumentation, which was significantly shortened by the prior preparation of glide path. However, null hypothesis was accepted regarding canal transportation and centring ability of reciprocal instruments with or without prior preparation of glide path.

In the current study, mesio- and disto-buccal roots of extracted maxillary molars were included. Schneider's (8) method is one of the methods frequently used in studies was preferred for canal curvature measurement (9,10), where root canal curvature greater than 25° is defined as severely curved root canals. Since the severity of the root canal curvature carrying a potential complication risk during root canal instrumentation (11); teeth with a curvature of 30°-40° were included. Nevertheless, radius of curvature was not measured, only teeth with visually similar radii of curvature were included. Due to the roots measured at the same angle in degrees can have different radii or abrupt curvature, using Schneider method can be seen as a possible limitation of the present study.

The most used method to compare before and after instrumentation is obtaining standard periapical radiographs and overlapping these radiographs with computer programs (12,13), however, the most important disadvantage of the method is that the radiographs are 2D. In most studies, this disadvantage has been avoided by using CBCT and micro-CT as imaging methods (9,10,14,15). Although micro-CT is considered as the most valid method in this field, it was reported that it is possible to examine and compare root canal anatomy before and after instrumentation using CBCT (14). For this reason, in the current study, CBCT, which is a more accessible method, allows 3D imaging and is frequently used in transportation studies, was preferred to use (7,15,16). It was observed that different analysis parameters

(FOV area, section thickness) were used in studies where instrumentation efficiency was examined using CBCT. Elnaghy and Elsaka (15) determined the FOV area to be 8x8 cm please and the slice thickness to be 0.125 mm, while Mouro-Netto et al. (16) determined the FOV area as 16x4 cm and the section thickness as 0.125 mm. In our study, the FOV area was used as 5x3.8 cm and the section thickness as 0.076 mm, providing higher resolution than thicker slices and larger FOVs.

When the time required for canal instrumentation was evaluated Reciproc Blue files was found to be significantly faster if the glide path was prepared. Similar to our study, Vorster et al. (17), was evaluated the duration for WaveOne Gold file instrumentation in root canals, reported that time required for instrumentation with the Wave One Gold files was significantly reduced after a glide path preparation.

In studies focusing transportation, the measurement regions differ while there are studies (15,18,19) that take cross-sections from apical, middle, and coronal parts, there are also studies that evaluate apical 1, 2, 3, 4, 5 mm sections by considering only apical transportation (20). In a study transportation was evaluated using 3, 5, 7 mm sections but, the degree of transportation was measured in apical 3 and 5 mm sections only (15). Bucco-palatal transportation in addition to the mesio-distal direction were also examined in the current study. In each group, small transportation with no significant differences between the groups were obtained both at 3 mm and 5 mm cross sections. Preparation of the glide path using the path-files had no effect on the transportation values at the apical 3 and 5 mm after the instrumentation using the Reciproc Blue files. While in some studies, it was reported that Reciproc and Reciproc Blue files would cause less transportation when a glide path was created before instrumentation in curved root canals (4,7), de Carvalho et al. (9) concluded that performing a glide path before using the Reciproc file had no effect on transportation like our study. The reason for the lack of difference between the groups and the very low transportation degrees in the current study may be the use of the Reciproc Blue canal file, which works with reciprocating motion and has advanced metallurgical properties. Additionally, no significant difference was observed between the groups in terms of centring ability both in mesio-distal and bucco-palatinal directions at both levels of cross sections which means that the use of path-file has no effect on centring ability of Reciproc Blue instrument. While Hage et al. (7) reported that glide path creation improved the centring ability of Reciproc and Reciproc Blue files, some studies showed that a glide path preparation has no effect on centring ability of reciprocating instruments (9,17). Actually, results of present study are in line with the manufacturer instructions in which M-Wire Reciproc Blue instruments are recommended to use without any prior step for root canal instrumentation.

Conclusion

Within the limitations of the current study, glide path preparation prior to shaping with Reciproc Blue file had no effect on centring ability and transportation. However, time required for root canal instrumentation was significantly decreased when the Reciproc Blue file was used after glide path preparation using path files.

Ethics

Ethics Committee Approval: The current study was approved by the Ege University Faculty of Medicine Clinical Research Ethics Committee (approval number: 18-10.2/42, date: 30.10.2018)

Informed Consent: *In vitro* study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: S.M.K., B.Ş., Concept: S.M.K., Design S.M.K., Data Collection or Processing: S.M.K., B.Ş., Analysis or Interpretation: S.M.K., B.Ş., Literature Search: S.M.K., Writing: S.M.K., B.Ş.

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Original Article / Özgün Araştırma

The Frequency of Use of Complementary and Alternative Medicine in Infantile Colic and Factors Affecting Method Selection

İnfantil Kolikte Tamamlayıcı ve Alternatif Tıp Kullanım Sıklığı ve Yöntem Seçimini Etkileyen Faktörler

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Abstract

Objective: Infantile colic (IC) is crying spells that begin in the first three months after birth, last longer than three weeks and occur at least three days a week. In this study, it was aimed to examine the use of alternative and complementary medicine applications for treating IC and the factors affecting the chosen method.
Materials and Methods: Infants were questioned for the diagnosis of IC using the "IC diagnostic criteria" defined in Rome IV. A total of 113 patients were included in the study. Demographic data, medication or (use of complementary and alternative drugs for IC, methods used and treatment responses) were asked by parents of infants with IC. The obtained data were analyzed statistically.
Results: It was found that 91.2% of the patients used at least one of the complementary and alternative medicine methods. It was found that those with a high level of education used hot application, hairdryer, car driving, and evil eye beads more ($p<0.05$), those with higher income levels used hot application, hairdryer and prayer more ($p<0.05$).
Conclusion: The education and income level of the family affect the alternative and complementary medicine methods used in IC. Evidence-based studies are needed on this subject.

Keywords
Alternative medicine, complementary medicine, infantile colic

Anahtar Kelimeler
Alternatif tıp, tamamlayıcı tıp, infantil kolik

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Öz

Amaç: İnfantil kolik (IC), doğumdan sonraki ilk üç ayda başlayan, üç haftadan uzun süren ve haftada en az üç gün meydana gelen ağlama nöbetleridir. Bu çalışmada IC tedavisinde alternatif ve tamamlayıcı tıp uygulamalarının kullanımı ve seçilen yöntemi etkileyen faktörlerin incelenmesi amaçlanmıştır.
Gereç ve Yöntemler: Bebekler, Roma IV'te tanımlanan "IC tanı kriterleri" kullanılarak IC tanısı için sorgulandı. Toplam 113 hasta çalışmaya dahil edildi. İnfantil kolikli bebeklerin ebeveynlerinden demografik veriler, ilaçlar veya IC için tamamlayıcı ve alternatif ilaçların kullanımı, kullanılan yöntemler ve tedavi yanıtları hakkındaki verileri istendi. Elde edilen veriler istatistiksel olarak analiz edildi.
Bulgular: Hastaların %91,2'sinin tamamlayıcı ve alternatif tıp yöntemlerinden en az birini kullandığı belirlendi. Eğitim düzeyi yüksek olanların daha çok sıcak uygulama, saç kurutma makinesi, araba kullanma ve nazar boncuğu kullandıkları ($p<0,05$), gelir düzeyi yüksek olanların sıcak uygulama, saç kurutma makinesi ve duayı daha fazla kullandıkları ($p<0,05$) bulundu.

Sonuç: Ailenin eğitim ve gelir düzeyi infantil kolikte kullanılan alternatif ve tamamlayıcı tıp yöntemlerini etkilemektedir. Bu konuda kanıta dayalı çalışmalara ihtiyaç vardır.

Introduction

Any practice that is not accepted by modern medicine and used instead of medical treatments is defined as "alternative medicine". Complementary medicine is expressed as methods applied in addition to medical treatment. These two terms are used together under complementary and alternative medicine (CAM) (1). Modern medicine accepts the evidence-based approach. CAM applications are generally unproven, based on assumptions and observations or, as in our country, based on some beliefs. These methods can be harmful both directly and indirectly by causing delays in medical treatment. Although studies on children are limited, CAM is frequently used in children, especially in difficult situations to cope with (2,3). Herbal preparations were found the most commonly used CAM method (4).

Infantile colic (IC) was first defined by Wessel et al. (5) as anxiety and crying episodes of more than three weeks, at least three days a week, and exceeding three hours a day, which are common in the first three months after birth. Although behavioral therapies, diet, and many other methods have been recommended, no definitive evidence-based treatment has yet been found. Although the prevalence of IC in Turkey is not known, it has been reported as 51.1% in Eastern Anatolia and 66% in Western Anatolia (6,7). In this study, it was planned to evaluate the frequency of CAM use, the methods used, and the factors affecting the use of CAM in infants with IC in the Aegean region.

Materials and Methods

The study was planned as a observational, descriptive prospective study and was conducted in University of Health Sciences Turkey, Dr. Behçet Uz Pediatric Diseases and Surgery Training and Research Hospital, between 01.09.2018-01.03.2019. The parents of babies under one year of age who were referred to the outpatient clinic for healthy child follow-up were asked whether their babies had excessive crying during early infancy. Babies who were reported to have excessive crying were questioned for IC's diagnosis using the "Diagnostic Criteria for Infant Colic" defined in Rome IV (8). According to these criteria, prolonged and repetitive crying periods with no apparent reason for crying in the first five months of life, no suspicion of illness, and not stopped by caregivers were defined as IC. A two-stage questionnaire was applied to parents who agreed to participate in the study and signed a consent form. In the first step, the diagnosis of IC in her babies was confirmed and diagnosed. In the second step, a questionnaire form consisting of 28 items regarding demographic data, use of medication or CAM for IC, methods used and treatment responses were applied face to face to the parents of babies diagnosed with IC. A total of 113 patients with a retrospective diagnosis of IC were included in the study. The parents' income level was based on the minimum wage.

The study was approved by ethics committee of University of Health Sciences Turkey, Dr. Behçet Uz Pediatric Diseases and Surgery Training and Research Hospital (approval number: 2018/212, date: 02.08.2018), and all our patients' parents' informed consent has been received.

Statistical Analysis

Statistical analyses were performed using the SPSS version 16.0. Descriptive statistics were expressed as frequency (%), mean ± standard deviation. The groups were compared using the Pearson chi-square test and Fisher's Exact test for categorical data. The p-values <0.05 were considered statistically significant.

Results

Sixty-five (57.5%) of the patients were male, and the mean age was 6.5±3.5 months at the time of the study. The general characteristics of the patients are presented in Table 1. While 90 (79.6%) of these patients received medication (all of them simethicone) for IC, 103 (91.2%) used one of the CAM methods. Of the cases questioned, 91 (88.3%) applied abdominal/back massage, 65 (63.1%) herbal tea, 48 (46.6%) car method. The least applied methods were prayer/amulet in 22 (21%) cases, wearing evil eye beads in 14 (13.6%), vegetable oil on the soles of 13 (12.6%) feet (lavender/apple/thyme), and 1 (1%) was

acupuncture. The used CAM methods are shown in Table 2 with details. Since 91.2% of the patients used CAM, an analysis of the factors affecting CAM use could not be performed. Therefore, factors affecting CAM methods used by patients were investigated. In the comparison of the CAM applications according to family income, warm application to the abdomen, use hairdryer and prayer/amulet were more frequent in high family income group (p-values are <0.001, <0.001, 0.011 respectively). The results were showed in Table 3. In the comparison of CAM applications according to mothers' education levels, warm application, hairdryer use, drive around, prayer/amulet were more frequent in high educated group than the low educated group (p-values are <0.001, <0.001, 0.002, 0.019 respectively) (Table 4). In the comparison of CAM applications according to fathers' education levels, foot sole massage with oils, warm application, hairdryer use, drive around were more frequent in high educated group than the low educated group (p-values are 0.031, 0.002, <0.001, 0.007 respectively). Drinking herbal tea was more frequent in low educated group (p=0.002). The results were showed in Table 5.

It was observed that the parents of the only patient who underwent acupuncture were in the group with high education and income levels.

Table 1. Socio-demographic characteristics of infants with infantile colic and their families

	Patient group n=113
Gender (female/male)	48/65
Maternal education level, n (%)	
Who never went to school	16 (14.2)
Primary school/secondary school	42 (37.2)
High school	37 (32.7)
University	18 (16.0)
Father's education level, n (%)	
Who never went to school	4 (3.5)
Primary school/secondary school	38 (33.6)
High school	37 (32.7)
University	34 (30.1)
Income level, n (%)	
Very bad	22 (19.5)
Bad	28 (24.8)
Middle	35 (31)
Good	28 (24.8)
Very good	0 (0)
Family type, n (%)	
Nuclear family	82 (72.6)
Extended family	31 (27.4)
Place of residence, n (%)	
Rural area	81 (71.7)
Urban	(28.3)

Table 2. Complementary and alternative medicine methods used*

Treatment method	Patient group n=110
Used medication, n (%)	90 (79.6)
Benefit from medication (none/partial/complete), n	15/68/7
Medication side effect, n (%)	0
Use of CAM, n (%)	103 (91.2)
Benefit from CAM (none/partial/complete), n	9/59/35
CAM side effect, n (%)	0
CAM and medication use together, n (%)	74 (65.5)

*More than one method could be specified for the same patient, CAM: Complementary and alternative medicine

Table 3. The comparison of CAM methods according to family income

	Family income		p-value
	Low n=43 n (%)	High n=60 n(%)	
Massaging the abdomen/back	37 (86.0)	54 (90.0)	0.537
Foot sole massage with herbal oils (lavender/apple/thyme)	4 (9.3)	9 (15.0)	0.391
Drinking herbal tea	29 (67.4)	36 (60.0)	0.440
Warm application to the abdomen	2 (4.7)	22 (36.7)	<0.001
Operating a hairdryer	7 (16.3)	32 (53.3)	<0.001
Drive around	24 (55.8)	40 (66.7)	0.263
Drinking sugar water	4 (9.3)	4 (6.7)	0.717
Wearing an evil eye bead	4 (9.3)	10 (16.7)	0.282
Prayer/amulet	4 (9.3)	18 (30.0)	0.011
Swaddle	13 (30.2)	20 (33.3)	0.739
Shake	20 (46.5)	28 (46.7)	0.988

CAM: Complementary and alternative medicine

Table 4. The comparison of CAM methods according to mothers' education levels

	Maternal education level		p-value
	Low n=51	High n=52	
Massaging the abdomen/ back	43 (84.3)	48 (92.3)	0.206
Foot sole massage with herbal oils (lavender/ apple/thyme)	4 (7.8)	9 (17.3)	0.148
Drinking herbal tea	37 (72.5)	28 (53.8)	0.049
Warm application to the abdomen	2 (3.9)	22 (42.8)	<0.001
Operating a hairdryer	7 (13.7)	32(61.5)	<0.001
Drive around	24 (47.1)	40 (76.9)	0.002
Drinking sugar water	6 (11.8)	2 (3.8)	0.160
Wearing an evil eye bead	6 (11.8)	8 (15.4)	0.592
Prayer/amulet	6 (11.8)	16 (30.8)	0.019
Swaddle	15 (29.4)	18 (34.6)	0.571
Shake	26 (51.0)	22 (42.3)	0.378
CAM: Complementary and alternative medicine			

Table 5. The comparison of CAM methods according to fathers' education levels

	Paternal education level		p-value
	Low n=36	High n=67	
Massaging the abdomen/back	30 (83.3)	61 (91.0)	0.335
Foot sole massage with herbal oils (lavender/ apple/thyme)	1 (2.8)	12 (17.9)	0.031
Drinking herbal tea	30 (83.3)	35 (52.2)	0.002
Warm application to the abdomen	2 (5.6)	22 (32.8)	0.002
Operating a hairdryer	5 (13.9)	34 (50.7)	<0.001
Drive around	16 (44.4)	48 (71.6)	0.007
Drinking sugar water	4 (11.1)	4 (6.0)	0.446
Wearing an evil eye bead	6 (16.7)	6 (11.9)	0.553
Prayer/amulet	4 (11.1)	18 (26.9)	0.063
Swaddle	12 (33.3)	21 (31.3)	0.836
Shake	19 (52.8)	29 (43.3)	0.357
CAM: Complementary and alternative medicine			

Discussion

This study's data showed that CAM methods had been used at a high rate in babies diagnosed with IC in İzmir province, and the most frequently used methods were abdominal and back massage.

A meta-analysis study about the frequency of CAM use in children reported that it is used at a rate of 52% in European countries (9). Two separate studies reported that the frequency of CAM use in children in Turkey was 58.6% and 56.5 (10,11). In our country, the most common reason for CAM use was IC (50%), and in a study in Afyon, CAM use in IC colic was reported as 66% (7,12). In our study, this rate was 91.2%, and it was higher than the literature. These increasing high rates determined in Turkey and İzmir were thought to occur since no effective medical treatment for IC and CAM methods became widespread.

CAM methods used for IC vary from region to region, both globally and in our country. In studies conducted in the southeast part of Turkey, the most common CAM method used by parents for IC was relieving baby gas, while in the study conducted in west part of Turkey, the most commonly used IC method was reported as massage (7,12). Our investigation determined that the most frequently used method was the abdominal and back massage, followed by drinking herbal tea and driving around. The massage is the most commonly used method for IC in Afyon, and İzmir, the west of Turkey, suggests that socio-cultural differences affect the CAM methods (7).

Although massage is thought to improve symptoms significantly and has no side effects, evidence-based data are limited. A study conducted in Germany stated that 50% of the mothers applied massage therapy; baby massage calmed the baby by providing many sensorial stimuli and improved mother-baby interaction (10,13,14). It is essential to warn parents about this condition, as methods such as massage with vegetable oils can cause serious side effects by absorption from the skin and accidental ingestion.

In the literature, it has been reported that some mothers consume some herbal teas such as chamomile, lemon balm, fennel, licorice root, and anise tea both themselves and their babies (11,15). Studies showing that herbal tea is effective in treating colic are rare. One of the most critical problems in the therapeutic use of herbal teas is that the doses

and side effects are not standardized, and the other is that it reduces the amount of nutrition (16). Herbal tea was found to be the second most commonly used CAM method for IC in our study.

The least used method in our study was found to be acupuncture (9,16). Currently, acupuncture is one of the recommended CAM methods for IC treatment. The results of studies on acupuncture are inconsistent. A systematic review of the practice concluded that needle percutaneous acupuncture treatment should not be recommended for IC babies (1).

In the literature, Tuncel et al. (17) reported that the parents' education level did not affect CAM's use in the Diyarbakır region. In the study on CAM methods used in IC treatment in Afyon, it was reported that the frequency of CAM use increased with the increase in the level of education, and the most common method they used was massage (7,18). In our study, the rate of CAM use was high, and the use of the hot application, hairdryer, driving around with a car, and the use of evil eye beads were statistically significantly different in the high-level education group. Although it is said that the use of CAM in IC treatment is higher in our country since it is a developing country, the high rate of CAM use by mothers with high education levels contradicts this view (14). The role of fathers in the selection of IC treatment may be related to the parental equitable behavior model in İzmir province.

Considering the families' socio-economic status, some studies in the literature showed that CAM was applied in 87% of the children of low-income families (19). There are studies reporting that mothers with higher education and high income also apply CAM methods (20). Contrary to in Europe and our country, it was reported in some studies conducted in the USA that the demographic characteristics and education level of the parents did not affect the use of CAM methods (21). In our study, the family's income level and economic status changed the CAM method selected, and parents with a good income significantly preferred hot application, using a hairdryer and praying methods. This result may be due to parents with better socio-economic conditions, ease of access to information and a doctor.

When the literature is examined, it was stated that many parents avoid talking to their doctors about CAM use because of their negative attitude towards the methods, but contrary to popular belief, 85% of the doctors displayed a positive attitude towards CAM methods (7,22,23). In our study, most of the parents who used medical treatment reported partial benefits from the medication, and the parents who used CAM had similar partial benefits. However, most of the patients applied CAM methods along with medical treatment, and some stated that they used several CAM methods together. In our study, CAM methods were used most frequently with the doctor's recommendation (30.1%), and the decision-makers were most often the family elders (31.1%). With these data, it can be concluded that doctors are the most critical resource for CAM use and that there is no opposing party to using these methods. It is currently said that the first step of IC management is counseling to parents (24,25). These results led us to think that doctors should inform families about the use of CAM, which is not based on evidence, and warn them regarding several issues.

This study was a descriptive study, so any outcome related to the causality is not possible. In addition it was a survey study, which might have the risks of perfunctory, exaggeration, concealment and short-term memory biases.

Conclusion

This study showed that the use of CAM rates for the treatment of IC is relatively high. The education and income level of the family affects the alternative and complementary medicine methods used. Physicians should have awareness and knowledge about CAM methods, which have been increasingly used in recent years.

Acknowledgment: The study was presented as an oral presentation at the International İzmir Demokrasi University Medical Congress 2020.

Ethics

Ethics Committee Approval: The study was approved by ethics committee of University of Health Sciences Turkey, Dr. Behçet Uz Pediatric Diseases and Surgery Training and Research Hospital (approval number: 2018/212, date: 02.08.2018).

Peer-review: All our patients' parents' informed consent has been received.

Authorship Contributions

Concept: N.H., Ö.B., T.T., Design: N.H., Ö.B., Ö.B.S., Data Collection or Processing: N.H., Ö.B.S., Analysis or

Interpretation: N.H., T.T., Literature Search: N.H., Ö.B., T.T., Ö.B.S., Writing: N.H.

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Original Article / Özgün Araştırma

Evaluation of Risk Factors, Clinical Characteristics, and Prognosis in Cerebral Venous Thrombosis: A Single Tertiary Center Experience

Serebral Ven Trombozunda Risk Faktörleri, Klinik Özellikler ve Prognozun Değerlendirilmesi: Üçüncü Basamak Tek Merkez Deneyimi

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Abstract

Objective: We evaluated the demographic and clinical characteristics, risk factors, involved vasculature, and prognosis in cerebral venous thrombosis.

Materials and Methods: We included 53 patients with cerebral venous thrombosis (CVT) who were followed up in our neurology inpatient clinic and neurology intensive care unit in the study. The demographic and clinical characteristics on admission, risk factors, results of laboratory tests and neuroimaging studies, treatment, outcomes at discharge, and 6th-month follow-up were reviewed.

Results: The mean age of the patients was 44.1±13.7 (19-71) years. Thirty-one (58.4%) of the patients were female. The most frequent symptom was headache (69.8%). Puerperium and malignancy were the most common risk factors for patients under 45 years of age; whereas anemia and malignancy were the most common risk factors for patients over 45 years of age. The most common localization of CVT was found to be the transverse sinus (69.8%). The modified Rankin scale (mRS) scores at discharge were 0 for 47 patients (88.6%), 1 for 4 patients (7.5%) and 4 for 2 patients. In the 6th-month follow-up, 48 (90.5 %) of the patients had an mRS score of 0 with normal neurological examination.

Conclusion: Our study, representing the experience of a single tertiary referral center, showed that CVT mostly affected women of reproductive ages. More than 90% of patients in our study had an mRS score of 0 at the 6th-month follow-up. The results of our study suggest that early diagnosis of CVT with advanced neuroimaging techniques recently has improved the outcomes and reduced the disability.

Öz

Amaç: Bu çalışmada serebral ven trombozunun (SVT) demografik ve klinik özelliklerinin, risk faktörlerinin, tutulan damar sisteminin ve prognozun değerlendirilmesi amaçlanmıştır.

Gereç ve Yöntemler: Nöroloji polikliniğimizde ve nöroloji yoğun bakım ünitemizde takip edilen SVT'li 53 hasta çalışmaya dahil edildi. Başvurudaki demografik ve klinik özellikler, risk faktörleri, laboratuvar testleri ve beyin görüntüleme çalışmalarının sonuçları, tedavi, taburculuk sonuçları ve 6. ay takipleri gözden geçirildi.

Bulgular: Hastaların yaş ortalaması 44,1±13,7 (19-71) yıl idi. Hastaların 31'i (%58,4)

kadındı. En sık görülen semptom baş ağrısıydı (%69,8). Kırk beş yaş altı hastalarda lohusalık ve malignite en sık görülen risk faktörleri iken, 45 yaş üstü hastalarda anemi ve malignite en sık görülen risk faktörleri idi. SVT'nin en sık yerleşim yeri transvers sinüs (%69,8) olarak bulundu. Taburculuk mRS skoru 47 hastada (%88,6) 0, 4 hastada (%7,5) 1 ve 2 hastada 4 idi. Altıncı ay takibinde hastaların 48'inde (%90,5) normal nörolojik muayene ile mRS skoru 0 olarak bulundu.

Sonuç: Tek bir üçüncü basamak sevk merkezi deneyimini temsil eden çalışmamız, SVT'nin çoğunlukla üreme çağındaki kadınları etkilediğini göstermiştir. Çalışmamızdaki hastaların %90'ından fazlasının 6. ay takibinde mRS skoru 0 idi. Çalışmamızın sonuçları, son yıllarda gelişmiş nörogörüntüleme teknikleri ile SVT'nin erken teşhisinin sonuçları iyileştirdiğini ve özürlülüğü azalttığını göstermektedir.

