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Editorial Office
Adnan Menderes University Faculty of Medicine, Aydın, Turkey
Phone: +90 256 444 12 56 | +90 256 214 64 95
E-mail: info@meandrosmedicaljournal.org

Publisher Contact
Address: Molla Gürani Mah. Kaçamak Sk. No: 21/1
34093 İstanbul, Turkey
Phone: +90 (530) 17730 97
E-mail: info@galenos.com.tr/yayin@galenos.com.tr
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Clinical Features of Reactive Hyperplastic Lesions of Oral Cavity: A Retrospective Study of 460 Cases from Iran

Ağız Boşluğunun Reaktif Hiperplastik Lezyonlarının Klinik Özellikleri: İran'dan 460 Olgunun Retrospektif Bir Çalışması

Mahsa Kalantari Khandani[1], Fatemeh Bagheri[2], Maryam Alsadat Hashemipour[3], Mohammad Reza Zarei[3], Sepideh Eslamipanah[2]

[1]Kerman University of Medical Sciences, Kerman Dental School, Oral and Dental Diseases Research Center, Department of Oral and Maxillofacial Pathology, Kerman, Iran
[2]Kerman University of Medical Sciences, Kerman Dental School, Kerman, Iran
[3]Kerman University of Medical Sciences, Kerman Dental School, Oral and Dental Diseases Research Center, Department of Oral and Maxillofacial Medicine, Kerman, Iran

Abstract

Objective: Reactive hyperplastic lesions (RHLs) are common in the oral cavity due to the high frequency of stimulating and traumatic factors. The present study aimed to evaluate the clinical manifestations of these lesions in an Iranian population.

Materials and Methods: This retrospective study was performed on 460 cases with the histopathologic diagnosis of RHLs during a 23-year period (1997-2020). The data were collected from the Oral and Maxillofacial Medicine Department of the Dentistry Faculty of Kerman University of Medical Sciences. The lesions were classified into the five groups including irritation fibroma (IF), pyogenic granuloma (PG), peripheral giant cell granuloma (PGCG), peripheral ossifying fibroma, and epulis fissuratum. Age, gender, location, histopathologic diagnosis, and the clinical manifestations of the lesions were collected and analyzed by chi-square test using SPSS software version 20.

Results: Our findings showed that PG (32.8%) and IF (24.1%) were the most common lesions, while PGCG (10.9%) had the lowest frequency. All the lesions were more common in women and the most affected location was gingiva. Moreover, the mobility of adjacent teeth and loss of underlying bone were reported in 15% and 10.2% of the cases, respectively. A recurrance rate of 5.7% was noted after treatment.

Conclusion: Most of the clinical aspects of RHLs were similar to the findings of the previous studies. The observed differences could be attributed to racial or geographical factors.

Keywords: Irritation fibroma, peripheral giant cell granuloma, peripheral ossifying fibroma, pyogenic granuloma, reactive lesions

Öz

Amaç: Reaktif hiperplastik lezyonlar (RHL'ler), yüksek uyarıcı ve travmatik faktörlerin sıklığı nedeniyle ağız boşluğunda yaygındır. Bu çalışma, bir İran popülasyonunda bu lezyonların klinik belirtilerini değerlendirmeyi amaçlamaktadır.

Gereç ve Yöntemler: Bu retrospektif çalışma 23 yıllık bir dönemde (1997-2020) RHL'lerin histopatolojik tanısı ile 460 olgu üzerinde gerçekleştirildi. Veriler Kerman Tıp Bilimleri Üniversitesi Diş Hekimliği Fakültesi Ağız Diş ve Çene Hastalıkları Anabilim Dalı'ndan toplandı. Lezyonlar, iritasyon fibroma (IF), piyojenik granülom (PG), periferik dev hücreli granülom (PGCG), periferik ossifiye fibroma ve epulis fissuratum olmak üzere beş gruba ayrıldı. Toplanan veriler yaş, cinsiyet, yerleşim yeri, histopatolojik tanı ve lezyonların klinik belirtilerini içeriyordu. Veriler, SPSS yazılım versiyonu 20 kullanılarak ki-kare testi ile analiz edildi.

Bulgular: Bulgularımız, PG (%32,8) ve IF'nin (%24,1) en sık görülen lezyonlar olduğunu, PGCG'nin (%10,9) en düşük frekansa sahip olduğunu gösterdi. Tüm lezyonlar kadınlarda daha yaygındı ve en çok etkilenen yer diş eti idi. Ayrıca, olguların sırasıyla %15 ve %10,2'sinde komşu dişlerin hareketliliği ve altta yatan kemik kaybı bildirilmiştir. Tedaviden sonra %5,7'lik bir nüks oranı kaydedildi.

Address for Correspondence/Yazışma Adresi: Sepideh Eslamipanah, Kerman University of Medical Sciences, Kerman Dental School, Kerman, Iran
Phone: +98 343 211 90 21 **E-mail:** e_sepid@yahoo.com
ORCID ID: orcid.org/0000-0001-7192-7362

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Sonuç: RHL'lerin klinik yönlerinin çoğu, önceki çalışmaların bulgularına benzerdi. Gözlenen farklılıklar, ırksal veya coğrafi faktörlere bağlanabilir.

Anahtar Kelimeler: Tahriş fibroma, periferik dev hücreli granülom, periferik kemikleşen fibroma, piyojenik granülom, reaktif lezyonlar

Introduction

Reactive hyperplastic lesions (RHLs) of the oral cavity are known as a variety of lesions with local overgrowth. The majority of RHLs occur as the result of long-term stimulations with low intensity, including chronic chewing, food impaction, dental plaque and calculus, fractured teeth, overhanging dental restorations, and ill-fitting dentures. Furthermore, hormonal factors could predispose to some of these lesions, especially during adolescence, pregnancy and also hyperparathyroidism (1,2).

The RHLs are of non-neoplastic nature and indicate a chronic process of granulation tissue and scar formation in response to a local stimulation (3,4). A wide range of these lesions are observed in the oral cavity due to specific anatomical position and diverse functions continuously exposing the oral mucosa to chronic stimulants. Irritation fibroma (IF), pyogenic granuloma (PG), peripheral giant cell granuloma (PGCG), peripheral ossifying fibroma (POF), and epulis fissuratum (EF) are among the most common RHLs of the oral cavity (5-8).

Clinical diagnosis of RHLs might be challenging because of the similarity of these lesions to other oral pathosis including malignancies. On the other hand, the early diagnosis and treatment of RHLs could diminish the complications related to the growth and progression of the lesion (9-12).

Most of the studies on the RHLs of the oral cavity are limited to the reports of frequency, age, gender, and location distribution of these lesions, while few investigations assessed their clinical manifestations (13-17). Consequently, the present study aimed to evaluate the clinical features of RHLs in 460 patients referring to the Oral and Maxillofacial Medicine Department of the Dental School of Kerman University of Medical Sciences, Iran during a period of 23 years and compare the findings with other investigations.

Materials and Methods

This retrospective study was carried out on the cases of RHLs biopsied between September 1997 and September 2020 in the Oral and Maxillofacial Medicine Department of Kerman School of Dentistry. The study protocol was approved by the Ethics Committee of Kerman University of Medical Sciences with the code of 1397.278.

All the documents of the archive were assessed and the data of cases with the definitive diagnosis of an RHL in histopathology report were extracted and recorded. The data included age, gender, location, duration, clinical manifestations (e.g. size, color, surface, texture, and lateral profile of the lesion), accompanying symptoms (e.g. bleeding, mobility and dislocation of adjacent teeth, underlying bone loss, and pain), local irritating factors (e.g. denture, dental plaque and calculus, biting habits, medication usage), treatment and recurrance.

Cases with repeated biopsies, double registered cases and cases with the non-definitive diagnosis were excluded from the study. Microscopic slides of all samples were reevaluated by a second pathologist to confirm the histopathologic diagnosis.

Statistical Analysis

The data were analyzed by chi-square test using SPSS software version 20. $P<0.05$ was considered significant for all tests.

Results

Out of 3,126 patients who referred to the Department of Oral and Maxillofacial Medicine, 460 cases (14.71%) were diagnosed as one of the RHLs on the basis of histopathologic evaluation. The frequency of RHLs was reported as 32.8% (n=151), 24.1% (n=111), 20.4% (n=94), 11.7% (n=54), and 10.9% (n=50) for PG, IF, EF, POF, and PGCG, respectively.

Gender, Age, and Anatomic Location

Among 460 RHLs, 315 cases (68.5%) were in women and 145 cases (31.5%) were in men (male to female ratio: 1:2.2). All lesions were more common in women compared to men. The mean age of patients was 40 years and the mean age of women and men was not significantly different (p=0.146). Moreover, the mean age of women and men did not have a significant difference for any of the lesions separately ($p>0.05$) (Table 1).

The most commonly affected regions were upper and lower gingiva both with a frequency of 22% (n=101) and vestibular mucosa with a prevalence of 15.7% (n=72) (Table 2).

Clinical Findings

The results demonstrated that the mean duration of lesions at the time of diagnosis was 1.39±2.55 years with a range of 0.2-20 years. Clinical and histopathologic diagnoses were consistent in 77.4% of the cases. The majority of lesions had a smooth surface (65.9%) with a darker red color compared to normal mucosa (43.3%). Furthermore, 46.1% of the lesions had a firm texture, 68.9% had a narrow base, and 73.7% had a size of smaller than 3 cm (Table 3).

The evaluation of accompanying symptoms revealed that 37.8% of lesions had bleeding, 15% led to tooth mobility,

and 10.2% dislocated the adjacent tooth. Moreover, 10% and 18.5% of the lesions were accompanied by underlying bone loss and pain, respectively. The highest rates of bleeding, tooth mobility and underlying bone loss, dislocation of the adjacent teeth, and pain were reported in PG, PGCG, POF, and EF cases, respectively (Table 4).

We observed that 9.2% (n=29) of the women were pregnant at the time of lesion occurrence. The majority of pregnant cases (n=25) had PG, while two patients had POF and two were affected by PGCG and IF. In adittion, wearing denture, biting habits and accumulation of dental calculus were seen in 13.7%, 5.9% and 9.8% of patients, respectively. 22% of patients took medication.

Treatment and Recurrance

Most of the cases had been treated by excisional biopsy (91.8%) and incisional biopsy was performed only for 1.5% of the patients. The treatment technique was not recorded for

Table 1. Distribution of reactive lesions according to mean age and sex

Lesion	No. of pationts	Mean age	Sex		
			Male	Female	Ratio
PG	151 (32.8%)	35/08±19/35	44	107	1:2/4
IF	111 (24.1%)	41/34±13/64	39	72	1:1/8
EF	94 (20.4%)	58/50±14/27	17	77	1:4/5
POF	54 (11.7%)	29/68±15/62	21	33	1:1/5
PGCG	50 (10.9%)	34/25±20/79	24	26	1:1/08
Total	460 (100%)	40/08±18/55	145	315	1:2/2

PG: Pyogenic granuloma, IF: Irritation fibroma, EF: Epulis fissuratum, POF: Peripheral ossifying fibroma, PGCG: Peripheral giant cell granuloma

Table 2. Distribution of reactive lesions according to location

Lesion / Location	PG	IF	EF	POF	PGCG	Total
Maxillary gingiva	58 (12/7%)	11 (2/4%)	0 (0%)	23 (5%)	9 (2%)	101 (22/1%)
Mandibular gingiva	41 (9%)	11 (2/4%)	0 (0%)	30 (6/6%)	19 (4/2%)	101 (22/1%)
Vestibular mucosa	1 (0/2%)	1 (0/2%)	70 (15/3%)	0 (0%)	0 (0%)	72 (15/8%)
Alveolar ridge	15 (3/3%)	6 (1/3%)	22 (4/8%)	0 (0%)	22 (4/8%)	65 (14/2%)
Buccal mucosa	6 (1/3%)	48 (10/5%)	0 (0%)	0 (0%)	0 (0%)	54 (11/8%)
Tongue	20 (4/4%)	15 (3/3%)	0 (0%)	0 (0%)	0 (0%)	35 (7/7%)
Labial mucosa	5 (1/1%)	10 (2/2%)	0 (0%)	0 (0%)	0 (0%)	15 (3/3%)
Palate	2 (0/4%)	8 (1/8%)	0 (0%)	0 (0%)	0 (0%)	10 (2/2%)
Lip	3 (0/7%)	1 (0/2%)	0 (0%)	0 (0%)	0 (0%)	4 (0/9%)
Total	151 (33%)	111 (24/3%)	92 (20/1%)	53 (11/6%)	50 (10/9%)	457* (100%)

PG: Pyogenic granuloma, IF: Irritation fibroma, EF: Epulis fissuratum, POF: Peripheral ossifying fibroma, PGCG: Peripheral giant cell granuloma, *The lesion location was not mentioned for three patients

6.7% (n=31) of the cases. A total of 26 patients experienced recurrance with the frequencies of 11, 6, 2, and 1 cases of PG, IF, POF, PGCG, and EF, respectively.

Discussion

The RHLs are relatively common lesions in the oral cavity (18). Kerman is the largest province of Iran located in the southeast of the country with a population of over three million people. Kerman School of Dentistry is the main center in this province for referring patients with oral diseases.

In the present investigation, 460 cases (14.7%) out of 3126 evaluated cases had RHLs based on definitive diagnosis in the histopathologic report. The lesions were found to be more prevalent in women, compared to men (2.2 times higher), which is consistent with the results of most of the studies (2,4,5,13,16,17). The higher frequency of these lesions in female patients might be due to the role of hormones as a predisposing factor in the development of these lesions. Furthermore, the latter difference could be attributed to the fact that women pay more attention to oral hygiene and dental care.

The mean age of the patients was 40 years, which is close to the results of other investigations (9,14,19). In addition, in line with the previouse (9,19), the mean age of affected women and men was not significantly different.

Similar to all the previous studies, gingiva was the most common affected location (44.2%) (7-20). The high prevalence of these lesions in gingiva might be because of the chronic stimulations due to the accumulation of bacterial plaque and improper dental restorations. Moreover, some of these lesions e.g. POF and PGCG

Table 3. Distribution of reactive lesions according to surface charachteristics and color

Lesion	Surface			Color			Size			Consistency			
	Smooth	Non-smooth	Ulcerative	Normal color	Darker than normal	Paler than normal	<3	3-5	>5	Firm	Elastic	Hard	Soft
PG	96 (20/9%)	23 (5%)	11 (2/4%)	24 (5/2%)	102 (22/2%)	19 (4/1%)	122 (26/5%)	8 (1/7%)	6 (1/3%)	49 (10/6%)	65 (14/1%)	0 (0%)	19 (4/1%)
IF	89 (19/3%)	7 (1/5%)	4 (0/8%)	77 (16/7%)	11 (2/4%)	20 (4/3%)	100 (21/7%)	5 (1/1%)	3 (0/6%)	62 (13/5%)	22 (4/8%)	1 (0/2%)	18 (3/9%)
EF	48 (10/5%)	10 (2/2%)	13 (2/8%)	50 (10/9%)	21 (4/7%)	9 (1/9%)	52 (11/3%)	10 (2/2%)	12 (2/6%)	45 (9/8%)	15 (3/3%)	0 (0%)	6 (1/3%)
POF	35 (7/6%)	8 (1/7%)	1 (0/2%)	25 (5/4%)	24 (5/2%)	3 (0/6%)	42 (9%)	4 (0/9%)	3 (0/6%)	34 (7/4%)	10 (2/2%)	7 (1/5%)	0 (0%)
PGCG	35 (7/6%)	8 (1/7%)	2 (0/4%)	3 (0/6%)	41 (9%)	4 (0/9%)	23 (5%)	11 (2/4%)	12 (2/6%)	22 (4/8%)	23 (5%)	2 (0/4%)	2 (0/4%)
Total	303 (65/9%)	56 (12/2%)	31 (6/6%)	179 (39%)	199 (43/3%)	55 (12%)	339 (73/7%)	38 (8/3%)	36 (7/8%)	212 (46/1%)	135 (29/3%)	10 (2/2%)	45 (9/8%)

PG: Pyogenic granuloma, IF: Irritation fibroma, EF: Epulis fissuratum, POF: Peripheral ossifying fibroma, PGCG: Peripheral giant cell granuloma

Table 4. Distribution of reactive lesions according to accompanied symptoms

Location \ Lesion	PG	IF	EF	POF	PGCG	Total
Bleeding	104 (22/6%)	10 (2/2%)	6 (1/3%)	25 (5/4%)	29 (6/3%)	174 (37/8%)
Tooth mobility	34 (7/4%)	2 (0/4%)	1 (0/2%)	15 (3/3%)	17 (3/7%)	69 (15%)
Tooth displacement	17 (3/7%)	6 (1/3%)	1 (0/2%)	16 (3/5%)	7 (1/5%)	47 (10/2%)
Bone loss	16 (3/5%)	0 (0%)	2 (0/4%)	11 (2/4%)	17 (3/7%)	46 (10%)
Pain	26 (5/6%)	8 (1/7%)	28 (6/1%)	11 (2/4%)	12 (2/6%)	85 (18/5%)

PG: Pyogenic granuloma, IF: Irritation fibroma, EF: Epulis fissuratum, POF: Peripheral ossifying fibroma, PGCG: Peripheral giant cell granuloma

may originate from the periodontal ligament and gingival connective tissue or the periosteum (17). Gingiva being affected as the most prevalent site of RHLs supports the theory that these lesions are of similar nature but within different evolutionary stages. Daley et al. (20) suggested that the vascular texture of PG is gradually replaced with fibrotic tissue resulting in fibrous hyperplasia or IF. In the current investigation, the mean age of patients affected by IF was higher than in PG cases, which supports the mentioned theory. However, some other studies did not report such variation (19).

We found PG as the most prevalent RHL in this study with a frequency of 32.8%. Although many investigations revealed IF as the most prevalent RHL (3-5,12,17,19), some studies completed in Iran (13) and India (9) reported findings similar to the latter result of the present study. PG had a higher prevalence in gingiva and was 2.4 times more common in women than men, which is in line with the previous studies (1,2,7,13,14,19). Similar to the investigation conducted by Zarei et al. (13), the majority of PG cases were observed in the third decade of life.

IF was found as the second prevalent RHL after PG in the present study. Most of the cases were observed in the buccal mucous and during the fifth decade of life. Similar to previous studies, this lesion was more frequent among female subjects (2,4,5,15,19).

EF with the prevalence of 20.4% were the third common RHL in our study. Most cases were found in the vestibule and among women during the sixth and seventh decades. The latter findings are in line with the previous studies (13-19).

We found POF as the fourth common lesion in our study with a prevalence of 11.7%. similar to the literature, all cases of POF exclusively affected gingiva with a higher frequency in the mandible. Moreover, the majority of cases were female patients in their second and third decades of life (7, 13, 14, 18, 19).

PGCG with a prevalence of 10.9% was reported to have a lower frequency, in comparison with the other reactive lesions in this study. Consistent with the previous studies, all of PGCG cases located in gingiva and alveolar ridge mucosa (7-22). This lesion was more common in the lower gingiva and the prevalence was not significantly different between female and male patients. Previous studies (9, 13, 23) did not find any difference between the two genders in terms of PGCG prevalence. In some studies, this lesion was reported to be more common among men (5, 17). Most of the PGCG cases were in the fifth decade of life, which is in line with the literature (4, 5, 19).

Our results confirmed the previous investigations that PG and IF can affect any part of oral mucous, while POF exclusively affects the gingiva and PGCG is seen only in the gingiva and alveolar ridge. Eversole and Rovin (24) recommended that the presence of PGCG and POF in the gingiva support the fact that these lesions may histologically originate from the adjacent periodontal ligaments or periosteum.

In terms of the role of local stimulating factors in the generation of lesions, as could be expected, denture, biting habits, and dental plaque were the most common factors in EF, IF, and PG, respectively (21). In the literature, only Babu and Hallikeri (14) investigated the role of local stimulating factors. They reported the frequency of smoking habits to be higher in patients with PG, compared to other reactive lesions.

In the current study, 91.8% of the lesions were cured by excisional biopsy. In order to prevent recurrance, it is essential to perform a precise histopathologic evaluation and assure the lack of affected lesion base (17). Furthermore, dentists should attempt to minimize the controllable stimulating factors, such as faulty dental restorations, ill-fit dentures, and dental plaques to prevent recurrance. We found the highest rate of recurrancefor PG among the 26 cases of the present study. Babu and Hallikeri (14) reported a higher recurrance rate of 10.9%, compared to the present investigation. Similar to the current study, the mentioned authors revealed the highest recurrance rate for PG, POF, and PGCG.

Conclusion

Overall, the most and least prevalent RHLs in the present study were PG and PGCG, respectively. In addition, the lesions were more common among female patients, compared to male subjects. The most frequent site of lesions was the gingiva and recurrance was reported for 5.7% of the lesions with the highest rate found for PG.

Ethics

Ethics Committee Approval: This study was approved by the Ethics Committee of Kerman University of Medical Sciences (1397.278).

Informed Consent: Informed consent is not required.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Concept: M.K.K., M.A.H., M.R.Z., Design: M.K.K., M.A.H., M.R.Z., Data Collection or Processing: F.B., S.E., Analysis or Interpretation: F.B., S.E., Literature Search: M.K.K., Writing: M.K.K., S.E.

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Original Article / Özgün Araştırma

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Evaluation of Shear Bond Strength of Different Dental Materials to Bovine Enamel Used as Trauma Splints

Travma Splint Olarak Kullanılan Farklı Dental Materyallerin Makaslama Bağlanma Dayanım Kuvvetinin Değerlendirilmesi

Zeynep Şahin[1], Emine Şen Tunç[2]

[1]Private Practice, Ankara, Turkey
[2]Ondokuz Mayıs University Faculty of Dentistry, Department of Pediatric Dentistry, Samsun, Turkey

Abstract

Objective: This research aimed to measure the shear bond strength (SBS) of different dental materials to bovine enamel, while using them as splints for two different time periods.

Materials and Methods: Bovine incisor enamel sections were randomly divided into 10 groups based on test material and surface pretreatment. Each group was further split into 2 subgroups based on the length of the splinting period. Trauma splint materials (Gradia Direct Anterior Composite Resin, Gradia Core, Vertise Flow, Riva Light-Cure) were applied to enamel surfaces using one of two modes of a universal adhesive [etch-and-rinse (E&R) or self-etch (SE)] according to the manufacturers' instructions or with no pre-treatment [self-adhering (SA)]. At the end of splinting period, SBS of samples were evaluated with a universal testing machine, and data was analyzed using one-way ANOVA, post-hoc Tukey's, and paired t-tests.

Results: SBS values for each of the trauma splint materials were significantly higher when used with an E&R protocol as compared to an SE or SA protocol. Moreover, SBS values for Vertise Flow and Riva Light Cure used with an SA protocol were significantly lower than those of all other groups tested ($p<0.05$).

Conclusion: All the materials tested may be considered appropriate for use as a trauma splint except for Vertise Flow and Riva Light Cure when applied using an SA protocol.

Keywords: Bond strength, dental material, trauma splint

Öz

Amaç: Bu çalışmanın amacı, iki farklı splintleme periyodu sonunda travma splint yapımında kullanılan farklı dental materyallerin sığır minesine makaslama bağlanma dayanım kuvvetini (MBDK) değerlendirmektir.

Gereç ve Yöntemler: Çalışmada taze çekilmiş sığır kesici dişlerinden mine kesitleri elde edildi. Örnekler test materyali, yüzey hazırlık işlemlerine göre rastgele 10 gruba ayrıldı, ardından splintleme süresine göre iki alt gruba daha ayrıldı. Travma splint materyali olarak dört farklı dental materyal (Gradia Direct Anterior Kompozit Rezin, Gradia Core, Vertise Flow, Riva Light Cure HV) test edildi. Dental materyaller üretici tavsiyeleri dikkate alınarak mine yüzeylerine bir universal adeziv kullanılarak etch&rinse (E&R), self-etch (SE) ve/veya self-adhering (SA) olarak bağlandı. Splintleme sürelerinin sonunda MBDK'ler evrensel test cihazı ile değerlendirildi. Elde edilen verilerin istatistiksel analizde t-testi, tek yönlü ANOVA ve Tukey testleri kullanıldı.

Bulgular: Tüm travma splint materyalleri için E&R modundaki gruplarda diğer uygulama gruplarından daha yüksek MBDK'ler gözlendi. En düşük MBDK'ler Vertise Flow ve Riva Light Cure HV'nin SA yani hiç bir yüzey işlemi uygulanmayan gruplarında elde edildi ($p<0,05$).

Address for Correspondence/Yazışma Adresi: Emine Şen Tunç Prof, Ondokuz Mayıs University Faculty of Dentistry, Department of Pediatric Dentistry, Samsun, Turkey
Phone: +90 362 312 19 19-8210 **E-mail:** etunc@omu.edu.tr
ORCID ID: orcid.org/0000-0002-4397-8952

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Sonuç: Vertise Flow ve Riva Light Cure HV'nin hiç bir yüzey işlemi uygulanmayan grupları dışındaki tüm uygulama gruplarının travma splint yapımında kullanılabileceği düşünülmektedir.

Anahtar Kelimeler: Bağlantı kuvveti, dental materyal, travma splinti

Introduction

Traumatic dental injuries are frequently encountered in maxillofacial trauma, and the primary aim of treatment is to preserve the health of the pulp and periodontium (1). Tissue management is crucial to avoid complications (2), and the use of a dental trauma splint (DTS) following tooth repositioning is the standard of care for stabilizing injured teeth and optimizing healing outcomes (3).

From past to present, many different techniques have been used as DTS in dentistry (4,5). Nowadays, The International Association of Dental Traumatology recommends using flexible splints for shorter durations (6), and wire-composite splints are a suitable option for passive mobilization of a traumatized tooth. The splinting materials must be removed from the enamel surface after treatment to prevent bacterial plaque buildup (7,8), without harming the injured tooth or periodontal tissues. Clearly, the high bond strength (BS) to enamel of etch-and-rinse (E&R) adhesives make it hard to remove wire-composite splints without damaging enamel (9). In order to overcome this problem researchers sought alternative splinting systems that had enough BS to endure splinting but were easily removable without damaging the enamel (10,11). Surprisingly, few studies have investigated the effectiveness of dental materials and adhesives in DTS applications, despite the wide range of available products that claim to be versatile and user-friendly. Therefore, this study was conducted to determine the shear BS (SBS) of 4 different dental materials [1 microhybrid composite, 1 dual-cure core, 1 self-adhering (SA) flowable composite (SAFC), 1 resin-modified glass ionomer (RMGIC)] applied to bovine enamel as splints using a universal adhesive (G-Premio Bond) in different modes for splinting periods of 2 different lengths (2 weeks vs 4 months). The tested null hypotheses were as follows: 1) There is no significant difference between the BS of the tested dental materials to bovine enamel; and 2) There is no significant difference between BS of a universal adhesive applied in E&R and self-etch (SE) modes.

Materials and Methods

This study has followed the Checklist for Reporting *In vitro* Studies guidelines for *in vitro* research as discussed in 2014 concept note (12).

Materials

The study was conducted with 4 commercially available dental filling materials: 1 microhybrid composite resin (Gradia Direct Anterior Composite Resin), 1 dual-cure core material (Gradia Core), 1 SAFC (Vertise Flow), 1 RMGIC (Riva Light Cure HV) and 1 universal adhesive (G-Premio Bond). All materials were applied according to the manufacturers' instructions (Table 1).

Preparation of Enamel Specimens

A total of 34 extracted, non-damaged, permanent bovine incisors were used in this study. Before the experiment, the teeth were polished and were then kept in a 0.1% thymol solution at room temperature. Teeth were examined under a stereomicroscope at ×30 magnification and excluded if they had cracks or stains.

Teeth roots were trimmed and 6 enamel specimens measuring approximately 6x6x3 mm were cut from each tooth, for a total of 200 specimens. These were randomly divided into 10 equal groups (n=20) according to test material and surface pretreatment, and each group was further divided into 2 subgroups (n=10 each) according to length of the immersion period (2 weeks vs 4 months) used to simulate minimum and maximum splinting length (6), as follows:

G/SE: Gradia Direct Anterior Composite Resin and G-Premio Bond in SE mode,

G/E&R: Gradia Direct Anterior Composite Resin and G-Premio Bond in E&R mode,

GC/SE: Gradia Core and G-Premio Bond in SE mode,

GC/E&R: Gradia Core and G-Premio Bond in E&R mode,

VF/SE: Vertise Flow and G-Premio Bond in SE mode,

VF/E&R: Vertise Flow and G-Premio Bond in E&R mode,

VF/SA: Vertise Flow in self adhering (SA) mode (any surface pretreatment)

RLHV/E: Riva Light Cure HV and %37 phosphoric acid

RLHV/PA: Riva Light Cure HV and %20 polyacrilic acid

RLHV/SA: Riva Light Cure HV in SA mode.

G-Power v.3.1.9.2 software (Heinrich Heine, University of Dusseldorf, Dusseldorf, Germany) was used to determine the required minimum sample size according to the data of a previous research (13). Based on the parameters of an alpha-type error of 0.05, a beta power of 0.95, and an effect size of 4.18 the minimal estimated sample size per group was found to be 4.

Enamel surfaces of samples were shear-bond tested by embedding them in acrylic resin cylinders. A cylindrical polyethylene tube was placed on the enamel surface after surface pretreatment, filled with the material to be tested. Specimens were cured with an LED (VALO, Ultradent Products Inc, South Jordan, UT, USA) unit for 20s at

1,000 mV/cm^2, stored at 37 ˚C and 100% humidity for 24 hours, and inspected for defects under a stereomicroscope at ×10 magnification. They were then aged in distilled water at 37 ˚C for 2 weeks or 4 months, with weekly water refreshment.

SBS Testing

SBS was calculated (in MPa) by shearing specimens with a knife-edge blade in a universal testing device (Lloyd LRX, Lloyd Instruments, Fareham, Hants, UK) at a crosshead speed of 1.0 mm/min. Pre-test failures were treated as 0 MPa for group mean calculation. Fractured specimens were examined at ×25 magnification under a microscope (Stemi 2,000-C; Carl Zeiss, Gottingen, Germany) and classified as cohesive (C), adhesive (A), or mixed (M) failures.

Statistical Analysis

One way analysis of variance (ANOVA) was used to detect significant differences ($p<0.05$) in SBS values among the groups, and post-hoc comparisons were made using the Tukey HSD test, with the storage period used as the repeated measure. Differences in SBS between subgroups (time periods) of the same group were evaluated using paired t-tests.

Results

Means and standard deviations for the groups are presented in Table 2. The 4-month subgroup of the GC/E&R group had the highest SBS (23.39±7.49), and the 2-week subgroup of the RLHV/SA group had the lowest SBS (0.36±0.15).