Introduction

Cerebral venous thrombosis (CVT) is a subtype of stroke which involves cerebral venous sinuses and cortical veins leading to hemorrhage or venous infarcts (1,2). It affects the young population with a female predominance. Previously, the frequency of CVT was underestimated because of diagnostic complexities; however, the development of advanced imaging modalities in recent decades allowed for prompt and accurate diagnosis of CVT, even in mild cases (3). Clinical features of CVT include headache, visual disturbances, epileptic seizures, focal neurological deficits, movement disorders, altered mental status related to involved cerebral venous structures (4). CVT can be provoked or unprovoked, and multiple risk factors can be present in patients. Most of the patients with CVT are known to have prothrombotic risk factors and thrombophilias are identified in more than one-third. In the young population, sex-specific risk factors including pregnancy, puerperium, and oral contraceptives are the most striking predisposing conditions, whereas malignancy is more common in the advanced ages (5). There are no certain blood parameters pathognomonic for CVT, but higher D-dimer levels strengthen the diagnosis. When the diagnosis of CVT is considered in the presence of cerebral hemorrhage or ischemic lesions on the initial radiological examination, advanced neuroimaging techniques should be performed in order to evaluate venous structures. Computed tomography (CT) venography and magnetic resonance venography (MRV) can be used alternatively to digital subtraction angiography for diagnosis. However, magnetic resonance imaging (MRI) is superior to CT for demonstration of the thrombosis and parenchymal lesions in suspected CVT (4,6). The goal of the treatment in CVT is to reverse the thrombosis and prevent cerebral herniation, as well as parenchymal injury (6,7).

In the present study, we aimed to evaluate the demographic and clinical characteristics, risk factors, clinical presentation, involved cerebral venous sinuses, and prognosis of CVT in a university hospital.

Materials and Methods

Fifty-three patients diagnosed with CVT in the neurology inpatient clinic and neurology intensive care unit of Gazi University Hospital between 2006 and 2015 were included in this retrospective study. Demographic and clinical characteristics, potential risk factors, neurological examination findings, modified Rankin Scale (mRS) scores at discharge and follow-up were reviewed. The laboratory studies including complete blood count, anticardiolipin antibodies, antiphospholipid antibodies, proteins C-S, antithrombin-III (AT-III), activated protein C resistance, Factor V Leiden and *methylenetetrahydrofolate reductase* (*MTHFR*) gene mutations, serum homocysteine levels were recorded when available. Patients were screened for Behçet's disease (BD) and the pathergy test was applied to patients, since BD is a prevalent chronic inflammatory disease in Turkey. Cranial MRI and MRV studies were conducted in our hospital (1.5 and 3 Tesla Magneton Aera and Verio; Siemens, Erlangen, Germany) and assessed by two experienced neuroradiologists (M.U., N.T.). Laboratory and radiological data were obtained from our hospital information management system. Ethical approval was obtained from the clinical research Ethics Committee of Gazi University Faculty of Medicine Hospital for the study (decision no: 252, date: 12.05.2014).

Statistical Analysis

Analysis of the data was performed by using IBM SPSS 21.0 statistical package program. Continuous variables were expressed as mean ± standard deviation or as median (interquartile range). Categorical variables were presented as frequencies and percentages. The normality of data was assessed

by the Kolmogorov-Smirnov test. Pearson chi-square (χ^2), Yates (χ^2), or Fisher's Exact (χ^2) tests were used to compare qualitative data where appropriate. The Independent Samples t-test (t-test in independent groups) was used to compare quantitative data in the study. A p-value smaller than α=0.05 was considered statistically significant.

Results

The mean age of 53 patients included in the study was 44.1±13.7 (19-71) years. Thirty-one (58.4%) patients were female and 22 (41.6%) were male. Clinical characteristics of the patients and potential risk factors for CVT are summarized in Table 1.

When risk factors were evaluated according to gender; the most frequent risk factor was anemia in women and local infections in men. Multiple risk factors were identified more frequently in women. Puerperium and malignancy were the most common risk factors for patients under 45 years of age, while anemia and malignancy were found to be the most common risk factors for patients over 45 years. The presence of multiple risk factors was notable for patients over 45 years of age.

The most common localization of CVT was found to be transverse sinus in 37 patients (69.8%) (Table 2). Venous infarction was detected in 28 of the patients, the majority of which were in the right hemisphere (35.7%). A statistically significant difference was found between the two genders regarding the presence of hemorrhage or hemorrhagic infarction ($p<0.05$) (Table 2). Mortality due to CVT was not observed in our cohort. The mRS score at discharge was 0 in 47 patients (88.6%). In the 6th month follow-up, 48 (90.5 %) of the patients had a mRS score of 0 with totally normal neurological examination.

Discussion

In this retrospective study of 53 patients with CVT at a tertiary hospital in Turkey, we evaluated the demographic and clinical characteristics, risk factors, clinical presentation complaints, involved cerebral venous structures, and short-term follow-up findings of CVT. The median age of patients was 44 years similar to large cohorts in which onset age distribution was found to be 3rd to 4th decades (2,8,9). Our findings demonstrating female predominance (58.7%) were also in accordance with previous studies (9-11). Female superiority in CVT is mainly attributed to sex-specific risk factors [puerperium, pregnancy, and the use of obsessive-compulsive disorder (OCD)] acting as both predisposing and precipitating conditions for CVT (11,12). The International Study on cerebral vein and dural sinus thrombosis (ISCVT) study showed that the prognosis of female patients with sex-specific risk

Table 1. Clinical characteristics and potential risk factors of the patients with CVT (n=53)

	Total n (%)
Age (mean ± SD) standard deviation	44.1±13.7 (19-71)
Female sex, n (%)	31 (58.4%)
Potential risk factors, n (%)	
Anemia	19 (33.9%)
Activated protein C resistance	16 (30.2%)
MTHFR gene mutation (heterozygous)	15 (28.3%)
Protein C/S deficiency	14 (26.4%)
Malignancy	8 (15%)
Puerperium	6 (11.3%)
Local infections	5 (9.4%)
Factor V Leiden mutation	3 (5.7%)
MTHFR gene mutation (homozygous)	2 (3.8%)
History of cerebral venous thrombosis	2 (3.8%)
Pregnancy	1 (1.8%)
Anti-thrombin III deficiency	1 (1.9%)
Trauma	1 (1.9%)
Dehydration	1 (1.9%)
Post lumbar punction	1 (1.9%)
Behçet's disease	1 (1.9%)
History of venous thromboembolism	1 (1.9%)
Neurological signs and symptoms, n (%)	
Headache	37 (69.8%)
Papilledema	20 (37.7%)
Seizures	19 (35.8%)
Nausea and vomiting	15 (28.3%)
Cranial nerve palsies	10 (18.9%)
Motor deficits	10 (18.9%)
Altered mental status	9 (17.0%)
Sensory deficits	2 (3.7%)
Dysarthria	2 (3.7%)
Gait disturbances	1 (1.8%)

CVT: Cerebral venous thrombosis, SD: Standard deviation, *MTHFR*: Methylenetetrahydrofolate reductase

Table 2. Radiological findings of patients with CVT				
	Total n=53 n (%)	**Female n=31**	**Male n=22**	
Involved venous structures, n (%)				
Transverse sinus	37 (69.8%)	23 (74.2%)	14 (63.6%)	
Sigmoid sinus	29 (54.7%)	17 (54.8%)	12 (54.5%)	
Superior sagittal sinus	20 (37.7%)	12 (38.7%)	8 (36.4%)	
Internal juguler vein	17 (32.1%)	8 (25.8%)	9 (40.9%)	
Cortical veins	5 (9.4%)	3 (9.7%)	2 (9.1%)	
Inferior sagittal sinus	1 (1.9%)	0 (0%)	1 (4.5%)	
Sinus rectus	1 (1.9%)	0 (0%)	1 (4.5%)	
Multiple sinus involvement	32 (60.4%)	19 (61.3%)	13 (59.1%)	
Parenchymal lesion n (%)				**p-value**
No lesion	25 (47.1%)	12 (38.7%)	13 (59.1%)	0.143
Venous infarcts	28 (52.8%)	13 (59.1%)	9 (40.9%)	
Hemorrhage or hemorrhagic infarcts	23 (43.4%)	17 (54.8%)	6 (27.3%)	0.046
CVT: Cerebral venous thrombosis				

factors was better than in other conditions (12). Nasr et al. (13) showed that pregnancy and puerperium were risk factors for 24.6% of 11,400 CVT patients who were followed between 2001 and 2008 and these patients had lower mortality rates when compared to others. In our study, no significant difference in terms of prognosis was found between patient groups according to risk factors. When we compared the risk factors according to gender in our study, the most common risk factor was anemia in 25.8% of women and local infection in 22.7% of men. In Coutinho et al.'s (12) study, the most common risk factors for CVT were genetic thrombophilia affecting 25% of men, and use of OCD affecting 46% of women. Duman et al. (2) found that prothrombotic conditions were the second causative risk factor for CVT after reproductive health-related factors in the Turkish population. Although the clinical impact of heterozygous (*MTHFR*), FV Leiden, and prothrombin gene mutations on CVT is uncertain, these mutations may contribute to the multifactorial causality in CVT.

In our study, we accepted anemia (hemoglobin <12 g/dL) and thrombocytosis (platelets >450x10.e3/uL) as risk factors for CVT. Hemogram abnormality was found in 45.2% of women and 22.7% of men with CVT. We suggest that the higher incidence of iron deficiency anemia among women in low-middle income countries contributes to the higher proportion of anemia in patients with CVT. However, the relationship between anemia and cerebrovascular diseases, particularly CVT, is not fully understood (14-16); several mechanisms have been proposed to date (15). Decreased iron levels induce megakaryocyte activity paradoxically, which provoke secondary thrombocytosis, thus leading to a hypercoagulable state (17). In addition, iron deficiency was found to cause increased levels of factor VIII as a prothrombotic risk factor (15,18). Although the relationship between iron deficiency and venous thromboembolism is controversial, anemia should be considered as a precipitating risk factor for CVT with underlying predisposing conditions. Eventually, CVT is a multifactorial disease, which means that the identification of a risk factor or cause should not interrupt the complete diagnostic workup (17). Patients diagnosed with CVT should be screened for all risk factors; especially for reproductive health-related risk factors (puerperium, pregnancy, use of OCD) which are important in terms of recurrence.

At least one serologic marker for vasculitis was detected in 11.3% of the patients. One of our patients had a previous diagnosis of BD. In the ISCVT study, 3% of patients were found to have systemic vasculitis especially systemic lupus erythematosus and BD. Hyperhomocysteinemia was detected as a risk factor for SVT in 11.3% of the patients included in our study. In the ISCVT study, less than 1% of CVT

cases had hyperhomocysteinemia (8). Although some meta-analyses showed a higher risk of venous thromboembolism with hyperhomocysteinemia (19,20), the latest study from the Netherlands failed to confirm this relationship (21).

The clinical picture of CVT varies broadly from mild headache to encephalopathy which can present with acute, subacute, or chronic symptoms. There are four important syndromes of CVT: isolated intracranial hypertension, focal neurological deficits, epileptic seizures, and encephalopathy (22). In the ISCVT study, the headache was observed in 88.8% of patients, which is followed by seizures with a rate of 39.3% and motor deficit with 37.2% (8). In our study, headache was the most common symptom with 69.8%, followed by multiple symptoms in 64.2% of patients; 35.8% of patients had seizures, and 18.9% of patients had motor deficits. Similar to large cohorts, the most common symptom was headache in both genders (2,12). The initial neurological examination revealed papilledema in 37.7% of our patients, whereas 18,9% had motor deficits. The most common finding in the neurological examination of the ISCVT cohort was a motor deficit in 37.2% of patients, which was followed by papilledema in 28.3% (8). When neurological signs of our study population were compared according to gender; papilledema was the most common finding in both groups. The corresponding parameter was reported to be motor deficit for either sex in the study of Coutinho et al. (12).

Cranial MRI followed by MRV is the most sensitive diagnostic tool while demonstrating the venous thrombus and parenchymal lesions in acute, subacute, and chronic stages in CVT (22). We detected transverse sinus thrombosis in 69.8% of the patients, sigmoid sinus thrombosis in 54.7%, and superior sagittal sinus thrombosis in 37.7% of the patients. In the ISCVT study, the most common localization was the superior sagittal sinus with 62% (8). Duman et al. (2) reported transverse and sigmoid sinuses were the most frequently involved cerebral veins in CVT among the Turkish population.

In the ISCVT study, mortality was observed in 8.3% of the patients and found to be associated with underlying risk factors such as malignancy (8). Coutinho et al. (9) reported a rate of 0-28% for CVT-associated deaths after 2000 in a systematic review of literature. Recently, Duman et al. (2) reported no death due to CVT in 1144 patients. In our cohort, no death was observed either at discharge or in 6 months. We found patients with CVT had a better prognosis and 96.2% were functionally independent with favorable mRS scores (0-1) at discharge; while the proportion of patients with mRS 0-1 at discharge was 65.7% in the ISCVT cohort (8). Reduced mortality and disability in CVT can be explained by higher recognition of CVT and advances in modern neuroimaging techniques. Furthermore, experiences in treatment options might result in less mortality together with a better prognosis.

There are some limitations to our study. First, the study population was small since it consisted of cases from a single center with a relatively rare diagnosis. Second, the retrospective design of the study might give rise to some missing clinical and laboratory data. Third, the follow-up period was relatively short to observe recurrences. And last, the lack of follow-up MR imaging studies restricted the interpretation of vascular recovery showing recanalization of thrombosed sinuses.

Conclusion

In conclusion, CVT is a rare subgroup of cerebrovascular diseases with a relatively better prognosis, of which early diagnosis is essential. Our study reflecting a single-center experience from a tertiary hospital showed that CVT affected mostly women of reproductive ages. The patients are often in the 3rd to 4th decades but CVT can be seen at any age from childhood to late adulthood. CVT is typically multifactorial and the identification of all precipitating and predisposing risk factors is essential to avoid recurrences. The clinical symptomatology comprises a broad spectrum varying from headache to coma. The most commonly involved venous structure in CVT was found to be transverse sinus in our study. Venous infarcts, hemorrhages, and hemorrhagic infarcts as parenchymal lesions occurred in nearly half of the patients during the clinical course. More than 90% of our patients had mRS score of 0 at the 6th-month follow-up. We suggest that early diagnosis of CVT with advanced neuroimaging techniques improved the outcomes and reduced the disability.

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Ethics

Ethics Committee Approval: Ethical approval was obtained from the clinical research ethics committee of Gazi University Faculty of Medicine Hospital for the study (decision no: 252, date: 12.05.2014).

Informed Consent: Retrospective study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: H.M.M., H.Z.B.Ç., B.N., N.T., M.U., Concept: H.M.M., H.Z.B.Ç., B.N., N.T., M.U., Design: H.M.M., H.Z.B.Ç., B.N., N.T., M.U., Data Collection or Processing: H.M.M., H.Z.B.Ç., B.N., N.T., M.U., Analysis or Interpretation: H.M.M., H.Z.B.Ç., B.N., N.T., M.U., Literature Search: H.M.M., H.Z.B.Ç., B.N., N.T., M.U., Writing: H.M.M., H.Z.B.Ç., B.N., N.T., M.U.

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Original Article / Özgün Araştırma

The Prevalence of University Students' Use of Tobacco Products, Their Opinions on Their Effects on Health, and the Factors Affecting Use

Üniversite Öğrencilerinin Tütün Ürünlerini Kullanım Prevalansı, Sağlık Üzerine Etkilerine İlişkin Görüşleri ve Kullanımı Etkileyen Faktörler

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Abstract

Objective: It was aimed to examine the prevalence of use of tobacco products, the reasons for the first use, opinions on tobacco products' effects on health, and factors affecting the waterpipe use of university students enrolled in technical, social, and health sciences in this study.

Materials and Methods: This cross-sectional study was conducted with a 43-item questionnaire among fourth-year students from three educational fields of a university with 72.3% participation rate was in Ankara, Turkey in March 2019.

Results: 21.1% of the students were current waterpipe users and 32.6% were current cigarette smokers. For both cigarette and waterpipe, "curiosity" was the most common reason first reported use, followed by socializing and related reasons. For both tobacco products, there was consensus on whether they were harmful to health and on the positive effects of quitting. Regarding the addictive effect, significantly more of the participants gave lower scores for waterpipe than for cigarettes (46.4% versus 83.5%). The risk factors for being current waterpipe use were the students' cigarette smoking and waterpipe use in the family or close friends.

Conclusion: Waterpipe use was relatively common among university students and socialization was important as a risk factor for being a current user. The addictive effect of waterpipe can be emphasized more in awareness campaigns. The fight against tobacco should be conducted by peer work and targeting all tobacco products and regardless of students' educational fields.

Keywords
Tobacco, cigarette, waterpipe, health, student

Anahtar Kelimeler
Tütün, sigara, nargile, sağlık, öğrenci

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Öz

Amaç: Bu çalışmada teknik, sosyal ve sağlık bilimlerinde öğrenim gören üniversite öğrencilerinin tütün ürünleri kullanım yaygınlığının, ilk kullanım nedenlerinin, tütün ürünlerinin sağlığa etkilerine ilişkin görüşlerinin ve nargile kullanımını etkileyen faktörlerin incelenmesi amaçlanmıştır.

Gereç ve Yöntemler: Kesitsel tipteki bu araştırma, 43 soruluk bir anket ile Mart 2019'da Ankara'da %72,3 oranında katılım ile bir üniversitenin üç eğitim alanında öğrenim gören dördüncü sınıf öğrencileri arasında gerçekleştirilmiştir.

Bulgular: Öğrencilerin %21,1'i halen nargile kullanıcısı ve %32,6'sı halen sigara içicisiydi. Hem sigara hem de nargile için "merak" ilk kullanım için en sık bildirilen neden iken, bunu "sosyalleşme" ve ilişkili nedenler izlemiştir. Her iki tütün ürünü için de sağlığa zararları ve bırakmanın olumlu etkileri konusunda fikir birliği vardı. Bağımlılık yapıcı etki konusunda, sigaraya nazaran nargile için katılımcıların daha

fazla bir kısmı anlamlı olarak daha düşük puanlar verdiler (%46,4 ve %83,5). Halen nargile kullanıyor olma durumu için risk faktörleri öğrencinin sigara kullanıyor oluşu ile ailede veya yakın arkadaşlarda nargile kullanımı olarak bulunmuştur.
Sonuç: Üniversite öğrencileri arasında nargile kullanımı görece yaygın bulundu ve sosyalleşme, halen nargile kullanıyor olmak için bir risk faktörü olarak önemliydi. Nargilenin bağımlılık yapıcı etkisi bilinçlendirme kampanyalarında daha çok vurgulanabilir. Tütünle mücadele, öğrencilerin eğitim alanları ne olursa olsun, tüm tütün ürünlerini hedef alan akran çalışmaları ile yürütülmelidir.

Introduction

Currently, 19.2% of the world population over 15-year-old are tobacco users (1). Eight million people die each year due to tobacco (2). Health expenditures due to diseases attributed to cigarette smoking were calculated as 422 billion USD for 2012 (1). Considering cigarette consumption, Turkey ranks second among Organization for Economic Co-operation and Development countries (3). The prevalence of daily tobacco use increased from 26.5% in 2016 to 28.0% in 2019 (4).

The use of alternative tobacco products other than cigarettes is gradually increasing. Waterpipe, also known as hookah, is consumed more than cigarettes in some Middle Eastern countries (5). Several studies have revealed that tobacco use begins between 16-18 ages (6,7). One in 5 young adults use at least one tobacco product in Turkey according to the latest Global Adult Tobacco Survey (8).

In the present study, we aimed to examine the prevalence of tobacco products use, the reasons for first use, opinions on the effects of tobacco products on health, and the factors affecting current waterpipe use among senior university students enrolled in technical, social and health sciences.

Materials and Methods

The population of this cross-sectional study was the fourth-year students in a university in Ankara, Turkey (n=1974). We used the sample size formula for stratified rate estimation for the waterpipe smoking rate estimation of the population. Accordingly, we calculated the minimum (min) sample size as 386 volunteers using stratified sampling from three education areas, with a 20% non-response rate, d=0.05 error and α=0.05 while ∀Ph = Qh = 0.50. We distributed this number (n) to the strata in proportion to their weight, and 63, 216, 107 volunteers were allocated in "technical sciences", "social sciences," and "health sciences", respectively. The departments were randomly selected to reach the n of students determined for the three scientific fields. Variables such as age and gender were not taken into account in the selection of students.

After the approval provided by Başkent University Institutional Review Board (project no: KA19/68, date: 26.02.2019), data collection was carried out in March 2019 through a 43-item questionnaire about the participant's educational background, some demographic characteristics, and opinions on the use of cigarettes and waterpipes prepared by the researchers. Modifications were made after pilot testing. Participants were asked to score their perceptions of (i) the harm, (ii) the addictive effects, (iii) the positive effects of quitting cigarette smoking and waterpipe use on health as the 5-point Likert scale with "no idea" option. The participation rate was 72.3% (n=279).

Current waterpipe smokers were considered those who used waterpipe at least once in the last year. Former waterpipe smokers were considered those who used waterpipe at least once but never used it in the last year. Never waterpipe smokers were considered those who have never used a waterpipe. Definition of current, former, and never cigarette smoking accepted by a glossary of Centers for Disease Control and Prevention (9).

Statistical Analysis

N and percentage (%), mean with standard deviation, and median with min-maximum values were used for descriptive statistics. Comparisons were conducted using the independent t-test and one-way analysis of variance (ANOVA) with Tamhane test for continuous variables and chi-square test for categorical variables. Binary logistic regression analysis with the Backward-Wald method were performed to present factors associated with current waterpipe use and the results are showed with odds ratios with a 95% confidence interval (CI). SPSS 26.0 was used in all analyses, and $\alpha<0.05$ was considered significant except for $\alpha=0.016$ for assessing post-hoc test results.

Results

A total of 279 students (39.8% health, 37.6% social and 22.6% technical sciences) were included in the study. The mean age of the participants was 22.8±1.2 years. Of the participants, 72.4% were women, and 65.9% was living with their family. Of the participants, 77.2% and 51.1% reported that none of the family members and five best friends used waterpipes. Of the participants, 67.0% had tried to smoke a cigarette and 57.3% had tried to use waterpipe at least once in their lifetime. No significant difference was found regarding the ages of first trials of cigarette and waterpipe by the educational fields (p=0.882 and p=0.386). No significant difference was found between the ages of the first trial of waterpipe regarding being a current waterpipe using status (p=0.571).

Of the participants, 65.4% used waterpipes and 59.5% smoked cigarettes firstly before 18 years old. The mean age of the first cigarette trial was 16.5±3.5 years, and it was 16.8±2.4 years for waterpipe. Of the participants, 32.6% were current cigarette smokers, 21.1% used waterpipes at least once in the last year (Table 1). Most (93.2%) waterpipe smokers stated that they smoke outside the house. More than half of the participants (56.1%) did not consider quitting waterpipe use.

"Socialization" (30.6%) for waterpipe and "stress relief" (20.9%) for cigarettes are the first ranks for reason of use. While 92.5% of the participants scored that hookah use is harmful to health, 46.4% scored it was addictive, also, 83.5% of them scored that quitting hookah use would have a positive effect on health. It has been shown these rates are quite high for cigarettes (Figure 1).

There was a significant difference between the students from educational fields regarding their opinions on the addictive effect of cigarette smoking (p=0.011; 4.35±0.94 in health sciences vs. 4.68±0.60 in social sciences), but not the waterpipe use (Table 2). In other cases, no statistical difference was observed by fields of education. The mean scores on the harm of waterpipe to health and addictive effect of waterpipe use were lower (p=0.019 and p=0.001) among the current than non-current users of waterpipe.

The waterpipe use prevalence was higher among men (p=0.003), those who have family members who use a waterpipe (p<0.001), whose father and brother use a waterpipe (p=0.005 and p=0.002, respectively), who have friends use a waterpipe (p<0.001), who have

Table 1. Characteristics of participants' use of tobacco products

Variable	Number (n)	Percent (%)
The age of first tobacco product use		
Cigarette (n=185)		
≤14	36	19.5
15-17	74	40.0
≥18	75	40.5
Mean ± SD	16.5±3.5	
Waterpipe (n=159)		
≤ 14	23	14.5
15-17	81	50.9
≥18	55	34.6
Mean ± SD	16.8±2.4	
The status of tobacco products use (n=279)		
Cigarette		
Never smoker	175	62.7
Former smoker	13	4.7
Current smoker	91	32.6
Waterpipe		
Never user	119	42.7
Former user	101	36.2
Current user	59	21.1
At least once a year, but less than once a month	39	14.0
At least once a month, but less than once a week	16	5.7
At least once a week	4	1.4

Smoking and waterpipe use were asked separately. Therefore, a participant may use both tobacco products. SD: Standard deviation

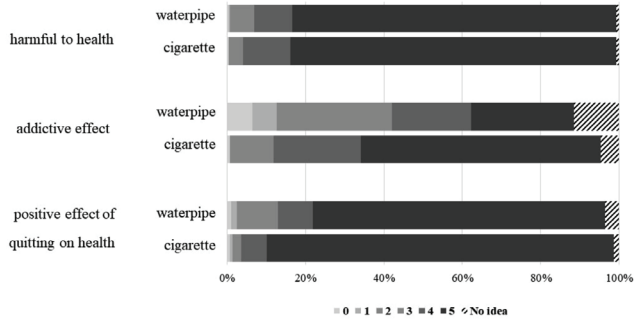

Figure 1. Distribution of participants' opinions on health effects of cigarette and waterpipe smoking

Table 2. Comparison of the participants' opinions on the health effects of tobacco products use by their educational field

Question	Educational field (Mean ± SD)			p-value
	Health	Social	Technical	
Cigarette smoking is harmful to health	4.75±0.65	4.85±0.43	4.73±0.63	0.281
Cigarette smoking is addictive	4.35±0.94	4.68±0.60	4.36±0.99	0.011*
Quitting cigarette smoking has a positive effect on health	4.73±0.89	4.89±0.48	4.86±0.44	0.178
Waterpipe using is harmful to health	4.79±0.61	4.71±0.76	4.59±0.96	0.228
Waterpipe using is addictive	3.30±1.62	3.44±1.57	3.12±1.43	0.468
Quitting waterpipe using has a positive effect on health	4.50±1.12	4.54±0.94	4.61±0.89	0.765

The harmful effects on health be scored as "0= No harm at all, 5= Very harmful", addictive effects as "0= Not at all addictive, 5= Highly addictive" and positive effects of quitting on health as "0= Not at all, 5= Very effective" as the Likert scale.

The analysis was made excluding the students who said they "have no idea". Tamhane test was preferred since post-hoc tests did not distribute the variances homogeneously.

*A statistical significance was observed among students studying in health and social sciences. SD: Standard deviation

smoked cigarettes and who were current cigarette smokers (p<0.001). The prevalence of waterpipe use among the students in health science was lower than the students in the technical and social sciences (p=0.005).

The binary logistic regression showed that the model accuracy of predicting the risk of being a current waterpipe user was 81.4% with excluding for missing data of five cases. Since the n of former cigarette smokers was low and its 95% CI was wide, this result may be excluded from the model. The presence of a waterpipe user in the family of the participant increased the risk of being a current waterpipe smoker 5.980 times (95% CI: 2.708-13.204; p<0.001). In addition, the presence of waterpipe users among friends increased the risk of being a current waterpipe smoker by 4.144 times (95% CI: 1.872-9.173; p<0.001). Also, being a current cigarette smoker increased the risk of being a current waterpipe smoker 3.756 times (95% CI: 1.710-8.252; p=0.001).

Discussion

In this study, it has been shown that current smoking (32.6%) and current waterpipe use (21.1%) can be evaluated as critical level. "Curiosity" was the first rank reported reason of use for cigarettes and waterpipe. Socialization and related choices were marked more in the individuals who use waterpipe than cigarettes. While there was a consensus among the participants regarding the harms of cigarettes and waterpipes and the positive effects of quitting on health, the waterpipe was taken a dramatically lower score compared to cigarettes regarding addictive effects (46.4% vs. 83.5%). In addition, current waterpipe smokers evaluated that the harm and addictive effect of waterpipe to health are lower than former and never smokers. Logistic regression results showed the importance of current smoking and waterpipe use in the social environment among the possible risk factors affecting waterpipe use.

In our study, the prevalence of current cigarette smoking was 32.6%, which was higher than the prevalence of waterpipe use (21.1%). The prevalence of cigarette smoking among university students in Turkey varies between 24.4% and 50% (10-12). The prevalence of waterpipe use among university students in Turkey, both in our study (21.1%) and in other studies from Turkey (10,11,13,14) can be seen approximately 10 times higher (12.7-31.1%) than the prevalence in adults (15-17). These data confirm that university students are a risk group for waterpipe use. Even though waterpipe use cannot be considered a major worldwide problem, it does show that it is a public health threat regarding the prevalence of its use among young people.

More than half of the participants have tried to use waterpipes or cigarette smoking at least once in their lifetime. Of the ever users, 65% tried waterpipe use, 59.5% tried cigarette smoking for the first time under the age of 18, which is prohibited by legal regulations in Turkey (18). Also, the commercial establishments offering waterpipe should be at least 100 meters away from the schools (19). The high prevalence of tobacco product trials in adolescence and 93.2% of

the students' preference of cafes and waterpipe cafes for waterpipe use may be indicated problems in the implementation of the law.

In our study, 43.9% of current waterpipe users wanted to quit. Considering the prevalence of waterpipe cessation desire (14-48%) in the northern part of the world (20), our result can be thought as an opportunity for interventions.

In the presented study, it was a striking finding that current cigarette smoking increased the risk for current waterpipe use by 3,756 times. In a study, approximately 59% of waterpipe users were found to use at least one more tobacco product (21). In a study from Turkey, cigarette smoking was indicated to increase the risk of waterpipe use by 7.1 times (22). This finding shows that a person who consumes one tobacco product is more likely to use another tobacco product.

Due to the nature of cross-sectional studies, it is difficult to establish a causal relationship. The literature review was carried out in both English and Turkish to minimize the publication bias and to observe the cultural and geographical differences in waterpipe use. To avoid forced-choice bias, yes/no questions were tried to be kept to a min, and other bias reasons related to the questionnaire were tried to be minimized by applying a pre-test and rearranging the questionnaire. There may be a family information bias for related questions, and also volunteer, recall and response fatigue biases.

Conclusion

In this study, like other studies in Turkey, we determined that the prevalence of waterpipe use among university students was quite high and that waterpipe smoking was used for socialization. While almost all students who used waterpipes reported that they used it in public places outside the home, it is also noteworthy that more than half of them started smoking under the age of eighteen despite legal regulations. It indicates that is necessary to closely monitor the implementation of legal regulations and to inspect more effectively the places where waterpipe is offered. Also, the struggle against the use of tobacco products by young people should be started before university age. Waterpipe use was relatively common among university students and socialization was important as the reason and as a risk factor for being a current user. The addictive effect of waterpipe can be emphasized more in awareness campaigns. The fight against tobacco should be conducted by peer work and targeting all tobacco products and regardless of students' educational fields.

Ethics

Ethics Committee Approval: The study was approved by the Başkent University Institutional Review Board (project no: KA19/68, date: 26.02.2019).

Informed Consent: Cross-sectional study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: N.B.A., Design: N.B.A., Data Collection or Processing: N.B.A., Analysis or Interpretation: H.Ö., E.T., Literature Search: N.B.A., H.Ö., E.T., Writing: N.B.A., H.Ö., E.T.

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Original Article / Özgün Araştırma

Examination of the Correlation of Distal End Parameters of the Humerus with Other Parameters

Humerus Distal Uç Parametrelerinin Diğer Parametrelerle İlişkisinin İncelenmesi

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Abstract

Objective: The aim of this study is to determine the correlation between all osteometric parameters obtained from the humerus and parameters obtained from the distal end of the humerus.

Materials and Methods: A total of 16 parameters were measured in 67 dry bones using the Image-J program.

Results: Groove for the ulnar nerve width (GUW) and the angle of the groove of the ulnar nerve (GUA) parameters were found to be higher on the left side and the difference was found to be statistically significant ($p<0.05$), GUA and intertubercular groove width were not significantly correlated with any parameter ($p>0.05$).

Conclusion: We believe that the results of the study will provide osteometric data of the humerus in distal end fracture treatment, comminuted fractures, and reconstructive surgery of the humerus, anthropological and forensic studies and increase the anatomical knowledge levels of experts interested in the region.

Öz

Amaç: Bu çalışmanın amacı humerustan elde edilen tüm osteometrik parametreler ile humerus distal ucundan elde edilen parametreler arasındaki korelasyonu bulmaktır.

Gereç ve Yöntemler: Toplam 67 kuru kemikten, 16 parametre ölçümü Image-J programıyla yapıldı.

Bulgular: Sulcus ulnaris genişliği (GUW) ve sulcus ulnaris açısı (GUA) parametreleri sol tarafta daha yüksek bulundu ve aradaki fark istatistiksel olarak anlamlı bulundu ($p<0,05$), GUA ve sulcus intertubercularis genişliği herhangi bir parametre ile anlamlı olarak korelasyon göstermedi ($p>0,05$).

Sonuç: Çalışmanın sonuçlarının humerus distal uç kırıklarının tedavisi, parçalı kırıklar ve humerus rekonstrüktif cerrahisi, antropolojik ve adli çalışmalarda osteometrik veriler sağlayacağı ve bölgeyle ilgilenen uzmanların anatomik bilgi düzeylerini artıracağı kanaatindeyiz.

Keywords
Dry bone, distal end of the humerus, osteometry

Anahtar Kelimeler
Kuru kemik, humerus distal ucu, osteometri

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Introduction

As in all biological structures, bone structure is driven by phylogenetic, structural, and functional factors (1,2).

The structure of the bone and its connection with its function is central to all inquiries that target or involve the bone. This includes deductive and inductive applications of skeletal biology both in medical science and also in archaeological or forensic sciences. Bone lengths and correlations have been studied by anthropologists, forensic scientists and orthopaedists for surgery and prosthetic design (3).

Distal humerus fractures account for 0.5-7% of all fractures, and approximately 30% of elbow fractures (4). Various implants are available for fracture patterns in the distal of humerus and these implants have been shaped according to the general anatomy knowledge of the elbow region (5).

The aim of this study is to investigate the relationship between humeral morphometry and humeral distal end parameters and other parameters of the humerus by digital measurements made on the dry bones of the humerus.

Materials and Methods

This study was initiated with 2021/797 numbered approval from Karabük University Ethics Committee (date: 24.12.2021). In the study, a total of 67 humerus bones, 35 left and 32 right, of unknown gender, were used from the bone collections in the anatomy laboratories of the medical faculty of the three universities. Deformed bones were not included in the study.

Morphometric Measurements

The bones placed on a measuring ruler were photographed with a professional digital camera (Nikon® - model D5300) parallel to the ground and from a height of 40 cm. The taken photos were transferred to Image-J (version 1.8 for Windows) program and they were calibrated. On the calibrated photos, 16 parameters were measured and recorded 2 times for each bone at different dates by the same observer.

Parameters;

The maximum length of the humerus (HML),

The vertical diameter of the humeral head (HVD),

Intertubercular groove width (GIW),

Deltoid tuberosity diameter (TDD),

Capitulum of the humerus width (CHW),

Trochlea of humerus width (THW),

Capitulum of humerus + trochlea of humerus transverse length,

Coronoid fossa width (TCW),

Radial fossa width,

The length between medial epicondyle and lateral epicondyle (FRW),

Groove for the ulnar nerve width (GUW),

The angle of the groove of the ulnar nerve (GUA),

Capitulum of humerus length (CHL),

Trochlea of humerus length (THL),

Olecranon fossa length (transverse plane) (FOL) and Olecranon fossa width (coronal plane) (FOW). All parameters showed in Figure 1.

Statistical Analyses

The data obtained in the study was analysed by using SPSS (Released: 2016, IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY: IBM Corp.) software. In data evaluation, mean, standard deviation, minimum and maximum values were used as descriptive statistical methods. Normality of the continuous variables was evaluated with Shapiro-Wilk test. Two Sample t-test and Mann-Whitney

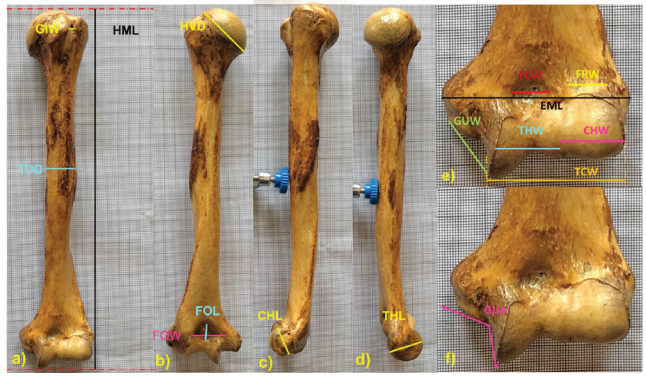

Figure 1. Parameters taken from the a) anterior, b) posterior, c) lateral, d) medial, e) and f) end of the humerus.
CHW: Capitulum of humerus width, EML: The length between medial epicondyle and lateral epicondyle, FCW: Coronoid fossa width, FOL: Olecranon fossa length (transverse plane), FRW: Radial fossa width, FOW: Olecranon fossa width (coronal plane), GIW: Intertubercular groove width, GUA: The angle of the groove of the ulnar nerve, GUW: Groove for the ulnar nerve width, HML: The maximum length of the humerus, HVD: The vertical diameter of the humeral head, TCW: Capitulum of humerus + trochlea of humerus transverse length, TDD: Deltoid tuberosity diameter, THL: Trochlea of humerus length, THW: Trochlea of humerus width

U test were used in the comparison of continuous variables between two groups. The linear correlation between the continuous variables was evaluated with Spearman and Pearson correlation test. P<0.05 level was considered as statistically significant.

In order to assess intra-observer precision and measurement accuracy, technical error of measurement (TEM), relative technical error of measurement (rTEM) and reliability coefficient (R) were calculated by using Microsoft Excel program.

RESULTS

The means of the first and second measurements were used in the study. TEM, rTEM, and R of the measurement parameters are shown in Table 1. TEM values are between 0.04 and 1.69. rTEM values are between 0.36% and 12.49%. R values are between 0.83 and 0.99, and the values are close to 1.

GIW, TDD, FCW, FOL, and FOW parameters were not distributed normally. No significant difference was found between the right and left side in these parameters (p>0.05) (Table 2). The other parameters were normally distributed; GUW and GUA parameters were found to be higher on the left side and the difference was found to be statistically significant (p<0.05) (Table 3).

Correlation Analysis Results

The correlation analysis is shown in Table 4. GUA and GIW were not significantly correlated with any parameter (p>0.05) (Table 4).

Table 1. Intra-observer error analysis (TEM, rTEM, R) (n=67)

Parameters	TEM	rTEM (%)	R
GIW	0.11	12.49	0.83
FRW	0.07	5.56	0.88
THW	0.08	3.63	0.90
THL	0.10	3.82	0.91
FOW	0.06	3.53	0.93
TDD	0.06	3.10	0.94
FCW	0.06	3.47	0.94
GUW	0.07	5.18	0.94
CHW	0.05	2.82	0.95
FOL	0.06	2.30	0.96
CHL	0.05	2.18	0.97
TCW	0.04	0.95	0.98
GUA	1.69	1.44	0.98
HVD	0.05	1.05	0.98
HML	0.11	0.36	0.99
EML	0.05	0.84	0.99

TEM: Technical error of measurement, rTEM: Relative technical error of measurement, R: Reliability coefficient, GIW: Intertubercular groove width, FRW: Radial fossa width, THW: Trochlea of humerus width, THL: Trochlea of humerus length, FOW: Olecranon fossa width (coronal plane), TDD: Deltoid tuberosity diameter, FCW: Coronoid fossa width, GUW: Groove for the ulnar nerve width, CHW: Capitulum of humerus width, FOL: Olecranon fossa length (transverse plane), CHL: Capitulum of humerus length, TCW: Capitulum of humerus + trochlea of humerus transverse length, GUA: The angle of the groove of the ulnar nerve, HVD: The vertical diameter of the humeral head, HML: The maximum length of the humerus, EML: The length between medial epicondyle and lateral epicondyle

Table 2. Non-normally distributed parameters (n=67)

Parameters	Left	Right	p-value
GIW (cm)	0.89 (0.25-1.93)	0.92 (0.35-1.69)	0.252
TDD (cm)	2.17 (0.60-2.83)	2.20 (1.74-2.72)	0.497
FCW (cm)	1.84 (1.11-2.19)	1.73 (1.40-2.45)	0.250
FOL (cm)	2.76 (1.82-3.38)	2.85 (1.82-3.38)	0.748
FOW (cm)	1.91 (0.94-2.50)	1.93 (1.40-2.65)	0.713

GIW: Intertubercular groove width, TDD: Deltoid tuberosity diameter, FCW: Coronoid fossa width, FOL: Olecranon fossa length (transverse plane), FOW: Olecranon fossa width (coronal plane)

Table 3. Normally distributed parameters (n=67)

	Left	Right	p-value
FRW (cm)	1.401±0.213	1.384±0.243	0.765
THW (cm)	2.458±0.265	2.446±0.320	0.868
THL (cm)	2.724±0.335	2.740±0.394	0.859
GUW (cm)	1.559±0.301	1.328±0.292	0.003
CHW (cm)	1.962±0.251	1.886±0.233	0.207
CHL (cm)	2.404±0.267	2.411±0.351	0.932
TCW (cm)	4.478±0.417	4.403±0.386	0.459
GUA (°)	121.4 ±14.6	113.0±15.4	0.028
HVD (cm)	4.678±0.479	4.813±0.511	0.270
HML (cm)	32.37±2.68	31.99±2.29	0.534
EML (cm)	6.129±0.563	5.988±0.594	0.333

FRW: Radial fossa width, THW: Trochlea of humerus width, THL: Trochlea of humerus length, GUW: Groove for the ulnar nerve width, CHW: Capitulum of humerus width, CHL: Capitulum of humerus length, TCW: Capitulum of humerus + trochlea of humerus transverse length, GUA: The angle of the groove of the ulnar nerve, HVD: The vertical diameter of the humeral head, HML: The maximum length of the humerus, EML: The length between medial epicondyle and lateral epicondyle

Table 4. Spearman-Pearson correlation test results of humerus parameters (n=67)

Variables	HML	HVD	GIW	TDD	CHW	THW	TCW	FCW	FRW	EML	GUW	GUA	CHL	THL	FOL
1. HML (cm)															
2. HVD (cm)	0.675**														
3. GIW (cm)	0.189	0.157													
4. TDD (cm)	0.496**	0.598**	-0.286												
5. CHW (cm)	0.454**	0.419**	0.072	0.379**											
6. THW (cm)	0.526**	0.553**	0.084	0.421**	0.073										
7. TCW (cm)	0.716**	0.665**	0.168	0.503**	0.600**	0.730**									
8. FCW (cm)	0.399**	0.383**	0.193	0.299*	0.153	0.502**	0.423**								
9. FRW (cm)	0.252*	0.279*	0.086	0.342**	0.295*	0.159	0.277*	0.092							
10. EML (cm)	0.665**	0.577**	0.220	0.537**	0.444**	0.654**	0.798**	0.492**	0.158						
11. GUW (cm)	0.442**	0.372**	0.033	0.253	0.254*	0.325**	0.400**	0.341*	0.065	0.541**					
12. GUA (°)	-0.108	-0.218	-0.141	0.045	0.050	-0.199	-0.196	0.074	0.005	-0.240	0.084				
13. CHL (cm)	0.442**	0.539**	0.012	0.543**	0.553**	0.433**	0.665**	0.381**	0.241	0.610**	0.334**	-0.116			
14. THL (cm)	0.591**	0.631**	0.164	0.489**	0.406**	0.462**	0.591**	0.453**	0.069	0.608**	0.514**	-0.062	0.563**		
15. FOL (cm)	0.378**	0.345**	0.253	0.196	0.305*	0.278*	0.441**	0.185	0.278*	0.376**	0.148	-0.135	0.351**	0.234	
16. FOW (cm)	0.210	0.219	0.218	0.186	0.095	0.271	0.275*	0.277	0.098	0.301*	0.179	0.013	0.192	0.210	0.597**

**p<0.01, *p<0.05, HML: The maximum length of the humerus, HVD: The vertical diameter of the humeral head, GIW: Intertubercular groove width, TDD: Deltoid tuberosity diameter, CHW: Capitulum of humerus width, THW: Trochlea of humerus width, TCW: Capitulum of humerus + trochlea of humerus transverse length, FCW: Coronoid fossa width, FRW: Radial fossa width, EML: The length between medial epicondyle and lateral epicondyle, GUW: Groove for the ulnar nerve width, GUA: The angle of the groove of the ulnar nerve, CHL: Capitulum of humerus length, THL: Trochlea of humerus length, FOL: Olecranon fossa length (transverse plane), FOW: Olecranon fossa width (coronal plane)

Discussion

This study aimed to examine the correlation between measurement parameters of the distal end of humerus and all humerus parameters by making anthropometric measurements on dry bones of the humerus showed that HML parameter was associated with most of the distal end parameters. It was also found that parameters on distal end were generally correlated with each other. Another result was the finding that GUW and GUA parameters were higher on the left side when compared with the right side.

In this study, the measurements of which were repeated twice, TEM, rTEM, and R values of 16 parameters were calculated. R values of all parameters were found to be between 0.83 and 0.99, and they were interpreted to have almost perfect reliability (6,7). The highest rTEM percentage was calculated as 12.49% in GIW parameter. The reason why this parameter had high rTEM value is the fact that it had a mean lower than 1 cm (8).

Fractures to the long bones are common (9). In such cases, samples are needed to evaluate the methods used in implants. In the literature, various fixation experiments have been performed on these specimens for clinical use based on screw-plate positioning. Knowing both the proximal and distal end of the humerus very well will increase the success in experiments such as biomechanical adjustment and durability on samples with various devices (10-13). Distal humeral prosthesis hemiarthroplasty used in comminuted distal humerus fractures should be designed to provide anatomical prerequisites and should be compatible with natural humeral anatomy. Having detailed knowledge of elbow anatomy will increase the design and success of new prostheses (14). At this point, the results obtained in this study will create an important anatomical resource.

Segmental studies of the humerus, especially in forensic medicine and archeology, there are studies that estimate the height from the humerus by using various formulas to determine the height of the people in the population (15,16). We think that HML will be a reference for such studies.

Kastamoni et al. (17) stated that GUW was positively significantly correlated with humeral retroversion angle and HML and negatively significantly correlated with olecranon fossa depth. Since the parameters of humeral retroversion angle and olecranon fossa

depth were not included in our study, no inferences were made in our study on this. Although Vettivel et al. (18) found positively significant correlation with GUW and HML in their study, no significant correlation was found in the present study. Wafae et al. (19) stated that intertubercular groove morphology was not correlated with humeral morphometry. Measurements were made from the middle level of intertubercular groove both in this study and in Wafae et al.'s (19) study, while measurement was made from the proximal of the groove in Kastamoni et al.'s (17) study. We think that the difference in the results may be due to this reason.

Kastamoni et al. (17) concluded that there were no significant differences between olecranon fossa width and HML, EML, and THW parameters. On the other hand, a statistically significant correlation was found between FOW and EML in our study.

There are studies in literature which report correlation between HML and EML (20-22). There are also studies reporting a significant correlation between HML and THW parameters (22, 23). Similar results found in our study support the existing literature. While Kastamoni et al. (17) stated that HML parameter is not significantly correlated with EML and THW parameters, this result is not consistent with the results of our study and other studies. On the other hand, Kastamoni et al. (17) found statistically significant positive correlation between EML and THW and this result was found to be consistent with the results of our study.

There are studies in literature which show statistically significant positive correlation between HML and HVD parameters and this result is consistent with the results of our study (21,22). Similar to the results of our study, Çini and Arı (21) found significant positive correlation between HML and CHW parameters. However, although they found significant correlation between HML and GUW parameters, no significant correlation was found between these parameters in our study.

de Queiroz Chaves et al. (24) conducted by using the computed tomography images of the right sides of 30 individuals, they found mean GUA parameter as 92.7±10. In our study, this parameter was found as 113.0±15.4° on the right side. The fact that we had no recorded information about general health state since our study was conducted on dry bones, uncertain gender distribution and age, demographic differences and differences in sample size may be the reasons for the difference between studies. GUA parameter was found to be higher on the left side when compared with the right side in our study and the difference was found to be statistically significant. Since the same person was not compared bilaterally in our study, this difference was weak in terms of evidence. This parameter was not found to be correlated with any of our parameters. It has been stated in literature that bone structure is important in cubital tunnel syndrome factors (25). GUA is also the parameter that can determine the shape of cubital tunnel and we believe that it is clinically important.

This study has some limitations. Not having information about the age and gender of the dry bones used, about general health state and about whether the right and left bones belonged to same people are important limitations of the study.

Conclusion

Nevertheless, the anatomical information found in this highly reliable study will guide clinicians in studies evaluating humeral segments, especially in the treatment of elbow instability, the treatment and reconstruction of distal humeral fractures. It will help the multidisciplinary team to produce a more suitable prosthesis both biomechanically and kinesiologically by correlating it with radiological data. The results of this study will contribute to studies in the fields of anatomy, biomechanics, forensic sciences, and anthropology.