In terms of surface pre-treatment, for both splinting periods, the G/E&R and GC/E&R groups had significantly higher ($p<0.05$) SBS values compared to their counterparts applied in SE mode. SBS values for the VF/E&R group were also significantly higher than those of both the VF/SE and VF/SA groups ($p<0.05$), whereas SBS values for the RLHV/E and RLHV/P groups were similar ($p>0.05$) and significantly

Table 1. Composition and application mode of the testing materials

Material	Constituents	Manifacturer	Application
Etch-37 w/BAC	35% phosphoric acid, 1% BAC	Bisco Inc., Schaumburg, USA	1. Gently air dry for 5 s 2. Apply etchant for 15 s 3. Rinse for 10 s and gently air dry for 3 s
G-Premio Bond	10-MDP, phosphoric acid ester monomer, dimethacrylate, 4-MET, MEPS, acetone, silicon dioxide, initiators	GC Corp., Tokyo, Japan	Self - etch mode: 1. Apply the adhesive for 10 s 2. Gently air dry for 5 s 3. Light cure for 10 s Etch - rinse mode: 1. 37% phosphoric acid was applied according to the manufacturer's instructions 2. Apply the adhesive as for the self-etching mode
GC Cavity Conditioner	20% poliacrilic acid, $AlCl_3$	GC Corp., Tokyo, Japan	1. Gently air dry for 5 s 2. Apply etchant for 10 s 3. Rinse for 10 s and gently air dry for 3 s
Gradia Direct Anterior Composite	UDMA, dimethacrylate co-monomers, silica (22%), pre-polymerize fillers (size: 0.85 µm, weight: 42%), pigments	GC Corp., Tokyo, Japan	1. Standardized 2-mm-height cylinders were built up on the specimens 2. Light cure for 20 s
Gradia Core	UDMA, dimetacrilat components (1-5%), Fe–III-oxide (Fe_2O_3)	GC Corp., Tokyo, Japan	1. Standardized 2-mm-height cylinders were built up on the specimens 2. Light cure for 20 s
Vertise Flow	GPDM, HEMA, MeHQ, pre-polymerize particles, Ba-glass, SiO_2, YbF_3	Kerr, Orange, CA, USA	1. Standardized 2-mm-height cylinders were built up on the specimens 2. Light cure for 20 s
RIVA Light cure HV	Poliacrilic acid, tartaric acid, HEMA, strontium, fluoroaluminium silicate	SDI, Victoria, Australia	1. Standardized 2-mm-height cylinders were built up on the specimens 2. Light cure for 20 s

BAC: Benzalkonium chloride, MDP: 10-methacryloyloxydecyl-dihydrogen-phosphate, 4-MET: 4-methacryloxyethyltrimellitate anhydride, UDMA: Urethane dimethacrylate, GPDM: Glycerol phosphate dimethacrylate, HEMA: 2-hydroxyethyl methacrylate, MeHQ: Hydrochinon monoethyl ether

higher compared to the RLHV/SA group ($p<0.05$). No significant SBS decreases were observed over time in any groups. In fact, the SBS values of GC/SE and RLHV/SA groups increased significantly ($p<0.05$).

Fracture modes for each group are given in Table 2. Most failures were adhesive, followed by mixed and cohesive failures. Adhesive failures were associated with lower BS values.

Discussion

This study found different BS for the different resin materials tested; therefore, the first null hypothesis was rejected. Similarly, the universal adhesive used in different modes (E&R and SE) resulted in different BS; therefore, the second null hypothesis was also rejected.

To avoid the drawbacks of wire-composite splints with E&R adhesive (14), new approaches are required for splinting traumatized teeth that are easy to implement and have easily removable materials (11). In the present study, 3 different materials were tested as possible alternatives to conventional composite resins.

Composite resin is an advantageous DTS due to its availability (4). Traditional composites bonded with E&R adhesive have high BS (11,15). In fact, there is no consensus regarding the ideal bond-strength for a trauma splint, difficult removal process of composite splints may harm to periodontal healing (14). This study found the conventional microhybrid G/E&R group to have relatively high SBS values (22.85 at 2 weeks and 20.32 at 4 months). Moreover, in line with previous studies (14,16,17), the SBS values of the G/SE group were significantly lower than those of the G/E&R group ($p<0.05$).

The SBS values of the dual-core GC/E E&R and GC/SE groups were similar to those of the G/E&R and G/SE groups, with the SBS values of the GC/SE group also significantly

Table 2. Mean ± standard deviations and the fracture modes of groups

Storing criteria					
Groups	2 weeks of water storage at 37 °C		4 months of water storage at 37 °C		
	Bond strength (Mean ± SD)	Mode of failure	Bond strength (Mean ± SD)	Mode of failure	p values for paired t-test
G/SE	10.42±4.27[b]	A=8 M=2	10.20±1.37[c]	A=8 M=2	0.896
G/E&R	22.85±3.94[a]	A=3 M=6 C=1	20.32±4.90[a,b]	A=3 M=6 C=1	0.267
GC/SE	8.11±1.95[a,b]	A=8 M=2	11.04±2.39[c]	A=8 M=2	0.028
GC/E&R	19.17±4.95[a]	A=5 M=4 C=1	23.39±7.49[a]	A=5 M=4 C=1	0.258
VF/SE	4.22±2.29[b,c]	A=10	3.21±1.63[d]	A=10	0.318
VF/E&R	19.69±7.75[a]	A=7 M=3	15.80±6.16[b,c]	A=7 M=3	0.215
VF/SA	0.57±0.30[c]	A=10	0.60±0.30[d]	A=10	0.745
RLHV/E	10.70±3.39[b]	A=8 M=2	12.88±4.85[c]	A=8 M=2	0.340
RLHV/PA	12.82±4.75[b]	A=10	14.31±4.13[c]	A=10	0.513
RLHV/SA	0.36±0.15[c]	A=8 M=2	2.18±0.94[d]	A=8 M=2	<0.001
	Time (T)	Group (G)	T*G		
P-values	0.426	<0.001	0.129		

*The letters a, b, c indicate the differences between the application groups within the same measurement time

higher than those of the GC/E&R group (p>0.05). As a dual-core material, Gradia Core adds a chemical cure to its light-curing properties to ensure full polymerization, and it can be used in conjunction with a universal adhesive. Giovannetti et al. (18) discovered that disrupted light transmission can reduce the bonding potential of light-cured materials. Dual-cure materials can solve this issue through chemical reaction-based polymerization. The study indicate that dual-cure core materials can using for DTS as an alternative to composite resin, as indicated by the SBS values obtained for the GC groups.

SAFC resin is a chair time reducing alternative to traditional composites, as it doesn't require surface preparation steps (19). However, this study found the BS of VF to have prohibitively lower than, both of the G/E&R and GC/E&R groups, at intact enamel when used in SA mode or with a universal adhesive mode. This is line with Sadek et al. (20), reported that despite the good surface adaptation of low viscosity flowable composites, their BS is lower than hybrid composites and core materials. Clinicians should only use SAFC for DTS construction if combined with an adhesive bonding agent.

The use of an orthodontic luting RMGIC as a DTS was recently described by Kahler et al. (11). In light of studies demonstrating that the use of an adhesive as a surface pretreatment didn't improve the quality of RMGIC restorations (21); this study evaluated RMGIC BS with no pretreatment (RLHV/SA) and with two types of acid pretreatment: polyacrylic acid (RLHV/PA) and phosphoric acid (RLHV/E). The SBSs of both acid-prepared RLHV sub-groups were similar and significantly higher than the self-adhesive sub-group. These results align with previous studies (22).

Splint materials must sustain bond durability for up to 4 months, while dental materials have a limited lifespan and may degrade due to physical, chemical, or biological stimuli (23). Non-etheless, the study found no significant reduction in the BS of any materials tested over time.

The mean SBS values in this study aligned with Table 2's fracture surface analysis. Only the G/E&R and GC/E&R groups showed cohesive fractures, possibly because universal adhesives' higher BS was achieved with extra phosphoric acid etching, resulting in mixed and cohesive fractures. Using the universal adhesive in SE mode resulted in mostly adhesive failures, also SA groups VF and RLHV had similar results too in our study. These observations align with previous *in vitro* studies (16,17,23).

It is important to note that in clinical situations, blood and other oral fluids may limit the BS of dental materials to intact enamel. The ideal minimum BS required for a DTS to intact enamel remains unclear. To determine the effectiveness of new methods/materials for trauma splinting in actual clinical settings, clinical trials are necessary.

Conclusion

Considering the restrictions of this study,

- Resin-based composites and dual-cure core materials, with universal adhesive, showed adequate SBS values for a semi-rigid trauma splint in both E&R and SE modes. However, the higher SBS values in E&R modes may difficult splint removal.

- A SAFC lacked sufficient SBS values for use as a trauma splint without surface pre-treatment. But, when used with a universal adhesive in E&R or SE mode, its SBS values increased.

- RMGIC samples didn't have suitable SBS values for use as a trauma splint without surface pre-treatment. However, its SBS values increased after pretreatment with phosphoric or polyacrylic acid.

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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: Since the materials used in this study do not related with any patient, informed patient approval was not required.

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Authorship Contributions

Surgical and Medical Practices: Z.Ş., Concept: E.Ş.T., Z.Ş., Design: E.Ş.T., Z.Ş., Data Collection or Processing: Z.Ş., Analysis or Interpretation: E.Ş.T., Z.Ş., Literature Search: E.Ş.T., Z.Ş., Writing: E.Ş.T., Z.Ş.

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Evaluation of Cytotoxicity and Antibacterial Effect of Different Types of Mineral Trioxide Aggregate

Farklı Mineral Trioksit Agregatların Sitotoksisite ve Antibakteriyel Etkilerinin Değerlendirilmesi

Özge Ünlü[1], Edibe Egil[2], Mehmet Demirci[3], Seda Kuşoğlu[4]

[1]İstanbul Atlas University Faculty of Medicine, Department of Medical Microbiology, İstanbul, Turkey
[2]İstanbul Aydın University Faculty of Dentistry, Departmant of Pediatric Dentistry, İstanbul, Turkey
[3]Kırklareli University Faculty of Medicine, Department of Medical Microbiology, Kırklareli, Turkey
[4]Üsküdar University Faculty of Engineering and Natural Sciences, Department of Molecular Biology and Genetics, İstanbul, Turkey

Abstract

Objective: Mineral trioxide aggregate (MTA) has been used as a filling material in endodontic procedures over decades. The newer formulations of MTA were launched in the dental market and their cytotoxic, proliferative and antimicrobial effects need to be revealed. This study compared the possible cytotoxic and proliferative effects on fibroblasts and the antimicrobial activity against *Streptococcus mutans*, *Lactobacillus acidophilus*, and *Enterococcus faecalis* of four different MTAs in the dental market.

Materials and Methods: Cytotoxicity assay was performed on 3T3 fibroblast cell lines were determined using yellow tetrazolium 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, while the antimicrobial activity was tested with the broth microdilution method.

Results: RetroMTA, AngelusMTA, and NeoMTA demonstrated a proliferative effect on 3T3 cells, suggesting induction in tissue repair. Moreover, NeoMTA showed the highest antimicrobial activity against all strains tested.

Conclusion: According to our study, NeoMTA and RetroMTA may be recommended for clinical applications in comparison with the conventional AngelusMTA.

Keywords: Mineral trioxide aggregate, MTA, cytotoxicity, proliferative effect, antimicrobial activity

Öz

Amaç: Mineral trioksit agregat (MTA), endodontik prosedürlerde dolgu malzemesi olarak yıllardır kullanılmaktadır. MTA'nın sitotoksik, proliferatif ve antimikrobiyal etkilerinin yeni formülasyonlarda test edilmesi ihtiyacı oluşturmaktadır. Bu çalışma, fibroblastlar üzerindeki olası sitotoksik ve proliferatif etkileri ile dört farklı MTA'nın *Streptococcus mutans*, *Lactobacillus acidophilus* ve *Enterococcus faecalis* üzerindeki antimikrobiyal aktivitesini karşılaştırmayı amaçlamaktadır.

Gereç ve Yöntemler: 3T3 fibroblast hücre hatları üzerinde sitotoksisite analizi sarı tetrazolyum 3-(4,5-dimetiltiyazol-2-il)-2,5-difeniltetrazolium bromid kullanılarak, antimikrobiyal aktivite ise sıvı mikrodilüsyon yöntemi ile test edildi.

Bulgular: RetroMTA, AngelusMTA ve NeoMTA, 3T3 hücreleri üzerinde proliferatif etki gösterdiği gözlemlenmiştir. Antibakteriyel etki açısından incelendiği zaman NeoMTA en yüksek etkiyi göstermiştir.

Sonuç: Çalışmamıza göre NeoMTA ve RetroMTA'nın klinik uygulamalarda klasik AngelusMTA'ya alternatif olarak önerilebileceği düşünülmektedir.

Anahtar Kelimeler: Mineral trioksit agregat, MTA, sitotoksisite, proliferatif etki, antimikrobiyal aktivite

Address for Correspondence/Yazışma Adresi: Assoc. Prof. Edibe Egil, İstanbul Aydın University Faculty of Dentistry, Departmant of Pediatric Dentistry, İstanbul, Turkey
Phone: +90 212 411 30 00 **E-mail:** edibeegil@aydin.edu.tr
ORCID ID: orcid.org/0000-0003-0889-0223

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Introduction

Majority of endodontic failures occur in a consequence of leakage of irritants into the periapical tissues. An ideal filling material should seal the root canal system and it should not have cytotoxic or genotoxic effects, also it should be compatible with host tissues and dimensionally stable (1). Therefore, an important aim of endodontic therapy is the elimination of microorganisms from the root canal. The amount of microorganisms inside the infected root canal is reduced by intracanal medication, instrumentation and irrigation (2,3). Thus, an endodontic sealer with antimicrobial effects may be beneficial in order to eliminate complications after endodontic processes.

Mineral trioxide aggregate (MTA) was developed during the 1990s. Although initially recommended as a root-end filling material, in the following years it has been used for pulp capping, pulpotomy, apexogenesis, apical barrier formation in teeth with open apexes, repair of root perforations, and as a root canal filling material (1,2).

Different types of MTA were released from different manufacturers since the first MTA production. Revealing the possible cytotoxic or proliferative effects of MTA is important as it is in close contact with the gingiva and the fibroblasts which are the predominant cell type in the area (3,4). This study aimed to investigate and compare different MTAs in the dental market according to their possible cytotoxic and proliferative effects on fibroblasts, also to reveal antibacterial activity against *Streptococcus mutans* ATCC 25175, *Lactobacillus acidophilus* ATCC 4356 and *Enterococcus faecalis* ATCC 29212, which are the most frequently recovered microorganisms from refractory periapical periodontitis. The null hypothesis is that there is no difference between different types of MTA according to their possible cytotoxic and proliferative effects, and antibacterial activity.

Materials and Methods

Tested cements

MTA-Angelus (AngelusMTA; Angelus Soluções Odontológicas, Londrina, Brazil), EndocemMTA (CemMTA; Maruchi, Wonju-si, Korea), RetroMTA (RetroMTA; BioMTA, Daejeon, Korea) and NeoMTA (NeoMTA; Avalon Biomed Inc. Bradenton, FL, USA) were tested to reveal their antibacterial activity and cytotoxicity. Since the materials used in this study do not related with any patient, ethical and informed patient approval was not required.

Bacterial strains

Streptococcus mutans American Type Culture Collection (ATCC) 25175, *Lactobacillus acidophilus* ATCC 4356 and *Enterococcus faecalis* ATCC 29212, which are primary dental pathogens were chosen in order to compare MTAs antibacterial activity and these strains purchased from Refik Saydam National Public Health Agency, Turkey.

Antimicrobial Broth Microdilution Test

Antimicrobial activity of the selected MTAs were performed according to the instructions of the Clinical Laboratory Standards Institute. Ten mg/mL of each MTA was dissolved in Brain Heart Infusion Broth, and 180 uL of each MTA dissolved medium was added to the first well of the relevant row of the 96 well plate. Serial dilutions were done for each MTA dissolved medium up to 8 fold. Overnight broth cultures of *S. mutans*, *L. acidophilus* and *E. faecalis* were adjusted to the turbidity of a 0.5 McFarland standard. Twenty uL of each strain were inoculated each well. Broth without MTA materials was served as controls for comparison. Plate incubated overnight and bacteria levels in each well were measured with a spectrophotometer at 600 nm (5).

Cytotoxicity Assay

The cytotoxic effects of RetroMTA, AngelusMTA, NeoMTA and CemMTAs on 3T3 embryonic mouse fibroblast cell lines were determined using yellow tetrazolium 3- (4-5-dimethyl thiazolyl-2) -2,5-diphenyltetrazolium bromide (MTT). The 3T3 cell line was cultured in "Dulbecco's Modified Eagle's Medium/High Glucose" (DMEM/High, Gibco 41966), containing 10% (v/v) fetal bovine serum (heat-inactivated), 1% (v/v) penicillin-streptomycin antibiotic. All incubations were done at 37 °C in a humidified atmosphere of 5% CO_2. MTT Assay was performed according to the recommended protoco (6). Flow-chart for MTT cytotoxicity assay peocedure was shown in Figure 1.

Statistical Analysis

When the tests were performed twice, 10 uL of distilled water was applied to the control group which was considered as 100% viable.

Cell viability % = [Absorbance570 (treated wells)/ Absorbance570 (control wells)] x 100

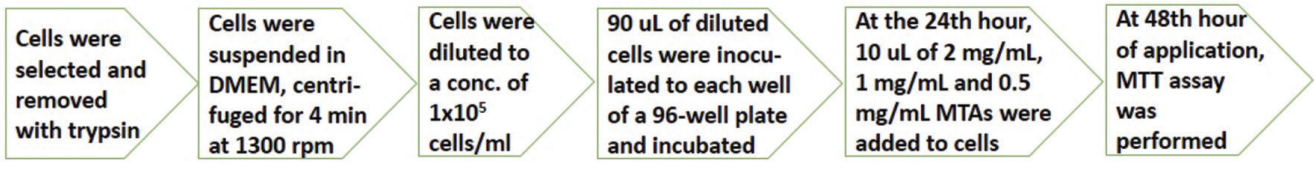

Figure 1. Flow chart for MTT cytotoxicity assay procedure
MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide

One-way ANOVA test was utilized to analyze the consistency between the data obtained from MTT tests to determine the effects of MTAs on 3T3 cell viability using IBM SPSS software version 22.0 (IBM Corporation, New York, USA). The significance of the experimental groups according to the control group was analyzed by Dunnett's test.

The concentrations of the substances were applied to the apse and the cell viability (%) data was placed on the ordinate and the graphics created with GraphPad Prism 8.0 program (Graph-Pad Software, Inc., San Diego, CA, USA).

Results

Antibacterial Broth Test

Antimicrobial susceptibility tests revealed that 10 mg/mL NeoMTA inhibited the growth of *S. mutans*, *L. acidophilus* %100 and *E. faecalis* at a rate of 89% and had the most powerful effect against bacterial growth. Also, it has been observed that NeoMTA had an inhibitory effect on the growth of *S. mutans* and *E. faecalis* at the lowest concentration tested which was 1.25 mg/mL.

In addition, 10 mg/mL AngelusMTA inhibited the growth of *L. acidophlius*, *S. mutans* and *E. faecalis* at the following rates 100%, 99% and 62%, respectively. Also, it had an inhibitory effect on the growth of *S. mutans* and *E. faecalis* until the lowest concentration tested which was 1.25 mg/mL.

Moreover, 10 mg/mL RetroMTA had an inhibitory effect on *S. mutans*, *L. acidophilus* and *E. faecalis* at the rates of 89%, 70% and 75%, respectively. RetroMTA's inhibitory effects were observed at descending rates until 1.25 mg/mL for *S. mutans* and *E. faecalis*, and 2.5 mg/mL for *L. acidophilus*.

Finally, according to antimicrobial susceptibility tests 10 mg/mL CemMTA had an inhibitory effect on *L. acidophilus* and *E. faecalis* at the rates of 94% and 11%. However, it did not show any inhibition on the growth of *S. mutans*.

All the results of antimicrobial susceptibility assays for *S. mutans*, *L. acidophilus* and *E. faecalis* were given in Table 1.

Cytotoxicity Assay

The highest application dose (2 mg/mL) of NeoMTA and RetroMTA's cytotoxic effect could not be measured in the spectrophotometer due to the sediment formed resulting from the interaction between MTA and the medium.

Table 1. Inhibitory effects (%) of different types of MTA against the growth of *S. mutans*, *L. acidophilus* and *E. faecalis*

	MTA	10 mg/mL	5 mg/mL	2.5 mg/mL	1.25 mg/mL
S. mutans ATCC 25175	NeoMTA	99.00%	93.48%	52.31%	28.14%
	RetroMTA	88.87%	38.16%	11.61%	10.65%
	AngelusMTA	98.73%	69.16%	14.15%	5.56%
	CemMTA	0.00%	0.00%	0.00%	0.00%
L. acidophilus ATCC 4356	NeoMTA	100.00%	83.02%	15.84%	0.00%
	RetroMTA	70.42%	30.73%	1.15%	0.00%
	AngelusMTA	100.00%	51.34%	-9.16%	0.00%
	CemMTA	94.27%	84.73%	31.49%	0.00%
E. faecalis ATCC 29212	NeoMTA	89.10%	67.29%	28.41%	22.53%
	RetroMTA	74.89%	26.54%	19.66%	16.64%
	AngelusMTA	62.41%	20.09%	8.90%	4.73%
	CemMTA	11.33%	-68.29%	30.42%	0.00%

MTA: Mineral trioxide aggregate

Table 2. Cell viability % results of four MTAs

	2 mg/mL	1 mg/mL	0.5 mg/mL
RetroMTA	Not measured	74.33±5.21	105.48±6.34
AngelusMTA	Not measured	86.52±3.97	104.89±4.49
NeoMTA	Not measured	73.29±4.78	106.35±8.39
CemMTA	Not measured	Not measured	88.32±3.5

MTA: Mineral trioxide aggregate

Cytotoxicity assays revealed that the application dose (1 mg/mL) of RetroMTA, AngelusMTA and NeoMTAs were determined to have 26%, 14% and 27% cytotoxic effect on 3T3 cells, respectively (**p<0.01, **p<0.01, ***p<0.001, respectively).

The lowest application dose (0.5 mg/mL) of RetroMTA, AngelusMTA and NeoMTA was determined to have 5%, 5% and 6% proliferative effect on 3T3 cells, respectively (***p<0.001, **p<0.01, ***p<0.001, respectively).

Moreover, CemMTA's cytotoxic effect in 2 mg/mL and 1 mg/mL concentrations could not be measured spectrophotometrically, because of the sediment formed in the medium. However, it was observed that 0.5 mg/mL CemMTA had a %12 toxic effect on 3T3 cell line. The cytotoxic effects of all tested MTA's were given in Figure 2. In addition, cell viability % results of four MTAs were given in Table 2.

Discussion

MTA was developed as a root-end filling material and it has been used for a long time. Different types of new MTAs were released from different manufacturers (1,2). However, these new MTAs should be investigating a focus on biocompatibility and antibacterial activity.

According to the findings of the present study, we tested three oral bacteria that were commonly associated with oral diseases. It was reported in previous studies that initial carious lesion is associated with *S. mutans* and cavitated lesion are associated with both *S. mutans* and *Lactobacillus* (1,3). MTA is used as a direct/indirect pulp capping material. the materials used in deep cavities should be able to maintain the vitality of the pulp, prevent the entry of residual bacteria into the root canal system and reduce the pulp inflammation (7,8).

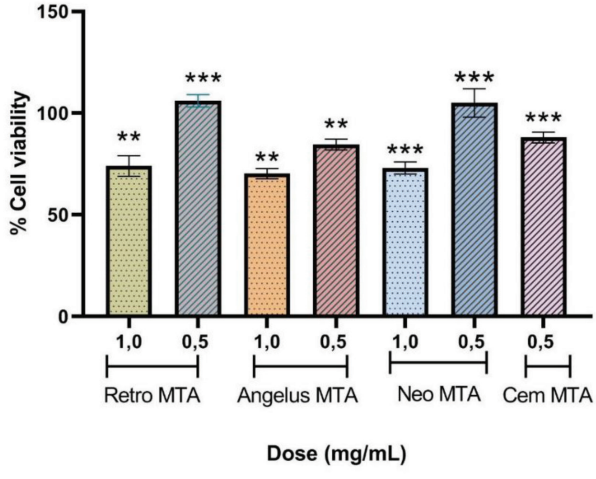

Figure 2. Cytotoxic effects of four kind of MTA's in different concentrations

*MTA: Mineral trioxide aggregate, **p<0.01, ***p<0.001, vertical bars demonstrating standard deviation values*

NeoMTA, AngelusMTA and RetroMTA have similar antibacterial effects to *S. mutans*. The only exception was the non-significant antibacterial activity of CemMTA against the *S. mutans*. These results agree with previous studies. Luczaj-Cepowicz et al. (9) reported that AngelusMTA had a good antibacterial effect against the standard strains of *S. mutans*. Donyavi et al. (10) reported that RetroMTA had antibacterial activities against the *S. mutans*. To the best of our knowledge, there is no study about the antibacterial activity of CemMTA and NeoMTA.

There are no reports in the literature of studies that have examined the antimicrobial properties of the MTAs against *L. acidophilus*. Our study was the first study to collect this data. NeoMTA and AngelusMTA had the greatest antibacterial effect against the growth of *L. acidophilus*. The concentration (CFU/mL) of this species in the presence of this biomaterial was zero (100% reduction). RetroMTA and CemMTA demonstrated acceptable antibacterial activity against the standard strains of *L. acidophilus*. Despite the fact that NeoMTA and AngelusMTA were very successful in inhibiting the growth of *L. acidophilus*, either RetroMTA and CemMTA could be used for pulp capping in deep caries lesion.

We investigated the antibacterial effect of *E. faecalis* because it is the most isolated microorganism from the infected root canals. Antibacterial properties of root-canal sealers gain importance in preventing the regrowth of bacteria in the root canals (11). It was reported that MTA has an antibacterial effect against the *E. faecalis* in previous studies (12,13). Donyavi et al. (10) reported that RetroMTA had antibacterial activities against the *E. faecalis*. Koçak et al. (14) reported that AngelusMTA had acceptable MBCs against *E. faecalis*. In the present study, NeoMTA, RetroMTA and AngelusMTA showed similar antibacterial activity against *E. faecalis*. Our results agree with previous studies.

The main components of the antibacterial effect of MTA are tricalcium silicate and dicalcium silicate. When these components are mixed with water, alkaline calcium silicate gel forms. The calcium hydroxide in the silicate matrix releases hydroxide ions. As a result, a highly alkaline environment is formed and bacterial growth is prevented (15-17). In addition to these two main components, materials with different properties have been added to the MTAs used today. Differences in the antibacterial activities of the four MTA types used in this study are probably the result of differences in structure and composition.

The cytotoxicity of end-root filling materials is a major concern for dentists. Antimicrobial components in the root-canal sealers do not have selective toxicity, they may show toxic effects on host cells. The toxic effects of these materials can cause degeneration of periapical tissue and delay wound healing (1-3,18,19). In the present study, the biocompatibility of RetroMTA, AngelusMTA, NeoMTA and CemMTA, was evaluated by using a MTT assay, comparing their cytotoxicity with well-studied AngelusMTA.

MTT test was utilized in order to evaluate the metabolic effects of AngelusMTA, CemMTA, RetroMTA and NeoMTA on 3T3 cells. In living cells, due to the presence of the mitochondrial dehydrogenase enzyme, the tetrazolium ring of the MTT (3- (4,5-dimethylthiazole-2,5-diphenyltetrazolium)) molecule is cleaved, resulting in formation of water-insoluble formazan crystals. Then, formazan crystals are dissolved by DMSO and their absorbances are measured with a spectrophotometer at 570 nm. MTT may activate apoptosis-related factors such as intracellular caspase-8, caspase-3, or intracellular leaks that may occur according to the formation of MTT formazan crystals. Thus, attention should be taken in order not to lose control of cell viability during the MTT test, which is one of the most widely used methods to analyze cell viability and proliferation. However, there may be deviations in the MTT test as a result of the interaction of metabolic rate and mitochondria number with various factors, which is the main disadvantage of the MTT method (20,21).

Eukaryotic cells isolated from animal tissues and having limited ability to reproduce under standard conditions are prevented from aging by providing continuous reproduction ability in cell culture (22). These cells have been used for many years in many biological and biochemical researches, such as drug/chemical agent-dose trials.

The cell line to be used to determine the toxicity of chemical agents on the cell should be related to the natural use of the chemical (23). ISO 10993-5 cytotoxicity tests, the study of the toxicity of dental materials on cells, the use of the cell type used in this study is recommended. Therefore, in our study, the cytotoxicity of MTA materials was analyzed using a 3T3 cell line.

Kouchak Dezfouli et al. (24) compared the cytotoxicity of RetroMTA with ProRootMTA and reported that both of them showed similar biocompatibility. In our study, AngelusMTA showed a better percentage of cell viability. RetroMTA and NeoMTA showed similar cell viability. RetroMTA, AngelusMTA and NeoMTA have a proliferative effect on 3T3 cells, suggesting induction in tissue repair. As a result of our findings, it can be recommended to use MTA as a pulp capping material.

Conclusion

In conclusion, the NeoMTA showed the best antibacterial activity against all strains we tested *in vitro* and it was also found to be the most biocompatible material according to our results. Therefore, based on our findings on the antibacterial effect of tested MTA materials against the main bacteria associated with dental diseases, NeoMTA and RetroMTA may be recommended for dental clinical applications when compared with conventional AngelusMTA in the dental market.

Ethics

Ethics Committee Approval: Since the materials used in this study do not related with any patient, ethical approval was not required.

Informed Consent: Since the materials used in this study do not related with any patient, informed patient approval was not required.

Peer-review: Externally and internally peerreviewed.

Authorship Contributions:

Concept: E.E., Design: Ö.Ü., M.D., Data Collection or Processing: E.E, Analysis or Interpretation: M.D., S.K., Literature Search: E.E., Writing: Ö.Ü., E.E., M.D., S.K.

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

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Salivary Protease Activity in Children with Cystic Fibrosis

Kistik Fibrozisli Çocuklarda Tükürük Proteaz Aktivitesi

Zeynep Pınar Keleş Yücel[1], Taina Tervahartiala[2], Angelika Silbereisen[3], Yavuz Tokgöz[4], Timur Köse[5], Georgios Tsilingaridis[6], Nagihan Bostancı[3], Timo Sorsa[2], Gülnur Emingil[7]

[1]Giresun University Faculty of Dentistry, Department of Periodontology, Giresun, Turkey
[2]University of Helsinki and Helsinki University Hospital, Head and Neck Center, Department of Oral and Maxillofacial Diseases, Helsinki, Finland
[3]Karolinska Institutet, Section of Periodontology and Dental Prevention, Department of Dental Medicine, Division of Oral Diseases, Stockholm, Sweden
[4]Aydın Adnan Menderes University Faculty of Medicine, Department of Pediatric Gastroenterology, Hepatology and Nutrition, Aydın, Turkey
[5]Ege University Faculty of Medicine, Department of Biostatistics and Medical Informatics, İzmir, Turkey
[6]Karolinska Institutet, Department of Dental Medicine, Division of Orthodontics and Pediatric Dentistry, Stockholm, Sweden
[7]Ege University Faculty of Dentistry, Department of Periodontology, İzmir, Turkey

Abstract

Objective: Patients with cystic fibrosis (CF) present with impaired protease-antiprotease balance in their lungs. However, salivary protease equilibrium in children with CF with poor oral health has not been reported. The current study investigated salivary matrix metalloproteinase-8 (MMP-8), tissue inhibitor of matrix metalloproteinases-1 (TIMP-1), neutrophil elastase (NE), and myeloperoxidase (MPO) levels in children with CF with or without gingivitis.