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Ethics

Ethics Committee Approval: This study was initiated with 2021/797 numbered approval from Karabük University Ethics Committee (date: 24.12.2021).

Informed Consent: Informed consent is not required.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Concept: R.S.B., Design: R.S.B., Data Collection or Processing: Ş.T., R.S.B., N.E.Ş., Analysis or Interpretation: Ş.T., R.S.B., N.E.Ş., Literature Search: Ş.T., R.S.B., N.E.Ş., Writing: Ş.T., R.S.B., N.E.Ş.

Conflict of Interest: No conflict of interest was declared by the authors.

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Original Article / Özgün Araştırma

Assessment of the Association Between Complete Blood Cell Parameters, Levels of Vitamin B_{12} and Folate, Decreased Iron Storage and Recurrent Vasovagal Syncope Episodes

Tekrarlayan Vazovagal Senkop Epizotları ile Vitamin B_{12} ve Folat Seviyeleri, Azalmış Demir Depoları ve Tam Kan Sayımı Parametreleri Arasındaki İlişkinin Değerlendirilmesi

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Keywords
Vasovagal syncope, fainting, serum ferritin, complete blood count, mean platelet volume, mean corpuscular hemoglobin concentration

Anahtar Kelimeler
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Abstract

Objective: To evaluate the relationship between recurrent vasovagal syncope (VVS) and complete blood cell parameters, serum levels of ferritin, vitamin B_{12}, and folate.
Materials and Methods: This retrospective study included patients with recurrent VVS and healthy controls. Children and adolescents presenting with at least two VVS episodes were included. Exclusion criteria were as follows; having an electrocardiogram abnormality, patients without an evident trigger before fainting, having an infection, having a history of chronic disease, and taking any medications or vitamin supplements, including folate, vitamin B_{12}, and iron.
Results: A total of 44 patients and 66 healthy controls were included. There were no significant differences between the groups in terms of age and gender. Mean corpuscular hemoglobin concentration (MCHC) (p=0.014), mean platelet volume (MPV) (p=0.020), and levels of ferritin (p<0.0001) were significantly lower in the patient group. No significant differences were found between the groups with respect to other laboratory parameters. Binary logistic regression analysis showed that every 1-unit decrease in serum ferritin constitutes a 0.972 [95% confidence interval (CI)= 0.954-0.990] fold risk. Also, every 1-unit decrease in MPV constitutes a 0.453 (95% CI=0.275-0.745) fold risk of VVS.
Conclusion: This study showed lower levels of serum ferritin, smaller platelet sizes, and lower levels of MCHC. Additionally, smaller platelet sizes and lower levels of ferritin were independent risk factors.

Öz

Amaç: Tekrarlayan vazovagal senkop (VVS) ile tam kan sayımı, serum ferritin, vitamin B_{12} ve folat düzeyleri arasındaki ilişkinin incelenmesi amaçlanmıştır.
Gereç ve Yöntemler: Çalışmamız tekrarlayan VVS ve sağlıklı çocuk ve adölesanları içermiştir. Hasta grubuna en az iki tipik VVS atağı geçirmiş olgular dahil edildi. Elektrokardiyogramında anormallik varlığı, bayılma öncesi VVS için belirgin bir tetikleyicinin yokluğu, enfeksiyon ya da kronik bir hastalık öyküsünün varlığı, vitamin B_{12}, folat ya da demir replasmanı alıyor olmak dışlama kriterleri olarak

belirlendi. Yaş, cinsiyet, senkop sonrası bakılmış olan tam kan sayımı, serum ferritin, vitamin B_{12} ve folat düzeyleri retrospektif olarak kaydedildi.
Bulgular: Bu çalışmaya 44 tekrarlayan VVS olgusu, 66 sağlıklı kontrol dahil edildi. Ortalama korpüsküler hemoglobin konsantrasyonu (p=0,014), ortalama platelet volümü (MPV) (p=0,020), ve serum ferritin düzeyleri (p<0,0001) VVS grubunda anlamlı olarak daha düşüktü. Gruplar arasında diğer laboratuvar parametreleri açısından anlamlı farklılık saptanmadı. Binary lojistik regresyon analizi serum ferritin düzeylerindeki her 1 birim düşüklüğün tekrarlayan VVS riskinde 0,972 kat [%95 güven aralığı (GA)= 0,954-0,990], MPV değerindeki her 1 birim düşüklüğün ise tekrarlayan VVS riskinde 0,453 kat (%95 GA=0,275-0,745) artışa neden olduğunu gösterdi.
Sonuç: Çalışmamız sonucunda serum ferritin, MPV ve ortalama hücresel hacim değerleri hasta grubunda anlamlı olarak düşük saptanmıştır. Bununla birlikte düşük MPV ve düşük ferritin düzeylerinin tekrarlayan VVS için bağımsız birer risk faktörü olduğu sonucuna varılmıştır.

Introduction

Syncope is a common disorder that is described as an abrupt and impermanent loss of consciousness. The cumulative frequency of occurrence is 35% during the lifetime and the incidence increases around the age of 15 years. Syncopes have been sorted out into three categories; i) cardiogenic, ii) non-cardiogenic, iii) undetermined causes (1). The major causes of the cardiac syncopes are arrhythmias, and abnormalities of the left and right sides heart. The causes of noncardiac syncope are reflex syncope, also known as neurally mediated syncope, hypothermia, cerebrovascular conditions, seizures, and metabolic conditions (hypoglycemia, hypoxia). Vasovagal syncope (VVS) is the most prevalent type of reflex syncope. It is a consequence of abnormal interactions of complex neurocardiovascular mechanisms (2). Emotional stress, pain, fever, prolonged standing, cough, sneezing, micturition, and defecation may trigger VVS. The diagnosis is of VVS based on symptoms, physical examination, and electrocardiogram (ECG) findings (3).

There are various studies regarding the relationship between VVS and routine laboratory results such as complete blood count (CBC), levels of serum vitamin B_{12}, folate, and ferritin (4). Reduced monoamine oxidase activity was demonstrated in rats with iron deficiency. Also, since higher plasma norepinephrine levels were demonstrated in pediatric patients with postural orthostatic tachycardia syndrome (POTS), it was proposed that there may be an association between iron deficiency and catecholamine metabolism (5). Vitamin B_{12} participates in the synthesis of catecholamines. Additionally, it is an important cofactor in myelin degradation. The levels of vitamin B_{12} and folate are important in some neurological disorders even without a proven insufficiency (6). Maybe, due to the connected functions of vitamin B_{12} and folate in myelination and/or synthesis of methionine from homocysteine, deficiency in one vitamin alters the other's functions (7). The linkage between VVS and levels of folate, and vitamin B_{12} may be result from hyperhomocysteinemia-related autonomic dysfunction (8).

Herein, we aimed to evaluate the association between VVS and CBC, levels of serum vitamin B_{12}, folate, and ferritin.

Materials and Methods

Study Design and Subject

We have obtained the approval of the Ethics Committee from Aydın Adnan Menderes University in line with the principles outlined in the second Declaration of Helsinki (protocol number: 2022/91, date: 12.05.2022). No informed consent was taken due to the retrospective design. This study was conducted from April 2021 to April 2022 in the departments of pediatrics, pediatric neurology, and pediatric cardiology of two universities. Fourty-four pediatric patients who were admitted with a history of at least 2 episodes of VVS, and 66 healthy controls were included. Data were consist of 11 patients and 28 healthy controls from Sivas Cumhuriyet University (diagnosed by a pediatrician) and 33 patients and 38 healthy controls from Aydın Adnan Menderes University (diagnosed by a pediatrician, two pediatirc neurologist, and a pediatric cardiologist). Only patients with an evident trigger were included. The control group consisted of 66 healthy children, who were admitted to the general pediatrics for examination before sportive activities and underwent CBC, vitamin B12, ferritin, and folate. Exclusion criteria were as follows; having an abnormality in the ECG, patients without an evident trigger before the fainting, having

a history of an infection in the last 4 weeks, having a history of chronic disease. Also, children who were on medication with any medications or vitamin supplements including folate, vitamin B_{12}, and iron were excluded. The results were extracted from the electronic database. Having hemoglobin levels <12 mg/dL and <13 mg/dL were accepted as anemia for females and males, respectively. Having ferritin levels <15 ng/mL and, folate levels <4 ng/mL were considered as deficiency. A level of <400 pg/mL is accepted as a low level for vitamin B_{12}. Normal values of mean corpuscular volume (MCV), red distribution width (RDW), mean platelet volume (MPV), and mean corpuscular hemoglobin concentration (MCHC) were accepted as 80-94 fL, 11.5-14.5%, 8-12 fL, and 34-36 g/dL (9,10).

Statistical Analysis

Statistical analyses were performed using the SPSS-22 (IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.). Kolmogorov-Smirnov test was applied to specify the normal distribution of numerical variables. Categorical data were presented with n and %. Normally distributed numerical data presented with mean ± standard deviation, non-normally distributed data presented with median interquartile range. Student t-test was applied for comparison of normally distributed data and the Mann-Whitney U test was used for comparison of non-normally distributed data. The chi-square test was used for the comparison of categorical data. After the prior analysis binary logistic regression analysis was used to determine the risk factors. A p-value <0.05 was accepted as statistically significant.

Results

The study group consisted of 44 patients and 66 healthy controls. Three patients had iron deficiency anemia. Among the patients with VVS and iron deficiency, 4 of them had both high RDW and low MCV. Of the 110 children, none of them had a lower MPV and only 4 children had higher MPV. Precisely, 34 of the 44 patients had a lower MCHC level than 34 g/dL and 14 of them had a lower level of ferritin. Folate, vitamin B_{12}, and iron deficiencies were also seen in the healthy controls. The numbers of children with vitamin B_{12} deficiency (<400 pg/mL), folate deficiency, iron deficiency, and iron deficiency anemia were 46, 10, 8, and 2, respectively. MCHC (p=0.014), MPV (p=0.020), and levels of ferritin (p<0.0001) were significantly lower in VVS group (Table 1). Binary logistic regression analysis showed that every 1-unit decrease in serum ferritin constitutes a 0.972 [95% confidence interval (CI) =0.954-0.990] fold risk. Also, every 1-unit decrease in MPV constitutes a 0.453 (95% CI=0.275-0.745) fold risk for VVS (Table 2).

Discussion

The current study showed that MCHC, MPV, and levels of serum ferritin were significantly lower in patients with VVS. Also, binary logistic regression analysis indicated that lower levels of serum ferritin and MPV were independent risk factors.

Table 1. The comparison of demographical features and laboratory findings between the groups

	Syncope (n=44)	Control (n=66)	p-value
Age**	14.12 (3.79)	15.00 (3.00)	0.272
Gender			
Female	24 (54.5%)	38 (57.6%)	0.754
Male	20 (45.5%)	28 (42.4%)	
WBC**	6.59 (2.42)	6.55 (2.28)	0.698
ANC**	3.29 (1.76)	3.59 (1.53)	0.514
ALC**	2.53 (0.48)	2.62 (0.92)	0.840
RBC*	5.03±0.49	4.89±0.59	0.193
Hb**	14.05 (1.08)	13.8 (2.35)	0.963
Htc**	42.10 (3.28)	41.45 (6.48)	0.548
MCV**	83.40 (6.45)	82.50 (4.53)	0.786
MCHC**	33.40 (1.68)	33.85 (1.80)	**0.014**
RDW**	13.05 (1.28)	13.10 (1.25)	0.447
PLT*	30.09±62.64	294.73±99.42	0.682
MPV**	9.60 (0.80)	10.00 (1.73)	**0.020**
PCT**	0.28 (0.09)	0.31 (0.08)	0.893
PDW**	10.70 (1.80)	11.70 (3.58)	0.088
NLR**	1.35 (0.83)	1.47 (0.99)	0.181
Ferritin**	17.9 (18.5)	38.5 (42.18)	**<0.0001**
Folate*	14.94±51.45	7.46±3.94	0.341
Vitamin B_{12}**	296 (248.75)	303.00 (184.75)	0.905

*Non-normally distributed data were given as median (IQR). **Normally distributed data were given as mean (± SD). WBC: White blood cell, ANC: Absolute neutrophil cell, ALC: Absolute lymphocyte cell, RBC: Red blood cell, Hb: Hemoglobin, Htc: Hemotocrite, MCV: Mean corpusculary volume, MCHC: Mean corpusculary hemoglobin concentration, RDW: Red distrubution width, PLT: Platelets, MPV: Mean platelet volume, PCT: Plateletcrit, NLR: Neutrophile lymphocyte ratio

Table 2. Binary logistic regression analysis for prediction of independent risk factors of vasovagal syncope

	OR	95% CI for OR lower limit	Upper limit	p-value
MPV**	0.453	0.275	0.745	**0.002**
Ferritin**	0.972	0.954	0.990	**0.002**
Constant	4806.80	-	-	**0.001**

**Normally distributed data. OR: Odds ratio, CI: Confidence interval, MCHC: Mean corpuscular hemoglobin concentration, MPV: Mean platelet volume

The pathophysiological mechanism of VVS is sophisticated and not well-understood. Usually, after a trigger, especially in combination with dehydration and/or a sudden upright posture, relative hypovolemia take place. Afterward, cardiac contractility increases, the mechanoreceptors are triggered in the ventricle and then the vagal nerve transmits the signal to the central nervous system. The increased activity in vagal tone results in a decrease in heart rate. Additionally, the decrease in sympathetic activity causes a decrease in vascular tone in both arterioles and venules occurs. The decreases in preload, venous return, and ventricular volume leads to a drop in mean arterial pressure (11,12). Therefore, sympathetic system activation occurs due to reduced venous return during the early phase, and a suppression occurs in the sympathetic system in the terminal vasodilatation phase. However, Vaddadi et al. (13) have proposed that in the terminal vasodilation phase, the parasympathetic system becomes active and suppression does not occur in the sympathetic system. Therefore, regardless of the controversy in the mechanism, the parasympathetic system becomes dominant over the sympathetic system in the terminal vasodilator phase. Head-upright-tilt test can be used to evaluate the syncope etiology and verify the diagnosis of VVS. According to the head-upright-tilt-table test, positive results in VVS can be categorized into cardioinhibitory type, vasodepressor type, and mixt type.

In the presence of sympathetic activation, the release of platelets with larger volume from the spleen to the systemic circulation and thrombopoiesis in bone marrow increase (14). Kabul et al. (15) demonstrated that platelet sizes were significantly larger in vasodepressor type VVS than in the other VVS groups and the control group. However, platelet sizes were significantly lower in patients with cardioinhibitory and mixt-type response groups than in the other groups. Therefore, it was proposed that MPV values may have a role in predicting increased vagal response in patients with cardioinhibitory and mixed-type VVS (16). In the present study, platelet sizes were significantly smaller in VVS group. Furthermore, lower MPV was an independent risk factor. However, since the head-upright-tilt-test could not be performed VVS subtypes could not be categorized. On the other hand, Yalçın et al. (17) proposed that mixt-type VVS is the most common type of VVS in younger patients (<25 years). Thus, in the current study, significantly lower platelet sizes in VVS group may be related to the higher frequency of mixt-type of VVS at younger ages.

Research showed that there may be an association between iron storage and syncope (5). Jarjour and Jarjour (18) suggested that patients with neurally mediated syncope had significantly lower iron storage. Guven et al. (19) demonstrated that lower ferritin levels were more prevalent in children with syncope. A clinical trial showed that fluctuations of epinephrin activated the syncope episodes (20). Therefore, some researchers proposed that lower iron storage may be related to alterations in catecholamine metabolism. This suggestion was also supported by the evidence that monoamine oxidase, which plays a key role in the metabolism of epinephrine and norepinephrine, activity reduced in rats with iron deficiency (5). Similarly, serum ferritin levels were significantly lower in the VVS group and lower levels of ferritin were an independent risk factor, in the current study.

MCHC is a measure of the concentration of hemoglobin in a given volume of the erythrocyte and a low MCHC is related to reduced iron storage (21). Moreover, it was demostrated that low MCHC and increased reticulocyte counts are the first findings indicating iron deficiency, even without anemia (22). In the present study, the most frequent abnormal laboratory parameter is low MCHC. This is the first study that demonstrated that MCHC was significantly lower in patients with VVS. This association may be due to the fact that iron deficiency may lead to a lower MCHC. Moreover, it should be kept in mind that the suggested levels of ferritin higher than 15 ng/mL are for iron deficiency anemia. The suggested levels for ferritin regarding other disorders related to iron

deficiency may be different. Therefore, numerical data of laboratory results were compared between the groups in the present study. There were no significant differences between the groups in terms of other CBC parametres. The absence of a relationship maybe associated with the fact that abnormality of these parameteres are not the first findings of iron deficiency and maybe normal in children with mild iron deficiency without anemia.

The relationship between VVS and serum levels of folate and vitamin B_{12} has been explained by the key role in the carbon transfer metabolism that is important for the production of serotonin, the other monoamine neurotransmitters, and catecholamines (4,5). Also, both vitamins have important roles in myelination and the synthesis of methionine from homocysteine and lower levels lead to hyperhomocysteinemia. Hyperhomocysteinemia has negative impacts on autonomic nerve functions, oxidative stress, inflammation, the proliferation of smooth muscle cells, and vascular endothelium (9). Aminorroaya et al. (4) found no significant differences in terms of folate and vitamin B_{12} deficiency between VVS group and healthy controls. However, serum vitamin B_{12} levels were significantly lower in patients with frequent VVS episodes (≥3 episodes) than in those with infrequent (<3 episodes) episodes (4). Kovalchuk et al. (23) found that serum levels of pyridoxine and vitamin B_{12} were significantly lower in the VVS group. Although no significant differences were found between the groups, lower levels of serum folate were associated with longer durations of VVS (23). Öner et al. (24) demostrated that vitamin B_{12} levels were significantly lower in children with POTS, also known as presyncope, than in the healthy controls. In the current study, there were no significant differences between the groups in terms of serum levels of vitamin B_{12} and folate. The absence of the relationship may be related to the small number of patients and the complexity of the VVS pathogenesis.

This is one of the few studies evaluating extensive laboratory result in VSS. However, its retrospective design with a small number of patients, and the absence of the results of total iron-binding capacity and serum levels of iron were the limitations. However, having an infection was an exclusion criterion to specify the serum ferritin results for the evaluation of iron storage of the patients. Although the head-upright-tilt-table test is not a certain requirement for the diagnosis, since the subtypes of VVS could be determined, the absence of the test was another limitation.

Conclusion

In conclusion, the current study showed that lower levels of MCHC, smaller platelet sizes and lower levels of serum ferritin were related to VVS. Furthermore, smaller platelet sizes and lower levels of ferritin were independent risk factors.

Ethics

Ethics Committee Approval: We have obtained the approval of the Ethics Committee from Aydın Adnan Menderes University in line with the principles outlined in the second Declaration of Helsinki (protocol number: 2022/91, date: 12.05.2022).

Informed Consent: Retrospective study.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: M.A., Concept: M.A., Design: M.A., A.K.T., E.Ç., A.T., Data Collection or Processing: M.A., A.K.T., E.Ç., S.F.Ç., A.T., Analysis or Interpretation: M.A., S.F.Ç., Literature Search: M.A., S.F.Ç., Writing: M.A., S.F.Ç., A.T.

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Original Article / Özgün Araştırma

Protective Efficacy of Thiamine (Vitamin B_1) Alone on LPS-induced Acute Kidney Injury

Tiamin (Vitamin B_1) Tedavisinin LPS ile Indüklenen Akut Böbrek Hasari Üzerine Koruyucu Etkileri

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Abstract

Objective: Sepsis-induced acute kidney injury (sAKI) is the leading cause of renal dysfunction and mortality in intensive care unit. We must target its multidimensional pathogenesis for new treatment strategies. Therefore, we decided to reveal the protective efficacy of thiamin on sAKI in terms of its antioxidant, anti-inflammatory and mitochondrial regulatory effects.

Materials and Methods: Four rat groups were formed as; healthy (HG), sepsis (SG), thiamine (TG), sepsis + thiamine (TSG) groups. In kidney tissue, oxidant [malondialdehyde (MDA), nitric oxide (NO)] and antioxidant (glutathione (GSH), catalase (CAT)] levels were measure. In serum, tumor necrosis factor-alpha (TNF-α), interleukin-1β (IL-1β), procalcitonin (PCT) and urea, creatine (Cr), lactate were also surveyed.

Results: There were significant decreases in MDA and NO levels ($p<0.001$, $p<0.001$, respectively) and increases in GSH and CAT levels ($p<0.001$, $p<0.001$, respectively) when TSG and SG groups were compared. Proinflammatory cytokine levels were significantly elevated in SG ($p<0.001$). In the TSG group, PCT, IL-1β and TNF-α levels were decreased compared with SG ($p<0.001$ $p<0.001$, $p<0.001$, respectively). In the SG group versus HG group results, lactate levels were found to be 4-fold higher ($p<0.001$) and urea, Cr levels were 3-4 fold higher ($p<0.001$, $p<0.001$, respectively). In the TSG group, there was an obvious decrease in lactate and urea, Cr levels compared with SG ($p<0.001$, $p=0.002$, $p<0.001$, respectively).

Conclusion: We revealed the preventive efficacy of thiamin against sAKI by reducing the oxidant parameters and proinflammatory cytokine levels and increasing the antioxidant parameter levels with the attainment of near normal kidney functions, simultaneously. With its cheap, readily available, and good safety profile in adults, thiamin appears like a tempting adjunctive therapy for sAKI.

Öz

Amaç: Sepsinin indüklediği akut böbrek hasarı (sABH), yoğun bakımda renal disfonksiyon ve mortalitenin önde gelen sebebidir. Yeni tedavi stratejileri için sepsisin çok yönlü patogenezini hedeflemeliyiz. Bu nedenle, sABH'da tiaminin antioksidan, antiinflamatuvar ve mitokondrial düzenleyici etkilerini kullanarak koruyucu etkilerini ortaya koymayı amaçladık.

Gereç ve Yöntemler: Ratlar sağlıklı (HG), sepsis (SG), tiamin, sepsis + tiamin (TSG) olmak üzere dört gruba ayrıldı. Böbrek dokusunda oksidan [malondialdehit (MDA),

nitrik oksit (NO)] ve antioksidan [glutatyon (GSH), katalaz (CAT)] seviyeleri ölçüldü. Serumda TNF-α, IL-1β, prokalsitonin (PCT) ve üre, kreatinin (Cr), laktat değerlendirildi.
Bulgular: TSG grubunda, SG grubuyla karşılaştırıldığında, MDA ve NO seviyelerinde anlamlı bir azalma (p<0,001, p<0,001, sırasıyla) ve GSH ve CAT seviyelerinde artış (p<0,001, p<0,001, sırasıyla) saptandı. SG grubunda, proinflamatuvar sitokin seviyelerinde anlamlı bir artış mevcuttu (p<0,001). TSG ve SG karşılaştırıldığında, PCT, IL-1β ve TNF-α seviyerinde belirgin bir düşüş saptandı (p<0,001, p<0,001, p<0,001, sırasıyla). SG grubunda HG grubuna kıyasla laktat seviyelerinde 4 kat artış (p<0,001) ve üre ve Cr seviyelerinde 3-4 kat artış gözlendi (p<0,001, p<0,001, sırasıyla). TSG grubunda SG grubuna kıyasla laktat, üre ve Cr seviyelerinde aşikar bir düşüş vardı (p<0,001, p=0,002, p<0,001, sırasıyla).
Sonuç: Tiaminin sABH üzerinde, oksidan parametreleri ve proenflamatuvar sitokinleri azaltarak ve antioksidan seviyelerini yükselterek neredeyse normal böbrek fonksiyonuna ulaşmayı sağlayabildiğini ortaya koyduk. Ucuz, kolay ulaşılabilir ve iyi bir güvenlik profiline sahip tiaminin sABH tedavisinde cazip bir ek tedavi seçeneği olabileceği kanısındayız.

Introduction

Sepsis is a life-threatening organ dysfunction shaped by host response and pathogen factors (1). Sepsis-induced acute kidney injury (sAKI) is the leading cause of renal dysfunction which accounts nearly 50% of all cases and has 41% mortality in intensive care units (2). Currently used early goal-directed strategies are reactive but nonspecific (1). The way to develop effective treatments for sAKI is through a good understanding of its pathogenesis.

Although renal ischemia is trusted on to be the main reason of sAKI, many conflicting studies have been presented. Animal models of sAKI revealed that kidney injury may develop without renal ischemia. Renal medullar microcirculatory disturbances may lead to medullar oxygen delivery failures resulting hypoxia which may be the major trigger point of renal injury (3).

In sepsis induced organ injury, tissue and vascular injury and the inflammatory host response initiates a multidirectional cascade resulting to endothelial dysfunction. The over expression of inducible nitric oxide synthase (iNOS) elevates nitric oxide (NO) production, tissue hypoxia and additionally the generation of reactive nitrogen species (RNS) and reactive oxygen species (ROS) (4).