Materials and Methods: Eleven CF and 11 systemically healthy children aged 3-12 years were evaluated. Clinical periodontal examinations including probing pocket depth (PPD), gingival index (GI), plaque index (PI), and bleeding on probing (BOP) were recorded and saliva samples were obtained. Salivary MMP-8, TIMP-1, NE, and MPO levels were analyzed by immunofluorometric assay and enzyme-linked immunosorbent assay.

Results: Salivary levels of MMP-8, TIMP-1, NE, MPO, and MMP-8/TIMP-1 molar ratios were similar in CF and systemically healthy children ($p>0.05$). Levels of MMP-8, NE, and MPO were significantly higher in the saliva of children with gingivitis compared to periodontally healthy children in both CF and systemically healthy groups ($p<0.05$). MMP-8 and MMP-8/TIMP-1 levels were positively correlated with GI, PI, and BOP ($p<0.05$), while NE and MPO levels were related to all periodontal parameters ($p<0.01$).

Conclusion: CF may not alter the activity of MMP-8, NE, and MPO. On the other hand, gingival inflammation had a pronounced effect on salivary levels of these enzymes and the MMP-8/TIMP-1 molar ratio in children, irrespective of CF. Further investigations in larger cohorts are needed to better clarify this issue.

Keywords: Gingivitis, matrix metalloproteinases, leukocyte elastase, cystic fibrosis, biomarkers, saliva

Öz

Amaç: Kistik fibrozisli (KF) hastalar, akciğerlerde bozulmuş proteaz-antiproteaz dengesi ile kendini göstermektedir. Bununla birlikte, ağız sağlığı kötü olan KF'li çocuklarda tükürük proteaz dengesini gösteren bir çalışma henüz bulunmamaktadır. Bu çalışmanın amacı, gingivitise sahip olan veya olmayan KF'li çocuklarda tükürük matriks metalloproteinaz-8 (MMP-8), matriks metalloproteinaz-1 doku inhibitörü (TIMP-1), nötrofil elastaz (NE) ve miyeloperoksidaz (MPO) seviyelerinin araştırılmasıdır.

Gereç ve Yöntemler: Üç - on iki yaşları arasında 11 KF ve 11 sistemik sağlıklı çocuk değerlendirildi. Klinik periodontal muayenede, sondalanabilir cep derinliği, gingival indeks (Gİ), plak indeksi (Pİ) ve sondalamada kanama (SK) değerleri kaydedildi; ve tükürük örnekleri alındı. Tükürük MMP-8, TIMP-1, NE ve MPO seviyeleri, immünoflorometrik analiz ve enzime bağlı immünosorbent analiz metodlarıyla değerlendirildi.

Address for Correspondence/Yazışma Adresi: Zeynep Pınar Keleş Yücel Assoc. Prof., Giresun University Faculty of Dentistry, Department of Periodontology, Giresun, Turkey
Phone: +90 532 067 98 88 **E-mail:** zeynepinar14@hotmail.com
ORCID ID: orcid.org/0000-0001-9139-8752

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Bulgular: KF ve sistemik sağlıklı çocuklarda tükürük MMP-8, TIMP-1, NE, MPO ve MMP-8/TIMP-1 molar oranı düzeyleri benzerdi (p>0,05). Gingivitise sahip olan çocuklarda tükürük MMP-8, NE ve MPO seviyeleri periodontal olarak sağlıklı çocuklara göre hem KF hem de sistemik olarak sağlıklı gruplarda anlamlı olarak daha yüksekti (p<0,05). MMP-8 ve MMP-8/TIMP-1 seviyeleri Gİ, Pİ ve SK ile pozitif korelasyon gösterirken (p<0,05), NE ve MPO seviyeleri tüm klinik periodontal parametrelerle ilişkili bulundu (p<0,01).

Sonuç: KF, MMP-8, NE ve MPO'nun aktivitesini değiştirmeyebilir. Ancak çocuklarda gingival enflamasyon, KF'den bağımsız olarak bu enzimlerin tükürük seviyeleri ve MMP-8/TIMP-1 molar oranı üzerinde belirgin bir etkiye sahipti. Bu konuyu açıklığa kavuşturmak için daha büyük kohort çalışmalarına ve ileri araştırmalara ihtiyaç vardır.

Anahtar Kelimeler: Gingivitis, matriks metalloproteinazlar, lökosit elastaz, kistik fibrozis, biyomarkerlar, tükürük

Introduction

Cystic fibrosis (CF) is an autosomal recessive inflammatory disease associated with the cystic fibrosis transmembrane conductance regulatory (CFTR) gene dysfunction affecting many organs, especially lungs, causing chronic destructive pulmonary infections as the main reason for early death (1). The CFTR dysfunction causes impaired ion channel function and thus leads to the hyper-viscous secretions from exocrine glands, including salivary glands, resulting in changed salivary properties and profile (2,3). Mucus hyper-viscosity furthermore facilitates increased colonization in the airways by Gram-negative bacteria like *Pseudomonas aeruginosa* leading to non-resolving neutrophilic inflammation (4). In the pathogenesis of CF, proteolytic enzymes released by neutrophils are believed to have a central role in processes causing tissue damage and abnormal tissue remodeling (5,6). In this context, matrix metalloproteases (MMPs), which exert numerous biological functions including both physiological remodeling of tissues and pathological tissue destruction in chronic inflammatory conditions including periodontal disease, seem to be of specific importance (7,8). MMP secretion and activity are strictly regulated by specific tissue inhibitors of metalloproteinases (TIMPs) and a dysfunction of TIMPs and/or a MMP-TIMP disbalance promotes the pathological destruction of gingival tissue (8).

Neutrophil elastase (NE) is well-known as one of the major destructive proteases degrading extracellular matrix (ECM) components, and is believed to have effects on increased MMP activity and degradation of TIMPs. NE has also been shown to be a biomarker of CF (7). In addition to NE, myeloperoxidase (MPO) is the other important destructive enzyme released by degranulation of neutrophils. MPO has a pivotal role in oxidative stress mechanisms and it can activate both latent proMMP-8 and proMMP-9 through this way as well as oxidatively inactivate TIMPs (8). Therefore, NE and MPO have the ability to modify inflammatory reactions by promoting MMPs.

Studies have shown that CF patients have increased MMP-9 and lower TIMP-1 concentrations due to the NE action in their sputum or bronchoalveolar lavage fluid (BAL) (7,9). On the other hand, clinical research clearly indicated altered salivary flow rate and its components during the course of CF (10). Saliva is an important fluid reflecting changes in the oral cavity and thus periodontal inflammation (11). It was hypothesized that changes in the salivary content and increased levels of MMPs in CF might affect the periodontal health. Therefore, this study aimed to investigate salivary MMP-8, TIMP-1, MMP-8/TIMP-1 molar ratio, NE and MPO levels in CF and systemically healthy children, in the presence or absence of gingival inflammation.

Materials and Methods

Study population

Eleven children with CF (aged 3 to 12 years) monitored by the Aydın Adnan Menderes University Faculty of Medicine, Department of Pediatric Gastroenterology, Aydın, Turkey between May 2016 and December 2018 were enrolled for the present study. All children with CF had a verified diagnosis by abnormal sweat test in combination with the presence of characteristic clinical properties compatible with the CF phenotype and/or a genotype with mutations. CF patients who were clinically and symptomatically stable with no acute respiratory infections for at least 4 weeks and with no other systemic disease were included. Patients were excluded if they used probiotics or any anti-inflammatory drugs, need for a lung transplantation, had pulmonary exacerbation and have taken oral/intravenous antibiotics or any other medications that may affect the periodontium and study findings in the previous four months (12). For the control group (C), eleven age and gender matched systemically healthy children applied for dental checkups to the Aydın Adnan Menderes University Faculty of Dentistry, Aydın, Turkey were included. In both groups, the exclusion criteria also included the received periodontal treatment, use of medications (antibiotic or other anti-inflammatory drugs) in the last four months, presence of caries and having less than 10 fully-erupted teeth. The ethical principles stated in the World Medical Association's Declaration of Helsinki were followed; and this study protocol was approved by the Ethics Committee on Clinical Researches of Ege University (decision number: 17-5/9, date: 11.09.2017). Following the aim and the procedures of this study were explained, informed consent was provided from all participants before the enrollment [parents (in writing) and children (orally)].

Clinical Periodontal Assessment

Clinical periodontal examination was performed for each participant in CF and C groups to identify their periodontal status. For the clinical periodontal examination, probing

pocket depth (PPD), gingival index (GI) (13), plaque index (PI) (14) and bleeding on probing (BOP) (15) parameters were measured and recorded at four sites (mesial, distal, buccal, lingual/palatinal) on each fully-erupted tooth present by a single calibrated periodontist (ZPKY) using a Williams periodontal probe (Hu-Friedy, Chicago, IL). Clinical attachment level was also evaluated, however data was not shown since none of the children had any attachment loss. Additionally, the determination of calculus formation was performed by a visual assessment on the surface of each tooth and noted as present or absent (16). This data was not presented either since no calculus was observed on any tooth surface in both CF and systemically healthy children. The children were defined as periodontally healthy (H) if they had clinically healthy gingiva (GI=0), good oral hygiene, and PPD ≤3 mm with no clinical attachment loss and radiographic bone loss. Children showing GI ≥1 and PPD ≤3 mm were diagnosed with gingivitis (G). After the completion of the periodontal examination, children were further divided into subgroups based on their periodontal conditions: CF and periodontally healthy (CF-H, n=6); CF and gingivitis (CF-G, n=5); systemically healthy and periodontally healthy (C-H, n=6); systemically healthy and gingivitis (C-G, n=5).

Saliva Sampling

Saliva samples (n=22) were obtained by asking the patients to expectorate into sterile 50 mL polypropylene tubes for five minutes in the morning hours (8.00-10.00 am) following an overnight fast one day after the clinical periodontal measurements (17). All children were requested to avoid eating, drinking and any oral hygiene procedures two hours before the sampling under supervision of their parents. Obtained saliva was centrifuged at 10,000 x g for 15 minutes at 4 °C and the supernatants were snap frozen (-20 °C) and stored at -80 °C.

MMP-8 Analysis by Immunofluorometric Assay

Salivary MMP-8 levels were measured by a time-resolved immunofluorometric assay (Medix Biochemica, Espoo, Finland), as previously described (18). The detection limit for MMP-8 was 0.08 ng/mL.

TIMP-1, NE and MPO Analyses by Enzyme-linked Immunosorbent Assay

Salivary TIMP-1, NE and MPO levels were determined by commercial enzyme-linked immunosorbent assays (ELISAs) (Human TIMP-1, Human Biotrak ELISA Systems, GE Healthcare, Buckinghamshire, UK; Human NE, Platinum ELISA, Bender MedSystemss GmbH, Vienna, Austria; Human MPO, Immunodiagnostic AG, Bensheim, Germany) according to the manufacturer's instructions, as previously described (19). The detection limit for TIMP-1 was 1.25 ng/mL, for NE 1.98 pg/mL and for MPO 1.6 ng/mL.

The calculation of MMP-8/TIMP-1 molar ratio was performed to convert ng/mL levels to mol/L (18).

Statistical Analysis

The distribution of all numerical variables was tested by Shapiro-Wilk normality test. For data of BOP and NE variables that were not normally distributed, normality was achieved by logarithmic transformation. Then, 2x2 factorial ANOVA was carried out for intergroup comparisons of all descriptive variables including the effect of periodontal status, systemic condition and interactions between CF and the periodontal status. Sex ratio was assessed using chi-square test. The possible correlations of biochemical variables with clinical periodontal parameters were analyzed using Pearson correlation test. All data analyses were performed using the statistical software program (SPSS, v22.0, IBM, Chicago, IL) at α=0.05 significance level.

Results

Demographic Characteristics and Clinical Findings

Demographics of the children with CF and systemically healthy and their related subgroups are shown in Table 1. Age and sex distribution were not significantly different among the study groups (CF and C) as the groups were matched ($p>0.05$). For subgroups, mean age (years) of CF-G, C-G, CF-H and C-H was 8.20±2.59, 8.60±2.51, 6.00±3.16 and 6.00±1.67, respectively. The mean age of CF-H and C-H was significantly lower than the CF-G and C-G ones ($p<0.05$). Male-to-female ratio was similar among the subgroups ($p>0.05$).

Clinical periodontal findings of CF and systemically healthy children and their related subgroups are outlined in Table 2. Clinical periodontal measurements including PPD, GI and PI values showed no significant difference between CF and C groups ($p>0.05$), whereas BOP scores of the CF children were significantly higher compared to the C children ($p<0.05$). In the subgroups, CF-G and C-G had higher scores of PPD, GI, BOP and PI compared to CF-H and C-H children ($p=0.003$ for PPD and $p<0.0001$ for GI, BOP, PI).

Biochemical Findings

The biochemical findings of the CF and systemically healthy children and their related subgroups are outlined in Table 3. No significant differences were found between the CF and C groups regarding salivary levels of MMP-8, TIMP-1, MMP-8/TIMP-1 molar ratio, NE and MPO ($p>0.05$). The CF-G and C-G subgroups exhibited elevated MMP-8, MMP-8/TIMP-1, NE and MPO in saliva when compared to the periodontally healthy subgroups (CF-H and C-H) ($p<0.05$); while no statistically significant difference was observed in salivary concentrations of TIMP-1 among all four subgroups ($p>0.05$).

Correlations Between Clinical Parameters and Biochemical Data

Correlations of age and clinical parameters with biochemical data are presented in Table 4. Salivary NE and MPO levels had a strong positive relationship with

all clinical periodontal parameters (PPD, GI, BOP and PI) ($p<0.01$). MMP-8 levels and MMP-8/TIMP-1 molar ratio were also positively associated with GI, BOP and PI ($p<0.05$). No correlation was detected between age and the biochemical parameters (MMP-8, TIMP-1, MMP-8/TIMP-1 molar ratio, NE and MPO) ($p>0.05$).

Discussion

This age matched case-control study evaluating the possible association between CF and periodontal health showed that salivary MMP-8, MMP-8/TIMP-1, NE and MPO levels were elevated in the presence of gingival inflammation in both CF and systemically healthy children. Moreover, salivary MMP-8, MMP-8/TIMP-1, NE and MPO levels were positively correlated with GI, BOP and PI and also NE and MPO levels in saliva were positively associated with PPD.

Earlier studies examining clinical periodontal status in CF children reportedly stated no significant differences in plaque accumulation (20,21), gingival bleeding (21-24) or calculus occurrence (22,23) between the patients of CF and systemically healthy controls. In contrast to the earlier reports, BOP scores were significantly increased in children with CF compared to systemically healthy ones. However, other periodontal parameters such as PPD and PI were not significantly different between CF children and systemically healthy controls. These results may be due to the differences in age ranges or clinical indexes evaluated in previous studies (20-24). On the other hand, the current findings of high BOP scores in CF patients suggested that the systemic inflammation of CF could exacerbate gingival inflammation and lead to the manifestation or worsening of the clinical signs of periodontal disease. This is supported by a recently published study that demonstrates a higher BOP as well as an increased pro-inflammatory host response in CF patients with gingivitis than those of non-CF (25).

There is a considerable evidence that function of salivary glands are affected in patients with CF due to the defective

Table 1. Demographic data of groups and related subgroups

	Cystic fibrosis (CF) (n=11)		Systemically healthy (Control) (C) (n=11)	
Age (years)	7.00±3.00		7.18±2.40	
Sex (F/M)	6/5		5/6	
	CF-H (n=6)	CF-G (n=5)	C-H (n=6)	C-G (n=5)
Age (years)	6.00±3.16	**8.20±2.59**	6.00±1.67	**8.60±2.51**
Sex (F/M)	3/3	3/2	3/3	2/3

CF-H: Cystic fibrosis-periodontally healthy, CF-G: Cystic fibrosis-gingivitis, C-H: Control-periodontally healthy, C-G: Control-gingivitis. Values in bold: Significant differences ($p<0.05$) compared to CF-H and C-H

Table 2. Clinical periodontal evaluation of groups and related subgroups

	Cystic fibrosis (CF) (n=11)		Systematically healthy (Control) (C) (n=11)		Cystic fibrosis effect p-value	
GI	1.13±0.67		0.83±0.68		0.070	
PI	0.97±0.45		0.81±0.44		0.140	
BOP (%)	1.33±0.39		1.10±0.43		**0.001**	
PPD (mm)	2.07±0.37		2.03±0.41		0.695	
	CF-H (n=6)	CF-G (n=5)	C-H (n=6)	C-G (n=5)	Periodontal status effect p-value	Cystic fibrosis-periodontal status interaction p-value
GI	0.61±0.21	1.74±0.45	0.32±0.15	1.44±0.53	**<0.001**	0.984
PI	0.63±0.20	1.37±0.32	0.46±0.15	1.24±0.22	**<0.001**	0.833
BOP (%)	1.01±0.13	1.72±0.10	0.75±0.17	1.52±0.16	**<0.001**	0.607
PPD (mm)	1.81±0.24	2.38±0.22	1.86±0.42	2.22±0.33	**0.003**	0.446

GI: Gingival index, PI: Plaque index, BOP: Bleeding on probing, PPD: Probing pocket depth, CF-H: Cystic fibrosis-periodontally healthy, CF-G: Cystic fibrosis-gingivitis, C-H: Control-periodontally healthy, C-G: Control-gingivitis. Values in bold for two main groups: Significant difference ($p<0.05$) from C group. Values in bold for subgroups: Significant differences ($p<0.05$) compared to CF-H and C-H

CFTR gene (26). Such genetic alteration can affect the composition, properties and flow of saliva in patients with CF (10,27-29). In addition, researchers have confirmed altered salivary protein profiles in patients suffering from CF (10,29). However, the literature dealing with periodontal status in CF is based solely on clinical assessments and specific proteins were not studied in saliva. In our previous report, we evaluated whether salivary triggering receptor expressed on myeloid cells-1, its putative ligand peptidoglycan recognition protein-1 and also calprotectin

Table 3. Biochemical findings of groups and related subgroups

	Cystic fibrosis (CF) (N=11)		Systematically healthy (Control) (C) (N=11)		Cystic fibrosis effect p-value	
MMP-8 (ng/mL)	671.49±208.91		499.35±327.88		0.101	
TIMP-1 (ng/mL)	358.41±213.02		277.50±145.84		0.316	
MMP-8/TIMP-1	1.11±0.72		0.92±0.77		0.534	
NE (ng/mL)	2.53±0.56		2.23±0.56		0.193	
MPO (ng/mL)	3623.65±1318.07		2817.82±1358.66		0.096	
	CF-H (n=6)	CF-G (n=5)	C-H (n=6)	C-G (n=5)	Periodontal status effect p-value	Cystic fibrosis-periodontal status interaction p-value
MMP-8 (ng/mL)	527.48 169.84±	844.31±67.90	359.83±382.69	666.77±146.46	**0.006**	0.961
TIMP-1 (ng/mL)	391.12±268.64	319.16±140.38	323.89±171.85	221.83±95.69	0.289	0.852
MMP-8/TIMP-1	0.87±0.67	1.40±0.74	0.41±0.38	1.52±0.67	**0.007**	0.284
NE (ng/mL)	2.34±0.65	2.76±0.36	1.93±0.50	2.59±0.41	**0.023**	0.588
MPO (ng/mL)	3021.02±1231.48	4346.81±1117.64	1856.39±777.91	3971.54±898.97	**0.001**	0.379

MMP-8: Matrix metalloproteinase-8, TIMP-1: Tissue inhibitor of matrix metalloproteinases-1, NE: Neutrophil elastase, MPO: Myeloperoxidase, CF-H: Cystic fibrosis-periodontally healthy, CF-G: Cystic fibrosis-gingivitis, C-H: Control-periodontally healthy, C-G: Control-gingivitis. For two main groups: No significant difference ($p>0.05$) from C group. Values in bold for subgroups: Significant differences ($p<0.05$) compared to CF-H and C-H

Table 4. Correlations between clinical and biochemical parameters

	Age (years)	PPD (mm)	GI	PI	BOP (%)	MMP-8 (ng/mL)	TIMP-1 (ng/mL)	MMP-8/ TIMP-1	NE (ng/mL)	MPO (ng/mL)
Age (years)	1	0.310	0.412	**0.600†**	**0.472***	0.310	0.133	0.320	0.352	0.203
PPD (mm)	0.310	1	**0.582†**	**0.591†**	**0.565†**	0.357	-0.253	0.313	**0.593†**	**0.485†**
GI	0.412	**0.582†**	1	**0.833†**	**0.930†**	**0.502***	-0.100	**0.475***	**0.554†**	**0.758†**
PI	**0.600†**	**0.591†**	**0.833†**	1	**0.876†**	**0.662†**	-0.163	**0.656†**	**0.541†**	**0.724†**
BOP (%)	**0.472***	**0.565†**	**0.930†**	**0.876†**	1	0.618†	-0.071	**0.549†**	**0.606†**	**0.704†**
MMP-8 (ng/mL)	0.310	0.357	**0.502***	**0.662†**	**0.618†**	1	0.089	**0.647†**	**0.687†**	**0.684†**
TIMP-1 (ng/mL)	0.133	-0.253	-0.100	-0.163	-0.071	0.089	1	**-0.547†**	0.074	0.001
MMP-8/TIMP-1	0.32	0.313	**0.475***	**0.656†**	**0.549†**	**0.647†**	**-0.547†**	1	0.357	**0.445***
NE (ng/mL)	0.352	**0.593†**	**0.554†**	**0.541†**	**0.606†**	**0.687†**	0.074	0.357	1	**0.748†**
MPO (ng/mL)	0.203	**0.485***	**0.758†**	**0.724†**	**0.704†**	**0.684†**	0.001	**0.445***	**0.748†**	1

GI: Gingival index, PI: Plaque index, BOP: Bleeding on probing, PPD: Probing pocket depth, MMP-8: Matrix metalloproteinase-8, TIMP-1: Tissue inhibitor of matrix metalloproteinases-1, NE: Neutrophil elastase, MPO: Myeloperoxidase. *Correlation (in bold) is significant at the 0.05 level (2-tailed); †Correlation (in bold) is significant at the 0.01 level (2-tailed)

were associated with CF; and observed that CF children showed a varying salivary biomarker profile, particularly regarding the levels of calprotectin, in addition to elevated gingival inflammation scores (30). We therefore investigated the salivary analysis of proteolytic mediators with clinical periodontal measurements to assess, for the first time, the relationship of the periodontal status with CF.

Proteolytic enzymes released from neutrophils are taken part in the pathophysiology of CF and the balance of MMPs with their inhibitors or the activators is believed to reflect the proteolytic processes of the disease (5). Increased MMP-9 levels and MMP-9/TIMP-1 molar ratios have been shown to correlate with NE activation in sputum (7) and BAL (9) samples of CF children. This is corroborated by elevated serum concentrations of MMP-8 and MMP-9 in cases with CF lung disease (1). Contrary to sputum, BAL or serum fluids, lower salivary MMP-9 concentrations have been shown in CF patients than controls (26). The current findings showed that CF does not appear to influence the salivary levels of proteinases significantly, although these enzymes tend to rise in CF. This observation may point out the distinctiveness of the oral cavity from the lower airway and blood or might be linked to the local responses of CF rather than the systemic inflammatory response. Yet, salivary MMP-8 and MMP-8/TIMP-1 molar ratio were significantly increased in the gingivitis subgroup of CF in comparison to the periodontally healthy CF children. Additionally, in the systemically healthy group, children with gingival inflammation had also higher levels of these molecules compared to periodontally healthy children. These findings are in accordance with the literature that reveals the involvement of MMP-8 with gingival inflammation (18,31-36).

It is also important to note that NE and MPO concentrations in saliva were significantly elevated in addition to MMP-8 in gingivitis subgroups in the present study. Evidence shows that neutrophils release NE and MPO which in turn activate MMP-8 and -9, respectively, in response to the accumulation of plaque (8). Therefore, the increased MMP-8 and MMP-8/TIMP-1 molar ratio might be a consequence of higher levels of NE and MPO activity in children with gingivitis. Our data also presented a significant correlation of MMP-8 levels and MMP-8/TIMP-1 molar ratio with GI, BOP and PI. Besides, the levels of NE and MPO also had a positive relationship with all clinical periodontal parameters. If all these findings of the study are taken into consideration, it is likely that the increased levels of these enzymes in saliva of children seems to be arised from the microbiological condition regardless of the systemic condition. An activated systemic inflammatory response by CF disease could have a tendency to influence the gingival health, however in the present study we evaluated these molecules solely in saliva and also with a relatively small sample size. The results of current study need to be verified with larger size of CF patients.

Conclusion

Within the limitations of this study, the current findings suggest that salivary MMP-8, TIMP-1, NE, MPO are similar between children with CF or without CF. Importantly, children with CF had elevated bleeding scores, and gingival inflammation had a pronounced effect on salivary proteolytic activity. Larger scale studies analysing neutrophilic enzymes in biofluids are needed to determine the validity of these results and to clarify the association between CF and gingival inflammation.

Ethics

Ethics Committee Approval: The ethical principles stated in the World Medical Association's Declaration of Helsinki were followed; and this study protocol was approved by the Ethics Committee on Clinical Researches of Ege University (decision number: 17-5/9, date: 11.09.2017).

Informed Consent: Following the aim and the procedures of this study were explained, informed consent was provided from all participants before the enrollment [parents (in writing) and children (orally)].

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Authorship Contributions

Surgical and Medical Practices: Z.P.K.Y., T.T., A.S., Y.T., Concept: N.B., T.S., G.E., Design: N.B., T.S., G.E., Data Collection or Processing: Z.P.K.Y., T.T., A.S., Y.T., Analysis or Interpretation: Z.P.K.Y., T.T., A.S., T.K., G.T., Literature Search: Z.P.K.Y., Y.T., T.K., G.T., Writing: Z.P.K.Y.

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Original Article / Özgün Araştırma

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Comparison of Endodontic Treatment Approaches Between Endodontists and General Dentists in Turkey

Türkiye'deki Endodontistler ve Genel Diş Hekimleri Arasındaki Endodontik Tedavi Yaklaşımlarının Karşılaştırılması

Safa Kurnaz, Gülsen Kiraz

Kütahya Health Sciences University, Faculty of Dentistry, Department of Endodontics, Kütahya, Turkey

Abstract

Objective: The purpose of this cross-sectional study was to compare the knowledge and treatment approaches related to endodontic treatment (ET), and the use of new techniques and materials during treatments between general dentists (GD) and endodontists.

Materials and Methods: This study was conducted with endodontists and GDs working in Turkey. The questionnaire consisted of 30 multiple-choice questions divided into three main categories, including demographic information, general approach to ET, and root canal treatment (RCT) procedures. The obtained data were analyzed using the chi-square test ($p<0.05$).

Results: A total of 454 completed questionnaires were collected from a total of 750 distributed questionnaires, resulting in a response rate of 60.5%. Most of the respondents (56.8%) reported performing more than 20 RCTs per week. Approximately 60% of the respondents stated that they never used rubber-dam isolation during ET. It was determined that 89.1% of GDs did not use any magnification system during ET ($p<0.001$). The great majority of the respondents (95.4%) reported using sodium hypochlorite as a primary irrigant. Endodontists used rotary systems more than GDs ($p<0.05$).

Conclusion: Endodontists and GDs take different approaches to ET, and endodontists use new techniques and materials more often than GDs. The results of this study point to the importance and necessity of continuous education programs to encourage the use of new systems and techniques in endodontics.

Keywords: Endodontists, dentists, root canal therapy, questionnaires

Öz

Amaç: Bu kesitsel çalışmanın amacı; genel diş hekimleri ve endodontistler arasında endodontik tedavi ile ilgili bilgi, tedavi yaklaşımları ve tedaviler sırasında yeni teknik ve materyallerin kullanımını karşılaştırmaktır.

Gereç ve Yöntemler: Bu çalışma Türkiye'de çalışan endodontist ve genel diş hekimleri ile gerçekleştirildi. Anket, demografik bilgiler, endodontik tedaviye genel yaklaşım ve kök kanal tedavisi prosedürlerini içeren üç ana kategoriye ayrılan çoktan seçmeli 30 sorudan oluşuyordu. Elde edilen veriler ki-kare testi kullanılarak analiz edildi ($p<0.05$).

Bulgular: Dağıtılan toplam 750 anketten toplam 454 tamamlanmış anket toplandı ve %60,5'lik bir yanıt oranıyla sonuçlandı. Katılımcıların çoğunluğu (%56,8) haftada 20'den fazla kök kanal tedavisi gerçekleştirdiğini bildirdi. Katılımcıların yaklaşık %60'ı endodontik tedavi sırasında rubber-damı hiç kullanmadığını belirtti. Genel diş hekimlerinin %89,1'inin endodontik tedavi sırasında herhangi bir büyütme sistemi kullanmadığı tespit edildi ($p<0,001$). Katılımcıların büyük çoğunluğu (%95,4) ana irrigasyon solüsyonu olarak sodyum hipoklorit kullandığını bildirdi. Endodontistler döner alet sistemlerini genel diş hekimlerine göre daha fazla kullanmaktaydı ($p<0,05$).

Sonuç: Endodontistlerin ve genel diş hekimlerinin endodontik tedaviye yaklaşımları farklılık göstermektedir ve endodontistler yeni teknik ve materyalleri genel diş hekimlerinden daha sık kullanmaktadır. Bu çalışmanın sonuçları, endodontide yeni sistem ve tekniklerin kullanımını teşvik etmek için sürekli eğitim programlarının önemine ve gerekliliğine işaret etmektedir.

Anahtar Kelimeler: Endodontistler, diş hekimleri, kök kanal tedavisi, anketler

Address for Correspondence/Yazışma Adresi: Safa Kurnaz Asst. Prof., Kütahya Health Sciences University, Faculty of Dentistry, Department of Endodontics, Kütahya, Turkey
Phone: +90 274 230 00 56 **E-mail:** safa.kurnaz@ksbu.edu.tr
ORCID ID: orcid.org/0000-0002-8079-7536

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Introduction

Root canal treatment (RCT) is an indispensable part of modern dentistry, and dentistry faculties should prepare their students to perform uncomplicated RCTs of acceptable quality. During their education, dentistry students can access up-to-date information through their academic programs. However, after graduation, dentists must follow developments in the field of endodontics out of their own interests and efforts. Applications related to endodontics are widely used in postgraduate training. General dentists (GD) who attend such seminars often want to learn how to perform RCT easier and more successful (1).

Dentists' knowledge of and experience with RCTs affect the success of endodontic treatment (ET) (2). In 2006, the European Society of Endodontology published guidelines for ET which outline the standard of care for endodontics according to scientific evidence (3). It has been reported that the majority of dentists worldwide do not follow these guidelines (4).

Many studies have examined the success rates of ET to date, reporting success rates in the range of 74-98% for RCT (5-7). However, the success rates of ETs for GDs have been lower, measured at 65-75% (8). In Turkey, the majority of RCTs are performed by GDs, making it is very important for GDs to follow developments in endodontics (9). Several studies have assessed the knowledge and practice of dentists in specific countries (10,11). However, few studies have examined dentists' attitudes toward new endodontic materials and techniques in Turkey (12,13).

The aim of this study was to assess and compare GDs and endodontists in Turkey in terms of their attitudes toward and knowledge and use of procedures, materials and recently developed techniques in ET. The scope of the study was also to evaluate whether endodontic specialty, years of professional experience or gender affected the choice of treatment procedures or the use of new technologies and materials.

Materials and Methods

This study was approved by the Clinical Research Ethics Committee of Kütahya Health Sciences University, Kütahya, Turkey (decision no: 2020/03-07, date: 06.02.2020). A questionnaire was designed on the basis of similar studies involving surveys of GD and endodontists (12,14-16). A survey was designed using Google Forms, and a link was mailed electronically to 750 GDs and endodontists. A reminder e-mail was sent two weeks later after the initial correspondence. Three hundred forty GDs and 114 endodontists returned the questionnaires. The questionnaire consisted of 30 multiple-choice questions. All the respondents received a written explanation about the study before participation and they were informed that their participation in the study was entirely voluntary. Informed consents were obtained before the onset of the survey.

The questions were divided into three main categories as follows:

1- General information: gender, age, specialty, years of professional experience and type of practice (i.e., private dental clinics or government hospitals)

2- General approach to ET: working hours per week; frequency of RCT, retreatment and trauma cases; types of the tooth treated; use of rubber-dam, magnification systems, cone-beam computed tomography (CBCT), ultrasonics and irrigation activation systems; number of treatment visits for vital and devital teeth; frequency of complications; perspective on regeneration and apical surgery

3- RCTs procedures: method of working length (WL) determination; preference of root canal files, root canal irrigants and intracanal medicaments; use of lubricants; choice of smear layer removal; most challenging step during ET and types of complications; and treatment choices for drainage and crown restorations

Statistical Analysis

The data were collected and analyzed using the statistical package SPSS 20 (Statistical Package for Social Sciences, Chicago, IL, USA). The obtained data were analyzed using the chi-square test, and the level of significance was set at 0.05.

Results

A total of 454 completed questionnaires were collected from a total of 750 distributed questionnaires, resulting in a response rate of 60.5%. The demographic information and characteristics of participants are presented in Table 1.

General approach to ET

The results of the general approach to ET are presented in Table 2. There was a statistically significant difference in working hours between GDs and endodontists ($p<0.05$). While the endodontists mostly worked 30-40 hours, the GDs usually worked over 40 hours a week.

The majority of the respondents reported performing more than 20 RCTs and 1-5 retreatment cases per week. While the endodontists performed a high rate of more than 20 RCTs, the GDs mostly performed 1-5 retreatment cases per week ($p<0.001$).

The vast majority of the participants reported that they mostly treated molar teeth (83.0%). Out of the endodontists, 97.4% reported that they treated molar teeth, while 78.2% of the GDs treated molar teeth.

It was determined that the majority of the respondents (59.7%) never used rubber-dam isolation during ET. Only 4.9% of the respondents reported that they routinely use rubber-dam during ET. The vast majority of the endodontists (82.5%) reported using rubber-dam in some cases. Most of the GDs (77.9%) reported that they never used rubber-dam.

While 28.1% of the endodontists used a dental operating microscope in some cases, 24.6% used dental loupes in some cases. Out of the GDs, 89.1% stated that they did not use any magnification system during ET ($p<0.001$). Likewise, 65.9% of the participants did not use CBCT for RCT or retreatment indications, while 84.2% of the endodontists stated that they used CBCT in some cases ($p<0.001$). Additionally, 57.1% of the participants never used ultrasonic systems during treatment, whereas 79.0% of the endodontists used ultrasonic systems in some cases ($p<0.001$). More endodontists than GDs used irrigation activation devices during irrigation, and this difference was statistically significant (59.7% and 31.2%, respectively) ($p<0.05$).

The majority of the participants (72.2%) stated that the frequency of complications, or the rate of procedural errors experienced during treatment, was 0-10%; however, whether patients were treated by endodontics specialists did not significantly influence the frequency of procedural errors ($p>0.05$).

It was determined that 68.4% of the endodontists performed regenerative ET, and 66.5% of the GDs had not yet performed this treatment but wanted to do it in the future. Moreover, only 16.5% of the participants reported that they performed apical surgery, while 51.1% reported that they referred patients to a maxillofacial surgeon for apical surgery.

Table 1. Characteristics of study respondents (n=454)

	n (%)
Gender	
Male	215 (47.4)
Female	239 (52.6)
Age	
25-35	329 (72.4)
35-45	72 (15.9)
45-55	47 (10.4)
>55	6 (1.3)
Years in practice	
<5	146 (32.2)
5-10	175 (38.5)
11-20	80 (17.6)
>20	53 (11.7)
Clinical speciality	
General dentist	340 (74.9)
Endodontist	114 (25.1)
Type of practice	
Private dental office	205 (45.1)
Government hospital	249 (54.9)

RCT Procedures

The results of the RCT procedures are presented in Table 3. The plurality of the respondents (48.0%) preferred a combination of both radiographs and electronic apex locators as the WL determination method. Continuous rotary systems were also among the most popular instruments (57.7%). The preference in type of root canal file was affected by clinical specialty. More endodontists than GDs generally used continuous rotary systems, and this difference was statistically significant (82.5% and 49.4%, respectively) ($p<0.05$).

The great majority of the practitioners (95.4%) used sodium hypochlorite as a primary irrigant. Most of the practitioners preferred root canal lubricants during ET. Likewise, 83.5% of the respondents preferred root canal medicaments for multi-visit treatments. Specialty did not affect the use of root canal medicaments during ET ($p>0.05$).

Most of the practitioners (65.0%) stated that they did not leave the teeth open for drainage. While 41.2% of the GDs stated that in some cases the teeth were left open for drainage, 95.6% of the endodontists stated that they did not leave teeth open for drainage ($p<0.001$)

The participants stated that the most challenging step during ET was chemo-mechanical preparation (30.0%). While a high rate of the endodontists likewise reported that the most challenging stage is chemo-mechanical preparation (47.4%), the GDs stated that they had more difficulty during isolation (27.1%). The majority of the participants (72.2%) stated that the complication they experienced most frequently during treatment was instrument separation (67.8%). Whether the practitioners were endodontic specialists or not did not significantly influence the type of procedural errors encountered during ET ($p>0.05$).

The participants stated that a high proportion of teeth required crown restorations when there was no wall left or only one wall remaining of a tooth (53.7%). Specialty did not influence the choice to treat with crown restorations.

Discussion

This survey aimed to evaluate the attitudes, knowledge and practice patterns of GDs and endodontists in Turkey. In the reviewed literature, there was no detailed information on the operating principles of GDs and endodontists in Turkey. The data collected in our study can thus serve as a basic source of information for future research on GDs and endodontists in the field of endodontics.

A large proportion of the respondents reported performing more than 20 RCTs and 1-5 retreatments per week. In 2012, Kaptan et al. (13) found that clinicians in Turkey performed only 10 RCTs per month. The rising ratio of RCTs performed in Turkey since 2012 is promising. Unfortunately, it was observed that some of the respondents, especially the GDs working in government hospitals, performed a small number of retreatments.

Table 2. Results of questions related to respondents' general approach to ET			
	General dentist	Endodontist	p-value
Weekly working hours			
0-20	11 (3.2%)	0 (0%)	
20-30	24 (7.1%)	9 (7.9%)	*
30-40	124 (36.5%)	65 (57.0%)	
>40	181 (53.2%)	40 (35.1%)	
Average number of RCTs per month			
0	12 (3.5%)	0 (0%)	
1-5	25 (7.4%)	0 (0%)	
6-10	61 (17.9%)	2 (1.8%)	**
11-15	48 (14.1%)	2 (1.8%)	
16-20	39 (11.5%)	7 (6.1%)	
>20	155 (45.6%)	103 (90.3%)	
Average number of endodontic retreatments per month			
0	103 (30.3%)	2 (1.8%)	
1-5	196 (57.7%)	12 (10.5%)	
6-10	16 (4.7%)	16 (14.0%)	**
11-15	9 (2.6%)	27 (23.7%)	
16-20	6 (1.8%)	15 (13.2%)	
>20	10 (2.9%)	42 (36.8%)	
Trauma cases per month			
0-5	329 (96.8%)	112 (98.2%)	
5-10	9 (2.6%)	2 (1.8%)	NS
>10	2 (0.6%)	0 (0%)	
Types of cases treated routinely			
Anterior	36 (10.6%)	0 (0%)	
Premolar	38 (11.2%)	3 (2.6%)	*
Molar	266 (78.2%)	111 (97.4%)	
Rubber dam isolation			
Always	8 (2.4%)	14 (12.3%)	
In some cases	67 (19.7%)	94 (82.5%)	**
Never	265 (77.9%)	6 (5.2%)	
Use of magnifications systems			
Always dental loupe	11 (3.2%)	10 (8.7%)	
Always dental operating microscope	2 (0.6%)	1 (0.9%)	
Dental loupe in some cases	22(6.5%)	28 (24.6%)	**
Dental operating microscope in some cases	2 (0.6%)	32 (28.1%)	
Never	303 (89.1%)	43 (37.7%)	

Table 2. Continued

	General dentist	Endodontist	p-value
Use of irrigation activation devices			
Yes	106 (31.2%)	68 (59.7%)	**
No	234 (68.8%)	46 (40.3%)	
Use of ultrasonic systems			
Always	12 (3.5%)	8 (7.0%)	**
In some cases	85 (25.0%)	90 (79.0%)	
Never	243 (71.5%)	16 (14.0%)	
Use of CBCT			
Always	8 (2.4%)	1 (0.9%)	**
In some cases	50 (14.7%)	96 (84.2%)	
Never	282 (82.9%)	17 (14.9%)	
Visit of RCT for vital cases			
Single-visit	170 (50%)	76 (66.7%)	*
Multi-visit	28 (8.2%)	0 (0%)	
Usually single-visit	99 (29.1%)	38 (33.3%)	
Usually multi-visit	43 (12.7%)	0 (0%)	
Visit of RCT for devital cases			
Single-visit	49 (14.4%)	9 (7.9%)	**
Multi-visit	114 (33.5%)	8 (7.0%)	
Usually single-visit	73 (21.5%)	57 (50.0%)	
Usually multi-visit	104 (30.6%)	40 (35.1%)	
Experience of complication or procedural errors (%)			
0-10	246 (72.4%)	82 (71.9%)	NS
10-30	83 (24.4%)	32 (28%)	
30-50	9 (2.6%)	0 (0%)	
>50	2 (0.6%)	0(0%)	
Regenerative treatment practice			
Performed	72 (21.2%)	78 (68.4%)	**
Not performed, but would like to	226 (66.5%)	32 (28.1%)	
Would not perform	42 (12.3%)	4 (3.5%)	
Apical surgery practice			
Performed	56 (16.5%)	19 (16.7%)	*
Would not perform	130 (38.2%)	17 (14.9%)	
Refer to an oral surgeon	154 (45.3%)	78 (68.4%)	

Pearson chi-square test, NS: Not-significant ($p>0.05$), *$p<0.05$, **$p<0.001$, RCT: Root canal treatment, CBCT: Cone-beam computed tomography

Table 3. Results of questions related to respondents' use of RCTs procedures			
	General dentist	Endodontist	p-value
Working length determination			
Tactile sensation	17 (5.0%)	0 (0%)	
Paper point technique	5 (1.5%)	0 (0%)	
Radiography	67 (19.7%)	2 (1.8%)	*
Electronic apex locator	97 (28.5%)	48 (42.1%)	
Electronic apex locator + radiography	154 (45.3%)	64 (56.1%)	
Root canal instruments routinely used			
Hand files	42 (12.4%)	2 (1.8%)	
Ni-Ti rotary systems	168 (49.4%)	94 (82.4%)	**
Ni-Ti reciprocating systems	130 (38.2%)	18 (15.8%)	
Use of a lubricant during canal instrumentation			
Yes	238 (70%)	93 (81.6%)	NS
No	102 (30%)	21 (18.4%)	
Choice of primary root canal irrigation solution			
Sodium hyphoclorite	319 (93.8%)	114 (100%)	
Saline solution	10 (2.9%)	0 (0%)	
Chlorhexidine	5 (1.5%)	0 (0%)	NS
Hydrogen peroxide	3 (0.9%)	0 (0%)	
Other	3 (0.9%)	0 (0%)	
Removal of smear layer			
Yes	233 (68.5%)	97 (85.1%)	NS
No	107 (31.5%)	17 (14.9%)	
Use of intracanal medicament between appointments for multiple-visit cases			
Yes	277 (81.5%)	102 (89.5%)	NS
No	63 (18.5%)	12 (10.5%)	
Leave teeth open for drainage			
Yes	14 (4.1%)	0 (0%)	
In some cases	140 (41.2%)	5 (4.4%)	**
No	186 (54.7%)	109 (95.6%)	
Most challenging step for ET			
Anesthesia	26 (7.6%)	15 (13.2%)	
Access cavity preparation and root canal location	82 (24.1%)	17 (14.9%)	
Isolation	92 (27.1%)	20 (17.5%)	*
Chemomechanical preparation	83 (24.4%)	54 (47.4%)	
Obturation	57 (16.8%)	8 (7.0%)	

Table 3. Continued

	General dentist	Endodontist	p-value
Most precedural error			
Instrument seperation	222 (65.3%)	86 (75.4%)	NS
Perforation	2 (0.6%)	2 (1.8%)	
Blockage	86 (25.3%)	19 (16.7%)	
Unable to locate canals	13 (3.8%)	0 (0%)	
Other	17 (5.0%)	7 (6.1%)	
Crown restoration preference			
If the tooth has no walls or only one wall	176 (51.8%)	69 (60.5%)	NS
If the tooth has two walls	109 (32.1%)	40 (35.1%)	
If the tooth has three walls	15 (4.4%)	0 (0%)	
Always	40 (11.7%)	5 (4.4%)	

Pearson chi-square test, NS: Not-significant (p>0.05), *p<0.05, **p<0.001, ET: Endodontic treatment

According to the guidelines of the European Society of Endodontology, RCT procedures should only be performed if the tooth is isolated by a rubber-dam. However, rubber-dams were used routinely by only 4.8% of the respondents in our study, and the vast majority of the practitioners (59.9%) reported never using rubber-dams during ET procedures. In order to spread awareness of the benefit of rubber-dams, sufficient training on this subject should be provided in undergraduate education; practitioners should be supported thereafter with training and should be informed about malpractice.

The use of magnification systems is important, especially for locating extra canals and evaluating the anatomy of the pulp chamber. In our study, 76.7% of the participants reported that they did not use magnification systems; 28.1% of the endodontists used dental operating microscopes, and 24.6% used dental loupes in some cases. Among the GDs, 89.1% did not use any magnification system (p<0.001). Based on these results, the use of magnification should be encouraged in Turkey to ensure successful ET. Similarly, 66.1% of the participants did not use CBCT for ETs, while 84.2% of the endodontists used CBCT in some cases (p<0.001). It appears that the importance of using CBCT is taught well in endodontics education in Turkey, and we hope that its use will become more common with the help of training and seminars (17).

Regenerative ETs have developed in the past decade and have become an effective treatment alternative for immature teeth (18). In our research, although 68.4% of the endodontists had performed regenerative procedures, 66.5% of the GD had not performed such procedures but wanted to do so if given the opportunity. As regenerative treatments are taught and practiced as part of the endodontics specialty degree in Turkey, there is no practical application of this subject in undergraduate education. GDs' avoidance of regenerative treatment can be attributed to this lack of education.

In our study, both the endodontists and the GDs reported that the most common complication was instrument separation (67.8%). The procedural error of instrument separation, common in Turkey, may be attributed to overuse of instruments for economic reasons. In addition, the GDs stated that isolation was the most challenging step during RCT. GDs may have difficulty with isolation since they do not usually use rubber-dams during RCT.

Conclusion

According to the findings of this cross-sectional study, it was showed that the general approaches to ET procedures in Turkey differ from the widely acknowledged quality guidelines in endodontics. Despite the introduction of new materials and techniques, most of the GDs surveyed chose conventional methods. GDs and endodontists have different approaches to ET, and the endodontists in this study used new techniques and materials more often than the GDs. The findings indicate the importance and necessity of postgraduate training and courses to improve standard ET quality in Turkey.

Ethics

Ethics Committee Approval: This study was approved by the Clinical Research Ethics Committee of Kütahya Health Sciences University, Kütahya, Turkey (decision no: 2020/03-07, date: 06.02.2020).

Informed Consent: Informed consents were obtained before the onset of the survey.

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Authorship Contributions

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

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Evaluation of the Relationship Between Bruxism, Temperament and Childhood Trauma History

Bruksizm Yakınması Olan Kişilerde Mizaç Özelliklerinin ve Çocukluk Çağı Travma Öyküsünün Değerlendirilmesi

Ayşe Döndü[1], Gökhan Özkan[2]

[1]Aydın State Hospital, Clinic of Psychiatry, Aydın, Turkey
[2]Aydın Adnan Menderes University, Faculty of Dentistry, Department of Dentomaxillofacial Radiology, Aydın, Turkey

Abstract

Objective: It is known that psychological factors play a role in the development of bruxism and temporomandibular joint disorders. The purpose of this study was to examine the effects of depressive disorder, anxiety disorder, childhood psychological trauma levels, and personality temperament characteristics on bruxism.

Materials and Methods: Patients who applied to the Aydın State Hospital, Psychiatry Polyclinic and Aydın Adnan Menderes University Faculty of Dentistry with a complaint of clenching were included in the study. Clinical interview, SCID-I, Beck Depression Rating Scale (BDRS) Beck Anxiety Rating Scale (BARS), Temperament Evaluation of Memphis, Pisa, Paris and San Diego Autoquestionnaire (TEMPS-A), childhood trauma scale (CTS) were applied.

Results: The mean BDRS was determined as 13.5 BARS 20.7. According to the cut-off values of the Beck depression scale, mild depressive symptoms were observed in 25 people, moderate depressive symptoms in 20 people, and severe depressive symptoms in 7 people. Twenty-five people had mild anxiety symptoms, 14 people mild, 21 people had severe anxiety symptoms. Hyperthymic (n=1), depressive (n=11), anxious temperament (n=15), irritable temperament (n=4), and cyclothymic temperament (n=1) were found. Almost no hyperthymic or cyclothymic temperament was found in patients with bruxism. No statistically significant difference was found between men and women in terms of temperament ($p>0.05$), the mean of CTS was 58.6, and no difference was found in the sub-dimensions of physical, sexual emotional abuse and emotional and physical neglect.

Conclusion: Although many factors are related in the etiology of bruxism, studies on the effect of personality and temperament are less. In our study, anxious temperament was found to be significantly higher in patients with bruxism.

Keywords: Bruxism, temperament, childhood trauma

Öz

Amaç: Diş sıkma ve temporamandibular eklem hastalıklarının etiyolojisinde psikolojik faktörlerin önemli olduğu bilinmektedir. Bu çalışmanın amacı diş sıkan hastalarda depresif bozukluk, anksiyete bozukluğu çocukluk çağı ruhsal travma düzeylerini ve kişilik mizaç özelliklerinin etkisini saptamaktır.

Gereç ve Yöntemler: Aydın Devlet Hastanesi Psikiyatri Polikliniği'ne ve Aydın Adnan Menderes Üniversitesi Diş Hekimliği Fakültesi'ne diş sıkma şikayeti ile başvuran hastalar çalışmaya alınmıştır. Katılımcılara, SCID-I, Beck Depresyon Derecelendirme Ölçeği (BDDÖ), Beck Anksiyete Derecelendirme Ölçeği, Memphis, Pisa, Paris ve San Diego Mizaç Değerlendirme Anketi Türkçe formu, Çocukluk Çağı Travma Ölçeği (ÇÇTÖ) uygulanmıştır.

Bulgular: Hastalarda BDÖ ortalaması 13,5, BAÖ 20,7 olarak belirlenmiştir. BDDÖ kesme değerlerine göre bakıldığında 25 kişide hafif, 20 kişide orta, 7 kişide şiddetli depresif belirtiler gözlenmiştir. Yirmi beş kişide hafif, 14 kişide orta, 21 kişide şiddetli anksiyete belirtileri vardı. Hipertimik mizaç (n=1), depresif mizaç (n=11), anksiyöz mizaç (n=15), irritabl mizaç (n=4), siklotimik mizaç (n=1) olarak saptanmıştır. Bruksizm olan hastalarda neredeyse hipertimik ve siklotimik mizaca rastanmamıştır. Mizaç açısından kadın ve

Address for Correspondence/Yazışma Adresi: Ayşe Döndü MD, Aydın State Hospital, Clinic of Psychiatry, Aydın, Turkey
Phone: +90 507 236 47 60 **E-mail:** aysdnd@hotmail.com
ORCID ID: orcid.org/0000-0003-0412-365X

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erkekler karşılaştırıldığında fark saptanmamıştır (p>0,05). ÇÇTÖ ortalaması 58,6 olup, fiziksel, cinsel, duygusal istismar, ve duygusal, fiziksel ihmal alt boyutlarında fark saptanmamıştır.

Sonuç: Bruksizm etiyolojisinde birçok faktörün ilişkisi olmakla birlikte kişilik ve mizaç etkisi ile ilgili çalışmalar daha azdır. Çalışmamızda bruksizmi olanlarda anksiyöz mizacın belirgin olarak daha yüksek olduğu saptanmıştır.

Anahtar Kelimeler: Bruksizm, mizaç, çocukluk travması

Introduction

Oral health and hygiene is an integral part of overall health. It is also an important role in quality of life, which affects an individual's appearance, speaking, eating and social status. Oral diseases qualify as major public health problems owing to their high prevalence and incidence in all regions of the world, and as for all diseases, the greatest burden of oral diseases is on disadvantaged and socially marginalized populations. It is known that the evaluation of oral health in individuals with psychiatric disorders has a weak priority. Psychiatric patients who report significant oral health problems due to the side effects of the drugs used and the nature of the disease are prone to more negative effects (1).

As a result of the chronicity of the psychiatric illness, there is reluctance to provide oral hygiene. Chronic psychiatric patients constitute a high risk group in terms of oral health due to inadequate dental visits, irregular eating habits, poor oral hygiene and side effects of the drugs used. The data on this subject show that individuals with psychiatric diseases have more oral health problems and treatment needs. Impairment in executive functions, reluctance towards dental treatment, financial difficulties resulting from job loss, long-term hospitalizations, family and community difficulties such as lack of support, stigma in the society, and the reluctance of some dentists to treat these individuals contribute to the high incidence of dental caries and periodontal disease in these patients. Therefore, the necessity of considering the possible negative effects of treatment options on oral and dental health during the treatment of psychiatric patients is emphasized in many studies (2).

However, apart from hygiene, it is known that psychological factors are play a role in the etiology of temporomandibular joint disorders and bruxism. Bruxism is a common, unusually increased jaw activity caused by strong jaw movements accompanied by teeth grinding and/or clenching.

In the past years, it has been suggested that morphological factors such as occlusal disorders and the anatomy of the bone structure of the orofacial region cause bruxism. However, nowadays, the role of various neurotransmitters in the central nervous system, especially in the dopaminergic system, dysfunction in the afferent and efferent thalamic and/or striatopallidal pathways, and the basal ganglia that play a role in the coordination of movements are emphasized (3).

If bruxism occurs during a neurological disease such as parkinsonism, a psychiatric disorder such as depression, schizophrenia or with the use of a drug, it is called "secondary bruxism". Dopamine antagonist drugs have the effect of inducing bruxism or worsening existing bruxism (4). Periodontitis may also be exacerbated in some patients using SSRIs because these drugs cause a motion sickness including bruxism. These drugs may cause bruxism by increasing extrapyramidal serotonin levels and thus suppressing the dopaminergic pathways of these control movements.

When the literature is examined, it has been noticed that there are not enough studies on this subject. The aim of this study is to detect the prevalence of depressive disorder, anxiety disorder, childhood trauma and personality temperament traits in people with bruxism.

Materials and Methods

This study was approved by Aydın Adnan Menderes University Non-Invasive Clinical Research Ethics Committee (protocol no: ADÜDHF2018/041, date: 15.08.2018). Patients between 18-79 years of age who applied to the Aydın State Hospital, Psychiatry Polyclinic and Aydın Adnan Menderes University, Faculty of Dentistry with a complaint of clenching were included in the study. Patients who applied to the clinic and accepted to participate in the study were first examined by an experienced dentist and those who could not find any underlying disease related to bruxism were included in the study. The psychiatric evaluation was made by the psychiatrist after the patients with the complaint of clenching were evaluated by the dentist.

Demographic and clinical variables such as age, gender, educational status, marital status, past psychiatric disease, drug use history, alcohol, smoking, substance use status, clenching and comorbid disease were collected through the sociodemographic data form created by the clinician.

Clinical interview, The Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-I), Beck Depression Rating Scale (BDRS), Beck Anxiety Rating Scale (BARS), Temperament Evaluation of Memphis, Pisa, Paris and San Diego Autoquestionnaire (TEMPS-A), childhood trauma scale (CTS) was applied by clinician.

Patients who did not read and sign the informed consent form, were diagnosed with schizophrenia, bipolar disorder, had a history of mental retardation and substance use disorder, and had systemic diseases that could cause bruxism were not included in the study.

Statistical Analysis

Independent Sample t-test was used for the comparison of normally distributed quantitative data between the groups, and Pearson chi-square test was used for the comparison of qualitative data.

Data Collection Tools

Diagnostic interviews with the Turkish version of SCID-I were conducted with each patient. The Structured Clinical Interview for DSM-IV Axis I Disorders was developed by First et al. (5) to diagnose according to the DSM-IV system.

TEMPS-A questionnaire was developed by Akiskal et al. (6) to identify affective temperament. The questionnaire consists of 99 items arranged to determine irritable, depressive, cyclothymic hyperthymic and anxious temperaments. Temperament is an individual's characteristic level of emotional excitability or intensity and is typically recognized within the first few weeks after birth. It is often assumed to be an early indication of personality, though personality combines temperament with experiences to shape life-long traits. Thinking about his whole life, the person answers the items as yes or no to determine the presence of dominant depressive (18 items), cyclothymic (19 items), hyperthymic (20 items), irritable (18 items) and anxious (24 items) temperaments in a person. The cut-off points (minimum "yes" response required to determine temperament) were 13, 18, 20, 13 and 13, respectively.

The Turkish validity and reliability studies of this form were carried out by Vahip et al. (7). The test-retest reliability of the Turkish form, calculated for each temperament trait, is between 0.73 and 0.93 and the Cronbach-alpha coefficients between 0.77 and 0.85.

BDRS measures the emotional, physical and cognitive symptoms of depression. It is a self-assessment scale that includes 21 symptom categories and used to assess the severity of depression. The highest score to be obtained is 63. It was developed by Beck et al. (8) and its validity and reliability study in Turkey was performed by Hisli (9).

BARS is used to evaluate the anxiety symptoms of individuals. It is a Likert-type self-assessment scale consisting of 21 items. The high total score indicates the high level of anxiety experienced by the person. It was developed by Beck and Steer (10) and its validity and reliability study in Turkey was performed by Ulusoy et al. (11).

CTS developed as 70 items by Bernstein in 1994 (12). The scale was adapted into Turkish in 1996 by Şar et al. (13). It is a five-point Likert type self-report scale. The scale, which includes questions evaluating emotional, physical, sexual abuse and verbal violence in childhood, has five factors: emotional, physical, sexual abuse and physical and emotional neglect. It is a self-report scale for retrospectively evaluating childhood and adolescence experiences of abuse and neglect. In the scale, physical or sexual abuse scores range from 7-35, emotional neglect scores range from 16-80, physical neglect scores range from 8-40, and emotional abuse scores range from 12-60.

Results

Of the 82 people who agreed to participate in the study, 70 (86%) were female and 12 (14%) were male, and the rate of females was found to be more frequent among the people who came to the psychiatry outpatient clinic with the complaint of teeth clenching randomly. In terms of their marital status, 46.3% were married, 41.5% were single and 3.7% were divorced, with an average age of 35.7 and an average education level of 9.9 years.

The mean BDRS was determined as 13.5 BARS 20.7. According to the cut-off values of the BDRS, mild depressive symptoms were observed in 25 people, moderate depressive symptoms in 20 people, and severe depressive symptoms in 7 people. Twenty-five people had mild anxiety symptoms, 14 people moderate, 21 people had severe anxiety symptoms.

Hyperthymic (n=1), depressive (n=11), anxious temperament (n=15), irritable temperament (n=4), cyclothymic temperament (n=1) were determined. Almost no hyperthymic or cyclothymic temperament was found in patients with bruxism.

No difference was found between men and women in terms of temperament ($p>0.05$), the mean of CTS was 58.6, and no difference was found in the sub-dimensions of emotional, physical, sexual abuse and physical, emotional neglect.

Discussion

Psychological factors have an important place in the etiology and progression of the bruxism. The present study attempted to highlight this role by evaluating the patients who applied to the dental clinic. According to the results of our study, psychiatric evaluation is very important in people with bruxism. Anxiety disorder and anxious temperament were found to be more common in patients with bruxism.

The most acceptable diagnostic criteria for bruxism are specified in the third edition of the International Classification of Sleep Disorders. In this classification, bruxism is included in the group of "sleep-related movement disorders". For a diagnosis of sleep-associated bruxism to occur, there must be abnormal wear on the tooth surfaces due to teeth grinding. In addition, at least one of the clinical conditions such as pain and fatigue in the jaw muscles that occur temporarily in the morning, temporal headache and jaw locking must be present (14). Sleep bruxism (nocturnal bruxism) is included in DSM-IV-TR as "parasomnias not otherwise named" among parasomnias. However, bruxism is not included in the DSM-5. In addition to sleep bruxism, the diagnosis of psychogenic bruxism (F45.8 Other somatoform disorders) is also included in the International Statistical Classification of Diseases and Related Health Problems 10th Revision. Psychogenic bruxism is defined as bruxism in which there is sensory, function, and behavioral impairment resulting from physical disorders, which is

closely related to stressful events or problems over time, but is not mediated by the autonomic nervous system.

Many theories have been proposed over the years, and a multifactorial model to explain bruxism etiology seems to be the most plausible hypothesis, according to which psychosocial and pathophysiological factors interact with morphological-peripheral ones (15).