Concurrently, neutrophils generate an oxidative stimulus initiating a cytokine storm including pro-inflammatory molecules especially like interferon-gamma, tumor necrosis factor-alpha (TNF-α) and interleukin-1β (IL-1β). This cascade triggers the downstream production of ROS and RNS and propagate sAKI (4).

As a result of all; oxidative stress, inflammation, mitochondrial dysfunction, renal medullar hypoxia and endothelial dysfunction seem to be interrelated mechanisms in the sAKI pathophysiology. By this way the term 'metabolic resuscitation' for sepsis treatment got in range of new studies (5,6). With its role as a constant co-factor of mitochondrial aerobic metabolism and redox cycle and with antioxidant/anti-inflammatory effects thiamin has caught the attention in sepsis-induced organ failure therapy studies (7).

Thiamine is a water-soluble vitamin. Some essential nutrients like thiamine may be deficient during sepsis (8,9). In septic patients, the prevalence of thiamine deficiency is between 20-71% (10,11). Thiamine deficiency may increase lactic acid generation and the existing metabolic stress in sepsis may increase consumption of antioxidant molecules like thiamine (7,9). Whether thiamin deficiency is the cause or consequent effect of sepsis is not clear. All these steps above, creates a vicious cycle between thiamine and sepsis pathophysiology.

Transketolase, α-ketoglutarate dehydrogenase, branched chain amino acid dehydrogenase and pyruvate dehydrogenase are all thiamine pyrophosphate (TP) -dependent enzymes having key roles in Krebs cycle (aerobic metabolism) and pentose phosphate pathway. Shortened TP levels lead to decreased enzymatic activity of these enzymes resulting decreased oxidative phosphorylation, lactic acidosis, decreased production of adenosine triphosphate (ATP) and causing dysfunctions in high metabolic demand organs (12). Additionally, thiamine is critical for; ATP energy production, metabolism of some oxidant molecules such as ROS and for aerobic metabolism (13). It is considered that with all these overlapping pathogeneses of both thiamine metabolism and sepsis; bioenergetic especially mitochondrial failure resulting to organ injuries in especially high metabolic demand organs like kidneys, brain and heart, may exist (14).

Considering all these alterations of sepsis, we decided to reveal the protective efficacy of thiamine on sAKI by the means of its antioxidant and anti-inflammatory and mitochondrial regulatory effects.

Materials and Methods

Animals

A total of 32 male, Wistar albino rats were provided from Experimental Animal Unite of Aydın Adnan Menderes University, Turkey. Animals were kept in colony room, 12 hours of light/dark cycle and let to reach freely to water and food. All experimental design was consistent with the guide for the care and use of laboratory animals. Study has been approved by Animal Experiments Local Ethical Commission of Aydın Adnan Menderes University, Medical Faculty (approval number: 64583101/2016/49, date: 25.02.2016).

Chemical Substances

Lipopolysaccharide (LPS), (obtained from serotype 055:B5 *Escherichia coli*, L-2880) and TP was both obtained from Sigma Chemicals.

Experimental Study Design

Rats were fasted night before experiment and separated into four different groups, randomly (8 in each) as; healthy (HG), sepsis (SG), thiamine (TG), LPS + TP group (TSG). TP alone (TG) was created to investigate if TP alone may cause any deleterious effect on study parameters. By intraperitoneal (ip) route, saline was applied to HG rats. We performed preliminary studies to determine the exact LPS and TP doses and appropriate time for gathering tissue and blood samples after drug administrations (15). We have been revealed that 100 mg/kg TP dose has a protective effect on the oxidative stress in rats, in our previous study (16). Consequently, LPS at a dose of 5 mg/kg was given by ip injection to the right side of abdomen of SG and TSG rats. Thirty minutes before the ip administration of LPS, TP was administered ip. to the left abdomen of TSG rats. Eighteen hours after these interventions, blood samples were taken by cardiac puncture and kidney tissues were gathered under ketamine and xylazine (50 mg/kg and 5 mg/kg, respectively) anesthesia.

Blood analyses

Collected serum samples were kept at -80 °C. Serum urea and creatine (Cr) and lactate levels were surveyed using an autoanalyzer (C8000, Abbott, USA).

In serum specimens, by some merchant rat ELISA kits (Biotech Co. Ltd, China); TNF-α, IL-1β and procalcitonin (PCT) levels were evaluated via an ELISA reader (ELX800). The limit of detection for TNF-α was 9.37 pg/mL, and for PCT was 18.75 pg/mL. Assay range was determined as 15.6-1000 pg/mL for PCT and TNF-α, 31.25-2000 pg/mL for IL-1β.

Preparation of Tissue Homogenates

All tissues were homogenized in 50 mM, pH 7.4 phosphate-buffered saline for the measurement of tissue malondialdehyde (MDA), NO and glutathione (GSH) levels and to set up catalase (CAT) activity.

MDA was determined by the standard Ohkawa method (17). If thiobarbituric acid exists, MDA creates a complex which may be detected via the measurement of 532 nm wavelength absorbance. Results were indicated as µmol/g protein.

NO; was analysed by Navarro-Gonzalves method (18). By diazotization of sulphanilamide the emerging nitrite was measured. Via a microplate reader, samples were quantified versus a KNO^3 curve and analysed spectrophotometrically. Results were indicated as µmol/g protein.

CAT; to measure the activity method of Aebi (19) was used. The H^20^2 reduction rate was administered for 30 seconds at 240 nm. Results were indicated as U/g protein.

Glutatione (GSH); Beutler method was used to measure the GSH content (20). Via Shimadzu UV-160 spectrophotometer at 412 nm wavelength, the absorbance was detected. Results were indicated as µM/g protein.

Statical Analysis

To evaluate the normal distribution of numeric variables Kolmogorov-Smirnov test was used. All data were analyzed by the PAWS Statistics version 18 software SPSS using Kruskal-Wallis test to determine the significant differences between groups of the independent variables. The results are defined as mean ± standard error of the mean. Further, one-way analysis of variance (ANOVA) tests with Tukey-Kramer HSD or Tamhane as post-ANOVA tests were operated to determine the group differences. Statistical significance was stated at $p \leq 0.05$.

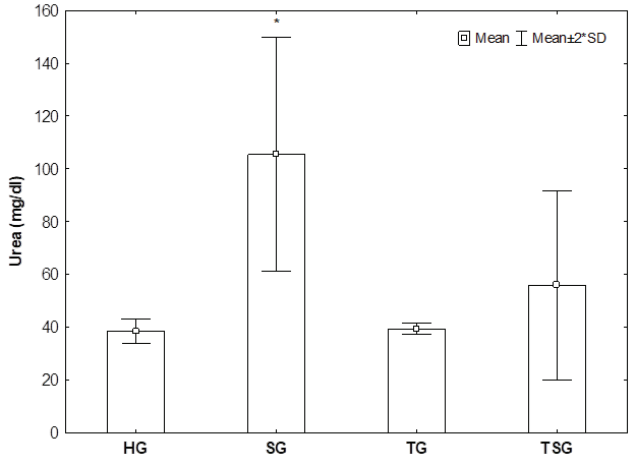
Results

The levels of oxidant/antioxidants in kidney tissues are shown in Table 1. When HG, TG and TSG were compared to SG, significant differences were revealed among all groups (p<0.001). A very prominent increase in all oxidants (MDA, NO) besides an evident reduction in antioxidants (GSH, CAT) was detected in SG. Statistically, no significant difference was defined among HG and TG (p>0.05) indicating TP alone had no harmful effect in renal tissue. There was an obvious decrease in MDA and NO levels (p<0.001, p<0.001, respectively) and a significant increase in GSH and CAT levels (p<0.001, p<0.001, respectively) in TSG when compared with SG. These results were indicating the antioxidant efficacy of thiamine alone on renal tissue with no harmless effect on healthy tissues.

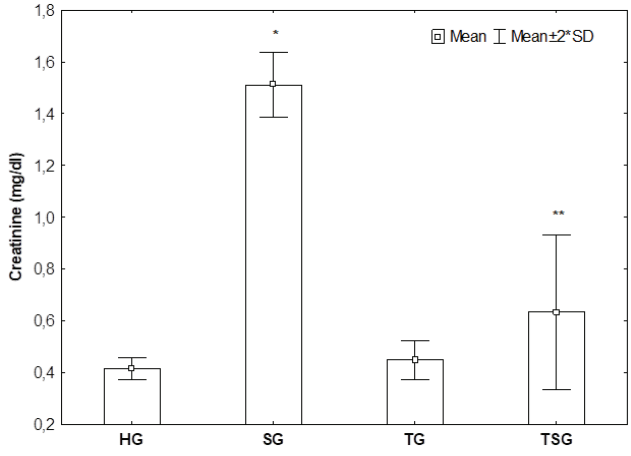
Table 2 shows the pro-inflammatory agents and lactate levels among groups. The markers of inflammation PCT, TNF-α and IL-1β levels were elevated significantly in SG versus HG (p<0.001). In TSG; PCT, IL-1β and TNF-α levels were all decreased when SG results were considered (p<0.001, p<0.001, p<0.001, respectively). There was no significant difference between HG and TSG in terms of proinflammatory agents (all p>0.05, respectively).

Lactate levels were nearly 4-fold higher in SG, when compared with HG (p<0.001). In TSG, lactate levels reduced nearly HG levels, as there was no significant difference between HG and TSG in terms of lactate levels (p>0.05).

Serum Cr levels were 0.41±0.00 mg/dL in HG, 1.51±0.02 mg/dL in SG, 0.44±0.01 mg/dL in TG and 0.63±0.05 mg/dL in TSG. There were nearly 4-fold increase in serum Cr levels in SG when compared with HG (p<0.001). With the comparison between SG and HG there were nearly 4-fold increase in serum urea levels in SG (p<0.001). These results were defining the establishment of severe sepsis induced AKI in our study model. When given alone TP had no adverse effect on kidney function tests as seen in TG results. A significant reduction was found in urea and creatinine levels of TSG when compared with SG (p=0.002, p<0.001, respectively). In TSG, Cr levels reduced nearly 2-fold with TP treatment when compared with sepsis group but there was still statistically difference between HG and TSG Cr levels (p=0.026). All these data may be seen in Graphic 1 and 2.



Graphic 1. Urea levels among groups
*All groups were compared with SG group. The bars were created using mean ± SEM values. Statistically significant value defined as p<0.05
SG: Sepsis group, SEM: Standard error of the measurement



Graphic 2. Creatine levels among groups
*All groups were compared with SG group. The bars were created using mean ± SEM values. Statistically significant value defined as p<0.05
**All groups were compared with TSG group. The bars were created using mean ± SEM values. Statistically significant value defined as p<0.05
SG: Sepsis group, TSG: Sepsis + thiamine pyrophosphate group, SEM: Standard error of the measurement

Discussion

In this study, our goal was to examine the protective efficacy of thiamine alone on sAKI in a model of LPS-induced sepsis. To the best of our knowledge, this is the first study examining thiamine efficiency alone

Table 1. The oxidant and antioxidant levels in kidney tissue among rat groups[1]

	MDA µmol/g protein	NO µmol/g protein	GSH µM/g protein	CAT U/g protein
HG n=8	1.23±0.14*	26.62±2.04*	15.64±1.39*	20.32±1.29*
SG n=8	2.83±0.10	69.92±3.73	4.85±0.62	7.78±0.69
TG n=8	1.10±0.10*	25.81±1.96*	16.80±1.24*	17.28±1.31*
TSG n=8	1.22±0.12*	31.51±2.65*	13.09±0.98*	15.38±0.91*

[1]The comparison of MDA, NO, CAT and GSH levels of each group with the SG group using one-way ANOVA and post-hoc Tamhane range test.

*Statistically significant value defined as p<0.001. All values are expressed as the mean ± standard error of the measurement.

MDA: Malondialdehyde, NO: Nitric oxide, GSH: Glutation, CAT: Catalase, HG: Healthy group, SG: Sepsis group, TG: Thiamine group, TSG: Sepsis + thiamine pyrophosphate group

Table 2. The pro-inflammatory agents and lactate levels among rat groups

	PCT ng/mL	TNF-α pg/mL	IL-1β pg/mL	Lactate U/L
HG n=8	35.25±2.94*	36.52±1.45*	49.58±3.10*	22.65±1.08*
SG n=8	107.62±4.33	116.86±7.20	140.18±11.14	90.38±2.83
TG n=8	33.62±3.76*	35.80±0.95*	46.42±2.50*	23.06±1.31*
TSG n=8	47.14±7.63*	47.81±3.18*	62.00±2.70*	26.35±1.26*

*Statistically significant value defined as p<0.001. All data are defined as the mean ± standard error of the measurement. All values were compared with SG group based on one-way ANOVA and post-hoc Tukey's range test.

PCT: Procalcitonin, TNF-α: Tumor necrosis factor alpha, IL-1β: Interleukin 1 beta, HG: Healthy group, SG: Sepsis group, TG: Thiamine pyrophosphate (TP) group, TSG: Sepsis + TP group

in rat sepsis model. We also determined to compare the efficacy of thiamine on oxidant/antioxidant parameters in kidney tissue. Furthermore, we tried to reveal the thiamine-related interactions on inflammatory parameters of sepsis and lactate level changes in LPS-induced septic rats.

In sAKI; there is an imbalance between oxidative host parameters like ROS/RNS and antioxidant defense mechanisms in the favor of oxidative parameters (4).

NO is a key molecule in the intersection point of oxidative stress, inflammation and vascular injury in sepsis (6). By the inflammatory mediators and proinflammatory cytokines, iNOS expression is increased. Consequently, iNOS leads the production of NO causing the oxidative stress in sAKI (21).

Besides, lipid peroxidation leads to a molecular cell damage. MDA is the last product of lipid production pathway. As an oxidative stress marker, MDA levels are elevated in LPS-induced sAKI patients (22).

As consistent, we have revealed a significant establishment of oxidative stress in our sepsis model. On the other hand, there was a significant decrease in MDA and NO levels in TSG group, demonstrating the renal antioxidant efficacy of thiamine.

GSH has a key role in the protection against septic oxidative stress. GSH may convert peroxides and ROS to innocuous compounds and mends oxidative tissue damage (12). In thiamine deficiency, pentose phosphate pathway may malfunction leading reduction in nicotinamide adenine dinucleotide phosphate (NADPH) production resulting in decreased GSH production (9).

CAT is an enzymic antioxidant protecting tissues from lipid peroxidation. Hence, the reduction of CAT levels in tissues may result in the accumulation of ROS (12). In a sAKI mice model, the prominent decrease of CAT levels has been revealed (23). In our study, we demonstrated the antioxidant efficacy of thiamine.

It is well known that one of the main pillars of sepsis is inflammatory host response (7). In renal tissues of septic mice, the marked elevation of TNF-α and IL-1β has been revealed (23). Increasing oxidative stimuli activates leucocytes more to generate a cytokine storm which may lead the overwhelming production of ROS. This inflammatory cascade plays a critical role in propagating the oxidative stress in sepsis causing negative effects on renal microcirculation (4).

An early marker of sepsis is PCT. PCT may also be used to predict the development of AKI in sepsis (24). As consistent, in our SG group serum PCT levels were significantly elevated, accompanied by a 3-4-fold

increase in urea and Cr levels, when compared with HG.

Mitochondrial dysfunction appears in early sepsis, determining survival (13,14). In sepsis cellular mitochondrial dysfunction prevents oxygen utilization and shifts the pathway to anaerobic metabolism. In thiamine deficiency accompanying sepsis, pyruvate is transformed to lactate leading to lactic acidosis and a deficit of ATP generation (25). This point of view opened a new era in septic organ injury treatment modalities called 'metabolic resuscitation'. Metabolic resuscitation focuses on augmenting oxygen utilization and properly maintenance of the redox balance (5). In this setting, thiamine has gained attention in sAKI with its pivotal role in mitochondrial respiration and cellular redox as a metabolic resuscitator.

High lactate levels are representative of tissue hypoperfusion and cellular hypoxia. Increased lactate levels are known to associate higher mortality in sepsis (25). Donnino et al. (10) revealed the high prevalence of thiamine deficiency in critically ill septic patients, and they also reported a negative correlation between lactic acidosis and thiamine levels. As consistent with literature, we found nearly 4-fold increase in lactate levels in SG.

In summary, despite early treatment goals and quick action plans mortality and sepsis induced organ failures still stands at high rates. Pathophysiologically, sepsis is a highly heterogenous clinical syndrome therefore, its treatment requires a multidimensional approach. Thiamine is a very important vitamin with powerful and beneficial effects on multiple metabolic steps. Thiamine acts like an antioxidant by preventing lipid peroxidation and propagates GSH production acting as a cofactor of NADPH production. Some thiamine dependent enzymatic pathways play a role in reducing intracellular ROS production and allowing the generation of antioxidants. Additionally, thiamine has key roles in the maintenance of aerobic respiration and cellular energy production. Owing to all the features of thiamine, we think that it has a pivotal role in sAKI.

Conclusion

We revealed the preventive efficacy of thiamine against sAKI by reducing the oxidant parameters and rising the antioxidant parameter levels with the attainment of near to normal kidney functions, simultaneously. Consequently, thiamine is a cheap, economic and readily available drug. It has a very good safety profile in adults with no significant side effects in long term. With this excellent profile thiamine looks like a tempting adjunctive therapy for sAKI.

Ethics

Ethics Committee Approval: Study has been approved by Animal Experiments Local Ethical Commission of Aydın Adnan Menderes University, Medical Faculty (approval number: 64583101/2016/49, date: 25.02.2016).

Informed Consent: Informed consent is not required.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: H.B.U., B.D., Concept: H.B.U., B.D., Design: H.B.U., B.D., Data Collection or Processing: H.B.U., M.Y., İ.K.Ö., Analysis or Interpretation: H.B.U., M.Y., İ.K.Ö., Literature Search: H.B.U., B.D., Writing: H.B.U., B.D.

Conflict of Interest: No conflict of interest was declared by the authors.

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Original Article / Özgün Araştırma

Assessment of Arterial Stiffness in Hemodialysis Patients, Using Speckle Tracking Carotid Strain Ultrasonography

Benek İzleme Karotis Gerilme Ultrasonografisini Kullanarak Arteriyel Sertliğin Hemodializ Hastalarında Değerlendirilmesi

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Keywords
Atherosclerosis, cardiovascular disease, carotid artery, end-stage renal disease, speckle tracking carotid strain, ultrasonography

Anahtar Kelimeler
Ateroskleroz, kardiovasküler hastalık, karotis arter, son dönem böbrek yetmezliği, benek izleme karotis gerilme, ultrasonografi

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Abstract

Objective: The newly introduced ultrasonographic based speckle tracking carotid strain (STCS) imaging method is a promising technique that enables cardiovascular risk assessment by measuring arterial stiffness and strain parameters in carotid arteries. The present study evaluates the application of this technique in an end-stage renal disease (ESRD) patient group.

Materials and Methods: In total, 100 patients (50 ESRD, 50 controls) were included in this study. Arterial stiffness and strain parameters were measured in the longitudinal and axial planes from both carotid arteries with the STCS technique. The measurements were analyzed between the groups and a multiple regression model was implemented to identify independent predictors of carotid stiffness and strain parameters.

Results: The study found a significant difference between ESRD and the control group in β-stiffness index, arterial distensibility (AD), elastic modulus and pulse wave velocity both in longitudinal and axial plane for stiffness parameters. Strain parameters for radial and circumferential strain parameters for both planes were significantly different between the two groups. In the multivariate linear regression analyse, estimated glomerular filtration rate was positively and independently associated with both axial and longitudinal AD and arterial compliance (all $p<0.05$).

Conclusion: The study demonstrated the feasibility and clinical value of the STCS method in the assessment of vascular stiffness, and its potential use as a tool in cardiovascular risk assessment in an ESRD patient group.

Öz

Amaç: Ultrasonografi zemininde geliştirilen yeni bir teknik olan benek izleme karotis gerilme (BİKG) görüntüleme yöntemi, karotis arterlerdeki arter sertliği ve gerilme parametrelerini ölçerek kardiyovasküler risk değerlendirmesini sağlayan umut verici bir tekniktir. Bu çalışma, bu tekniğin son dönem böbrek yetmezliği (SDBY) hasta grubunda uygulanabilirliğini değerlendirmektedir.

Gereç ve Yöntemler: Bu çalışmaya toplam 100 olgu (50 SDBY, 50 kontrol) dahil edildi. Arter sertliği ve gerinim parametreleri, BİKG tekniği ile her iki karotis arterden uzun aks ve aksiyal planda ölçüldü. Bu ölçümler gruplar arasında analiz edildi ve ayrıca karotis sertliği ve gerinim parametrelerinin bağımsız öngörücülerini belirlemek için çoklu regresyon modeli uygulandı.

Bulgular: Sertlik parametreleri için hem uzun aksta hem de aksiyal düzlemde β-sertlik indeksi, arteriyel esneyebilirlik, elastik modül ve nabız dalga hızında SDBY

ve kontrol grubu arasında anlamlı fark vardı. Her iki düzlem için radyal ve çevresel gerinim parametreleri iki grup arasında önemli ölçüde farklıydı. Çok değişkenli lineer regresyon analizinde, tahmini glomerüler filtrasyon hızı, hem aksiyal hem de uzun aksta arteriyel genişleyebilirlik ve arteriyel uyum ile pozitif ve bağımsız olarak ilişkiliydi (tümü p<0,05).
Sonuç: Çalışma, SDBY'li hastalarda vasküler sertliğin değerlendirilmesinde BİKG yönteminin uygulanabilirliğini ve klinik değerini göstermiştir ve bu teknik bu hasta grubunda kardiyovasküler risk değerlendirmesinde kullanılabilecek potansiyele sahip bir yöntemdir.

Introduction

End-stage renal disease (ESRD) is a serious public health issue that causes premature deaths. Half of the deaths in ESRD patients are caused by cardiovascular diseases, at a rate 20 times higher than the normal population (1).

Carotid artery system is an important indicator of the atherosclerotic status of the whole body (2). Standard carotid ultrasonograpy (USG) is an imaging modality that allows us to evaluate morphological changes in atherosclerosis such as carotid intima-media thickness (CIMT) and carotid plaque. Whereas speckle tracking carotid strain (STCS) USG is a newly introduced USG tecnique that allows us to measure not only morphological information but also functional information about the carotid system by measuring stiffness and strain parameters (3,4). These functional changes occur before the morphological changes (5). These changes, allowing for earlier detection of cardiovascular disease, especially for patients at risk (6,7).

Arterial analysis software using the STCS method measures differences in arterial diameter generated by each heartbeat. With the diameter and pressure measurements, variables related to arterial stiffness such as arterial compliance (AC), arterial distensibility (AD), β-stiffness index (β-SI), elastic modulus (EM), and pulse wave velocity (PWV) and variables related to arterial strain such as displacement, strain and strain rate can be calculated. The literature indicates that the STCS method can identify the changes in arterial stiffness parameters due to increased cardiovascular risk at a subclinical level in different patient groups (5,6,8-12).

Measurement of arterial stiffness and strain parameters with the STCS technique helps in the early detection of potential cardiovascular incidents. The present study evaluates the application of this technique in an ESRD patient group.

Materials and Methods

Study Population

A total of 100 subjects (50 ESRD who received hemodialysis and 50 controls) who underwent a carotid USG examination between 1 April 2021 to 31 May 2022 were included in the study. Both groups were paired according to age, gender and body mass index (BMI) (Table 1). Patients with severe common carotid artery (CCA) stenosis (>70%), a history of intervention to the CCA or to the neck region were excluded from the study. Approval from the Institutional Ethics Committee of Aydın Adnan Menderes University was provided (protocol no: 2022/78, date: 28.04.2022) with informed consent obtained from those included in the study.

Calculation of Stiffness and Strain Parameters

Carotid USG was performed with a Samsung RS80 USG device using a L3-12A (Samsung Medison Co., Ltd. Seoul, Korea) linear array transducer. The arterial analysis software programme (Samsung Medison Co., Ltd., Seoul, Korea) was applied to measure strain and stiffness parameters (Figure 1). CCA displacement was automatically calculated to evaluate the functional capabilities. The segment 5-10 mm beneath the carotid bulb was analyzed. Control points on the CCA were determined by the user. The frame created by the control points was set to automatically follow the optical flow algorithm.

Before the USG examination, subjects were rested in the supine position, in dim lighting and at ideal room temperature for a minimum of 10 minutes. Then the systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured with pulse wave analysis of the brachial artery using a Reister sphygmomanometer (Reister 1312 minimus II, Rudolf Riester GmbH, Jungingen Germany). The SBP and DBP were entered into the software. Two different planes (longitudinal and axial) used for each CCA were performed. The mean value of each plane measurements were noted for right and left CCA. In each plane, all stiffness parameters and radial strain parameters were measured by the software. The circumferential strain parameters for the axial plane were also calculated (Figure 1a, b).