In our study, patients with schizophrenia, bipolar disorder, substance use disorder and etc. were excluded by applying SCID 1. In studies with a larger number of patients, the frequency of comorbidity with other psychiatric diseases in people with bruxism can be examined. In order to eliminate confounding factors, conditions such as drug use were determined as exclusion criteria in this study.

Although many factors are related in the etiology of bruxism, studies on the effect of personality and temperament are less. In our study, anxious temperament was found to be significantly higher in patients with bruxism.

The disquiet temperament of the schoolchildren was shown to be associated with the occurrence of this parafunctional activity (16).

In particular, studies based on the clinical diagnosis of bruxism have provided a detailed description of temperamental features that characterize bruxism (e.g., perfectionism aggressiveness, hostility and sensitivity to stress), also pointing out a high prevalence of psychosocial disorders in populations of bruxers. These studies seem to reinforce the widely held view that a bruxism-psychosocial factors relationship exists among practitioners (17,18).

Further studies are needed to understand the link between anxiety and wake bruxism. Indeed, wake bruxism may be the result of a transient anxious response to stressful life events or a phenomenon related to a more complex psychopathological disorder (trait anxiety). Findings based on the use of questionnaires associated bruxism with both types of anxiety (19). These considerations seem to suggest that existence of a wake bruxism personological profile, strictly related to the sphere of mood and anxiety disorders, exist, even though it has not been defined yet.

The prevalence rate found especially for hyperthymic temperament is much higher than that reported in the healthy population (20) and unipolar disorders (21) in line with the results of studies conducted in our country and abroad. This finding supports the long-held assumption that there are specific temperamental characteristics of depression and mania. In other words, depression is primarily associated with depressive temperament and mania with hyperthymic temperament (22).

In our study, it was determined that anxious and depressive temperaments were more common in people with bruxism, but hyperthymic temperament was rare. Those with serious mental disorders such as bipolar disorder and schizophrenia were excluded.

In a study by Şahpolat et al. (23), the mean CTS score of the group of individuals with bruxism was found to be similar to the values in our study (66.88±12.41). However, it was stated that there was no statistically significant difference with the healthy control group. Friedrich (24) indicated that childhood physical and sexual abuse increased somatic complaints in victims. In a study conducted in the adult age group, it was reported that somatization disorder and chronic gastrointestinal disorders were more common in cases with a history of abuse (25).

The small sample size is the main limitation of our study. The lack of control group is another important limitation. In this respect, repeating the study with a larger sample may yield more accurate results.

Conclusions

In our study, childhood abuse and neglect experiences were determined based on the self-report of the patients and with the help of the scale as it was defined. Therefore, it may have caused the patients to have lower psychological traumatic life scores.

Ethics

Ethics Committee Approval: This study was approved by Aydın Adnan Menderes University Non-Invasive Clinical Research Ethics Committee (protocol no: ADÜDHF2018/041, date: 15.08.2018).

Informed Consent: Informed consent was obtained.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Concept: A.D., Design: A.D., G.Ö., Data Collection or Processing: A.D., G.Ö., Analysis or Interpretation: A.D., Literature Search: A.D., G.Ö., Writing: A.D.

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The Metabolic Effects of High-fructose Corn Syrup in Dental Tissues and Parotid Glands of Rats and the Therapeutic Effect of Alpha Lipoic Acid

Yüksek Fruktozlu Mısır Şurubunun Sıçanların Diş Dokularında ve Parotis Bezlerinde Metabolik Etkileri ve Alfa Lipoik Asitin Tedavi Edici Etkisi

Özge Kam Hepdeniz[1], Uğur Burak Temel[1], Fatma Nihan Çankara[2], Özlem Özmen[3]

[1]Süleyman Demirel University Faculty of Dentistry, Department of Restorative Dentistry, Isparta, Turkey
[2]Süleyman Demirel University Faculty of Medicine, Department of Pharmacology, Isparta, Turkey
[3]Mehmet Akif Ersoy University Faculty of Veterinary Medicine, Department of Pathology, Burdur, Turkey

Abstract

Objective: This study aimed to examine the metabolic effects of high fructose corn syrup (HFCS) in dental tissues and parotid glands of rats and antioxidant defense of alpha-lipoic acid (ALA) in HFCS-induced effects.

Materials and Methods: Female Wistar rats (n=24) divided into three groups (group 1: negative control, group 2: positive control, and group 3: ALA) with each group consisting of 8 animals. Rats in groups 2 and 3 were subjected to HFCS for 10 weeks. ALA was given to the animals in group 3 for the last 6 weeks of the experiment. The rats were euthanized by cervical dislocation. The tissues were prepared for histopathological, and immunohistochemical [caspase-3, caspase-8 and bone moprhogenetic protein-2 (BMP-2)] evaluations. Data were analyzed by Kruskal-Wallis (non-parametric data) and one-way ANOVA tests (parametric data) ($p<0.05$).

Results: Hyperemia and neutrophil leukocyte infiltrations in dental tissues and sialadenitis characterized by severe inflammatory reactions in the parotid gland were observed in group 2. After the ALA treatment, degenerative changes were decreased in both tissues, and the gland recovered an essentially normal appearance. Group 3 exhibited significantly lower inflammatory scores than the group 2 in terms of dental pulp ($p=0.044$). Both BMP-2 values and caspase-3 cell counts of the gingival tissue were significantly lower in group 3 compared to group 2 ($p<0.01$).

Conclusion: The vascular and pulpal tissue damage caused by corn syrup was determined in this study. It was concluded that ALA could be a potential antioxidant against the harmful consequences of corn syrup consumption.

Keywords: Alpha-lipoic acid, dental pulp, high-fructose corn syrup, parotid gland, rats

Öz

Amaç: Bu çalışmanın amacı, yüksek fruktozlu mısır şurubunun (HFCS) sıçanların diş dokularında ve parotis bezlerindeki metabolik etkilerini ve alfa lipoik asitin (ALA) HFCS kaynaklı etkilerdeki antioksidan savunmasını incelemektir.

Gereç ve Yöntemler: Dişi Wistar sıçanları (n=24), her biri 8 hayvandan oluşan üç gruba (grup 1: negatif kontrol, grup 2: pozitif kontrol ve grup 3: ALA) ayrıldı. Grup 2 ve 3'teki sıçanlara 10 hafta boyunca HFCS uygulandı. Grup 3'teki hayvanlara deneyin son 6 haftasında ALA verildi. Sıçanlara servikal dislokasyon ile ötenazi uygulandı. Dokular histopatolojik ve immünohistokimyasal [kaspaz-3, kaspaz-8 ve kemik morfogenetik protein-2 (BMP-2)] değerlendirmeler için hazırlandı. Veriler Kruskal-Wallis (parametrik olmayan veriler) ve tek yönlü ANOVA testleri (parametrik veriler) ile analiz edildi ($p<0.05$).

Bulgular: Grup 2'de diş dokularında hiperemi ve nötrofil lökosit infiltrasyonları ve parotis bezinde şiddetli enflamatuar reaksiyon ile karakterize sialadenit gözlendi. ALA tedavisi sonrası her iki dokuda da dejeneratif değişiklikler azaldı ve bez normal bir görünüm kazandı. Pulpa açısından grup 3 grup 2'ye göre anlamlı olarak daha düşük enflamatuar skorlar sergiledi ($p=0,044$). Diş eti dokusunun hem BMP-2 değerleri hem de kaspaz-3 hücre sayıları grup 3'te grup 2'ye göre anlamlı derecede düşüktü ($p<0,01$).

Address for Correspondence/Yazışma Adresi: Özge Kam Hepdeniz Asst. Prof., Süleyman Demirel University Faculty of Dentistry, Department of Restorative Dentistry, Isparta, Turkey
Phone: +90 533 368 26 27 **E-mail:** ozgekam@gmail.com
ORCID ID: orcid.org/0000-0002-6198-8839

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Sonuç: Bu çalışmada mısır şurubuna bağlı vasküler ve pulpal doku hasarlarının gerçekliği belirlendi. ALA'nın mısır şurubu tüketiminin zararlı sonuçlarına karşı potansiyel bir antioksidan olabileceği sonucuna varıldı.

Anahtar Kelimeler: Alfa-lipoik asit, pulpa, yüksek fruktozlu mısır şurubu, parotis bezi, sıçanlar

Introduction

Today, interest in the health effects of excessive high fructose corn syrup (HFCS) intake has increased due to its widespread commercial use and reporting of severe adverse metabolic effects in rodents, humans, and rats (1,2). Among these effects, oxidative stress (OS) which is observed due to HFCS-induced insulin resistance and the result of excessive production of reactive oxygen species (ROS) is an important risk factor that causes dysfunction of various tissues and organs of the body (3). However, there are hardly few studies analyzing OS and cytopathological consequences especially such as apoptosis in the salivary glands in insulin resistance (4).

Saliva has multifunctional roles, such as protecting the teeth surface and the mucous membranes of the oral cavity towards to chemical, biological, and mechanical attacks and antioxidant capacity, in maintenance of oral health (5). Therefore, saliva is considered an important element of the oral defense mechanism, as the antioxidants in saliva are the first line of defense against OS due to free radicals (6). Thus, the factors that impair the function of the salivary glands affect the oral health and quality of life negatively by changing the amount and content of saliva (7).

The dental pulp is a vascularized tissue with a dense capillary plexus and vulnerable to damage by inflammation, chemicals, and mechanical trauma (8). In an inflammatory state, odontoblasts produce copious amounts of proinflammatory mediators and various molecular and cellular signaling pathways are activated (8,9). However; OS, another consequence of a long-term inflammation, induces apoptosis of pulp cells and causes proliferation failure to replace damaged odontoblasts (10). Apoptosis markers such as caspase-3 have previously been studied in parotid gland and pulp (9,11). However, so far, no attention has been paid to their role in HFCS intake.

An alternative approach to minimize the effects caused by OS is to maintain a proper diet accompanied by regular antioxidant supplementation. It has been reported that alpha-lipoic acid (ALA), a mitochondrial coenzyme and natural antioxidant, has beneficial effects on OS parameters such as elimination of free radicals and reduction of OS in various tissues (12). It was previously reported that ALA has the ability to scavenge ROS, increase the intracellular antioxidant enzyme activity, and reduce the proinflammatory marker levels (13).

The aim of this study was to find out a) the possible metabolic effects of HFCS in parotid glands, gingiva and pulp of rats, b) the antioxidant defense of ALA in parotid glands and dental tissues of rats subjected to HFCS intake. The null hypothesis was that systemic application of ALA had no effect on the severity of inflammation induced by HFCS.

Materials and Methods

The animal experiments were approved by the Experimental Animal Committee on Animal Research of the Süleyman Demirel University, Isparta (decision no:3, date: 22.08.2013).

Female Wistar rats (n=24), weighing 250-300 g, were housed at a temperature of 21±2 °C with 60±5% humidity in a controlled room. All rats were fed with standard ad libitum chow (Korkuteli yem, Antalya, Turkey). F30 corn syrup (Toposmanoğlu, Isparta, Turkey), containing approximately 24% fructose and 28% dextrose in the syrup of 73% total solids, was prepared in 30% similar to the corresponding publications (14). The rats were randomly divided into three groups (group 1: negative control, group 2: positive control and group 3: ALA), with each group consisting of 8 animals. Standard diet and water were given only to group 1 for 10 weeks. F30 corn syrup was given to rats in group 2 and 3 within the drinking water for 10 weeks (2). ALA (100 mg/kg) (Thioctazid 600 mg tab, Meda Pharma, Turkey) was given only to rats of group 3 by oral gavage for the last 6 weeks of the experiment (14). Twenty-four hours after the last ALA application, rats were euthanized by cervical dislocation. The parotid glands were removed bilaterally. Subsequently, the jaws were defleshed and collected for histopathological examination.

During necropsy, the parotid glands, jaws were fixed in 10% buffered formalin solution (Sigma Diagnostics, St. Louis, MO, USA). After the fixation, the hemimandibulas were decalcified for 8 weeks in 18% EDTA (Cerkamed, Stalowa Wola, Poland). Five serial sections of each hemimandibula with 5 µm thickness were cut and stained with hematoxylin-eosin. Routine pathology procedures were also used for the parotid gland examined under light microscope (Olympus CX41, Tokyo, Japan). Histopathological lesions of parotid gland scored as (15);

0: Normal gland histology; 1: Only hyperemia; 2: Inflammatory cells ≤5 under a magnification of x400; 3: Inflammatory cells >6 under a magnification of x400.

For evaluating the number of polymorphonuclear leukocytes (PMNs) of the gingival junctional epithelium and connective tissue subjacent to the epithelium, an area of 0.05 mm x 0.05 mm was examined and counted under a magnification of x400 for each rats (16). Semi-quantitative histological scoring of pulp was performed according to the McClanahan criteria for histopathology (17).

Selected jaw tissue sections were immunostained for caspase-3 [Anti-caspase-3 antibody (ab4051), Abcam, Cambridge, UK] to demonstrate the apoptotic activity and bone morphogenetic proteins-2 (BMP-2) [(Anti-BMP2 antibody [65529.111] ab6285), Abcam, Cambridge, UK], parotid section was immunostained by caspase-3 and caspase-8 [Anti-Caspase-8 (ab25901), Abcam, Cambridge, UK] by routine streptavidin-biotin peroxidase technique. Tissues were counterstained with Harris hematoxylin. All slides were analyzed for immunopositivity, and a semi-quantitative analysis was carried out. The average percentages of both epithelial and submucosal immunopositive cells were then calculated. Morphometric evaluation was performed using the database manual cell sens life science imaging software system (Olympus Corporation, Tokyo, Japan).

Statistical Analysis

Statistical analysis was carried out using SPSS 13.0 software program pack (SPSS Inc., Chicago, IL, USA). The Kolmogorov-Smirnov test was used to establish the normal distribution of variables. The histopathological scores of the groups were compared with the non-parametric Kruskal-Wallis test. The immunohistochemical data were analyzed using a parametric one-way analysis of variance (ANOVA) test. Statistical significance was determined at $p<0.05$.

Results

No pathological changes were observed in gingival tissue and dental pulp of the group 1 (Figure 1A). Increased vessel count, hyperemia and neutrophil leukocyte infiltrations were observed in gingival tissue of group 2. The PMN infiltrations were increased in both gingival epithelium and submucosa of group 2 compared to the group 1 (Figure 1B).

As a result of histopathological evaluation of the pulp, group 3 exhibited significantly lower inflammatory scores than group 2 ($p=0.044$, Table 1), while group 3 and group 1 exhibited statistically similar inflammatory scores ($p=1.000$, Table 1).

The number of caspase-3 + cells of gingiva in group 3 were significantly lower than group 2 ($p<0.05$, Table 1). Similarly, BMP-2 immunoreaction was increased by HFCS-treated rats in group 2 and significantly decreased with ALA treatment in group 3 (Table 1). Caspase-3 immunoreaction of gingiva in the groups was demonstrated in Figure 1D-1F. BMP-2 expression of the groups was demonstrated in Figure. 1G-1I.

No pathological changes were observed in the parotid glands of group 1 (Figure 2A). In group 2, slight histopathological findings such as slight degeneration in some acinus and degranulation in some acinar cells were observed. The first sign of degeneration was the appearance of nuclear psychosis in some cells, especially in the parenchyma. The cell imaging showed shrinkage of nucleus more than the cells of group 1. Sialadenitis characterized by inflammatory reaction was observed in group 2 (Figure 2B). In group 3, the parotid gland recovered an essentially normal appearance. The contour of the nuclei became rounded and the vesicular appearance was returned (Figure 2C). Degenerative changes were decreased but no statistically significant difference was observed among the group 2 and 3 in terms of inflammatory index ($p=1.000$, Table 2).

The caspase-3 and caspase-8 reaction of the parotid gland in group 1 was low. The immunopositive cells in group 2 were significantly higher than the number of positive cells of group 3 (Table 2). Caspase-3 and caspase-8 immunoreaction of the parotid glands in the groups was also demonstrated in Figure 2D-2F and 2G-2I, respectively.

Discussion

It is well documented that HFCS induces metabolic disturbances in rats (2). One of the organs affected by OS, which is an important parameter in these metabolic disorders, is the parotid gland. Mata et al. (11) mentioned the effect of ROS on rat parotid gland and reported that ROS could stimulate the activity of caspase in parotid gland tissue leading to apoptosis. It was reported that in the presence of high glucose concentrations, ROS levels and apoptosis rates were significantly increased in a dose-dependent manner (18). Increased caspase reaction demonstrating apoptotic activity in this study was confirmed the information mentioned above. The number of caspase-3 + cells of the gingiva and caspase-3 + and caspase-8 + cells of the parotid gland observed in group 2 was significantly greater than the number of caspase - positive cells in group 3. These results also confirm that ALA treatment attenuates the increase in apoptotic activity associated with HCFS ingestion.

The effect of sucrose and correspondingly OS is partly systemic and dose-dependent (19). Therefore, these effects don't remain limited with parotid glands. Several studies in rats have also confirmed the effects of a diet rich in sucrose on dentin apposition (19,20). However, it has also been noted that high glucose levels could restrict macrophage function (chemotaxis and bacterial death), resulting in an inflammatory state that damages host cellular proliferation (10). The microscopic and histopathological analysis verified the efficacy of the experimental protocol used in this study for inducing inflammation. Notably, this inflammatory state was observed in group 2 of the present study. The sign of the inflammatory reaction by increased PMN infiltrations was seen in both the gingiva and parotid glands of group 2. High concentrations of glucose may be responsible for reduced cell growth, which may have contributed to this outcome. And this result may also be attributed to a rise in apoptosis and/or cell-cycle arrest.

Neovascularization and advanced cellular differentiation of dental pulp are crucial in terms of pulp healing. The pulp's healing potential is related to the dental pulp cells ability of secreting growth factors (21). One of these growth factors is BMPs. BMPs are signaling proteins and play an important

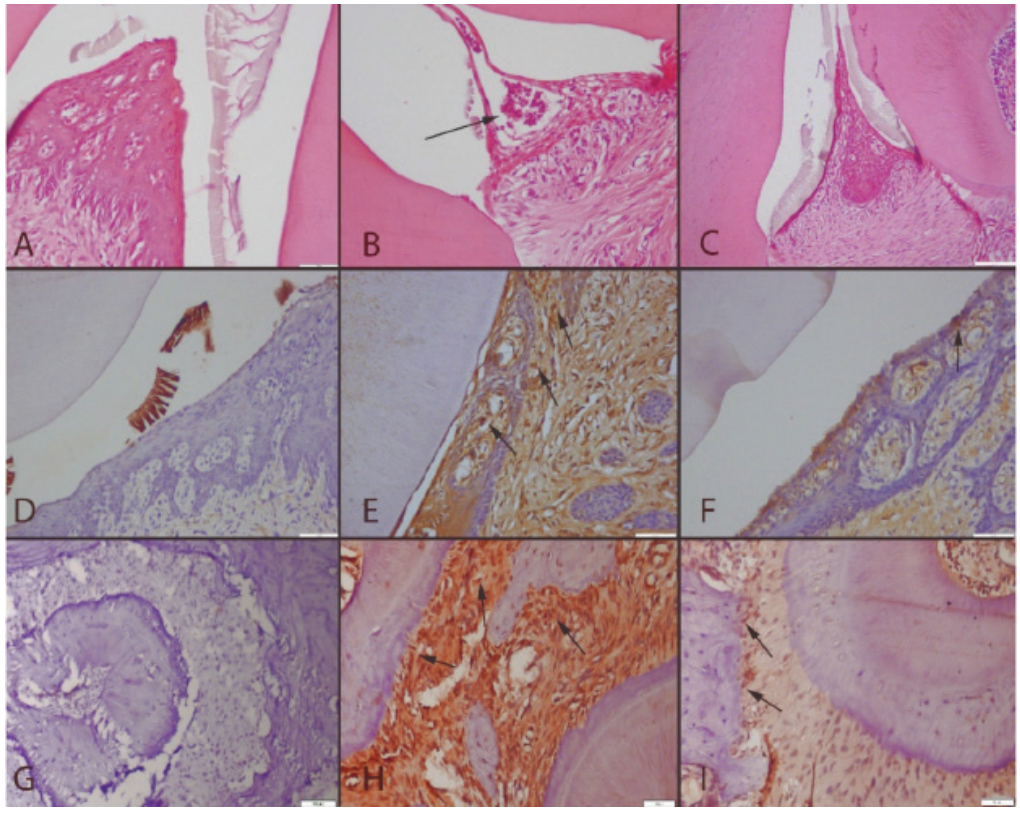

Figure 1. Representative histopathology of the oral mucosa in the groups (upper row).
(A) Normal appearance of the tooth, normal thickness with intact gingival mucosa in group 1, **(B)** Marked PMN infiltrations in gingival epithelium and sub adjacent connective tissue (arrows), marked decrease of thickness of the epithelial layer in group 2, **(C)** Ameliorated gingival lesions in group 3
Caspase-3 immunoreaction of the gingival tissue in the groups (medial row). **(D)** Negative immunoreaction both epithelial and connective tissue cells in group 1, **(E)** Marked increase in caspase-3 expression in gingival epithelium and connective tissue cells (arrows) in the group 2, **(F)** Decreased immunoreaction of caspase-3 (arrow) in group 3
BMP-2 expression between the groups (below row). **(G)** Negative immunoreaction in group 1. **(H)** Marked expression in cells (arrows) in group 2. **(I)** Decreased expression in cells (arrows) in group 3. Bars =50 μm.

Table 1. Histopathological and immunohistochemical results (mean ± SD) of gingiva and pulp

	Group 1	Group 2	Group 3	p-value
Inflammation scores of pulp	0.42±0.29	3.12±-1.15	1.37±0.32	1-2 (0.007)* 1-3 (1.000) 2-3 (0.044)*
PMN number at junctional epithelium of gingiva	1.28±0.35	6.75-0.36	3.50-0.56	1-2 (0.000)* 1-3 (0.008)* 2-3 (0.000)*
PMN number at connective tissue of gingiva	2.00±0.43	8.25-0.31	3.75-0.45	1-2 (0.000)* 1-3 (0.020)* 2-3 (0.000)*
Cas-3 positive cell (%)	0.28±0.18	11.25±2.21	2.75±0.70	1-2(0.000)* 1-3 (0.006)* 2-3 (0.000)*
BMP-2 positive cells (%)	1.14±0.45	29.75±2.54	7.75±1.28	1-2 (0.000)* 1-3 (0.000)* 2-3 (0.000)*

PMN: Polymorphonuclear leukocyte, BMP: Bone morphogenetic protein, SD: Standard deviation, *Statistically significant ($p<0.05$)

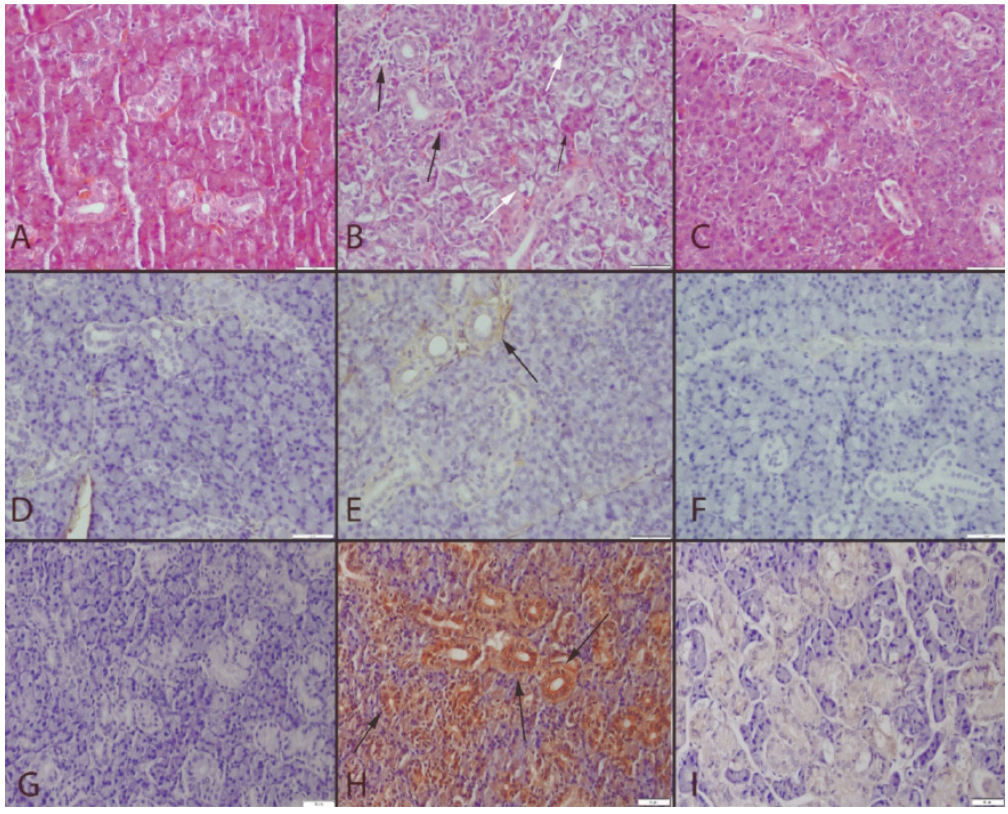

Figure 2. Representative histopathology of the parotid glands in the groups (upper row). **(A)** Normal histology of the parotid gland in group 1, **(B)** PMN infiltrations (black arrows), vacuolization at the cytoplasm (white arrows) and psychosis (thin arrow) at the nucleus of parotid gland cells in group 2, **(C)** Amelioration of the pathological findings of the group 3.
Caspase-3 immunoreaction of the parotid glands in the groups (medial row). **(D)** Negative caspase-3 reaction of the parotid gland in group 1, **(E)** Increased caspase-3 immunopositivity at the duct of parotid gland (arrow) in group 2, **(F)** Negative caspase-3 reaction of the parotid gland in group 3
Caspase-8 immunoreaction of the parotid glands in the groups (below row). **(G)** Negative caspase-3 reaction of the parotid gland in group 1, **(H)** Increased caspase-3 immunopositivity at the duct of parotid gland (arrows) in group 2, **(I)** Negative caspase-3 reaction of the parotid gland in group 3. Bars =50 μm.

Table 2. Histopathological and immunohistochemical results (mean ± SD) of parotid gland

	Group 1	Group 2	Group 3	p-value
Inflammation scores	0.00±0.00	2.12±0.22	1.87±0.29	1-2 (0.000)* 1-3 (0.000)* 2-3 (1.000)
Cas-3 positive cells (%)	0.85±0.35	22.75±2.54	7.75±1.28	1-2 (0.000)* 1-3 (0.000)* 2-3 (0.000)*
Cas-8 positive cell (%)	1.85±0.67	29.75±1.83	9.00±1.06.	1-2 (0.000)* 1-3 (0.000)* 2-3 (0.000)*

*Statistically significant ($p<0.05$), SD: Standard deviation

role in functions such as matrix synthesis, cell proliferation, embryogenesis, bone formation, and mesenchymal stem cell differentiation (22). In recent studies, it has been reported that BMPs play an important function in glucose homeostasis as a bridge between glucose metabolism and bone metabolism (23,24). A previous study noted that endothelial cells readily respond to high glucose therapy through upregulation of BMPs and their inhibitors and receptors (23). In present study, the BMP-2 reaction was significantly increased as a result of inflammation induced by taking fructose syrup for 10 weeks as aforementioned studies.

ALA is a component that is produced in very small amounts in the body and acts as a coenzyme in various reactions, is involved in glycolysis, and is responsible for converting blood sugar into energy (25). Nevertheless, the protective effects of ALA in different tissues have long been studied (3,12,13,18,25). The results of this study have supported the potential of therapeutic approaches of ALA. The group treated with ALA presented a lower inflammatory index and lower number of caspase-3+, caspase-8+ cells, and BMP2 than the group 2. ALA prevents microvascular damage and has a protective effect on neurons (3). This effect of ALA may provide the return of a modified appearance of cells and glands in group 2 to normal appearance in group 3 essentially in present study. As a result of all these results, the null hypothesis was rejected. To the best of our knowledge, in the literature, there are no previous studies evaluating the anti-inflammatory effects of the ALA in rat dental tissues and parotid glands, so it is not possible to make a comparison with other study models. The limitation of this study is that there were no markers of OS induced ROS. The inclusion of OS markers in this study may diversify the results of this study.

Conclusion

It was concluded that intercellular ROS formation and OS in oral mucosa, pulp and salivary gland cells enhanced by high glucose was attenuated by treatment with ALA due to the results of decreased degenerative changes and the reduced number of caspase + cells. Further studies are necessary to investigate the possible effects of corn syrup and ALA with a larger number of samples using longer treatment duration to evaluate not only the reduction in symptoms, but also the toleration.

Ethics

Ethics Committee Approval: The animal experiments were approved by the Experimental Animal Committee on Animal Research of the Süleyman Demirel University, Isparta (decision no: 3, date: 22.08.2013).

Informed Consent: Informed consent is not required.

Peer-review: Externally peer-reviewed.

Authorship Contributions

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

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Anatomical and Morphometric Evaluation of the Cranial Index and Its Relevance to Clinical Syndromes

Kraniyal İndeksin Anatomik ve Morfometrik Olarak Değerlendirilmesi ve Klinik Sendromlarla İlişkisi

Eren Öğüt[1], Özge Güzelad[2], Fatoş Belgin Yıldırım[2], Esra Sayılar[3]

[1]Bahçeşehir University Faculty of Medicine, Department of Anatomy, İstanbul, Turkey
[2]Akdeniz University Faculty of Medicine, Department of Anatomy, Antalya, Turkey
[3]Bahçeşehir University Faculty of Medicine, Medical Faculty Student, İstanbul, Turkey

Abstract

Objective: The most widely used method to define the craniofacial complex is the cranial index (CI). This index is calculated by determining the ratio between the maximum cranial width (CW) and the cranial length (CL), and is one of the clinically accepted anthropometric parameters. This study investigates the CI of Turkish dry skulls and its effects on sex, population affinity, and clinical syndromes.

Materials and Methods: One hundred adult Turkish dry skulls (57 male, 43 female) were investigated. The CI was calculated by the ratio of CL to CW and multiplied by 100 according to Martin's methods. SPSS 25 was used for statistical analysis.

Results: CI values were 88.75±1.40 mm in males and 84.90±1.13 mm in females, and differences between them were significant (p=0.045). Ultradolicocephalic and hyperdolichocephalic types were not detected. Brachiocephalic types (28%) were more frequent in females than in males, whereas ultra-brachycephalic types (33%) were more frequent in males (p<0.05). The most frequent type in Turkish dry skulls was brachycephalic (26%) and ultrabrachycephalic (26%), followed by the hyperbrachycephalic (22%), mesocephalic (21%), and dolichocephalic types (5%).

Conclusion: It can be said that the brachiocephalic type is a more frequent type in Turkish adult dry skulls. The differences in CI and type between the sexes may be indicative of sexual dimorphism. Skull types can be useful in demonstrating craniofacial abnormalities or clinical syndromes.

Keywords: Cranial index, craniometry, skull, craniofacial syndrome

Öz

Amaç: Kraniyofasiyal kompleksi tanımlamak için en yaygın kullanılan yöntem kraniyal indekstir (Cİ). Bu indeks maksimum kafatası genişliği (CW) ile maksimum kafatası uzunluğu (CL) arasındaki oran belirlenerek hesaplanır ve klinik olarak kabul edilen antropometrik parametrelerden biridir. Bu çalışma, Türk kuru kafataslarının Cİ'sini ve bunun cinsiyet, popülasyon yakınlığı ve klinik sendromlar üzerindeki etkilerini araştırmayı amaçlamaktadır.

Gereç ve Yöntemler: Yüz adet yetişkin Türk kuru kafatası (57 erkek, 43 kadın) incelendi. Cİ, CL'nin CW'ye oranıyla hesaplandı ve Martin'in yöntemine göre 100 ile çarpıldı. İstatistiksel analiz için SPSS 25 kullanıldı.

Bulgular: Cİ değerleri erkeklerde 88,75±1,40 mm, kadınlarda 84,90±1,13 mm ve aralarındaki farklılık anlamlı bulundu (p=0,045). Ultradolikosefalik ve hiperdolikosefalik tipler tespit edilmedi. Brakiosefalik tipler (%28) kadınlarda erkeklere göre daha sık görülürken, ultra-brakisefalik tipler (%33) erkeklerde daha sıktı (p<0,05). Türk kuru kafataslarında en sık görülen tip brakisefalik (%26) ve ultra brakisefalik (%26) olup, bunu hiperbrakisefalik (%22), mezosefalik (%21) ve dolikosefalik tipler (%5) izlemiştir.

Address for Correspondence/Yazışma Adresi: Eren Ögüt Assoc. Prof. MD, Bahçeşehir University Faculty of Medicine, Department of Anatomy, İstanbul, Turkey
Phone: +90 216 579 82 39 **E-mail:** erenogut@yahoo.com.tr
ORCID ID: orcid.org/0000-0003-2506-9883

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Sonuç: Çalışmamızın parametreleri ile brakiyosefalik tipin Türk erişkin kuru kafataslarında daha sık görülen bir tip olduğu söylenebilir. Cinsiyetler arasındaki Cİ ve tip farklılıkları, cinsiyet dimorfizminin bir göstergesi olabilir. Kafatası tipleri, kraniyofasiyal anormallikleri veya klinik sendromları göstermekte faydalı olabilir.

Anahtar Kelimeler: Kraniyal indeks, kraniyometri, kafatası, kraniyofasiyal sendrom

Introduction

Anders Retzius (1796-1860), developed the cephalic index, which was initially applied in physical anthropology to categorize prehistoric remains discovered in Europe (1). The cranial index (CI) is the term used by Retzius to describe his measurements when applied to dried skulls (2). Numerous variables affect the shape, size, density, and positioning of the skull and its components. Acquired diseases, posttraumatic abnormalities, neoplasia, deformations, or sutural synostosis can all be signs of a malformed skull (3). Osteopetrosis and frontometaphyseal dysplasia are skeletal dysplasias that can result in increased calvarial density, whereas hypophosphatasia and osteogenesis imperfecta can result in decreased calvarial density. The calvarial density that is diffusely diminished or elevated is typically linked to a process that affects the entire skeleton. Therefore, other features are required for accurate differentiation between these dysplasias (4).

CI is one of the most crucial factors in determining sexual dimorphism and population affinity (5). A wide range of parameters is used to determine CI, depending on age, sex, environment, ethnicity, and methodology (5). Positional plagiocephaly or sutural synostosis are the leading causes of skull malformation. The closure may not always cover the depth of the suture; therefore, the length of the sutures must be considered (4). CI must be standardized across all disciplines of expertise to improve researcher communication and enable accurate comparisons between studies (6).

Several methods have been used to investigate the form of the skull, each with its benefits and drawbacks (5). A method of systematic investigation was performed by photographing the skull in several regular planes. This had certain benefits, such as the capacity to see the landmarks of the skull in great detail. However, this approach has certain drawbacks in the form of distortions of near and far aspects of an object (5). Martin's method, which involved distributing caliper and steel tapes to establish the dry skull form, was the most commonly applied way of measuring skulls (7). These techniques and databases for craniofacial measurements have been used in several disciplines (8).

The CIs are thought of as clinical anthropometric indicators utilized in the examination of cranial anomalies (8). Therefore clinicians may change their course of action to determine whether there are any craniofacial anomalies (5). It could help forensic anthropologists classify human skulls, which can be crucial for identifying unknown skeletal remains (8). The current research aims to examine the CI in Turkish adult dry skulls and its effects on sex, population affinity, and certain clinical disorders.

Materials and Methods

In this study, 100 adult dry skulls were used. Skulls were obtained from the osteological collection of the Department of Anatomy, Akdeniz University. It was approved by the ethics committee of Akdeniz University on 26 August 2020 with protocol number 597. Twenty-one skulls with fractures, abnormalities, and pathological lesions were excluded from the study. Skull length and width measured by Digital Microcaliper. The maximum cranial length (CL) and breadth were measured according to Martin's method (7). CI is defined as the ratio of cranial width (CW) (maximum transverse diameter between two fixed points) to the CL (greatest anteroposterior diameter and the distance between the glabella and inion) and multiplied by 100 (9). To achieve intraobserver precision, three widely used precision estimates were calculated: the technical error of measurement (TEM), the relative TEM, and the coefficient of reliability (R) (10-13).

Statistical Analysis

The data were analyzed by SPSS 25 (IBM SPSS software, USA). Values are presented as mean ± standard deviation. Comparisons between the sexes were analyzed by Student's t-test. For all statistical comparisons, $p<0.05$ was assumed to indicate statistical significance.

Results

R values of the variables were near 1, indicating that most of the variables' variance was caused by other than measurement error. These findings imply that the measurement's intra-observer accuracy was acceptable. The skull types were classified according to the previous classification system described by Williams et al. (9). The detected types were presented in Figure 1.

The maximum CL and CW were 157.13 mm, 157.03 mm (males) and 157.12 mm,166.08 mm (females), respectively. The mean CI was 88.75±1.40 (males) and 84.90±1.13mm (females). The CI of males was more significant than the CI of females (Table 1). Significant differences were found according to the CI between the sexes ($p=0.045$). The mean CL was 146.53±1.38 mm in males and 148.91±1.51 mm in females. The mean CW was 129.31±1.26 mm in males and 126.14±1.65 mm in females. The most frequent type in Turkish dry skulls was brachycephalic (26%) and ultra brachycephalic (26%), followed by the hyperbrachycephalic

(22%), mesocephalic (21%), and dolichocephalic types (5%). The ultradolichocephalicphalic and hyperdolichocephalic types were not detected in both sexes. The ultra brachycephalic (33%) type was more frequent in males, while the brachycephalic (28%) type was more common in females. The ultrabrachycephalic type (33%) was followed by the brachycephalic (25%), hyperbrachycephalic (21%), mesocephalic (16%), and dolichocephalic types (5%) in males. The brachycephalic type (28%) and mesocephalic (28%) types were followed by the hyperbrachycephalic (23%), ultrabrachycephalic (16%), and dolichocephalic types (5%) in females.

Discussion

The present study revealed that adult Turkish dry skulls were brachycephalic type, which refers to a skull that is shorter than typical skulls. Females have shorter skulls when compared to males according to the CW and CI. This result is consistent with some studies conducted in Europe and Brazil. It has been reported that the mean CI in females was 71.48±5.81 and it was 69.75±4.02 in males, and the differences between them were significant. Gupta et al. (14) and Pandey et al. (15) reported that both CL and CW were more significant in males than females, indicating sexual dimorphism (Table 2). Salve et al. (16) reported that most males in the Andhra were dolichocephalic or mesocephalic, and females were mesocephalic, and the differences between sexes were significant (p=0.001). Similarly, Kumar and Nagar (2) (India), and Chaudhary et al. (17) (Nepal) reported that the difference between sexes according to the CI was significant (15). Chaudhary et al. (17) reported that CI could be an essential indicator to determine sex if the samples belong to the same population (CI: 71.48±5.81 in females and 69.75±4.02 in males) (17). Kumar and Nagar (2) found that male skulls tend to be dolichocephalic (53.33%) and mesocephalic (42.22%). The current study found significant differences in CI between sexes (p=0.045). The most frequent type was an ultrabrachycephalic type (33%), and it was followed by the brachycephalic (25%), hyperbrachycephalic (21%), mesocephalic (16%), and dolichocephalic types (5%) in males. However, the brachycephalic type (28%) and mesocephalic (28%) types were followed by the hyperbrachycephalic (23%), ultrabrachycephalic (16%) and dolichocephalic types (5%) in females. The findings of current study consistent with the previous studies and confirmed the sexual dimorphism. Therefore it can be said that the CI and type differences between the sexes may be an indicator of sexual dimorphism in Turkish dry skulls.

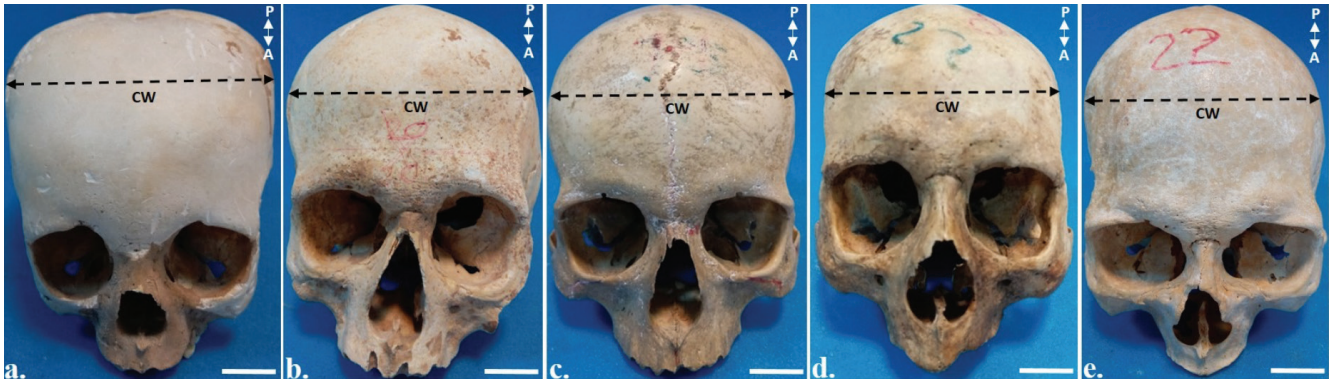

Figure 1. The five types of the skull were detected. **a.** Ultrabrachycephalic type (CW≫CL and CI ≥90 mm), **b.** Hyperbrachycephalic type (CW>CL and CI ≥85-89.9 mm) of skull has round/broad head, **c.** Brachycephalic type (CW>CL and CI=80 to 84.9 mm). The breadth greater than the length, **d.** Mesocephalic type (CL=CW and CI=75-79.9 mm). The intermediate lengths of the skull, **e.** Dolichocephalic type (CL>CW and CI=70-74.9mm). The anteroposterior diameter and length are greater than the breadth.

A: Anterior, P: Posterior, CI: Cranial index, CL: Cranial length, CW: Cranial width

Table 1. Analysis of mean cranial length (CL), cranial width (CW) and cranial index (CI) by sex

	Sex	N	Mean	Minimum	Maximum	SD	p-value
CL	Male	57	146.5342	106.77	157.13	1.38741	0.252
	Female	43	148.9175	105.74	157.12	1.51608	0.249
CW	Male	57	129.3115	97.92	157.03	1.26812	0.125
	Female	43	126.1437	96.79	166.08	1.65064	0.132
CI	Male	57	88.7530	73.86	104.37	1.40127	0.045
	Female	43	84.9052	72.96	109.37	1.13641	0.035

Independent Samples t-test. SD: Standard deviation

Table 2. Comparison of studies by years

Authors	Place	Year	N	CL (mm)	CW (mm)	CI (mean)	Cranial types
Salve et al. (16)	Andhra	2011	320	177.75±7.32 F: 172.68±4.4 M: 182.25±6.04	136.61±3.43 F:134.98±3.5 M:138.25±2.44	76.94±2.53 F: 78.2±2.33 M: 75.68±2	Dolicocephalic 42.18% Mesocephalic 54.06% Brachycephalic 3.75%
Howale et al. (18)	Maharastra	2012	75	171.1±8.9	129.8±5.4	75.49±3.95	Dolichocephalic 42.66% Mesocephalic 46.66% Brachycephalic 8% Hyperbrachycephalic 2.66%
Gupta et al. (14)	North India	2013	600	F: 177.74±8.44 M: 186.88±6.33	F: 136.19±6.13 M: 139.51±6.33	F: 76.83±5.5 M: 74.74±4.3	Dolichocephalic F: 34.67% M: 55% Mesocephalic F: 47.33% M: 32.67% Brachyphalic F: 14% M: 11.67% Hyperbrachycephalic F: 4% M :0.67%
Kumar and Nagar (2)	North India	2015	80	171.3±8.7 F: 169.1±7.4 M: 177.6±7.8	126.9±5.3 F: 126.9±6 M: 130.8±4	74.27±4.36 F: 75.22±5.15 M: 73.75±3.56	Dolicocephalic M: 53.33% F: 31.42% Mesocephalic M: 42.22% F: 62.85% Brachicephalic M: 2.22% F: 2.85% Hyperbrachicephalic M: 2.22% F: 2.85%
Pandey et al. (15)	Nepal	2016	292	F: 171±3 M: 182±6	F: 134±4 M: 138±3	F: 78.36±5.06 M: 75.82±4.43	Dolicocephalic 55.13% Mesocephalic 36.64% Brachycephalic 08.22%
Nascimento et al. (22)	Paraiba	2016	166	F: 171±7 M: 176.7±9	F: 141.9±6 M: 145±7	F: 82.9±3.2 M: 82.3±4.3	Dolichocephalic 2.41% Mesocephalic 17.47% Brachycephalic 80.12%
Madadi et al. (23)	Iran	2018	200	181.6±12.4	151.1±7.7	83.51±6.85 F: 85.87±7.33 M: 81.15±5.41	Dolichocephalic 3.5% Mesocephalic 21.5% Brachycephalic 40% Hyperbrachycephalic 35%
Woo et al. (21)	Thailand	2018	185	F: 166.85±7.76 M: 174.25± 6.52	F: 138.25±5.49 M: 142.38±5.83	F: 82.99±4.37 M: 81.81±4.23	Dolichocephalic 4.32% Mesocephalic 27.03% Brachycephalic 42.70% Hyperbrachycephalic 25.95%
Chaudhary et al. (17)	Nepal	2019	256	182.7±8.4 F: 177.1±6.0 M: 187.8±6.9	150.4±6.4 F: 147.9±5.7 M: 152.8±6.2	82.48±4.44 F: 83.62±4.08 M:81.41±4.52	Dolicocephalic 3.90% Mesocephalic 25.39% Brachycephalic 43.75% Hyperbrachycephalic 26.95%
Ay et al. (3)	India	2021	50	170.4±7.8	130.4±6.9	76.81±4.72	Hyperdolichocephalic 5.1% Dolicocephalic 13.26% Mesocephalic 20.4% Brachycephalic 11.22% Hyperbrachycephalic 1.2%
Botwe et al. (20)	Ghana	2021	300	179.1±8.6 F: 175.6±7.4 M: 182±8.5	139.4±5.4 F: 138±4.9 M: 140.5±5.6	78±13 F: 79±3.3 M: 77.3±3.6	Dolichocephalic 17.7% Mesocephalic 54.3% Brachycephalic 23.7% Hyperbrachycephalic 3% Hyperdolichocephalic 1% Ultrabrachycephalic 0.3%

Table 2. Continued

Authors	Place	Year	N	CL (mm)	CW (mm)	CI (Mean)	Cranial types
Present	Turkey	2022	100	F: 148.9175±9.94 M: 146.5342±10.47	F: 126.1437±10.82 M: 129.3115±9.57	88.75±10.57 84.90±7.45	Dolichocephalic F: 2 (%5) M: 3 (%5) Mesocephalic F: 12 (%28) M: 9 (%16) Brachycephalic F: 12 (%28) M: 14 (%25) Hyperbrachicephalic F: 10 (%23) M: 12 (%21) Ultrabrachicephalic F: 7 (%16) M: 19 (%33)

The CI is an essential tool that might be used to identify the population affinity in various populations (2,18). Previous studies revealed that the CI among Asians differed significantly by region, such as Koreans were classified as brachiocephalic, Japanese were mesocephalic, and Siberians were mesocephalic/brachycephalic (19). African skulls were predominantly dolichocephalic, while the Europeans were categorized as brachiocephalic. However, Botwe et al. (20) indicated that the majority of Ghanaians were mesocephalic (n=163, 54.3%) and it was followed by brachycephalic (n=71; 23.7%), dolichocephalic (n=53; 17.7%), hyperbrachycephalic (n=9; 3%), hyperdolichocephalic (n=3; 1%) and ultrabrachycephalic (n=1; 3%). It has been stated that the CL and CW significantly differed from the Central and Northeast Thai and Korean females. The most frequent type was brachycephaly, followed by the mesocephalic and hyperbrachycephalic types, and the rarest type was the dolichocephalic type (21). In a study, it was revealed that the CI of females was higher than that of males in the Andhra population in India (16). It was reported that North Indian males had dolichocephalic (74.74%) and females had mesocephalic (76.83%) (14). It has been stated that the CL of Latvia and Nigeria populations were higher than North Indians (14). However, Latvia, Nigeria, Malaysians, Japanese and Sri Lankans had broader skull when compared to the North Indians, according to the CW (14). Similarly, Pandey et al. (15) reported that 68.35% of males were dolichocephalic, and 50.74% of females were mesocephalic in Nepal. However, Chaudhary et al. (17) reported that 43.75% had brachycephalic followed by hyperbrachycephalic (26.95%), mesocephalic (25.39%), and dolicocephalic (3.90%) in Nepal. It was reported that Australians and Southern Africans had dolicocephalic, Europeans and Chinese had mesocephalic, and Mongolians had brachicephalic (2). It was reported that the majority of the Brazilians had brachycephalic (80.12%) and small part of them had dolicocephalic (2.41%) (22). Madadi et al. (23) detected that the most frequent types were brachycephalic and hyperbrachycephalic in Iranians on both sexes. The parameters of our study were similar to the Europeans and Brazilians, therefore it can be said that the brachiocephalic type is more frequent in Turkish adult dry skulls. It can be due to the asymmetric growth in the displacement of the sutures, sutural synostosis of the skull to the affected side or population affinity (14).

Howale et al. (18) reported that the most frequent types were mesocephalic (46.66%) and dolichocephalic (42.66%). It has been reported that the males were predominantly dolichocephalic (55%), and the females were mesocephalic (47.33%) (14). Ay et al. (3) reported that 20.4% of South Indians were brachycephalic and 13.26% of them had mesocephalic types. It was reported that various age groups, including children, all had the same CI, and there was no absolute consistency in CI (20). The current study revealed that the most frequent type was the brachycephalic in Turkish adult dry skulls, and less common was dolicocephalic (5%) on both sexes. Ultra brachycephalic (33%) was dominated in the males and brachycephalic (28%) was dominated in females. It may be caused by several factors including osteomyelitis, alterations in fontanelles, neurofibromatosis, langerhans cell histiocytosis, skull bifidum, metastases or underlying clinical syndromes. Skull types and CI may provide valuable diagnostic data to identify local and systemic impairments. Types of the skull can be helpful for monitoring, diagnosing, and reconstruction surgery to treat craniofacial abnormalities or syndromes (20). It has been reported that dolichocephalic skulls had fewer otitis media than brachiocephaly (24). And dolichocephalic skull is more common in autosomal dominant diseases. While more hyperbrachycephalic type has been reported in Apert syndrome, more mesocephalic type has been reported in Crouzon syndrome (25). Additionally, it has been stated that CI is lower in patients with sickle cell anemia than in healthy individuals (16). A brain injury, including fetal alcohol syndrome, is usually implied by a smaller cranial vault at birth. Macrocephaly can be brought on by skeletal dysplasia or an increase in intracranial volume. It may be possible to diagnose various dysplasias with the use of skull anomalies and a variety of acquired disorders, such as trauma and abuse. Therefore, skull anomalies and their types should be evaluated to diagnose various syndromes and disorders independent of surgery.

It should be supported by radiological imaging methods. The use of 3D imaging techniques can be useful in detecting different types of skulls according to sex. Large-scale studies are required in different populations to identify the underlying clinical syndromes.

Conclusion

It can be said that the brachiocephalic type is more frequent type in Turkish adult dry skulls. The differences in CI and type between the sexes may be indicative of sexual dimorphism. Skull types can be useful in demonstrating craniofacial abnormalities or clinical syndromes.

Ethics

Ethics Committee Approval: It was approved by the Ethics Committee of Akdeniz University on 26 August 2020 with protocol number 597.

Informed Consent: Informed consent is not required.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: E.Ö., Ö.G., Concept: E.Ö., Ö.G., F.B.Y., Design: E.Ö., Ö.G., F.B.Y., Data Collection or Processing: E.Ö., Ö.G., E.S., Analysis or Interpretation: E.Ö., Ö.G., Literature Search: E.Ö., Ö.G., E.S., Writing: E.Ö., Ö.G., F.B.Y., E.S.

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Screening TSH in Obstructive Sleep Apnea Syndrome: Null or Beneficial?

Tıkayıcı Uyku Apne Sendromunda TSH Taraması: Faydasız ya da Faydalı?

Utku Oğan Akyıldız[1], Aslı Akyol[2]

[1]Aydın Adnan Menderes University Faculty of Medicine, Department of Neurology, Aydın, Turkey
[2]Gazi University Faculty of Medicine, Department of Neurology, Division of Clinical Neurophysiology, Ankara, Turkey

Abstract

Objective: The link between obstructive sleep apnea syndrome (OSAS) and hypothyroidism, which is associated with mucopolysaccharide infiltration and swelling of the soft tissues of the upper airway, is known for years. However, reports regarding the frequency of hypothyroidism in OSAS, as well as the requirement of screening thyroid stimulating hormone (TSH), have inconsistent results. This study aims to reveal the actual frequency of hypothyroidism among individuals with complaints suggestive of OSAS and provide a rationale for whether or not to screen.

Materials and Methods: Two hundred eighty-two patients evaluated due to complaints suggesting OSAS and underwent overnight diagnostic polysomnography were retrospectively reviewed. Demographic, clinical, and polysomnographic parameters and serum TSH levels were analyzed and compared between OSAS patients and those with a normal apnea-hypopnea index (AHI). A multiple linear regression model was used to adjust for potential confounders. A two-tailed p-value of <0.05 was accepted as statistically significant.

Results: No patient from the entire study population was diagnosed with overt hypothyroidism. Pairwise comparisons between OSAS patients and non-OSAS controls revealed similar results in terms of TSH levels (1.62 mIU/L vs 1.44 mIU/L, p=0.258) and subclinical hypothyroidism frequency (3.2% vs 3.4%, p=0.934). There was no association between the TSH levels and AHI values.

Conclusion: According to these results, a TSH screen does not seem to be a mandatory part of routine workups in patients with sleep-disordered breathing symptoms for uncovering clinical hypothyroidism. Limiting TSH screening mainly to patients with marked symptoms regarding hypothyroidism would be appropriate in this population.

Keywords: Obstructive sleep apnea syndrome, clinical hypothyroidism, subclinical hypothyroidism, TSH

Öz

Amaç: Tıkayıcı uyku apne sendromu (TUAS) ve hipotiroidizm arasında yıllardan beri iyi tanımlanmış bir ilişki mevcuttur. Hipotiroidizmin üst solunum yollarını da içine alan yumuşak dokuda diffüz mukopolisakkarid birikimi üzerinden TUAS patofizyolojisine katkıda bulunduğu bilinmektedir. Bununla birlikte TUAS'de hipotiroidizmin sıklığı ve dolayısıyla da tiroid stimule edici hormon (TSH) taramasının gerekliliğini değerlendiren çalışmalarda çelişkili sonuçlar olduğu görülmektedir. Bu çalışmada amaç, TUAS şüphesiyle değerlendirilen hastalarda hipotiroidizmin gerçek sıklığını belirlemek ve bu popülasyonda TSH taramasının gerekli olup olmadığını ortaya koymaktır.

Gereç ve Yöntemler: TUAS şüphesi ile değerlendirilerek bir gecelik tanısal polisomnografiye tabi tutulan 282 hastanın kayıtları geriye dönük incelendi. Olguların klinik, demografik, polisomnografik bulguları ve TSH düzeyleri kaydedilerek OSAS saptanan ve saptanmayan grupta karşılaştırıldı. TSH düzeyleriyle ilişkisi olabilecek potansiyel değişkenler için çoklu hiyerarşik regresyon modeli uygulandı. P<0,05 istatistiksel olarak anlamlı kabul edildi.

Bulgular: Çalışma popülasyonundaki hiçbir olguda klinik hipotiroidizm saptanmadı. TUAS saptanan ve saptanmayan grupta TSH düzeyleri (1,62 mIU/L vs 1,44 mIU/L, p=0,258) ve subklinik hipotiroidizm oranlarının (%3,2 vs %3,4, p=0,934) benzer olduğu görüldü. TSH değerleri ile apne-hipopne indeksi arasında bir ilişki saptanmadı.

Address for Correspondence/Yazışma Adresi: Lect. Utku Oğan Akyıldız MD, Aydın Adnan Menderes University Faculty of Medicine, Department of Neurology, Aydın, Turkey
Phone: +90 533 654 15 69 **E-mail:** uakyildiz@adu.edu.tr
ORCID ID: orcid.org/0000-0001-6452-0492

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Sonuç: Mevcut çalışmada elde edilen bulgular, uyku ilişkili solunum bozukluğu şüphesi ile değerlendirilen olgularda rutin TSH taramasının klinik hipotiroidizmi saptamak yönünden ek bir katkı sağlamadığını göstermektedir. Bu popülasyonda TSH taramasının, hipotiroidizmle ilişkili belirgin semptomlar tanımlayan olgularda tercih edilmesi uygun görünmektedir.

Anahtar Kelimeler: Tıkayıcı uyku apne sendromu, klinik hipotiroidizm, subklinik hipotiroidizm, TSH

Introduction

Obstructive sleep apnea syndrome (OSAS) is basically characterized by partial and/or complete obstructions of the upper airway - frequently in association with intermittent hypoxia and arousals- during sleep and often presented with the typical clinical triad of snoring, excessive daytime sleepiness and witnessed apnea. It is a prevalent disorder among public which results in a number of cardiovascular and metabolic consequences and the common comorbidities include hypertension, obesity/metabolic syndrome, diabetes mellitus, coronary artery disease, cardiac arrhythmias, cerebrovascular diseases, pulmonary hypertension, cor pulmonale and etc. (1,2). The estimated prevalence approximates 2-5% among women and 3-7% among men which necessitates early diagnosis and identification of the population at high risk for screening (3). Hypothyroidism, which is another common disorder with prevalence rates of 3.7-5%, is accounted as a predisposing factor for years because of its undesirable contributions to the collapsibility of upper airway (4,5). Nevertheless, studies evaluating the frequency of hypothyroidism in OSAS state conflicting results. Although average rates between 0.7-3.4% have been reported in large cohorts; some researchers described a frequency up to 11.5% among 78 obese/overweight individuals referred to a sleep clinic (6,7). Beyond that, the situation gets more complicated while talking about subclinical hypothyroidism (SH) which have prevalence rates of 4.3-5% approximately (8,9). Since relevant data regarding the predisposing role of SH in OSAS is divergent and the disorder represents an asymptomatic state as in the name; the need for screening or potential benefits of documentation are either not clear.

The objective of this retrospective study is to reveal the actual frequency of hypothyroidism among individuals with complaints suggestive of OSAS and provide a rationale for whether or not to screen.

Materials and Methods

Medical charts of patients who were evaluated with at least one of the complaints including snoring, excessive daytime sleepiness, and witnessed apnea, and who underwent overnight polysomnography in the Sleep Laboratory of a single center between July 2017 and February 2019 were retrospectively reviewed. Demographic parameters (age and sex), anthropometric measures [body mass index (BMI)], scores of the Epworth sleepiness scale (ESS), polysomnographic indices, and laboratory data regarding serum thyroid stimulating hormone (TSH) levels were recorded. This retrospective study was approved by the Ethics Committee of Aydın Adnan Menderes University (protocol no: 2019/50, date: 21.03.2019).

Patients with a diagnosis of overt hypothyroidism before admission [previous TSH levels of >10 mIU/L (10), and/or receiving levothyroxine -LT4- replacement therapy], patients that use any medication that may influence TSH levels like lithium, dopamine agonists, amiodarone, etc., and patients without available laboratory data were excluded.

Nox Medical Programme was used for polysomnographic data acquisition and analysis. The recordings included electrooculogram, six-channel electroencephalogram, electrocardiogram, chin and leg electromyogram, oronasal thermal and nasal airflow, pulse oximetry, thoracic and abdominal respiratory effort and body position. The polysomnographic data were scored manually following the rules of AASM 2014 v2.4 guidelines (11). The severity of OSA was graded depending on the apnea-hypopnea index (AHI). An AHI value between ≥5 and <15 events/hour was categorized as mild OSAS; while the values between ≥15 and <30 events/hour were categorized as moderate and ≥30 events/hour as severe OSAS (12). Individuals with an AHI value of <5 events/hour did serve as the non-OSAS control group.

During the initial visit to the outpatient clinic, a complete physical and neurological examination was performed in addition to respiratory function tests, and serum TSH levels were either measured on the same morning after an overnight fast via the automatic chemiluminescence method. Overt/clinical hypothyroidism was diagnosed in the case of a newly detected TSH level >10 mIU/L as mentioned above (10).

An elevated TSH level indicative of SH was defined as >4.0 mIU/L and <10 mIU/L for this study, since 90% of patients with SH are known to have TSH levels between 4 and 10 mIU/L (13,14), and the usual goal of replacement therapy with levothyroxine in overt hypothyroidism is to achieve a range between 0.4-4.0 mIU/L regarding optimal TSH values (5).

Statistical Analysis

IBM.SPSS.20 package program (SPSS, Inc., Chicago, Illinois) was used for statistical analysis. The Shapiro-Wilk test was performed to assess the normality of numeric data. Continuous variables were presented as mean ± standard deviation or median (minimum-maximum) according to the normality. Categorical variables were presented as percentages and analyzed by the chi-square test. For pairwise comparisons, Independent samples t or Mann-

Whitney U test was performed and when the number of groups is more than two, one-way ANOVA or Kruskal-Wallis variance analysis was used together with posthoc tests. A multiple linear regression model was used to adjust for potential confounders. A two-tailed p-value of <0.05 was accepted as statistically significant.