The mean value of the CIMT was calculated with the same software for each CCA in the longitudinal

plane. Both the anterior and posterior wall interfaces that define the blood-intima boundaries in the carotid artery (minimum 5 spots in all) were selected on a still image, then the movement of the points were automatically monitored by the software (Figure 1c, d).

Statistical Analysis

The Statistical Package for Social Science version 21.0 was applied for the statistical processing of the data (International Business Machine Corporation, Armonk, NY, USA). Descriptive statistics were presented as numbers, percentages, and mean ± standard deviation values. The conformity of continuous variables to normal distribution was evaluated with descriptive statistics, kurtosis and skewness coefficients, histogram, and Shapiro-Wilk test. For categorical data a chi-square test was used. Where data had normal distribution a t-test was used, but otherwise the Mann-Whitney U test was used for comparing two independent groups. To identify the independent predictors of CCA stiffness and strain parameters, a multiple regression model was implemented. After adjusting for BMI, presence of diabetes mellitus (DM) and presence of hypertension (HT), the impact of estimated glomerular filtration rate (eGFR) on stiffness and strain parameters was given. The model fit was evaluated using proper residual and goodness-of-fit statistics. The type 1 error level was determined as 0.05.

Results

Baseline Characteristics

A total of 50 control subjects (56.8±15.5 years, 60% male, 40% female) and 50 ESRD (57.2 years ±15.9, 60% male, 40% female) were included in the study (Table 1). Both groups were matched in terms of gender and age. In terms of the biochemical data in blood, eGFR, total cholesterol and low-density lipoprotein in the ESRD group was lower, while creatinine, blood urea nitrogen and triglyceride levels were higher than the normal group (Table 1).

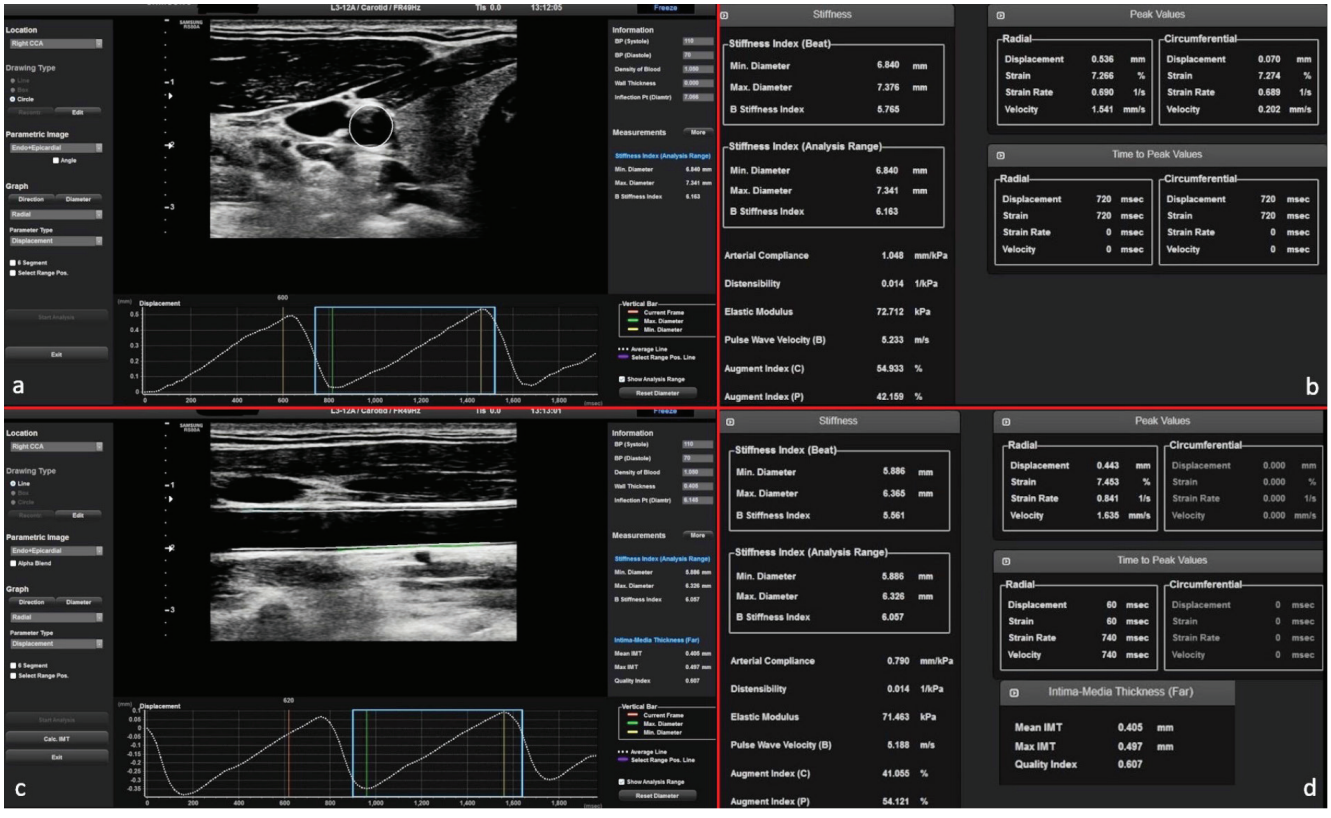

Figure 1. Evaluation of the right CCA in normal patient in (a) axial plane with related (b) stiffness and strain parameters report page, in (c) longitudinal plane with related (d) CIMT, stiffness and strain parameters report page
CCA: Common carotid artery, CIMT: Carotis media thickness

Carotid Stiffness and Strain Findings

Strain examination was successfully performed on all participants. In the evaluation of the stiffness parameters in the longitudinal plane (Table 2); while there was a significant increase in β-SI, EM, PWV parameters, in AD parameter there was a significant decrease in the ESRD group compared to the control group (all p<0.05). With only the AC stiffness parameter, no significant difference was observed between the groups. In the radial strain parameters in the longitudinal plane, only with the strain parameter there was a significant decrease in the ESRD group (p=0.014), while there was no difference between the two groups with other strain parameters (Table 2).

The results in the axial plane are consistent with the results in the longitudinal plane (Table 3). A statistical difference was observed between the ESRD and control group in all of the stiffness parameters in the axial plane. A significant increase was observed in β-SI, EM and PWV stiffness parameters, on the other hand there was a significant decrease in AC and AD stiffness parameters in ESRD group (all p<0.001). In the radial strain parameters in the axial plane, as in the longitudinal plane, there was a significant decrease in ESRD group in the strain parameter (p=0.001), while there was no difference with displacement and strain rate parameters. In the axial circumferential strain parameters, there was a significant decrease in displacement and strain parameters in ESRD group (all p<0.05) and no difference was observed for the strain rate parameter (Table 3).

In the multivariate linear regression analysis adjusted for BMI, the presence of DM, HT, and eGFR

Table 2. Comparison of the parameters in the longitudinal plane between the groups

	Control (n=50)	ESRD patients (n=50)	p-value
Right Mean CIMT* (mm)	0.67±0.2	0.75±0.26	0.095
Left Mean CIMT* (mm)	0.68±0.21	0.82±0.29	0.013
β-SI*	6.67±1.96	9.44±4.46	0.001
AC* (mm/kPa)	0.79±0.35	0.67±0.31	0.071
AD* (/kPa)	0.0135±0.0053	0.0097±0.0053	<0.001
EM* (kPa)	85.58±29.35	131.51±62.12	<0.001
PWV* (m/s)	5.58±0.93	6.72±1.51	<0.001
Strain parameters (radial)			
D** (mm)	0.46±0.13	0.45±0.18	0.459
S** (%)	7.35±1.92	6.66±3.01	0.014
SR** (1/s)	0.75±0.22	0.94±0.69	0.158

ESRD: End stage renal disease, *CIMT: Carotis media thickness, β-SI: β-stiffness index, AC: Arterial compliance, AD: Arterial distensibility, PWV: Pulse wave velocity, EM: Elastic modulus, **D: Displacement, S: Strain, SR: Strain rate

Table 1. Baseline characteristics and laboratory works of both groups

	Control (n=50)	ESRD (n=50)	p-value
Age (years)	56.8±15.5	57.2±15.9	0.904
Sex (male)	30 (60%)	30 (60%)	1
Sex (female)	20 (40%)	20 (40%)	1
Central systolic blood pressure (mmHg)	118.9±16.5	134.5±23	<0.001
Central diastolic blood pressure (mmHg)	75.4±11.1	81±16.8	0.064
Body mass index (kg/m^2)	26±4.3	25.1±5.4	0.186
Hypertension	15 (30%)	26 (52%)	0.025
Diabetes mellitus	8 (16%)	22 (44%)	0.002
eGFR (mL/min/1.73 m^2)	94.1±13.2	6.7±1.9	<0.001
Creatinine (mg/dL)	0.8±0.1	7.8±2.1	<0.001
Blood urea nitrogen (mg/dL)	15.3±11	51.5±15.6	<0.001
Total cholesterol (mg/dL)	203.8±42.1	167.9±47.6	<0.001
High-density lipoprotein (mg/dL)	54.4±15.9	49.5±16.3	0.076
Low-density lipoprotein (mg/dL)	124.8±36.4	87.4±37.5	<0.001
Triglyceride (mg/dL)	124.1±58.9	155.3±84.5	0.019

eGFR: Estimated glomerular filtration rate, ESRD: End stage renal disease

was negatively and independently associated with both axial and longitudinal β-SI, EM and PWV. In the multivariate linear regression analysis adjusted for BMI, the presence of DM, HT, and eGFR was positively and independently associated with both axial and longitudinal AD and axial AC (all p<0.05). From the strain parameters perspective, eGFR was positively and independently associated with axial radial displacement, axial radial strain and axial circumferential strain, and also was only negatively and independently associated with longitudinal radial strain rate (all p<0.05). On the other hand, eGFR had no impact on one stiffness parameter (longitudinal AC) and on most of the strain parameters (all p>0.05) (Table 4).

Discussion

The STCS technique was successfully performed on all participants of the present study. Both groups were compared by evaluating the stiffness and strain parameters of CCAs. A significant difference was observed between the groups in all stiffness parameters in both the longitudinal and axial planes except AC in the longitudinal plane. A significant difference was also observed in terms of the strain parameter in the longitudinal plane, displacement and strain parameters in the axial plane.

Vascular remodeling characterized by increased vascular stiffness was observed in patients with ESRD. This remodeling process also affects the elastic properties of the vascular structure (13,14). Increase in fibroelastic intimal thickness, calcification in vascular walls, inflammation and increased collagen cause changes in intrinsic elastic properties in vascular walls and this causes an increase in stiffness in the CCA of ESRD patients (15,16).

To the author's knowledge, there is only one study in the literature by Lee et al. (13) in which arterial stiffness of the CCA was evaluated using the STCS method in patients with ESRD. With respect to stiffness parameters, Lee et al. (13) observed statistically significant differences in AD, EM and PWV parameters (all p<0.005). In terms of strain parameters, Lee et al. (13) observed that there was a significant decrease in the strain ratio in both the longitudinal and axial planes in the ESRD group. As a result, it has been determined that the STCS method is much more valuable than the conventional aortic stiffness indices in cardiovascular risk classification in the ESRD patient group (13). The present study returned similar results to Lee et al. (13). These results show that many stiffness parameters (especially AD, EM and PWV) and some strain parameters (strain and strain ratio) are signifincantly different between the two groups and provide valuable information in cardiovascular risk classification of ESRD patients.

In order to support and reinforce this result, we performed a multivariate analysis evaluation.

Table 3. Comparison of the parameters in the axial plane between the groups

	Control (n=50)	ESRD patients (n=50)	p-value
β-SI*	6.97±1.82	11.03±5.64	<0.001
AC* (mm/kPa)	0.94±0.37	0.67±0.29	<0.001
AD* (/kPa)	0.0126±0.0047	0.0088±0.0049	<0.001
EM* (kPa)	89.44±27.02	150.18±74.08	<0.001
PWV* (m/s)	5.71±0.86	7.15±1.69	<0.001
Strain parameters (radial)			
D** (mm)	0.48±0.12	0.41±0.17	0.006
S** (%)	6.93±1.68	5.80±2.66	0.001
SR** (1/s)	0.76±0.24	0.77±0.51	0.145
Strain parameters (circumferential)			
D** (mm)	0.063±0.016	0.058±0.035	0.018
S** (%)	6.91±1.67	5.76±2.62	0.001
SR** (1/s)	0.75±0.24	0.77±0.51	0.135

ESRD: End stage renal disease, *β-SI: β-stiffness index, AC: Arterial compliance, AD: Arterial distensibility, PWV: Pulse wave velocity, EM: Elastic modulus, **D: Displacement, S: Strain, SR: Strain rate

Table 4. Multivariate linear regression analysis*

Parameters	β	95% CI	SE	p
Axial β-SI**	-0.316	-0.164 to -0.043	0.031	0.001
Axial AC**	0.359	0.053 to 0.175	0.031	<0.001
Axial AD**	0.30	0.056 to 0.176	0.030	<0.001
Axial EM**	-0.349	-0.182 to 0.063	0.030	<0.001
Axial PWV**	-0.330	-0.084 to 0.027	0.014	<0.001
Longitudinal β-SI**	-0.260	-0.142 to -0.020	0.031	0.009
Longitudinal AC**	0.153	-0.017 to 0.114	0.033	0.147
Longitudinal AD**	0.316	0.046 to 0.170	0.031	0.001
Longitudinal EM**	-0.312	-0.167 to -0.045	0.031	0.001
Longitudinal PWV**	-0.297	-0.077 to -0.019	0.015	0.002
Axial Radial D**	0.211	0.002 to 0.108	0.027	0.044
Axial Radial S**	0.205	0.003 to 0.108	0.026	0.039
Axial Radial SR**	0.002	-0.063 to 0.064	0.032	0.984
Axial Circumferential D**	0.183	-0.008 to 0.111	0.030	0.088
Axial Circumferential S**	0.209	0.004 to 0.108	0.026	0.036
Axial Circumferential SR**	-0.002	-0.064 to 0.062	0.032	0.985
Longitudinal Radial D**	-0.004	-0.052 to 0.051	0.026	0.974
Longitudinal Radial S**	-0.124	-0.020 to 0.081	0.025	0.235
Longitudinal Radial SR**	-0.222	-0.128 to -0.004	0.031	0.036

*Adjusted for body mass index, presence of diabetes mellitus, presence of hypertension, CI: Confidence interval, ESRD: End stage renal disease, SE: Standart error, **β-SI: Stiffness index, AC: Arterial compliance, AD: Arterial distensibility, PWV: Pulse wave velocity, D: Displacement, S: Strain, SR: Strain rate

In the analysis performed after correction of BMI, presence of DM and presence of HT, especially since the two groups were not homogeneous, all stiffness parameters except for longitudinal AC, were negatively or positively correlated with eGFR independently. Only axial radial displacement, axial radial strain, axial circumferential strain, and longitudinal radial strain rate parameters were found to be negatively or positively correlated with eGFR from a strain parameters perspective. Multivariate analysis showed that even after correcting for the secondary differences of the groups, the relationship between the depth of the ESRD and especially the stiffness parameters showed continuity.

The limitations of the study include the inability to evaluate the prognostic value of CCA strain parameters on clinical outcomes due to the cross-sectional nature of the study. Since measurements were made only once by a single radiologist, no intra- and inter-observer variability evaluation could be made. Further limitations of the study include the fact that STCS values are affected by smoking, age, hyperlipidemia, DM and other metabolic diseases, and the lack of clinical homogeneity between the groups in this respect.

Conclusion

The study demonstrated the feasibility and clinical value of the STCS method in the assessment of vascular stiffness in patients with ESRD. STCS is a method that has the potential to offer benefits in cardiovascular risk assessment and even in cardiovascular risk control in an ESRD patient group. Further multicenter randomized studies with a higher number of patients in this patient group will make significant contributions to the literature in this area.

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Ethics

Ethics Committee Approval: Institutional Ethics Committee of Aydın Adnan Menderes University

was provided (protocol no: 2022/78, date: 28.04.2022).

Informed Consent: Informed consent was obtained from those included in the study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: M.G., H.A., Concept: M.G., Z.G.A., Y.Y., Design: M.G., H.A., Y.Y., Data Collection or Processing: M.G., Z.G.A., Analysis or Interpretation: M.G., G.T., Y.Y., Literature Search: M.G., Y.Y., Writing: M.G., Y.Y.

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Original Article / Özgün Araştırma

Comparison of Fracture Strength of Modified PEEK, Nanohybrid Ceramic, Monolithic Zirconium Endocrowns Produced with CAD/CAM System

CAD/CAM Sistemi ile Üretilen Modifiye PEEK, Nanohibrit Seramik, Monolitik Zirkonyum Endokronların Kırılma Dayanımlarının Karşılaştırılması

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Abstract

Objective: The aim of this study; comparison of fracture strength of monolithic zirconium, nanohybrid ceramic and modified polyetheretherketone (PEEK) endocrowns produced with computer-aided design/computer-aided manufacturing (CAD/CAM) system.

Materials and Methods: Thirty permanent human first molar teeth of identical anatomical size were collected. After root canal treatment and endocrown preparation were applied to the teeth, they were divided into three groups (n=10). PEEK coping, nanohybrid ceramic and monolithic zirconium, endocrowns were produced by digital methods for each group. The infrastructure of the PEEK group was completed with 1 mm composite to be the same size as the other groups. Cement gap was determined as 100 µm. After cementation of the endocrowns, the specimens were placed in a chewing simulator, equivalent to 6 months of clinical use. For the fracture strength test, the specimens placed on the universal test device were loaded with a head speed of 1 mm/minute until they broke and the specimens were examined under a stereomicroscope to determine the failure type. Statistical analysis of test data was performed.

Results: The highest mean fracture strength of the monolithic zirconium endocrown group (2496.5±189.12 N), secondly, modified peek endocrown group (1728.2±139.26 N) and nanohybrid ceramic endocrown group (1248.8±107.6 N) were determined as the lowest. A statistically significant difference was found between the groups in terms of fracture strength ($p<0.05$).

Conclusion: Modified peek, nanohybrid ceramic, monolithic zirconium endocrowns can be an effective option for the restoration of root canal treated molar teeth with excessive material loss.

Öz

Amaç: Bu çalışmanın amacı bilgisayar destekli tasarım/bilgisayar destekli üretim (CAD/CAM) sistemi ile üretilen monolitik zirkonyum, nanohibrit seramik ve modifiye polietereterketon (PEEK) endokronların kırılma dayanımlarının karşılaştırılmasıdır.

Gereç ve Yöntemler: Anatomik boyutları özdeş olan 30 adet daimi 1. molar insan

dişi toplandı. Dişlere kanal tedavisi ve endokron preparasyonu uygulandıktan sonra üç gruba (n=10) ayrıldı. PEEK coping, nanohibrid seramik ve monolitik zirkonyum ve endokronlar her grup için dijital yöntemlerle üretildi. PEEK grubunun alt yapısı diğer gruplarla aynı boyutta olacak şekilde 1 mm kompozitle tamamlandı. Siman aralığı 100 µm olarak belirlendi. Endokronların simantasyonu sonrasında örnekler 6 ay klinik kullanıma eş değer olacak şekilde çiğneme simülatörüne yerleştirildi. Kırılma dayanımı testi için universal test cihazına yerleştirilen örneklere kırılıncaya dek 1 mm/dakika kafa hızıyla yükleme gerçekleştirildi ve numuneler, başarısızlık tipini belirlemek için bir ışık mikroskobu altında incelendi. Test verilerinin istatistiksel analizi yapıldı.
Bulgular: En yüksek kırılma dayanımı ortalaması monolitik zirkonyum endokron grubunun (2496,5±189,1 N), ikinci olarak modifiye peek endokron grubunun (1728.2±139.2 N) ve en düşük olarak nanohibrit seramik endokron grubunun (1248.8±107,6 N) belirlenmiştir. Gruplar arasında kırılma dayanımı açısından istatistiksel olarak anlamlı derecede farklılık bulunmuştur (p<0,05).
Sonuç: Modifiye PEEK, nanohibrit seramik, monolitik zirkonyum endokronlar aşırı madde kaybı olan kanal tedavili molar dişlerin restorasyonu için etkili bir seçenek olabilir.

Introduction

The structure of the endodontically treated tooth is weakened as a result of previous caries, fractures and treatment attempts. In addition, endodontic treatment causes the removal of intracoronal and intraradicular tooth structures. As a result of all these endodontic interventions, the fragility of the tooth increases (1). Traditionally, the restoration of endodontically treated teeth is provided by full-crown restorations applied on the intracanal post-core. However, the biomechanical properties of residual dental tissues change during conventional treatment. Complications such as perforation and fracture in the root during post treatment have led to the search for alternative methods (2).

As a result of a 10-year clinical study on endocrown restorations, they stated that the success rate of endocrown restorations is high in teeth with excessive substance loss and in individuals with parafunctional habits. Clinicians have reported that endocrown restorations can be applied as an alternative to traditional fiber-post restorations (3). Endocrown restorations should be especially preferred in cases with different morphology or in angled and calcified canals, in teeth with insufficient clinical crown length, narrow interocclusal distance, large loss of material, and in cases where there is not enough ferrule (4).

The first definition of endocrown was made by Bindl and Mörmann (5) in 1999. These researchers defined endocrown as adhesive restorations consisting of a central retention cavity covering the pulp chamber with a circumferential 90° butt margin, applied to root canal treated teeth with excessive substance loss. The endocrown preparation consists of a 1.0-1.2 mm peripheral butt margin and a central retention cavity extending into the pulp chamber. Endocrown restorations are monoblock structures with core and crown that do not receive support from the root canal cavity (5). The butt margin design allows the prevention of microleakage at the restoration-tooth interface, the prevention of shear stresses, and the preservation of the peripheral enamel layer around the margin (6).

Materials and Methods

This study was carried out in Fırat University Faculty of Dentistry, Department of Prosthetic Dentistry, with the 06.02.2020 decision dated and 2020/03-06 numbered, taken from the Fırat University Non-Interventional Research Ethics Committee. In the study, 30 mandibular first molar teeth with 7-8 mm cervico-occlusal distance and 13-15 mm root length, extracted for periodontal or orthodontic reasons, were used. Detertrage and ultrasonic cleaning processes were applied to remove tissue residues or dental calculus on the teeth. The coronal parts of the teeth were removed under water cooling with a steel separator 2 mm above the cemento-enamel junction. Then, root canal treatment was applied to all of the sample teeth in accordance with the standards.

After the root canal treatment procedures were completed, the root canal paste residues were cleaned. After the root canal entrances and canal mouth dentin were etched with acid, the 5th generation adhesive system Any-Bond (MD Clus, South Korea) was applied and left to act for 20 seconds. It was dried for 5 seconds with low air pressure and polymerized for 10 seconds with a light-emitting diode (LED) light device (Coxo, Guangdong, China). Then nanohybrid flowable composite (Grandio Flow, Cuxhaven, Germany) was applied to the canal entrances and pulp chamber and cured with LED light for 20 seconds. The teeth were embedded in acrylic resins (Imicryl; Konya, Turkey)

poured into polyvinyl chloride pipes with a diameter of 3 cm and a height of 5 cm, with the long axis of the roots perpendicular to the ground and 2 mm below the enamel-cementum composition (representing the bone level). The teeth were kept in distilled water for 1 week to harden the endodontic canal sealer.

Standard endocrown preparations were performed by a single operator. In order to ensure standardization, the cavity depth was measured with the aid of a periodontal probe and determined as 4 mm. The pulp chamber floor was prepared flat. Cavity inner edge angles were finished as a butt margin with an angle of 90°. The preparation was carried out with a cavity wall thickness of 2 mm (Figure 1).

Digital impressions of the samples were taken in the laboratory with an extraoral scanner (Dwos 3 Series, Dental Wings Inc, Montreal, Canada). The surface of the samples was coated with titanium dioxide powder [Dr. Mat Dental computer-aided design/computer-aided manufacturing (CAD/CAM) White Scan Spray, İstanbul, Turkey] to eliminate light reflections that may occur during impression taking. Polyetheretherketone (Peek) endocrowns were designed with 6 mm to be veneered with 1 mm composite later, endocrowns in the other group 7 mm cervico-occlusal height with the CAD program (DWOS software, Dental Wings Inc, Montreal, Canada). Cement gap was determined as 100 µm. The inner surfaces of the samples, which were adapted to the teeth after being produced in a milling device (K5; vhf camfacture AG, Germany), were roughened with Al_2O_3 powder in a sandblasting device (Rotaks, İstanbul, Turkey) before cementation.