Results

Two hundred eighty-two patients were included in the study. Two hundred fifty-three patients with characteristic symptoms satisfied the diagnostic criteria for OSAS (AHI ≥5/hour), whereas the remaining 29 had normal AHI values (<5/hour) and served as the non-OSAS control group. The median TSH level in the non-OSAS control group was 1.44 mIU/L, whereas the corresponding value was 1.62 mIU/L in the OSAS group (p=0.258). Neither an individual out of the OSAS patient group nor non-OSAS controls had a current TSH value of >10mIU/L compatible with overt hypothyroidism.

12% of OSAS (n=30) patients were diagnosed with mild disease, while 27% (n=68) had moderate and 61% (n=155) had severe disease. Age, sex, ESS scores and TSH values were similar among three grades of disease regarding OSAS patients, however severe OSAS group had higher BMI values when compared to the patients with mild and moderate disease (p<0.001*). In a similar manner, both sleep and hypoxia related polysomnographic indices demonstrated significant difference against severe disease group (Table 1). No significant correlation was observed between AHI values and TSH levels among OSAS patients (r=0.045, p=0.477).

Pairwise comparisons between OSAS patients and non-OSAS controls revealed similar results in terms of male rate, ESS score and TSH levels (p>0.05). However median age was younger (43.5 yrs vs 52 yrs, p<0.001) and median BMI was lower among non-OSAS controls. (30,45 kg/m^2 vs 32 kg/m^2, p=0.012) (Table 2).

The frequency of SH was 3.2% among OSAS patients (n=8), whereas the relative rate was 3.4% for the non-OSAS control group (n=1) (p=0.931) (Figure 1).

A multiple stepwise regression analysis revealed that TSH level was significantly associated with age and BMI. However, a similar association was not true for AHI (Table 3).

Discussion

In the current study, our results demonstrated that screening a considerable number of individuals with complaints suggesting OSAS failed to detect any case of overt hypothyroidism. Besides, no difference was established between OSAS patients and non-OSAS controls in terms of TSH levels or rates of SH.

The link between hypothyroidism and OSA syndrome, which arises from the narrowing of the upper airway, has been identified clearly for years, and hypothyroidism seems to act through several mechanisms during this pivotal process. Mucopolysaccharide infiltration of anatomical

Table 1. Baseline characteristics and polysomnographic findings in OSAS patients

	Mild OSAS (n=30)	Moderate OSAS (n=68)	Severe OSAS (n=155)	p-value
Male rate (%)	56.7% (n=17)	73.5% (n=50)	74.2% (n=115)	0.139
Age [yrs. (range)]	52.5 (18-76)	46 (22-80)	53 (24-81)	0.173
BMI [kg/m^2 range)]	28.85 (21.4-37.3)	30.05 (20.8-45.7)	34 (19-68.7)*	<0.001*
ESS	4 (0-13)	4 (0-21)	6 (0-19)	0.116
TSH levels (mIU/L)	1.27 (0.64-6.07)	1.81 (0.23-3.53)	1.88 (0.03-6.89)	0.537
Polysomnography - sleep parameters				
TST (min)	375 (281.5-446)	368 (177.5-477.1)	332 (97.5-448)*	0.001*
Sleep eff. (%)	83.3 (59.9-95.7)	80.7 (41.3-95)	73.4 (26.8-95)*	<0.001*
N3 sleep (%)	16.8 (6.3-31.6)	17.6 (1.1-41.5)	8.3 (0-37.1)*	<0.001*
Polysomnography - respiratory parameters				
AHI (events/hour)	9.55 (5.3-14.4)	21.48 (15.2-29.8)	59.2 (30.1-11.64)	<0.001*
ODI	9.1 (0.8-44.3)	21.95 (5.3-47.3)	66.6 (17.6-141.5)*	<0.001*
spO_2<90% dur (%)	1.45 (0-41.6)	1.45 (0-83.5)	16.7 (0-100)*	<0.001*
Average spO_2	93.15 (89.5-96.6)	93.1 (87.9-96.7)	91.5 (61.5-96.1)*	<0.001*

OSAS: Obstructive sleep apnea syndrome, BMI: Body mass index, ESS: Eppworth Sleepiness Scale, TSH: Thyroid stimulating hormone, TST: Total sleep time, Sleep eff: Sleep efficiency, AHI: Apnea-hypopnea index, ODI: Oxygen desaturation index, spO_2<90% dur: Time spent <90% spO_2. P<0.05 denotes statistical significance

structures throughout the upper airway and swelling of these soft tissues, as a result, presence of goiter, impaired function of upper airway dilator muscle due to neuropathy and increased weight as a consequence of expansion in water compartment, or obesity itself could all contribute to narrowing of the upper airway, which becomes more evident during sleep (15). Also, severe hypothyroidism is known to be associated with a negative influence on hypoxic and hypercapnic responses of chemosensors, thus respiratory drive. The diaphragm was demonstrated to be weak in hypothyroid patients which give rise to decreased lung volumes and this also deteriorates the clinical picture of sleep apnea (15,16). Replacement therapy with LT4 has been demonstrated to reverse sleep-disordered breathing in some studies; in some of which the selection bias could not be completely excluded (16-18). However, management of overt hypothyroidism with proper replacement therapy in the presence of comorbid OSAS seems essential. On the other side, the co-existence of these two disorders in an individual appears to be incidental since both have a high prevalence among the general population (16). In a study cohort of 418 individuals, Bahammam et al. (19) revealed no difference regarding clinical hypothyroidism between OSA patients and non-OSAS controls (0.4% vs 1.4%). Among 203 patients, who underwent polysomnography due to sleep-disordered breathing symptoms, Ozcan et al. (20) defined an overt hypothyroidism rate of 1.9%, which was not higher than the general population. Mete et al. (21) detected clinical hypothyroidism in 4% of their OSAS patients, whereas the related rate was 6.2% for non-OSAS controls.

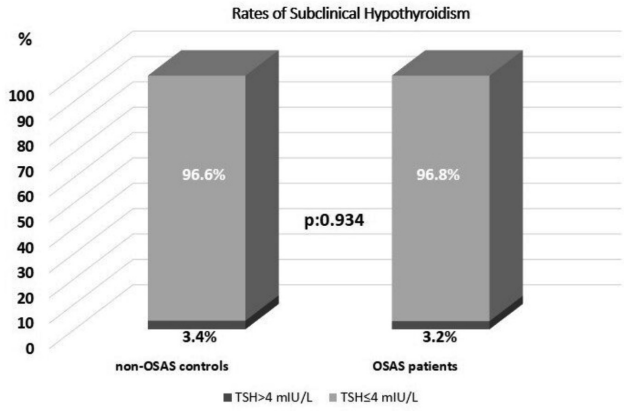

Figure 1. Rates of subclinical hypothyroidism among OSAS patients and non-OSAS controls

OSAS: Obstructive sleep apnea syndrome, TSH: Thyroid stimulating hormone

In the context of SH, the results are contradictory though. Despite the proportion of cases with SH being 11.1% and 10.8% respectively for the first two of the abovementioned studies (19,20); Mete et al. (21) reported a 4.7% rate of SH for OSAS, whereas the corresponding value was 6.2% for

Table 2. Baseline characteristics of study population

	Non-OSAS controls (n=29)	OSAS patients (n=253)	p-value
Male rate (%)	55.2%	71.9%	0.062
Age [yrs. (range)]	43 (18-61)	52 (18-81)*	<0.001*
BMI [kg/m^2 (range)]	30.4 (18.9-36.1)	32 (19-68.7)*	0.012*
ESS	6 (1-17)	5 (0-21)	0.534
TSH levels (mIU/L)	1.44 (0.26-4.32)	1.62 (0.03-6.89)	0.258

BMI: Body mass index, ESS: Eppworth sleepiness scale, OSAS: Obstructive sleep apnea syndrome, TSH: Thyroid stimulating hormone. P<0.05 denotes statistical significance

Table 3. Coefficients of stepwise multiple linear regression analysis for TSH

	TSH											
Variables	Model 1				Model 2				Model 3			
	B	Sh	β	t	B	Sh	β	t	B	Sh	β	t
Age	-0.012	0.005	-0.152	-2.556*	-0.012	0.005	-0.152	-2.556*	-0.013	0.005	-0.158	-2.643**
BMI					0.022	0.009	0.140	2.379*	0.020	0.011	0.123	1.860
AHI									0.001	0.003	0.038	0.566
Adjusted R2	0.019				0.036				0.033			
F change	6.535*				5.662*				0.320			
F	6.535*				6.153**				4.199**			

TSH: Thyroid stimulating hormone, BMI: Body mass index, AHI: Apnea-hypopnea index
*Correlation is significant at the 0.05 level. **Correlation is significant at the 0.01 level (two-tailed)

non-OSAS controls in their cohort. Similarly, Kapur et al. (22) identified SH in only 4 out of their 284 patients (1.4%), which was not higher than the general population either.

In our cohort, none of the patients from either the OSAS or non-OSAS group was newly diagnosed with clinical hypothyroidism, and SH rates were similar between the two. SH is known to attribute to an abnormal laboratory finding in the absence of clinical symptoms. Although overt hypothyroidism has been linked to OSA pathophysiology through several pathways, accumulating data has not verified the majority of these interactions for SH. Moreover, LT4 replacement did not demonstrate any improvement regarding respiratory disturbance in OSAS patients with SH (14). Hence there is not enough evidence yet, for screening SH in an asymptomatic population.

Our results support the restriction of TSH screening mainly to patients with marked symptoms. Since the rate of SH rather than overt form is evident in epidemiologic data regarding OSAS, and patients with SH have been demonstrated not to benefit from LT4 treatment in terms of respiratory disturbance, screening TSH does not seem to be beneficial in patients with a suspected diagnosis of OSAS.

Study Limitations

Our study has some limitations due to its retrospective design and absence of follow-up data. The magnitude of patients and controls, as well as the number of patients in each group regarding disease severity, was different in the current study, which was in favor of the severe group. Although this can be considered a disadvantage, it is also compatible with the accumulating epidemiological data in the literature. In comparison to mild and moderate patients, severe OSA patients comprise the majority of cases examined with sleep-disordered breathing complaints in sleep clinics (23,24). Also, the accepted cut-off value of TSH in our study might result in the underdiagnosis of some SH cases since recent reports by some experts suggest more strict upper limits for TSH (2.5-3 mIU/L) (25).

Conclusion

However, lowering the TSH cut-off would probably not influence the current results, as the subsequent increase in diagnosis would mainly include SH cases rather than overt hypothyroid ones, who already would not undergo treatment with LT4 replacement that rationalize TSH screening.

Ethics

Ethics Committee Approval: This retrospective study was approved by the Ethics Committee of Aydın Adnan Menderes University (protocol no: 2019/50, date: 21.03.2019).

Informed Consent: Retrospective study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

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

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Oxyhemoglobin Dissociation Curve in COVID-19 Patients

COVID-19 Hastalarında Oksihemoglobin Disosiasyon Eğrisi

Hilal Üstündağ[1], Cuma Mertoğlu[2], Mehmet Tahir Huyut[3]

[1]Erzincan Binali Yıldırım University Faculty of Medicine, Department of Physiology, Erzincan, Turkey
[2]İnönü University Faculty of Medicine, Department of Biochemistry, Malatya, Turkey
[3]Erzincan Binali Yıldırım University Faculty of Medicine, Department of Biostatistics, Erzincan, Turkey

Abstract

Objective: Coronavirus disease-2019 (COVID-19) is a disease that can progress with hypoxemia and severe respiratory distress in some patients. The oxyhemoglobin dissociation curve (ODC) is critical to understanding the effects of O2 exchange. This study aimed to evaluate the relationship between the ODC and oxygen-carrying capacity of hemoglobin (Hb) in COVID-19 patients.

Materials and Methods: In the study, ODCs were created by examining the data obtained from the arterial blood gas analyses of 686 intensive care unit (ICU) and non-ICU COVID-19 patients retrospectively.

Results: It was concluded that patients with COVID-19 and other respiratory distress patients had a slight right-leaning trend in the ODC compared with the standard curve. The P50 value of the ICU group was higher than the other groups (mean: 30.74 mmHg, n=131, p=0.047). While the percentage of oxyhemoglobin (mean: 65.44% vs 69.81%, p=0.015), the amount of glucose (mean: 163.39 mg/dL vs 195.36 mg/dL, p=0.002) and pH (median: 7.38 vs 7.41, p=0.007) in the non-ICU group was higher compared with the control group, the carboxyhemoglobin percentage (mean: 1.66% vs 1.13%, p=0.000), PCO_2 (42.02 mmHg vs 39.44 mmHg, p=0.015), potassium (mean: 4.33 mmol/L vs 4.04, p=0.026), and sodium (mean: 138.10 mmol/L vs 135.80 mmol/L, p=0.000) were lower. The methemoglobin percentage of the ICU group was lower (p=0.000) than the other groups.

Conclusion: The ODC of COVID-19 and other respiratory distress patients shifts slightly to the right, indicating that patients have partial respiratory difficulties.

Keywords: COVID-19, hematological parameters, oxygen affinity, SARS-CoV-2

Öz

Amaç: Koronavirüs hastalığı-2019 (COVID-19), hipoksemi ve bazı hastalarda ciddi derecede solunum sıkıntısı ile seyredebilen bir hastalıktır. Oksihemoglobin disosiasyon eğrisi (ODC), O2 değişiminin etkilerini anlamak için çok önemlidir. Bu çalışmanın amacı; COVID-19 hastalarında ODC ve hemoglobin (Hb) arasındaki oksijen taşıma kapasitesi ilişkisini değerlendirmektir.

Gereç ve Yöntemler: Çalışmada, yoğun bakım ünitesi (YBÜ) COVID-19, yoğun bakım dışı (non-YBÜ) COVID-19 ve COVID-19 olmayan diğer solunum sıkıntılı toplam 686 hastanın arter kan gazından elde edilen veriler retrospektif olarak incelenerek oksihemoglobin eğrileri oluşturuldu.

Bulgular: COVID-19 tanılı ve diğer solunum sıkıntılı hastaların ODC'lerinin standart eğriye göre hafif sağa eğilim gösterdiği belirlendi. YBÜ grubunun P50 değeri, diğer gruplara kıyasla daha yüksekti (ortalama: 30,74 mmHg, n=131, p=0,047). Kontrole kıyasla non-YBÜ grubunun; oksihemoglobin yüzdesi (ortalama: %65,44 vs %69,81, p=0,015), PO_2'ı (46,98 mmHg vs 48,98 mmHg, p=0,001), glikoz miktarı (ortalama: 163,39 mg/dL vs 195,36 mg/dL, p=0,002) ve pH'sı (medyan: 7,38 vs 7,41, p=0,007) daha yüksek iken karboksihemoglobin yüzdesi (ortalama: %1,66 vs %1,13, p=0,000), PCO_2'ı (42,02 mmHg vs 39,44 mmHg, p=0,015), potasyum (ortalama: 4,33 mmol/L vs 4,04, p=0,026) ve sodyum (ortalama: 138,10 mmol/L vs 135,80 mmol/L, p=0,000) seviyesi daha düşüktü. YBÜ grubunun methemoglobin yüzdesi ise diğer gruplara kıyasla daha düşüktü (p=0,000).

Sonuç: COVID-19 ve diğer solunum sıkıntılı hastaların ODC'si hafif sağa kaymaktadır, bu sonuç hastaların kısmen solunum güçlüğü çektiğini göstermektedir.

Anahtar Kelimeler: COVID-19, hematolojik parametreler, oksijen afinitesi, SARS-CoV-2

Address for Correspondence/Yazışma Adresi: Lect. Hilal Üstündağ MD, Erzincan Binali Yıldırım University Faculty of Medicine, Department of Physiology, Erzincan, Turkey
Phone: +90 541 969 98 47 **E-mail:** hurcar@gmail.com
ORCID ID: orcid.org/0000-0003-3140-0755

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Introduction

Coronaviruses form a large family of viruses that can cause diseases in humans and animals (1). The coronavirus disease-2019 (COVID-19) pandemic, from severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), emerged in 2019 and spread globally. It's primarily transmitted through droplets and contact with mucous membranes after exposure to infected surfaces (2). COVID-19 symptoms are nonspecific and can't be reliably distinguished from other viral respiratory infections. Initial patients had fever (98%), cough (76%), fatigue/myalgia (44%), sputum (28%), headache (8%), hemoptysis (5%), diarrhea (3%), and half reported shortness of breath (2).

SARS-CoV-2's damage mechanism to cells, tissues, and organs is unclear. COVID-19 patients exhibit severe atypical respiratory distress with hypoxemia, preceding other symptoms like radiological changes and dyspnea (3). Hypoxemia is critical in COVID-19, causing organ failure and death (4). The virus enters cells via ACE2 receptors, found in alveolar epithelial and vascular endothelial cells, triggering a strong immune response and widespread endothelial dysfunction (5).

Hemoglobin (Hb) is a heterotetramer with two alpha and two beta chains, an iron ion, and a porphyrin ring, essential for oxygen transport in vertebrates. About 97% of oxygen is transported from lungs to tissues by Hb in erythrocytes, while 3% dissolves in plasma and blood cells. Oxygen binds to Hb at high partial pressure (PO_2) in lungs and releases at low PO_2 in tissues due to consumption (6,7) Hb tetramer structure changes impact oxygen affinity and tissue oxygenation. Oxygen affinity relates to PO_2 and can be read from the oxyhemoglobin dissociation curve (ODC), with P50 representing 50% Hb oxygen saturation. Hb's molecular cooperation results in ODC's sigmoid shape. ODC shifts left or right in clinical situations. A right shift decreases oxygen affinity, improving tissue oxygenation; a left shift does the opposite. Decreased affinity raises P50, increasing tissue oxygenation. Factors like 2,3-DPG, pH, and temperature affect Hbs oxygen affinity (6-8).

With COVID-19 affecting over 22 million globally, theories explore the pathophysiology. One suggests that SARS-CoV-2 proteins interact with human Hb, facilitating iron removal, leading to functional Hb loss and iron accumulation (9).

Understanding respiration and gas exchange principles is key for diagnosing and treating respiratory illnesses. Some diseases stem from poor ventilation, membrane diffusion disorders, or gas transport issues (6). Arterial blood gas analysis assesses lung function and oxygenation, providing crucial information on patient's respiratory and metabolic status to guide treatment decisions (6,10,11)

The aim of this study was to determine whether there is a direct interaction between the viral proteins that cause COVID-19 and Hb that may lead to loss of oxygen carrying capacity in the oxygen Hb dissociation curve obtained from the artery blood gas.

Materials and Methods

Study Design

In the study, lab data of patients admitted to Erzincan Binali Yıldırım University Mengücek Research Hospital's intensive care unit (ICU) between May 2020 and February 2021, diagnosed with COVID-19 (polymerase chain reaction positive for SARS-CoV-2), non-ICU, and non-COVID-19 respiratory distress (control) were retrospectively examined. The study was approved by the Ethics Committee for Clinical Research at Erzincan University Faculty of Medicine (decision no: 05/05, date: 22.03.2020). Patients' demographic information and arterial blood gas values were recorded. Arterial blood gas samples were analyzed using ABL 700 (Radiometer, Copenhagen, Denmark).

Statistical Analysis

Descriptive statistics for patient and control groups' demographic and laboratory findings were presented. Quantitative variables were defined by average, median, interquartile range, and standard deviation; categorical variables as frequency and percentage. Categorical variables were analyzed using the χ^2 test. Shapiro-Wilk test checked normality hypothesis for quantitative variables between groups. Levene test was used to hypothesize variances' homogeneity. Parameters meeting parametric test assumptions were analyzed with one-way ANOVA, while Kruskal-Wallis analyzed those without. Tukey and Dunnett post-hoc tests determined significant changes' sources. Differences between groups were denoted by symbols, with different symbols signifying significant differences. Box-Plot charts summarized deterministic statistical characteristics, distribution, and parameter differences by groups. SPSS (version 20.0) was used for data analysis, with a p-value <0.05 considered significant.

Results

The study involved 343 COVID-19 patients and 343 control patients with different respiratory etiologies without COVID-19. O_2-Hb dissociation curves were generated using COVID-19 patient data and control group data, then compared (12). Table 1 shows average ages of ICU, non-ICU, and control groups as 71.51, 68.79, and 66.00 respectively; gender distributions were 80 males/51 females, 122 males/90 females, and 209 males/139 females. In ODC evaluations (Figure 1), non-ICU and control group curves without COVID-19 were similar; ICU group's ODC slightly tilted right, and all groups trended right compared to the standard curve. ICU group's P50 value was higher (mean: 30.74 mmHg, n=131, p=0.047). Non-ICU group had higher oxyhemoglobin percentage (mean: 65.44% vs 69.81%, p=0.015), PO_2 (46.98 mmHg vs. 48.98 mmHg, p=0.001), glucose (mean: 163.39 mg/dL vs 195.36 mg/dL, p=0.002),

Table 1. Summary of results from patients with ICU COVID-19, non-ICU COVID-19 and control group without COVID-19																
	Non-ICU					ICU					Control					p-value
Sex																
Male N (%)	122 (57.5)					80 (61.1)					209 (60.9)					0.699
Female N (%)	90 (42.5)					51 (38.9)					134 (39.1)					
Parametreler	**Mean**	**Median**	**SD**	**Min.**	**Max.**	**Mean**	**Median**	**SD**	**Min.**	**Max.**	**Mean**	**Median**	**SD**	**Min.**	**Max.**	
Age	68.79*	71.00	14.47	20.00	96.00	71.51*	73.00	13.57	20.00	96.00	62.31#	66.00	20.07	19.00	102.00	**0.000**
P50 (mmHg)	29.25#	28.79	3.71	21.28	53.74	30.74*	29.66	5.86	21.74	74.39	29.38*#	28.52	6.67	20.65	110.98	**0.047**
Bicarbonate plasma (mEq/L)	24.18	24.25	4.19	10.30	37.70	24.48	23.80	5.84	11.60	51.00	23.66	23.90	4.42	5.30	42.00	0.252
Bilirubin (mg/dL)	1.60	1.45	1.06	0.00	8.00	2.43	1.50	4.04	0.00	33.00	1.57	1.50	0.93	0.10	7.00	0.838
Deoxyhemoglobin (%)	27.67	21.40	20.73	1.20	92.00	28.62	25.70	22.63	0.50	83.20	31.51	28.30	20.63	0.70	94.30	0.107
Glucoce (mg/dL)	195.36*	147.50	123.35	40.00	695.00	185.44	158.00*#	104.38	64.00	879.00	163.39#	131.00	96.39	55.00	743.00	**0.002**
HCO_3 (mEq/L)	24.46	24.35	3.92	15.50	33.60	23.84	25.05	6.32	7.40	43.50	24.00	23.90	3.81	13.20	38.70	0.771
Hematocrit (%)	42.48	41.95	12.41	0.90	82.60	40.35	40.40	11.78	5.50	73.90	42.69	43.00	10.25	10.10	78.60	0.118
Hemoglobin (g/dL)	13.85	13.70	4.10	0.01	27.20	13.06	12.90	3.91	4.00	24.10	13.94	14.10	3.40	3.10	25.70	0.086
Carboxyhemoglobin (%)	1.13#	1.00	0.61	-0.30	4.80	1.02#	1.00	0.52	-0.30	2.90	1.66*	1.30	1.45	-0.40	11.40	**0.000**
Chlorine (mmol/L)	106.26	106.00	6.85	74.00	136.00	107.35	107.00	9.65	86.00	145.00	106.09	106.00	8.58	2.60	142.00	0.367
Lactate (mmol/L)	2.09	1.80	1.21	0.10	6.90	2.23	1.80	1.52	0.50	9.30	2.07	1.70	1.35	0.00	13.70	0.506
Methemoglobin (%)	1.36*	1.30	.45	-1.10	3.30	1.20#	1.20	0.49	0.10	3.20	1.42*	1.40	0.44	-1.40	4.60	**0.000**
Oxyhemoglobin (%)	69.81*	75.70	20.60	5.70	96.50	69.20#*	72.15	22.54	15.40	96.80	65.44#	68.10	20.52	4.00	97.10	**0.015**
Oxygen saturation (%)	71.73	78.20	21.16	5.80	98.80	70.76	73.75	23.06	15.60	99.50	67.53	70.90	21.15	4.10	99.30	0.076
Osmalarite (mOsmol/L)	283.36	283.60	11.61	233.50	340.90	286.14	283.65	18.28	243.20	353.50	285.24	285.65	11.70	237.60	359.30	0.147
pH (7.35-7.45)	7.407*	7.415	0.079	7.019	7.557	7.390#*	7.408	0.101	7.047	7.590	7.384#	7.383	0.080	6.819	7.649	**0.007**
Potassium (mmol/L)	4.04#	3.90	1.37	2.30	16.80	4.14#*	4.00	0.86	2.40	7.00	4.33*	4.10	1.55	2.40	20.10	**0.026**
Sodium (mmol/L)	135.80#	136.00	5.92	111.00	164.00	137.61#*	137.00	8.11	117.00	171.00	138.10*	139.00	5.51	113.00	163.00	**0.000**
Standard base (±3 mmol/L)	0.12	0.25	5.10	-17.50	18.70	0.17	0.20	6.62	-18.80	22.80	0.03	0.30	5.71	-24.50	36.00	0.970
Total O_2 (mEq/L)	13.80#	13.80	5.19	2.30	30.10	12.47*	12.10	5.40	2.70	25.90	12.71#*	12.80	4.98	0.90	30.50	**0.033**
pCO_2 (mmHg)	39.44*	39.00	9.78	12.20	113.00	41.78#*	39.70	12.92	12.70	101.00	42.02#	41.90	9.79	5.40	89.30	**0.015**
pO_2 (mmHg)	48.98#*	44.05	24.26	11.00	177.00	56.86#	45.80	36.30	16.00	295.00	46.98*	40.00	31.08	5.70	281.00	**0.001**
Ionized calcium (mg/dL)	1.15*	1.16	0.12	0.58	1.86	1.10#	1.10	0.10	0.82	1.47	1.15*	1.15	0.12	0.69	1.96	**0.000**

The values in Table 1 are presented as mean and standard deviation (SD). Differences between groups with the same symbol were not statistically significant, while differences between groups with different symbols were found to be significant at a p-value of less than 0.05. ICU: Intensive care unit, COVID-19: Coronavirus disease-2019, SD: Standard deviation, min-max: Minimum-maximum

and pH (median: 7.38 vs 7.41, p=0.007) than the control group, but lower carboxyhemoglobin percentage (mean: 1.66% vs 1.13%, p=0.000), PCO_2 (42.02 mmHg vs 39.44 mmHg, p=0.015), potassium (mean: 4.33 mmol/L vs 4.04, p=0.026), and sodium (mean: 138.10 mmol/L vs 135.80 mmol/L, p=0.000). ICU group had lower methemoglobin percentage (p=0.000).

ICU and non-ICU patients had similar deoxyhemoglobin, Hb, hematocrit, total bilirubin, bicarbonate plasma, chlorine, lactate, and osmolarity levels compared to control patients (no significant differences, Table 1, Figure 2).

Discussion

The ODC, which connects oxygen saturation (SO_2) and PO_2 in blood, is crucial for understanding blood's oxygen transport and release (13). P50 measures Hb's oxygen affinity, determining oxygen release from microcirculation to tissues. An increased P50 (rightward ODC shift) indicates reduced Hb-oxygen binding affinity, promoting oxygen release into tissues (7). Normal Hbs P50 is around 26 mmHg at 40 mmHg PCO_2 pressure (14).

In this study, examining arterial blood gas samples, all groups had higher P50 values compared to the standard. ICU COVID-19 patients had a higher P50 value than non-ICU COVID-19 patients (mean: 29.25 mmHg, p=0.047), with a slight ODC right shift. This suggests ICU COVID-19 patients require greater tissue oxygenation due to low O_2-Hb affinity. While Severinghaus's standard curve (12) indicates systemic arterial blood oxygen saturation separating from lungs is around 95 mmHg, Figure 1 shows all three groups' dissociation curves averaging 98 percent systemic arterial blood oxygen saturation.

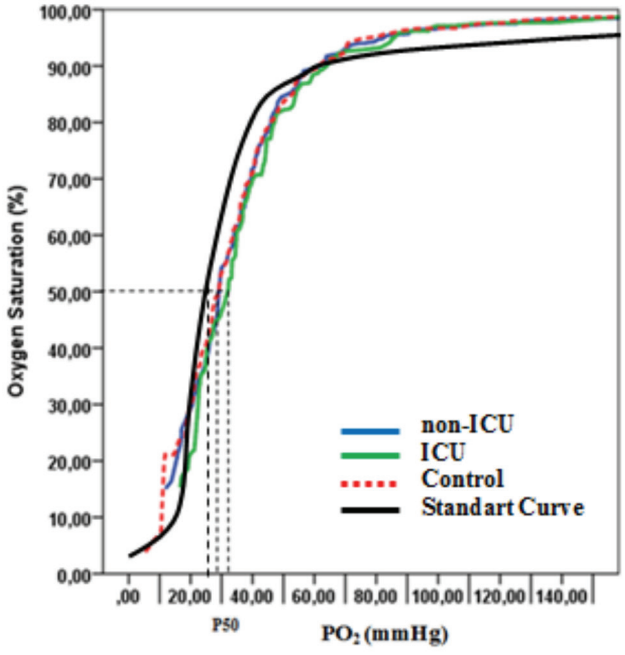

Figure 1. Comparative oxygen dissociation curves to the standard curve for all groups: the standard curve as described by JW Severinghaus (black curve), values of oxygenation saturation plotted versus PaO2 for patients admitted to the intensive care unit (ICU) with coronavirus disease-2019 (COVID-19) (green curve), and control patients without COVID-19 (control; red curve)

Daniel et al. (15) found no difference in Hb-O_2 affinity between 14 COVID-19 patients and 11 controls using an *in vitro* Hemox analyzer with standardized pH and temperature. P50 values were directly obtained from blood gas analyzers without adjustment for CO_2 or pH changes in COVID-19. They hypothesized that *in vivo* Hb-O_2 affinity could be affected by other factors in COVID-19. Vogel et al. (16) conducted a retrospective, observational study of blood gas analyses (n=3,518) from COVID-19 patients to investigate changes in Hb-O_2 affinity. They reported that this condition may play a role in adjusting to hypoxemia due to the lengthy disease process. Compared to patients with other causes of severe respiratory failure, COVID-19 patients had significantly higher Hb-O_2 affinity. Our findings show higher P50 and lower Hb-O_2 affinity in ICU COVID-19 patients. This may result from patients receiving oxygen support via ventilators, masks, or nasal cannulas. There are limited ODC-related studies in COVID-19 patients in the literature.

In critical illnesses, arterial blood gas (ABG) tests are vital for assessing lung function, diagnosis, and patient follow-up (17). Our study showed patients' pH values were within the normal range, allowing ODC evaluation at normal pH. All three groups displayed hypoxemia with ICU and non-ICU groups having higher PO_2 values than controls. Oxygen therapy may have increased arterial blood PO_2 in COVID-19 patients, which is a study limitation. No significant abnormalities were found in partial oxygen pressure against Hb oxygen saturation. All groups showed a slight right shift in ODC. Lower PCO_2 levels in non-ICU and ICU groups may be related to respiratory support therapy (18). Further *in vitro* and *in vivo* studies are needed to validate our hypothesis and understand the ODC mechanism during COVID-19 infection.