After the production of monolithic zirconium endocrowns was completed from blocks (KATANA UTML Zirconia, Kuraray Noritake INC., Okayama, Japan), they were sintered in a sintering furnace (Protherm Furnaces, İstanbul, Turkey) for 1.5 hours at 1,550 °C. Endocrowns fabricated using nanohybrid ceramic blocks (Cerasmart, GC Corp., Tokyo, Japan) were finished with silicone discs and polishing paste (Diapolisher GC Corp., Tokyo, Japan) according to the manufacturer's instructions. Visio.Link (Bredent, Germany) was applied to the upper surface of the substructures produced from PEEK block (JUVORA, Juvora Ltd. Thornton Cleveleys, Lancastershire, England) with a small brush and cured for 120 seconds in the polymerization device (Labolight DUO, GC, Europe). Thanks to the transparency of SX plates, manipulation and polymerization can be facilitated, and thus homogeneous and smooth composite veneering process can be performed with the same size as other samples. Indirect composite resin (Crea.lign, Bredent, Germany) was applied to the adhesive-applied PEEK substructure surface using the previously prepared SX plaque index and 30 seconds prepolymerization was carried out. Final polymerization was carried out in the device for 4.5 minutes.

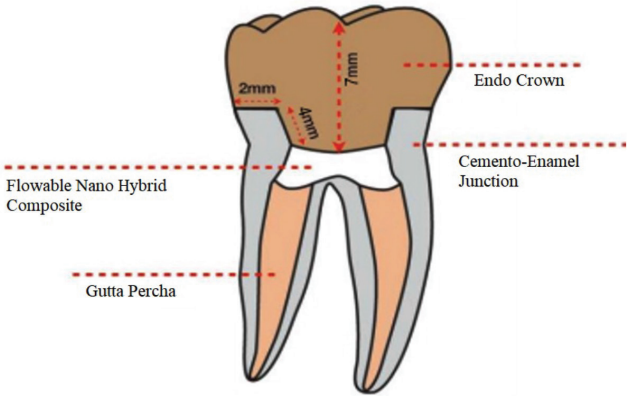

Figure 1. Endocrown preparation design

In order to ensure standardization, 50 N force was applied to the restorations for 60 seconds with a dynamometer (Algol, Japan), and the overflow resin cements (Panavia F 2.0, Kuraray Noritake INC. Okayama, Japan) were removed. Then, polymerization was performed on each surface for 20 seconds by means of a LED light device, and cementation was completed. Endocrown restorations belonging to 3 different groups were loaded into the dual axis chewing simulator (Esetron Smart Robotechnologies, Mod Dental, Ankara, Turkey) (Table 1). In the chewing simulator, 120,000 cycles were applied to the samples, corresponding to 6 months of clinical use. After the aging process, the specimens were tested for fracture strength by means of a universal test device (Instron device 3345, Norwood, MA, USA). A spherical steel tip with a diameter of five millimeters is positioned on the occlusal surface (central fossa) of the restorations. The force (N) values applied until the samples were broken along the long axis of the tooth with a head speed of 1 mm/min were recorded. The failure mode of each specimen was evaluated by observation under the stereomicroscope, 10X magnification (Leica MZ 12; Leica Microsystems GmbH, Wetzlar, Germany).

Table 1. Study groups and sample numbers		
Groups	**n**	**Contents**
Group 1	10	Endocrowns produced by CAD/CAM from monolithic zirconium blocks
Group 2	10	Endocrowns fabricated by CAD/CAM from nanohybrid ceramic blocks
Group 3	10	Modified PEEK endocrowns obtained by veneering of the substructures produced with CAD/CAM from PEEK blocks with composite
TOTAL	30	
CAD/CAM: Computer-aided design/computer-aided manufacturing, PEEK: Polyetheretherketone		

Burke classification was used while performing the fracture type analysis (7):

Type 1: Fracture occurring only in endocrown restoration.

Type 2: Fracture involving a small tooth fragment with endocrown restoration.

Type 3: Fracture involving more than half of the tooth with endocrown restoration (above the enamel-cementum border).

Type 4: Fractures below the cemento-enamel junction.

Statistical Analysis

IBM SPSS Statistics 22 program was used for statistical analysis. Parameters were suitable for normal distribution that determined by Kolmogorov-Smirnov and Shapiro-Wilks tests. The One-Way ANOVA test was used to compare the parameters between groups, and the Tukey HSD test was used to determine the group that caused the difference. Significance was evaluated at the $p<0.05$ level.

Results

The fracture strength results of the groups used in the study are summarized in Table 2. The average fracture strength of the monolithic zirconium endocrown group (2496.5±189.12 N) was statistically significantly higher than the modified PEEK endocrown (1728.2±139.26 N) and nanohybrid ceramic endocrown groups (1248.8±107.61 N) ($p1<0.001$; $p2:0.001$). The average fracture strength of the modified PEEK endocrown group was statistically significantly higher than the nanohybrid ceramic endocrown group ($p<0.001$) (Figure 2). No breakage was observed in any of the samples after the chewing simulator. Type 1 fracture (100%) in the entire modified PEEK endocrown group, type 1 fracture (70%) and type 2 fracture (30%) in the nanohybrid ceramic endocrown group, type 2 (10%), type 3 (40%) and type 4 (50%) fracture in the monolithic zirconium endocrown group were observed (Table 3).

Discussion

It is aimed that dental restorations are stable against the forces occurring during function, and it has

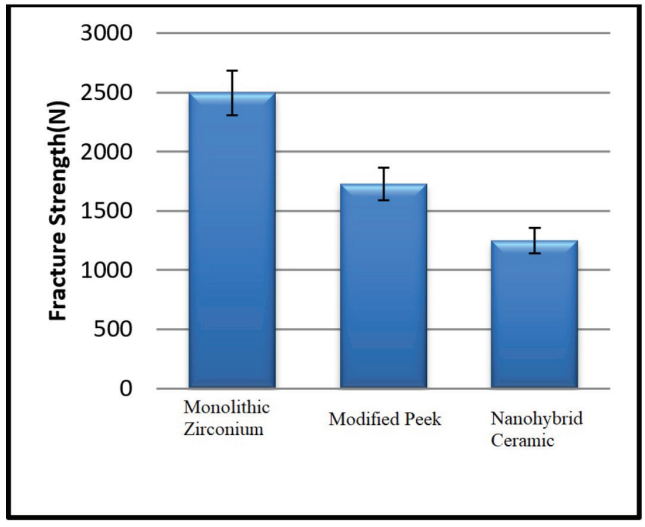

Figure 2. Average fracture strength graph of the groups

Table 2. Fracture strength values and statistical analysis results for the study groups		
Fracture strength (N)		
	Minimum-Maximum	**Mean ± SD**
Monolithic zirconium	2,152-2,792	2496.5±189.12
Modified PEEK	1,513-1,938	1728.2±139.26
Nanohybrid ceramic	1,073-1,437	1248.8±107.61
SD: Standard deviation		

Table 3. Percentage values of fracture types of endocrown groups				
	Type 1	**Type 2**	**Type 3**	**Type 4**
Modified PEEK	100%	0%	0%	0%
Nanohybrid ceramic	70%	30%	0%	0%
Monolithic zirconium	0%	10%	40%	50%
PEEK: Polyetheretherketone				

been reported in studies that the maximum bite force in the posterior region varies between 200-880 N (8). Kiliaridis et al. (9) that there is variation in maximum bite force between the sexes; reported this value as 807 N for men and 650 N for women. The fracture strengths of the CAD/CAM endocrown systems tested in our in vitro study were found to be well above the average of the maximum chewing forces.

In the clinical study of Tammam (10), it was reported that the endocrowns had a high durability rate (94.87%) as a result of 3-year follow-up of modified PEEK, monolithic zirconium and lithium disilicate endocrowns. In our study, in which we used monolithic zirconium and modified PEEK groups similarly, 120,000 cycles were applied in the simulator, equivalent to 6 months of clinical use, and no breakage was observed in any of the samples.

Rojpaibool and Leevailoj (11) investigated the effects of different resin cements on the fracture strength of lithium disilicate ceramics in the range of 100 µm and 300 µm cement gap. They reported that ceramics with 100 µm cement gap showed higher fracture strength in both resin cements. In our study, the cement gap of the endocrowns produced with CAD/CAM technology was designed to be 100 µm.

Elashmawy et al. (12), compared the fracture strength of monolithic zirconium, Vita Enamic, and veneered PEEK endocrowns. The highest fracture strength was found in monolithic zirconium endocrowns (1810.20±119.56 N) and the lowest value was found in veneered PEEK endocrowns (502.60±11.53 N). This difference was found to be statistically significant, similarly, in our study.

In their study, Al-Shibri and Elguindy (13) determined the fracture strength of Cerasmart endocrowns to be 1522.64±352.52 N, and Kassis et al. (14) 1300.53 N, Taha et al. (15) reported it as 1508.5± 421.7 N. In our study, the fracture strength of Cerasmart endocrowns was found close to these values (1248.8±107.61 N).

Beleidy and Ziada (16) compared the fracture strength of crown restorations produced from PEEK material using different veneering techniques. The fracture strength of PEEK crowns veneered with Crea. lign composite was found to be 1674 ±224.48 N. These values are similar to our study.

Shams et al. (17) investigated the endocrowns fracture strength of modified PEKK formed by veneering with IPS e.max CAD and IPS e.max CAD applied to extracted premolar teeth. In the study, the fracture strength value of the modified PEKK endocrowns was 1831.37±240.69 N, and it was found to be statistically significantly higher than the IPS e.max CAD endocrown. The fracture strength of the modified PEEK endocrowns in our study was 1728.2±139.26 N, which is close to Shams et al. (17) study.

Tartuk et al. (18) compared the fracture strength of monolithic zirconium, hybrid ceramic, PEEK crowns in their study. The fracture strength value of the monolithic zirconium group (3292±192 N) was found to be significantly higher than PEEK (2214±236 N) and hybrid ceramic (2325±264 N). However, no significant difference was found between PEEK and hybrid ceramics. In our study; a significant difference was detected between all groups. The difference between our study and Tartuk et al.'s (18) may be due to their working on crown restorations, using of PEEK without veneering in their study and preferring different block brands.

Elashmawy et al. (12) also evaluated the types of fracture in their study. Restorable fractures occurred in all veneered PEEK endocrowns. Irreversible fractures occurred in 80% of monolithic zirconium endocrowns. Similar results were obtained with our study in terms of failure types.

The elastic modulus of the materials used in our study is different from each other [dentin: 14.7 GPa, zirconium: 200 GPa, peek: 3-4 GPa (19), nanohybrid ceramic 12.16 GPa (20)]. If a material which has a higher elastic modulus compared to dentin is chosen, the restoration may become more rigid than the tooth structure. However, if a material close to the elastic modulus of dentin is chosen for the restoration, the restoration exhibits biomechanical behavior similar to tooth structure. As a result, the material used in the production of the endocrown affects the performance of the restoration (21). Although the modulus of elasticity values of nanohybrid ceramic and peek materials are close, the difference in the fracture strength test results in our study may be due to the use of peek structure with composite veneered (non-monolithic). The reason for the fracture type involving the tooth tissue in the monolithic zirconium group is also due to the fact that the elastic modulus of the

material is higher than the surrounding dentin tissue and it creates stress in this region.

Ghajghouj and Taşar-Faruk (22) noticed in their study that PEEK endocrown restorations provide reducing of the crack formation of tooth due to its low elastic modulus close to dentin tissue. They reported that peek is a material with sufficient fracture resistance for endocrown production. The results of our study also support this view.

Conclusion

Although monolithic zirconium endocrown restorations show high durability, they should not be preferred in patients with excessive chewing forces such as bruxism, due to their high modulus of elasticity compared to dentin.

Nanohybrid ceramic endocrowns can be used clinically safely due to their modulus of elasticity close to dentin. However, they have superior aesthetic properties.

In our study it was observed that the fracture strength values of endocrown groups produced with CAD/CAM technology were much higher than the average of the intraoral chewing forces. This shows that all of the endocrown groups in our study can resist intra-oral forces.

Ethics

Ethics Committee Approval: This study was carried out in Fırat University Faculty of Dentistry, Department of Prosthetic Dentistry, with the 06.02.2020 decision dated and 2020/03-06 numbered, taken from the Fırat University Non-Interventional Research Ethics Committee.

Informed Consent: Informed consent is not required.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: G.S.Ç., Concept: E.A., Design: G.S.Ç., Data Collection or Processing: G.S.Ç., Analysis or Interpretation: E.A., Literature Search: G.S.Ç., Writing: E.A.

Conflict of Interest: No conflict of interest was declared by the authors.

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Original Article / Özgün Araştırma

The Effect of Atherosclerotic Load on Transmetatarsal Amputation Failure in Patients with Diabetic Foot

Diyabetik Ayak Hastalarında Aterosklerotik Yükün Transmetatarsal Amputasyon Yetmezliğine Etkisi

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Abstract

Objective: The aim of this retrospective study was to investigate predictabilty of transmetatarsal amputation (TMA) failure in patients with diabetic foot with peripheral arterial disease.
Materials and Methods: Between January 2015 and November 2018, patients with diabetic foot, who had been followed up for 12 months after having TMA were evaluated. Atherosclerotic load was calculated from conventional angiographic images by using the modified Bollinger angiogram scoring method. Patients were classified as reamputation and non-reamputation groups after TMA at 12 months. Statistical difference analysis of atherosclerotic load was made between two groups.
Results: Twenty-seven diabetic foot patients whom have had TMA and angiographies before TMA were included in the study cohort. In the 12 month study period, 6 (22.2%) patients have had reamputation after TMA. The mean angiogram scores of reamputation group and non-reamputation group were 47.3 (±11.4) and 15.5 (±9.2), respectively. There was a statistically significant difference between the mean angiogram scoring of the two groups ($p<0.001$). Correlation was found between atherosclerotic load and TMA failure (r:-0,694, $p<0.001$). Receiver-operating characteristic curve analysis suggested that the optimum angiographic score cut-off value for reamputation at 12 month period was 29.5, with 100% sensitivity and 90.5% specificity.
Conclusion: Atherosclerotic load of lower limb arteries is associated with TMA failure. Atherosclerotic load that was calculated by using modified Bollinger angiographic score system can be used to predict TMA failure.

Keywords
Transmetatarsal amputation, atherosclerotic load, diabetic foot

Anahtar Kelimeler
Transmetatarsal ampütasyon, ateroskleroz, diyabetik ayak

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Öz

Amaç: Bu retrospektif çalışmanın amacı, periferik arter hastalığı olan diyabetik ayaklı hastalarda kısa dönemde oluşabilecek transmetatarsal ampütasyon (TMA) yetersizliğinin öngörülebilirliğini araştırmaktır.
Gereç ve Yöntemler: Ocak 2015-Kasım 2018 tarihleri arasında TMA yapılmış diyabetik ayak hastalarının 12 aylık takipleri değerlendirildi. Ameliyat öncesi aterosklerotik yük, modifiye Bollinger anjiyogram skorlama yöntemi kullanılarak hesaplandı. Hastalar, TMA sonrası 12 ay içinde yeniden ampütasyon olan ve olmayan gruplar olarak sınıflandırıldı. Aterosklerotik yükün yeniden ampütasyona etkisi istatistiksel analiz yapılarak hesaplandı.

Bulgular: Çalışma grubuna TMA öncesi anjiyografi yapılmış 27 diyabetik ayak hastası dahil edildi. On iki aylık çalışma döneminde 6 (%22,2) hastaya TMA sonrası yeniden ampütasyon uygulandı. Yeniden ampütasyon yapılan grubun ve yeniden ampütasyon yapılmayan grubun ortalama anjiyogram skorları sırasıyla 47,3 (±11,4) ve 15,5 (±9,2) idi. İki grubun ortalama anjiyografi skorları arasında istatistiksel olarak anlamlı fark vardı (p<0,001). Aterosklerotik yük ile TMA yetmezliği arasında korelasyon olduğu bulundu (r:-0,694, p<0,001). On iki aylık dönemde yeniden ampütasyon için optimum anjiyografik skor kesme değeri 29,5 olarak hesaplandı (%100 duyarlılık ve %90,5 özgüllük).
Sonuç: Alt ekstremite arterlerinin aterosklerotik yükü TMA yetmezliği ile ilişkili olabilir. Modifiye Bollinger anjiyografik skor sistemi kullanılarak hesaplanan aterosklerotik yük, kısa dönemde TMA yetersizliği olabileceğini tahmin etmek için kullanılabilir.

Introduction

Diabetic patients have high risk of complications. One of the most important complication is peripheral vascular disease, which may lead to diabetic foot ulcers (1). Diabetic foot ulcers may cause osteomyelitis or gangrene and cause lower extremity amputation. About 15% of all diabetic patients will develop a foot ulcer during their lifetimes (2). Diabetic patients with nonhealing ulcers of the midfoot, hindfoot, forefoot or ankle can be treated by transtibial amputations (TTA). However, TTA has high postoperative morbidity and mortality (3). Diabetic patients with nonhealing foot ulcers have operative alternatives that allow amputation at a more distal level. Transmetatarsal amputation (TMA) is an another effective surgical alternative method. However, TMAs are associated with significant failure or reamputation rates of between 26% and 63% (4,5). Peripheral arterial disease (PAD) was a risk factor for TMA failure (6). Although noninvasive ankle-brachial index measurements are often used for determining PAD, angiography is the gold standard method for PAD at lower extremity. Bollinger angiographic score system (7) is one of the way to analyze atherosclerotic load, and the bypass vs. Angioplasty in Severe Ischemia of the Leg trial showed its usability in patients with limb ischemia (8,9). In this retrospective study, we aimed to investigate the predictability of TMA failure in diabetic foot patients using the modified angiogram scoring method that detects atherosclerotic burden.

Materials and Methods

This retrospective study conducted between January 2015 and November 2018 on diabetic foot patients, who have had TMA and angiography before TMA procedure. The study was approved by the Adnan Menderes University Ethics Committee (protocol number: 2019/198, date: 19.12.2019). Informed written consent was received from all patiets. Inclusion criterias were patients who were undergone TMA for diabetic foot in study period and who were undergone conventional angiography and/or percutaneous balloon angioplasty (PBA) before TMA and patients who had monitoring 12 months. Exclusion criterias were initial amputation, patient who had not undergone conventional angiography and/or PBA before TMA. Each patient's assessment for decision of TMA and/or angiographic intervention were done in multi-disciplinary diabetic foot council.

Vascular interventions were performed via antegrade approach from the superficial femoral artery. PBA was performed when hemodynamically significant stenosis or occlusion was observed. All procedures were performed by an interventional radiologist. All angiography procedures were performed in operating room equipped with a C-arm brillance (Artis zee; Siemens, Erlangen, Germany).

TMA attempt was begun with removal of infected tissues and preserve plantar skin and soft tissue flaps for dorsal foot tissues. The metatarsals' sharp edges are smoothed. The first two metatarsals were amputated at the same level. The third to fifth metatarsals were amputated in as a cascade to the lateral border of the foot (Figure 1). All procedures were performed by an orthopedic surgeon.

Modified Bollinger scoring system (7) was used to assess the angiographies. Bollinger angiographic scoring system is based on the number and presence of stenoses and occlusions of each arterial segment. It consist of 13 infrainguinal arterial segments. Each of these arterial segment was scored with the severity of the disease. Four severities of disease are recognized in this method: occlusion, stenosis ≥50%, stenosis between 25% and 50%, and plaques affecting ≤25% of the luminal diameter. Each type of lesion was characterized by either a single lesion, multiple lesions affecting less than half of the artery

or multiple lesions affecting greater than half of the artery. For each severity of disease, only one extent of disease category is scored (Table 1). In this study, we made some modifications to Bollinger angiographic scoring system because of the antegrade approach of angiographies in our study. Five target infrainguinal arteries were assessed: superficial femoral artery, popliteal artery, anterior tibial artery (ATA), posterior tibial artery (PTA) and peroneal artery (Figure 2). Plantar arch was not evaluated because of unsatisfactory quality images in some angiographies. Also, in our modified Bollinger scoring system arteries were not divided as proximal and distal segments. Total angiographic scores were cumulative scores from each artery scores (maximum: 75; minimum: 0). Scoring was performed from post PBA images, in patients who had PBA.

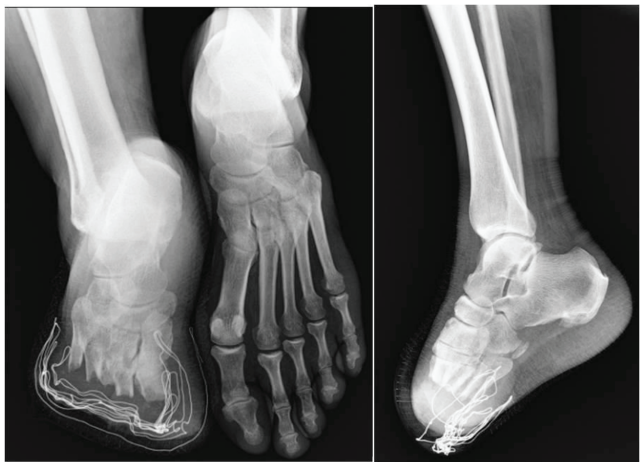

Figure 1. Sixty two year old male who had transmetetarsal amputation of right foot. Antero-posterior and lateral radiography show amputation level. This patient has no re-amputation at 12 mounths

Table 1. Bollinger scoring matrix*

Occlusion	Stenosis >50%	Stenosis <50%	Plaques	Location
	4	2	1	Single
13	5	3	2	Multipl <h
15	6	4	3	Multipl >h

*The vertical columns represent the different severities of atherosclerotic lesions observed, the horizontal ones the location of the lesions detected in each of the arterial segments. The numbers appering in the single field correspond to the score number. The additive score for each artery is obteined by adding the scores (see text for details).

h: Half the segment length

Patients were clinically evaluated after the procedure every 2 months by diabetic foot care team and were followed up for 12 months.

The patients were classified as reamputation and non-reamputation group after TMA at 12 months. We investigated whether there was any statistically significant difference of angiographic scoring system between the two groups. The predictive value of angiographic scoring system in TMA failure was analyzed by receiver-operating characteristic (ROC) curve. Optimal cut-off value for angiographic score was determined; sensitivity and specificity were calculated.

Statistical Analysis

Statistical Package for the Social Sciences (SPSS) 17.0 statistical software for Windows (SPSS Inc, Chicago, IL, USA) was used for statistical analyses. Descriptive statistics for continuous parameters were expressed as arithmetic means ± standard deviation or median (interquartile range). Spearman's rank test was used to determine correlation. Mann-Whitney U test was used to determine statistical difference between groups. The effect of angiographic score was studied by constructing a ROC curve with TMA failure as the primary variable. Also area under the curve (AUC) value for ROC curve was determined. Optimal cut-off value for angiographic score was determined; sensitivity and specificity were calculated. P-values <0.05 were considered statistically significant.

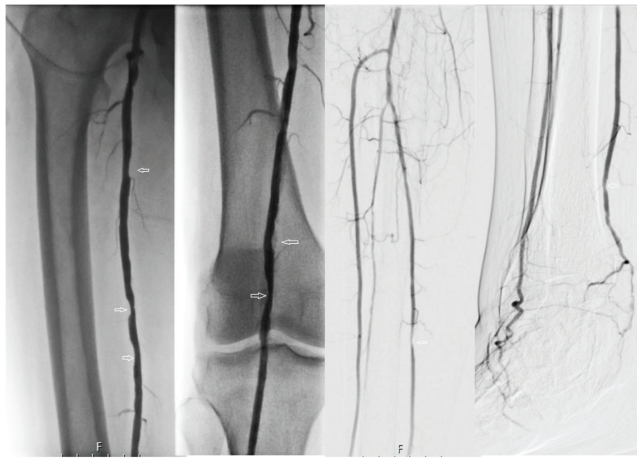

Figure 2. Conventional angiogram of the patient in Figure 1. Superficial femoral, popliteal and tibialis posterior artery have plaques and stenoses (arrows indicate the plaques and stenotic areas) Patients' angiogram score was 15

Results

Twenty-seven patients with diabetic foot matching the study criteria were found between January 2015 and November 2018. Of these patients, 20 (74.1%) were men and 7 (25.9%) were women. Mean age of patients were 65.4 years (range: 52-86). The mean duration of diabetes mellitus (DM) was 19.2 years (range: 9-28). Five (18.5%) severe stenosis, 7 (25.9%) mild stenosis and 15 (55.6%) total occlusions were detected on 27 angiograms. The mean total angiogram score was 22.5 (±16.5) in all patients. Based on affected arteries, ATA was the most affected artery and it affected in 11 (40.7%) patients. ATA and PTA were the most affected binary arteries 6 (22.2%). Infrapopliteal arteries were more affected than suprapopliteal arteries 24 (88.8%), 3 (11.1%), respectively. PBA was conducted in 17 (63%) patients (reamputation group 6 (100%), non-reamputation group 11 (52.4%). At post-PBA, 4 patients in reamputation group and 7 patients in non-reamputation group had an in-line flow to the foot, with at least a single-vessel below knee run-off. Remaining 10 (37%) patients either did not have severe stenosis-occlusion or did not accept PBA. In the 12 month study period, 6 (22.2%) patients have had reamputation due to TMA failure. In all of these patients, TMA failure had occured in the first 6 months. The mean angiogram score in reamputation group and non-reamputation group were 47.3 (±11.4) and 15.5 (±9.2), respectively (Table 2). Statistically significant difference was found between the mean angiogram scoring of the groups ($p<0.001$) (Figure 3). There was correlation between atherosclerotic load that was calculated by angiographic scoring system and TMA failure (r:-0,694, $p<0.001$). ROC curve analysis showed that the optimum angiographic score cut-off value for reamputation at 12 month was 29.5, with 100% sensitivity and 90.5% specificity. The AUC value was 0.98 (95% confidence interval: 0.95-1.00).

Discussion

TMA is an effective surgical approach in diabetic patients with forefoot gangrene and infection. In 1949, McKittrick first reported TMA as a method with 67% satisfactory outcomes in patients with DM (10). However, several studies have shown showed a high rate of TMA failure (4,5,11-13). In this situation, patients require more proximal amputation. In a meta analysis that include 24 studies with 1453 TMAs, Thorud et al. (14) found 33.2% rate of major amputation after TMA. Despite the high rates of failure, TMA is the preferred method because it maintains the ambulatory state of the foot.

Some studies have suggested risk factors associated with failure of amputations, such as sepsis, emergency surgery, renal disease, high body mass index, and longer operating duration (5,15,16). Also, abnormalities in leukocyte count, lymphocyte count, hemoglobin and albumin levels were found risk factors

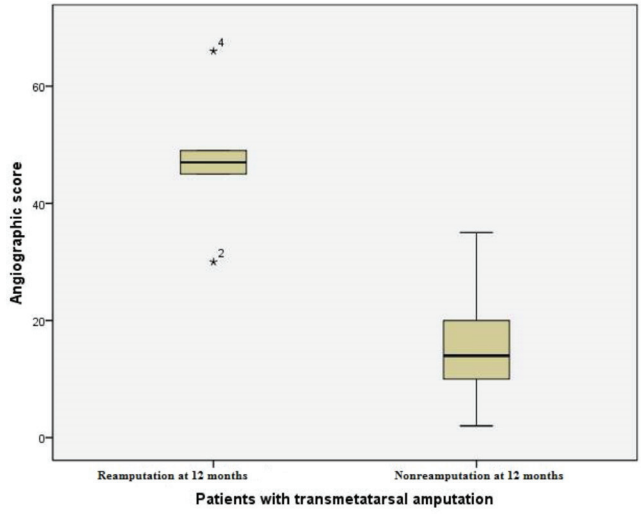

Figure 3. Graph showing a significant difference for angiographic score between patients with re-amputation and non re-amputation at 12 mounths

Table 2. Demographic characteristics, occlusive artery numbers and mean angiogram score of patients with reamputation and non-reamputation at 12 mounths after transmetatarsal amputation

	Patients with reamputation (n=6)	Patients without reamputation (n=21)
Gender (%male)	4 (66.7%)	16 (76.2%)
Mean age (years)	70.8 (±11.0*)	63.3 (±8.88*)
Steotic or occlusive artery number		
One artery	0	12
Two artery	3	7
Three artery	2	2
Four ≤ artery	1	0
Mean angiogram score	47.3 (±11.4*)	15.5 (±9.2*)

*Standard deviation, n: Number

for TMA failure (17). Another predictive risk factor for TMA failure is PAD of the lower extremity. Pollard et al. (16) suggest that TMA is associated with high complication rates in patients with vascular disease and they showed that palpable pedal pulse was a good predictor of healing (16). Some studies reported that forefoot amputations in patients with diabetus mellitus who had vascular disease were followed by subsequent proximal amputations (6,18,19). Humphrey et al. (6) showed 11% reoperation and 8% major amputation rate in their small cohort study with 41 patients, and found all patients who required reoperation, had peripheral vascular disease. Another study by Mandolfino et al. (19) analyzed 218 TMAs and reported presence of severe PAD is a significant risk factor for TMA failure. Shi et al. (20) showed that patients who underwent open bypass surgery have a better outcome than patients who underwent endovascular treatment. They found 44% rate of limb loss after TMA in a 3-year follow-up. Tan et al. (21) performed revascularization via open surgery or PBA and they showed complete healing of TMA in 63% of patients. In our study, endovascular treatment was performed in 17 (63%) patients (100% of reamputation group and 52.4% of non-reamputation group), and also 6 patients (22.2%) had reamputation after TMA procedure and all those TMA failures occured in the first 6 months.

In diabetic patients, PAD involves more distal arteries with diffuse calcification than non-diabetic patients (22). Although the importance of atherosclerotic burden in the treatment of patients with pulmonary arterial hypertension has been demonstrated, there is insufficient data on the effect of atherosclerotic burden in patients with TMA failure. The angiogram scoring method is feasible for showing the atherosclerotic load in infrapopliteal arteries (8,9). Matsukura et al. (23), used modified Bollinger scoring system of paramalleolar region arteries for investigate its usefulness at operative outcome of critical limb ischemia. Like Matsukura et al. (23), we made a new modified Bollinger scoring system on infrainguinal arteries in this retrospective study.

In our study, contrast to previous studies, we focused not only presence of vascular disease but also on the effects of atherosclerotic load in lower limb with TMA failure in 12 months period. We calculated atherosclerotic load by using modified Bollinger angiogram scoring system and we showed that patients with TMA failure have significantly higher atherosclerotic load than patients without TMA failure in the 12 month period. Patient with angiographic score of more than 29.5 is more likely to have TMA failure with 100% sensitivity and 90.5% specificity rates.

In contrast to our study, a study conducted by Toursarkissian et al. (24) reviewed 41 patients with TMA and 35 patients who had revascularization before TMA procedure, and foot vessels were assigned run off scores and this study showed that angiographic findings were insufficient to predict TMA failure.

Retrospective design of study, small number of cohort and limited arterial segmental evaluation (superficial femoral, popliteal, ATA, PTA and peroneal arteries) due to antegrade approach were limitations of this study. Also, in some patients, having PBA may lead to errors in scoring, because that angiographic scoring was done after PBA in these patients. The retrospective design and long term of study restricted our investigation into other possible predictive risk factors for TMA failure in our cohort.

Conclusion

This study showed that atherosclerotic load of lower limb arteries may be associated with TMA failure. Atherosclerotic load that was detected by using Bollinger angiographic score system may have a good prediction with high sensitivity and specificity values for TMA failure in diabetic foot patients. Additional angiographic studies with a large cohort group are needed to identify the effect of atherosclerotic load on the TMA failure in diabetic patients.

Ethics

Ethics Committee Approval: The study was approved by the Adnan Menderes University Ethics Committee (protocol number: 2019/198, date: 19.12.2019).

Informed Consent: Informed written consent was received from all patiets.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: E.C., Ş.Ö.Ş., Concept: M.B.Ç., T.Ş., E.C., Ş.Ö.Ş., Design: M.B.Ç., T.Ş., E.C., Ş.Ö.Ş., Data Collection or Processing: M.B.Ç.,

Analysis or Interpretation: M.B.Ç., Literature Search: M.B.Ç., Writing: M.B.Ç.

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Original Article / Özgün Araştırma

Complementary and Supportive Practices Used for Children with Dental Pain by Parents

Çocuklarda Diş Ağrısı ile Baş Etmede Ebeveynlerin Kullandıkları Tamamlayıcı ve Destekleyici Uygulamalar

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Abstract

Objective: This study aimed to determine the complementary and supportive practices used by parents for their children with dental pain.

Materials and Methods: The sample of this cross-sectional study consisted of 1,551 parents who had 6–12-year-old children, and whose children had previously experienced dental pain. The data were collected using questionaries prepared by the researchers. Data were analyzed by descriptive statistics and regression analysis.

Results: In all, 61.7% of parents used only complementary, 18.4% only supportive, and 19.9% both complementary and supportive practices to deal with their children dental pain. Child age, previous dental experience, age at developing the habit of tooth brushing, fear of the dentist, father's employment status, and number of children in the family were statistically significant predictive factors on using only complementary practices by parents.

Conclusions: Parents should be informed about the harmful practices that their children use to cope with dental pain and should be directed to methods whose benefits have been proven by evidence-based studies.

Keywords
Child, dental pain, parent, complementary and supportive practices

Anahtar Kelimeler
Çocuk, diş ağrısı, ebeveyn, tamamlayıcı ve destekleyici uygulama

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Öz

Amaç: Bu araştırma, çocuklarının diş ağrısı ile baş etmede ebeveynlerin kullandıkları tamamlayıcı ve destekleyici uygulamaları belirlemek amacıyla yapıldı.

Gereç ve Yöntemler: Kesitsel tipteki araştırmanın örneklemini, 6-12 yaş grubu çocuğu olan ve çocuğu daha önce diş ağrısı deneyimleyen 1.551 ebeveyn oluşturdu. Veriler, araştırmacılar tarafından hazırlanan anketler kullanılarak toplandı. Tanımlayıcı istatistikler ve regresyon analizi verilerin değerlendirilmesinde kullanıldı.

Bulgular: Çocuklarının diş ağrısı ile baş etmede ebeveynlerin %61,7'sinin yalnızca tamamlayıcı, %18,4'ünün yalnızca destekleyici ve %19,9'unun hem tamamlayıcı hem de destekleyici uygulamaları kullandığı saptandı. Çocuğun yaşı, önceki dental deneyimleri, diş fırçalama alışkanlığı kazanma yaşı, diş hekimi korkusu, babanın çalışma durumu ve ailedeki çocuk sayısı ebeveynlerce sadece tamamlayıcı uygulamaların kullanılmasında istatistiksel olarak anlamlı yordayıcı faktörler olarak tespit edildi.

Sonuç: Ebeveynler çocuklarının diş ağrısı ile başetmede kullandıkları zararlı uygulamalar konusunda bilgilendirilmeli ve yararı kanıta dayalı çalışmalarla kanıtlanmış yöntemlere yönlendirilmelidir.

Introduction

Dental pain is one of the common causes of pain during childhood that negatively affects the performance of activities of daily living and the quality of life in children and adolescents (1,2). Uncontrollable pain leads to deterioration in sleep quality, psychological problems, suppression of the immune system, and prolonged recovery time (3,4). Additionally, dental pain experienced by children negatively affects their families and leads to disruptions in the parents' physical, social, and psychological activities, and has a negative effect on the financial status of the family (5). Therefore, keeping the pain experienced due to orodental problems under control is important for both the child and the family.

In recent years, interest in complementary or supportive methods to protect health and remedy health problems has also been increasing in the field of orodental health (6). However, when not followed responsibly, these methods may exert more harmful than beneficial effects on children's health (7). For parents, the pain, anxiety, and fear experienced by their children are alarming. Therefore, parents resort to various methods that mostly are not based on a scientific basis, and that they know of based on their culture, see in their families, or are recommended by their immediate friends' circle to reduce their children's pain (8).

Management of dental pain among school-age children is crucial for the protection and development of children's health. One of the primary roles of nurses is to identify practices that directly affect children's health and to instill healthy lifestyle behaviours among children. Therefore, it is very important that nurses recognize such methods used by society and know the practices that will affect the health behaviour of individuals so that they can provide effective healthcare services (9,10). To the best of our knowledge, there is a gap in the literature that investigate complementary and supportive care practices used for the management of dental pain among children. This research aimed to determine the complementary and supportive practices used by parents for coping with dental pain of their children.

Materials and Methods

Participants

This study was carried out as a cross-sectional study in 2017-2018 and 2018-2019 academic years. Before starting the study, approval was obtained from the institution, Bozok University Non-Invasive Clinical Research Ethics Committee (date and decision number: 2017-04/01), and written consent from the participants.

The population of the study consisted of 1,600 parents who were registered in the 10 Family Health Centers (FHCs) in a provincial center, and who applied under the school health screening program to FHCs on the specified dates. The parents who had 6-12 year old children, whose children had previously experienced dental pain, and who agreed to participate in the study were included in the sample. In this regard, 49 parents who did not meet the inclusion criteria, were excluded from the study and the sample of the study occurred of 1,551 parents.

Data Collection

The questionnaires were prepared by researchers in accordance with the relevant literature (7,11). Firstly, a pilot study was conducted with participants who were not included in the study, and the questionnaires were finalized. Afterward, questionnaires were filled out during face-to-face interviews, and the interviews took a median of 20 minutes. The data collection tools were following;

Introductory information form: The form consisted of questions regarding the sociodemographic characteristics of parents, and childrens' orodental health and dental pain history, etc.

Complementary and supportive practices form: The form included questions on complementary and supportive practices used for the management of dental pain among children, whether they received information from health personnel before using it, whether they found the practice useful, etc.

Statistical Analysis

IBM SPSS Statistics 22.0 (IBM Corp., Armonk, New York, USA) package program was used to evaluate the data. Number and percentage values were used to assess the descriptive data. Factors that were considered to influence using only complementary practices were evaluated using simple binary logistic regression analysis. Hosmer and Lemeshow test was

used in the final model (chi-square=6.843, df=8, p=0.554). Significant variables were evaluated using multiple binary logistic regression analysis with backward wald elimination. P<0.05 was considered statistically significant in all the analyses.

Limitation of the Study

The data obtained is limited to the questions asked in the questionaries. The methods used are limited to children aged 6-12 years.

Results

According to the introductory characteristics of parents, the age distribution of mothers and fathers was 28 years and under (n=292, 18.8%; n=137, 8.8%), 29-39 age group (n=745, 48.0%; n=615, 39.7%), and 40 years and above (n=514, 33.2%; n=799, 51.5%). The educational status of mothers and fathers was literate (n=55, 3.5%; n=19, 1.3%), primary school (n=443, 28.6%; n=255, 16.4%), secondary school (n=300, 19.4%; n=272, 17.5%), high school (n=438, 28.2%; n=580, 37.4%), university (n=315, 20.3%; n=425, 27.4%). In all, 31.6% of the mothers (n=490) and 92.6% of fathers (n=1436) were employed, and 68.4% of the mothers (n=1061), and 7.4% of fathers (n= 115) were unemployed. Number of children in the family were one child (n=385, 24.8%), two children (n=652, 42.0%), and three children and above (n=514, 33.2%). Further, a total of 41.2% of the children were between 6-9 and 58.8% of them were between 10-12 years included in this study.

In all, 45.1% developed the habit of brushing their teeth between the ages of 4 and 6 years. Moreover, 51.7% feared the dentist and 50.5% had previous dental experience. The cause of dental pain was tooth eruption in 46.2%, tooth decay in 32.0%, tooth abscess in 8.3%, and unknown in 13.5% of the children (self-reported). In this sample, 61.7% of parents used only complementary, 18.4% followed only supportive, and 19.9% used both complementary and supportive practices together (mixed methods) for managing dental pain (Table 1).

As an only supportive practice, 50.2% of parents used mouthwash, 26.7% increased their child's tooth-brushing frequency, and 23.1% used floss for their children. In terms of complementary practices, rubbing poppy (Papaver somniferum), onions, garlic, potatoes, and cloves on the tooth, and using saltwater mouthwash were most commonly used methods. Further, 30.5% of the parents obtained the result they expected from the practice, whereas two-thirds did not see any benefit or did not obtain the expected result, and the rate of the side effects were 1.4% (Table 2).

Table 1. Oral-dental health and dental pain history of their children of parents

Features	n	%
Having a separate toothbrush		
Yes	1435	92.5
No	116	7.5
Having a separate toothpaste		
Yes	1160	74.8
No	391	25.2
Age at developing the habit of tooth brushing		
2-3 years	434	28.0
4-6 years	700	45.1
7-12 years	417	26.9
Number of times the child brushes the teeth		
1 time a day	798	51.5
2 times a day	328	21.1
3 times a day	122	7.9
Once a week	14	0.9
Occasionally	289	18.6
The child's fear of the dentist		
Yes	802	51.7
No	749	48.3
Having previous dental experience		
Yes	784	50.5
No	767	49.5
Reason for dental pain		
Tooth eruption	717	46.2
Tooth decay	496	32.0
Tooth abscess	128	8.3
Unknown	210	13.5
Use of pharmacological methods to deal with dental pain of child without consulting a physician		
Yes	540	34.8
No	1,011	65.2
Practices followed by parents to deal with dental pain		
Following only complementary practices	957	61.7
Following only supportive practices	285	18.4
Following mixed practices	309	19.9
Total	1,551	100.0

Table 2. Complementary and supportive practices used for managing dental pain of their children by parents

Features	n	%
Using only supportive practices (n=285)		
Using a mouthwash	143	50.2
Increasing tooth-brushing frequency	76	26.7
Flossing	66	23.1
Using only complementary practices* (n=957)		
Rubbing poppy	478	49.9
Rubbing onion	144	15.0
Rubbing garlic	126	13.2
Rubbing potatoes	130	13.6
Rubbing cloves	128	13.4
Mouthwash with salt	102	10.7
Rubbing olives without seeds	68	7.1
Mouthwash with carbonate	64	6.7
Rubbing cucumbers	45	4.7
Chewing black cumin	43	4.5
Rubbing carrots	40	4.2
Pressing with cologne-soaked cotton	19	2.0
Applying fluorine	18	1.9
Pressing with alcohol-soaked cotton	17	1.8
Rubbing soda	15	1.6
Applying cold	15	1.6
Vinegar water	10	1.0
Using both complementary and supportive practices/mixed methods (n=309)		
Increasing tooth-brushing frequency + rubbing poppy	50	16.1
Flossing + mouthwash with carbonate	46	14.9
Increasing tooth-brushing frequency + mouthwash with salt	159	51.5
Using a mouthwash + applying cold	54	17.5
Reason for using only complementary care practices to deal with dental pain of child* (n=957)		
To support the treatment	595	62.2
Thinking it is safer	219	22.9
Thinking it is natural	171	17.9
Thinking it is more useful	127	13.3
As it is easier to obtain	90	9.4
As such treatments are common	80	8.4
Ensuring everything is done to relieve the pain	65	6.8
Out of desperation	42	4.4
Because it has fewer side effects	40	4.2
To relax psychologically	39	4.1
Because of fear of the side effects of medication	25	2.6
Due to lack of money	21	2.2
Time and frequency of following complementary/supportive care practices		
Once a day	154	9.9
2 times a day	161	10.4
3 times a day	62	4.0
When it hurts	1,118	72.1
After each tooth-brushing session	56	3.6
Receiving information from health professionals before/while using complementary/supportive practices		
Yes	333	21.5
No	1218	78.5
Outcome of the method used		
I obtained the expected result	473	30.5
There was no improvement, but I was relieved to think I was doing my best	608	39.2
I didn't obtain the expected result	93	6.0
I didn't see any benefit or harm because I didn't practice it regularly	96	6.2
I didn't see any benefit	281	18.1
Side effects of complementary/supportive practices		
Yes	22	1.4
No	1,529	98.6
Recommending complementary/supportive practices to others		
Yes	297	19.1
No	660	42.6
Partly	594	38.3
Total	1,551	100.0

*Multiple responses were given, n: Number

Table 3 shows the results of multiple binary logistic regression analysis. In the final model, child age, previous dental experience, age at developing the habit of tooth brushing, fear of the dentist, father's employment status, and number of children in the family were statistically significant predictive factors on using only complementary practices. According to wald statistics, the most important factor influencing the practice of using only complementary practices was developing the habit of brushing teeth between the ages of 7 and 12 years (Table 3).

Table 3. Multiple logistic regression analysis results on factors influencing the practice of using only complementary practices

Variables	β	SE	Wald statistics	p	Exp (β)	95% CI for exp (β)	
						Lower	Upper
Age of children							
6-9 years	Ref						
10-12 years	0.378	0.124	9.234	0.002	1.460	1.144	1.863
Previous dental experience							
No	Ref						
Yes	0.334	0.117	8.168	0.004	1.397	1.111	1.757
Age at developing the habit of tooth brushing							
2-3 years	Ref						
4-6 years	0.400	0.129	9.543	0.002	1.492	1.157	1.923
7-12 years	0.539	0.160	11.312	0.001	1.714	1.252	2.346
Dentist fear							
No	Ref						
Yes	0.280	0.110	6.537	0.011	1.323	1.068	1.641
Father's employment status							
Employed	Ref						
Unemployed	0.601	0.236	6.471	0.011	1.824	1.148	2.899
Number of children in the family							
One	Ref						
Two	0.214	0.140	2.317	0.128	1.238	0.940	1.631
Three and above	0.404	0.155	6.805	0.009	1.498	1.106	2.029
Constant	-0.678	0.144	22.231	<0.001	0.508		

SE: Standart error, CI: Confidence interval, Ref: Reference/comparation group

Discussion

This study was conducted to determine the complementary and supportive practices employed by parents to deal with dental pain in their school-age children. The results showed that the most common causes of dental pain were tooth eruptions and decay (dental caries) (Table 1). A systematic review and meta-analysis emphasized that dental pain could occur among children and adolescents because of various reasons such as eruption, exfoliation, carious teeth, dentinal hypersensitivity, and abscess (12). In another study, dental caries was detected in 2,218 of 2,358 children aged 4-15 years (13).

In this study, mouthwash and floss were used as supportive practices for dental pain among children (Table 2). Mouthwashing, flossing, and tooth brushing to improve oral hygiene are recommended as supportive oral care practices, as they help with mechanical cleaning, such as tongue cleaning (14). In the present study, increasing the frequency of tooth brushing was another supportive care practice employed by the parents. Regarding oral health habits and behaviors, Honkala et al. (15) observed that toothbrushing less than once a day increases the likelihood of dental pain.

In the present study, the complementary practices most used to deal with dental pain among children included applying poppy (Papaver somniferum), onions, garlic, potatoes, and cloves on the tooth and using saltwater mouthwash (Table 2). Some practices reported in the previous studies were similar to those in the present study. Efe et al. (7), found that mothers rubbed aspirin on the aching teeth; used mouthwash with salt or lemon salt; put cotton soaked with vinegar, alcohol, or cologne; or placed raw garlic or onions on the aching tooth to alleviate dental pain.

Gürsoy and Gürsoy (16) reported the widespread use of thyme, garlic, mint, sage, chamomile, and cloves for a toothache. Owing to its antibiotic or anti-inflammatory properties, garlic and clove may have been used reducing the bacteria causing incidence of tooth decay and periodontal disease activities (17-19). However, further studies are needed before toothpaste or dental preparations containing herbs can be used among children (20).

Some methods used by parents can have harmful effects. In the present study, 1.4% of the parents reported side effects (Table 2). Especially, as plants contain active substances with very different and significant pharmacological effects, there is a serious risk of toxicity because of the variety of active substances and the possibility of drug-drug interactions when mixed with other plants (21,22). Some plants interact with certain drugs through various mechanisms, causing hepatotoxicity, renal toxicity, abnormal bleeding, and circulatory system problems. The use of garlic increases the risk of bleeding among people using aspirin (22). Therefore, parents should not follow complementary care practices without the advice of health professionals. Doing so without professional advice may put their children in danger.

In this study, the previous dental experience, and fear of dentists were predictors of following complementary care practices to alleviate dental pain (Table 3). Dental anxiety among children with previous dental experience or fear of dentists may delay seeking medical help and lead the family to consider using alternative methods. In a study conducted in Brazil with children aged 8-11 years, children with toothache had significantly higher levels of dental anxiety than children without toothache (23). Dental anxiety observed during childhood can persist until adulthood, causing people to avoid dental treatments and, as a result, may negatively affect oral dental health. In addition, having more children and father's employment status were found to be predictors of following complementary care practices to alleviate dental pain. It was attributable to the fact that families have insufficient finances to go to the dentist and thus resort to complementary methods initially.

Conclusion

This study is important in terms of revealing the individual methods used by parents in coping with the dental pain of their children. To protect and improve children's health, parents should be directed to evidence-based methods when dealing with dental pain and problems. In line with the results of this study, identifying risk groups through organized home visits by family nurses and increasing the awareness and knowledge levels of both parents and school-age children regarding dangerous and harmful practices are recommended.

Ethics

Ethics Committee Approval: The study was approved by the Bozok University Non-Invasive Clinical Research Ethics Committee (date and decision number: 2017-04/01).

Informed Consent: Cross-sectional study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: S.P., A.G., Design: S.P., A.G., Data Collection or Processing: S.P., A.Ş.T., A.G., Analysis or Interpretation: S.P., A.Ş.T., Literature Search: S.P., A.G., Writing: S.P., A.Ş.T., A.G.

Conflict of Interest: No conflict of interest was declared by the authors.

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ERRATUM

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The authors have retracted this article. After publication, it came to the authors' attention that Associate Prof. Mustafa Unubol and Prof. Dr. Zahit Bolaman were included in the Ethics Approval Form, but were not inadvertently among the original article's authors. All authors agree to this retraction.
The retracted article will be digitally watermarked on each page as "Retracted" to maintain the scholarly record.

Kind regards,
Meandros Medical and Dental Journal Editorial Board