In our study which also evaluated the glucose, electrolyte, bicarbonate, bilirubin, Hb and hematocrit levels in the ABG analysis; had high blood glucose levels (mean: 195.36 mg/dL, 185.44 mg/dL, 163.39 mg/dL respectively) outside the normal range were detected in all patients in the ICU, non-ICU and control groups. This indicates that patients glucose metabolism is impaired. In the cross-group comparison of other data, there were similar levels of Hb, hematocrit, total bilirubin, bicarbonate plasma, chlorine, lactate and osmolarity.

Most coronavirus non-structural proteins are mainly found in infected cells, playing a key role in RNA replication (19). The virus is unlikely to access significant Hb, and there is no evidence of infiltration into red blood cells (20). Liu and Li (9) suggest interactions may occur after immune hemolysis, but some studies report no significant hemolysis in COVID-19 patients (21-23). Our study's clinical data

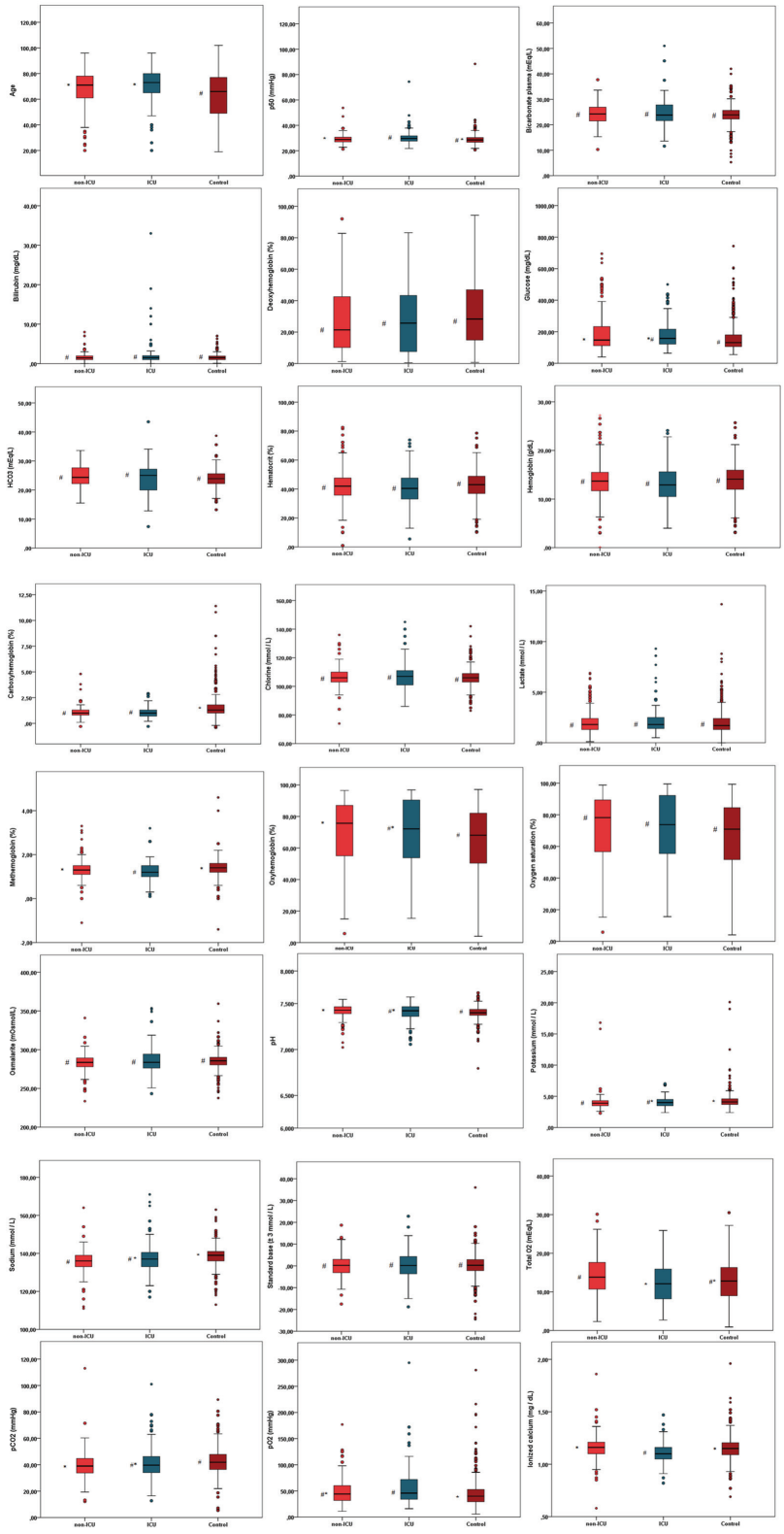

Figure 2. Corresponding laboratory values of age, P50, bicarbonate plasma, bilirubin, deoxyhemoglobin, glucose, HCO_3, hematocrit, hemoglobin, carboxyhemoglobin, chlorine, lactate, methemoglobin, oxyhemoglobin, oxygen saturation, osmolarity, pH, potassium, sodium, standard base, Total O_2, pCO_2, pO_2, and ionized calcium. The importance of the differences between the groups was indicated by the symbols. While the differences were found to be insignificant between groups with the same symbol, the differences between groups with different symbols were found to be significant

doesn't show significant hemolysis or abnormal Hb-oxygen decomposition. Similarly, another study evaluating ABG data of thirty COVID-19 patients found no significant clinical effect (24). Recent reports show similar mortality rates and mechanical ventilation needs for COVID-19 as other respiratory failure forms (3,25). Additionally, there's no evidence in the literature of significant anemia or excessive iron load caused by COVID-19 (21-23).

Our study has limitations due to small sample size, single-center, and retrospective design. The patient population receiving respiratory support and various medications in the service and intensive care unit may not fully reflect the impact of oxygen on Hb in COVID-19. The Control group was created from other patients with respiratory distress, limiting statistical significance. Therefore, larger sample sizes and *in vivo* and *in vitro* experimental studies are required for further verification.

Conclusion

The medical field and the global scientific community are making rapid strides in comprehending the underlying mechanisms of COVID-19 to effectively control its spread, provide proper care for patients, and ultimately discover definitive treatment options. In our study, which was carried out in order to contribute to the enlightenment of the physiological mechanism of the disease, it was concluded that patients diagnosed with COVID-19 and other respiratory distress patients were slightly right-leaning in the Hb-O_2 dissociation curve and had a higher percentage of oxygen saturation of arterial blood in all three groups.

Ethics

Ethics Committee Approval: The study was approved by the Ethics Committee for Clinical Research at Erzincan University Faculty of Medicine (decision no: 05/05, date: 22.03.2020).

Informed Consent: Retrospective study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: H.Ü., Design: H.Ü., C.M., Data Collection or Processing: C.M., M.T.H., Analysis or Interpretation: H.Ü., C.M., M.T.H., Literature Search: H.Ü., C.M., M.T.H., Writing: H.Ü., M.T.H.

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Knowledge and Practice Towards Family Planning Among Reproductive Age Women in Herat City - Afghanistan

Afganistan'ın Herat Şehrinde Üreme Çağındaki Kadınların Aile Planlaması Yöntemlerine Yönelik Bilgi ve Davranışları

Aziz-ur-Rahman Niazi[1], Khadejah Osmani[2], Nasar Ahmad Shayan[1,3], Filiz Abacıgil[4]

[1]Herat University Faculty of Medicine, Department of Public Health and Infectious Diseases, Herat, Afghanistan
[2]Herat University Faculty of Medicine, Department of Surgery, Herat, Afghanistan
[3]University of Western Ontario, Schulich School of Medicine and Dentistry, Department of Epidemiology and Biostatistics, London, Ontario, Canada
[4]Aydın Adnan Menderes University Faculty of Medicine, Department of Public Health, Aydın, Turkey

Abstract

Objective: Family planning, general fertility rate, and total fertility rate are the first three determinants of maternal health in Afghanistan. This study assessed knowledge, practice, and predictors of contraceptive use among women of reproductive age in Herat city of Afghanistan.

Materials and Methods: This descriptive study was conducted among 1,610 women who were selected by a convenient sampling technique. Multivariate logistic regression analyses were performed to identify factors related to the current use of any family planning methods among participants.

Results: The mean age of participants was 30.3±6.9 years, mean age of participants at first marriage was 17.6±3.6 years. The first three information sources about contraceptives were family members (35.5%), television (26.4%), and health staff (19.9%). The most common types of contraceptives (used by the participants) were combined oral contraceptives (18.1%), male condom (17.6%), and withdrawal methods (17.5%). Logistic regression analyses showed that adolescent age increased the risk by 2,159 [95% confidence interval (CI) (1,238-3,765); p=0.007], high parity (≥4) increased the risk by 1,525 [95% CI (1,162-2,002); p=0.002], husband who was unemployed increased the risk by 1.764 [95% CI (1,221-2,549); p=0.003], and lack of knowledge about family planning methods increased the risk by 11,674 [95% CI (7,067-19,285); p<0.001], in terms of not using any family planning methods.

Conclusion: We found that the use of any family planning method was low in risk groups. It is critical for health policymakers, healthcare workers, and family members to support and educate women, especially those in the risk group, about the benefit of family planning methods for their family and society.

Keywords: Family planning, reproductive health, contraceptive agents, Afghanistan

Öz

Amaç: Aile planlaması, genel doğurganlık hızı ve toplam doğurganlık hızı Afganistan'da anne sağlığının ilk üç belirleyicisidir. Bu çalışma, Afganistan'ın Herat şehrinde üreme çağındaki kadınların kontraseptif kullanımı ile ilgili bilgi, davranış ve belirleyicilerini değerlendirmiştir.

Gereç ve Yöntemler: Bu tanımlayıcı çalışma uygunluk örnekleme yöntemi ile seçilen 1.610 kadın üzerinde gerçekleştirilmiştir. Araştırmaya katılanların mevcut kullandığı aile planlaması yöntemleri ile ilişkili faktörleri belirlemek için çok değişkenli lojistik regresyon analizi yapılmıştır.

Address for Correspondence/Yazışma Adresi: Nasar Ahmad Shayan Assoc. Prof. MD, Herat University Faculty of Medicine, Department of Public Health and Infectious Diseases, Herat, Afghanistan; University of Western Ontario, Schulich School of Medicine and Dentistry, Department of Epidemiology and Biostatistics, London, Ontario, Canada
Phone: +1 226 582 88 07 **E-mail:** n.a.shayan@gmail.com
ORCID ID: orcid.org/0000-0002-8857-7765

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Bulgular: Katılımcıların yaş ortalaması 30,3±6,0 yıl, ilk evlilik yaşı ortalaması 17,6±3,6 yıl idi. Kontraseptif yöntemlerle ilgili ilk üç bilgi kaynağı aile üyeleri (%35,5), televizyon (%26,4) ve sağlık çalışanlarıdır (%19,9). Katılımcılar tarafından en yaygın kullanılan kontraseptif yöntemler kombine oral kontraseptifler (%18,1), erkek kondomu (%17,6), geri çekme yöntemidir (%17,5). Lojistik regresyon analizi sonuçları, herhangi bir aile planlaması yöntemi kullanmama durumunu, adolesan yaş grubunda olmanın 2,159 kat [%95 güven aralığı (1.238-3.765); p=0,007], yüksek paritenin (≥4) 1,525 kat, eşin çalışmamasının 1,764 kat, aile planlaması yöntemleri hakkında bilgi sahibi olunmamasının 11,674 kat artırdığını göstermiştir.

Sonuç: Risk gruplarında herhangi bir aile planlaması yöntemi kullanımının düşük olduğunu bulduk. Sağlık politikası yapıcıların, sağlık çalışanlarının ve aile üyelerinin, özellikle risk grubundaki kadınları, aile planlaması yöntemlerinin aileleri ve toplum için yararları konusunda desteklemesi ve eğitmesi kritik öneme sahiptir.

Anahtar Kelimeler: Aile planlaması, üreme sağlığı, kontraseptif yöntemler, Afganistan

Introduction

Despite significant achievements in health gains, especially in maternal and child health outcomes in Afghanistan since 2001, maternal deaths in the country is yet among the highest in the world (1,2). Among the five determinants of maternal health in Afghanistan, family planning, the general fertility rate, and the total fertility rate are the first three (2). Contraceptive prevalence rate (CPR) in Afghanistan is the lowest in the region (1), resulting in a high fertility rate in the country (5.2 per women), the highest in southeast Asia (3).

According to Afghanistan mortality survey 2010, about 91.6% of Afghan women aged 15-49 years understand about at least one modern family planning method, but only 20% of whom have ever used any modern contraceptive methods, exhibiting a huge gap between knowledge and practice of family planning methods in the country (4). In 2017, the national average of CPR in Afghanistan was 23.0%, with provincial level varying from 60.5% in Herat province to 0.5% in Noorestan (4). Recent studies investigating factors that influence the use of contraceptives by Afghan women recognized three contraceptive uptake predictors in the country: 1) those at the individual, couple and family level; 2) those relevant to service delivery; and 3) those relevant to the socio-cultural, religious and policy environment (1,4,5). More specifically, having more children, more number of living sons, better economic status, higher education, regular attendance to antenatal care facility, exposure to electronic media and child mortality experience were among the key factors that influenced contraceptive use in Afghan women. (1,5) Contraceptive pills were the most prevalent (39%) and intrauterine devices were the least prevalent (3%) methods used (4,6).

On the other hand, it is well known that discontinuation of contraceptives results in unwanted pregnancy and abortion (7). Most contraceptive users in Afghanistan reported switching to another method due to their unbearable side effects, and/or disagreement of husband and family (4).

This study aimed in assessing knowledge and practice of family planning methods and determine the predictors of use and non-use of contraceptives among women of reproductive age in Herat city, in west of Afghanistan.

Materials and Methods

Design and Setting

This descriptive, community-based, cross-sectional study was conducted between July and October 2018, among women aged 15-49 years, living in Herat city of Afghanistan. Herat city is the second largest city in the country, home to 574,276 people, of whom 287,919 (50.1%) are female. The age distribution of Herat population shows that 44.3% of residents are between 15 and 49 years old (8). Herat borders Iran in the west and Turkmenistan in the north and is the cultural and economic capital of the country.

Sampling and Sample Size

A sample size of 1,075 women was calculated using Raosoft Sample Size Calculator with 5% degree of precision, 99.9% confidence interval and 50% response distribution. Researchers decided to add 50% to the calculated sample size. Therefore, 1,610 samples, selected by convenient sampling technique were included in the study.

Inclusion Criteria

This study included 15-49-year-old women, who were residents of Herat city and were willing to participate in the study after an informed consent. Exclusion criteria included female residents of other provinces of Afghanistan and those who were not willing to participate in the study.

Data Collection

Participants were interviewed using a pre-tested, interview-based structured questionnaire, which included questions about participants' socio-demographic characteristics, knowledge, and practice of contraceptives, sources of information about contraceptives, history of contraceptive use in the past, history and reasons for discontinuation or switching between contraceptive methods, the most preferred and the least preferred methods ever used. Privacy of participants and confidentiality of information were maintained throughout the study. Data were collected by grade five and six students of the Herat University Faculty of Medicine. All surveyors were intensively trained for data collection and data entry both in the classroom and in the field for three weeks; after which a preliminary data collection assessment was conducted to ensure consistency

and accuracy of data collection techniques. The database was screened at the end of each week by the principal investigator for accuracy of data entry. Any questionnaire with missing data was excluded from the study.

Statistical Analysis

All statistical analyses were performed in IBM SPSS Statistics (version 25). A Kolmogorov-Smirnov test was performed to determine whether variables were normally distributed. Descriptive statistics were shown as mean (± standard deviation) for normally distributed variables, or medians and minimum-maximum values for the non-normally distributed variables. For non-normally distributed variables, non-parametric-tests (Mann-Whitney U test) was used to compare these parameters with the current use of family planning methods. Chi-square tests were performed for categorical variables. The univariate regression analyses were performed to examine associations between current use of any family planning methods with demographic parameters and knowledge about pregnancy prevention methods. For the multivariate regression analyses, the possible factors identified with univariate analyses were further analysed to determine factors related with current use of any family planning methods among participants. In this analysis, current use of family planning methods was taken as dichotomous variable, whereas age (≤18 years or above), parity (≤3 or above), husband's job (unemployed or employed), economic status of the family (poor or good), knowledge about pregnancy prevention methods (yes or no) and duration of marriage were taken as independent variables. The results of logistic regression analyses were showed as relative risk (odds ratio) and 95% confidence interval (CI). The Backward-Wald method was used. Association were considered statistically significant when p-value was less than 0.05.

The study protocol was reviewed and approved by the Human Ethics Committee of Herat University (approval number #180501, date: 01.05.2018). An informed consent was obtained from each participant prior to data collection.

Results

A total of 1,610 women of reproductive age were included in this study. The mean age of participants was 30.3±6.9 (range 15-45 years), mean age of participants at first marriage was 17.6±3.6 (range 7-34 years); duration of marriage was 12.6±7.7 (range 1-35 years), and mean number of births of women was 4.1±2.7 (range 0-16). Of the 1,610 participants included in this study, 57.6% had one or two sons and 56.5% had one or two daughters. Only 10.0% and 11.3% participants had four or more sons or daughters, respectively (Table 1). Women who had a history of dead children constituted 15.8%; one-third (34.1%) of participants were illiterate, while 21.9% were university students or graduate. Results show that 73.5% of participants and 12.0% of their husbands were unemployed, while 57.8% had an average economic status (Table 1).

The majority of participants (94.8%) had knowledge about family planning methods. The first three information sources were family members (35.5%), television (26.4%), health staff/doctors (19.9%), respectively. Of the 1,610 participants in this study, 92.0% ever used family planning methods in the past, while only 86.7% used contraceptives at the time of the study. The most common types of family planning methods reported by study participants were combined oral contraceptives (COC; 18.1%), male condom (17.6%) and withdrawal methods (17.5%). Participants were most satisfied from male condoms (22.2%), and least satisfied from injections (7.3%; Table 2). Among 1,210 women who discontinued or switched a family planning method, 55.7%

Table 1. Socio-demographic characteristics of women

	n	%
Education of women		
Illeterate	549	34.1
Primary	292	18.1
Secondary	193	12.0
High school	223	13.9
University	353	21.9
Job status		
Unemployed	1184	73.5
Employed	426	26.5
Husband's job status		
Unemployed	193	12.0
Employed	1417	88.0
Economic status of the family		
Bad	401	24.9
Average	930	57.8
Good	279	17.3
Family size (no of living boys)		
0	286	17.8
1	533	33.1
2	394	24.5
3	235	14.6
≥4	162	10.0
Family size (no of living girls)		
0	337	20.9
1	503	31.2
2	407	25.3
3	182	11.3
≥4	181	11.3

stated that they left the previously-used contraceptives because of their side effects.

In this study, age (≥19 years), parity (≥4), education (literate), husband's employment, good family economy, and knowledge about family planning methods were factors related with current contraceptive practice (Table 3); while women's employment was not associated with the current contraceptive practice (Table 3).

Logistic regression analyses showed that adolescent age increased the risk by 2.159 [95% CI (1.238-3.765); p=0.007], high parity (≥4) increased the risk by 1.525 [95% CI (1.162-2.002); p=0.002], husband's unemployment increased the risk by 1,764 [95% CI (1.221-2.549); p=0.003], and lack of knowledge about family planning methods increased the risk by 11,674 [95% CI (7.067-19.285); p<0.001] in terms of not using any family planning methods (Table 4).

Discussion

This study was conducted to evaluate the level of knowledge and practice of contraceptives among women of reproductive age in Herat city of Afghanistan. Moreover, factors related with the use and non-use of contraceptives were explored.

Table 2. Use of FP methods among women

	n	%
Ever use of FP methods		
Yes	1481	92.0
No	129	8.0
Current use of FP methods		
Yes	1396	86.7
No	214	13.8
Type of FP methods used (the first five methods)		
COC	291	18.1
Male candom	283	17.6
Withdrawal method	281	17.5
IUD	144	8.9
Injection	130	8.1
Most satisfied methods (the first five methods)		
Male condom	357	22.2
Withdrawal method	297	18.4
COC	285	17.7
IUD	172	10.7
Injection	118	7.3

FP: Family planning, COC: Combined oral contraceptive, IUD: Intrauterine device

The current study showed that 94.8% of women stated they had knowledge about contraceptives. This finding is comparable with the results of several other studies conducted in Kandahar, Afghanistan (94.5%), Saudi Arabia (99.2%), Sikkim of India (98.0%), Dodoma of Tanzania (96%), and Nepal (92.3%) (6,9-14); but was higher than results of studies conducted in Pakistan (88%; 81%) and Maghalaya, India (87%) (10,15,16); and much higher than those from Dar-es-Salam, Tanzania (75.0%), Nigeria (67.5%) and Ethiopia (42.3%) (12,17,18). This indicates that the knowledge towards contraceptives among women of reproductive age in Herat is high and satisfactory.

The study found that 92.0% participants had ever used at least a family planning method. This is higher than the level of contraceptive ever-use reported from Kandahar, Afghanistan (54.2%), Tanzania (47.4%), Nigeria (31.1%), and India (11%) (6,11-13). Furthermore, 86.7% participants in this study were current contraceptive users. This is higher than the rate of current contraceptive use reported from Saudi Arabia (75.4%), Nepal (64.6%), Ethiopia (50.4%), Pakistan (53%), Kandahar, Afghanistan (39.1%), India (38%), Tanzania

Table 3. Factors related to current use of any family planning methods among women

	Use of FP methods			
	Yes	No	X^2	p-value
Age				
≤18 years	57.1	42.9	26.020	<0.001
≥19 years	81.8	18.2		
Parity				
≤3	77.0	23.0	13.365	<0.001
≥4	84.2	15.8		
Education status of women				
Illiterate	78.3	21.7	2.975	0.0085
Literate	81.9	18.1		
Women's employment				
Unemployed	79.9	20.1	1.767	0.187
Employed	82.9	17.1		
Husband's employment				
Unemployed	72.5	27.5	9.333	0.002
Employed	81.8	18.2		
Economic status of the family				
Poor	76.3	23.7	6.555	0.01
Good	82.1	17.9		
Knowledge about family planning methods				
Yes	83.6	16.4	154.421	<0.001
No	28.6	71.4		

Table 4. Logistic regression of study variables that impact on current non-use of contraceptive practice among participants

	B	S.E.	Sig.	OR	95% CI	
Adolescent age (≤18 years)	0.770	0.284	0.007	2.159	1.238	3.765
Parity (≤3)	0.422	0.139	0.002	1.525	1.162	2.002
Husband's job (unemployed)	0.568	0.188	0.003	1.764	1.221	2.549
Knowledge about FP methods (insufficient)	2.457	0.256	<0.001	11.674	7.067	19.285
Constant	-2.259	0.390				

FP: Family planning, Sig.: Signature, OR: Odds ratio, CI: Confidence interval, S.E.: Standard error

(34%), and nation-wide Afghanistan (21.8%) (5,6,9,10,13,16-18). The differences between the rate of ever and current contraceptive use observed in this study and studies cited herewith may be due to cultural differences of study participants, access to family planning methods, and the study design. The current study was conducted in Herat city center, the residence of which have an easy access and may probably be more open to the use of family planning methods than participants in other studies. In fact, several studies indicated that feeling embarrassed to buy or ask for contraceptive was the most important barrier to buy or ask for family planning methods (11,12). Also, a positive association was reported between contraceptive use and distance from the nearest health center (14). Other studies demonstrated that rural residence is a predictor of lower contraceptive use in the country (5,14,18). Moreover, a positive correlation between the level of contraceptive knowledge and practice has been reported previously (1,5,6,13). In this study, we also found a significant association between the economic status and the use of family planning method. Therefore, the good knowledge on family planning method among women in Herat and their better economic status may have been translated to the increase use of contraceptives in this study.

In the current study, no significant gap was observed between the level of knowledge and the rate of contraceptive ever-use (94.8% knowledge vs 92.0% contraceptive ever-use). However, an 8.1% gap was seen between the level of knowledge and current CPR among participants (94.8% vs 86.7%). This indicates that a variety of perceived barriers prevented participants from using contraceptives continuously.

In this study, 55.7% participants who discontinued a specific contraceptive, left it due to its side effects. In fact, studies indicate that side effects impact on many daily activities leading to discontinuation or switching of the contraceptive (19); For example, 16.8% of study participants of a study in Kandahar city of Afghanistan, and 10.8% of participants in a study in Iran stopped using contraceptives because of their side effects (6,7). However, a study from Saudi Arabia revealed that desire of more children was the major reason for discontinuation of a specific contraceptive (9).

The most-commonly used family planning methods practiced in this study were COC (18.1%), male condom (17.6%), and female condom (17.5%). This is in accordance with the results of a study conducted in Kandahar city of Afghanistan, in which the most common contraceptive used by study participants was COC (24.8%) (6). Another study conducted in India revealed that most-commonly-used contraceptives were condoms (38.2%) and COCs (27.6%) (16). A study in Pakistan demonstrated that the most common contraceptives used by study participants were condoms (33.9%), tubal ligations (22.6%) and injections (18.8%) (10). Another study in Saudi Arabia indicated that 32.2% of participants used COC as their most common contraceptives (9). This highlights differences in tendency towards various types of contraceptives in different geographical and/or socio-cultural regions. A substantial finding of this study was the significantly-rare use of female condom among participants. This indicates that despite its accessibility in healthcare centers, female condom is not as favorable as male condom in Herat.

In the current study, the most abundant source of information about family planning methods were family members (35.5%), television (26.4%), and healthcare workers (19.9%). This is in contradiction with the findings of study from Kandahar, Afghanistan, in which the main source of information were health facilities (61.2%), parents (14.7%), and media (6.2%) (6). However, other studies emphasized the role of media as a major source of contraceptive information (10,15); on the other hand, this study more strongly highlights the role of family members in disseminating information about family planning in Herat. While it displays stronger family ties between family and the women of reproductive age in Herat, this also indicates that the source of information about family planning is not much scientific and reliable, and is based on personal experiences. It is advised that media and healthcare personnel make more effective efforts in providing counseling about the use of family planning methods to women in Herat.

The current study showed that the age of study participants was strongly related with the practice of family planning methods. Participants aged 19 years or above used contraceptives 2.159 times more than those aged 18 years or less. Previously, the role of age on contraceptive use was

also emphasized in Nepal (14). This may be due to the fact that when age increases, the level of women's knowledge about contraceptive increases and families have more children. Since pregnancy in women ageing 18 years or less is much riskier than those who age 19 years and above, it is advisable to educate and encourage younger women to use contraceptives to avoid risky pregnancies.

The current study also revealed that education level is significantly associated with contraceptive use among study participants. This is similar to findings of studies from Nepal, India and Afghanistan (5,14,20). It shows that with higher education levels, more women utilize family planning methods. This highlights that a higher education is correlated with a higher level of knowledge about effects and advantages of family planning methods.

Another related factor of contraceptive practice in this study was economic status of the family. Those with good economic status used contraceptives over four times more than those with lower economic status. Previously, the positive impact of family monthly income on the rate of contraceptive practice was reported (1,5,20). This may be because people with good income can afford buying contraceptives more easily and continually. However, the study conducted in Kandahar city of Afghanistan found no association between family income and the modern contraceptive use (6).

We also found that knowledge about family planning methods were significantly associated with current contraceptive practice. Those with better knowledge about family planning methods used contraceptives 11.674 times more than others. This is in accordance with results of similar studies conducted elsewhere (14,18). This indicates that having more knowledge about family planning methods is associated with initiating and maintaining the use of contraceptives among women.

The participants of this study were selected by convenient sampling, due to logistics and cultural reasons. Moreover, one-third of study participants were illiterate. Although this is in line with literacy statistics in the country, it may impact on obtaining thorough and methodical information in the study.

Conclusion

This study adds to body of current literature about the CPR and factors related with the use and non-use of family methods in developing countries. We found that the level of knowledge about family planning methods among women of reproductive age in Herat city of Afghanistan is satisfactorily high. There is no big gap between the level of knowledge and rate of contraceptive ever-practice; however, an 8.1% gap exists between the level of knowledge and current contraceptive use, mainly due to side effects. The most significant factors associated with contraceptive use were age, parity, family economic status and knowledge about contraceptives.

It is very important for policymakers, healthcare workers, family elders and spouses to support women of reproductive age and educate them about the benefits and advantages of family planning methods for their family and society.

Ethics

Ethics Committee Approval: The study protocol was reviewed and approved by the Human Ethics Committee of Herat University (approval number #180501, date: 01.05.2018)

Informed Consent: An informed consent was obtained from each participant prior to data collection.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: N.A.S., A-u-RN., K.O., F.A., Design: N.A.S., A-u-RN., F.A., Data Collection or Processing: N.A.S., K.O., F.A., Analysis or Interpretation: N.A.S., A-u-RN., F.A., Literature Search: N.A.S., K.O., F.A., Writing: N.A.S., A-u-RN., K.O., F.A.

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Effect of Different Irrigation Activation and Condensation Techniques on the Marginal Adaptation of White MTA

Farklı İrrigasyon Aktivasyon ve Kondenzasyon Tekniklerinin Beyaz MTA'nın Marjinal Adaptasyonuna Etkisi

Ali Türkyılmaz[1], Yağız Özbay[2], Gözde Akbal Dinçer[3], Ali Erdemir[1]

[1]Kırıkkale University Faculty of Dentistry, Department of Endodontics, Kırıkkale, Turkey
[2]Karabük University Faculty of Dentistry, Department of Endodontics, Karabük, Turkey
[3]Okan University Faculty of Dentistry, Department of Endodontics, İstanbul, Turkey

Abstract

Objective: To evaluate the effect of different irrigation activation and condensation techniques on the marginal adaptation ability of white ProRoot mineral trioxide aggregate (MTA).

Materials and Methods: Eighty single-rooted human teeth were prepared to a ProTaper Universal F4 file. Initially, the roots were randomly divided into four groups as follows: conventional syringe irrigation in group 1, sonic activation (EDDY) in group 2, passive ultrasonic irrigation in group 3, and Er:YAG laser in group 4. Then, ProRoot MTA was set with hand condensation or ultrasonic condensation techniques, and the adaptation ability of MTA was investigated using SEM.

Results: EDDY activation had the lowest gap scores ($p<0.001$). No statistical differences were observed between the regions ($p>0.05$), and condensation techniques ($p>0.05$).

Conclusion: The marginal adaptation of ProRoot MTA to root dentine increased with EDDY activation, and the marginal adaptation ability of the material was not affected by the condensation technique.

Keywords: Adaptation, EDDY, mineral trioxide aggregate, PIPS, PUI

Öz

Amaç: Farklı irrigasyon aktivasyon ve kondenzasyon tekniklerinin ProRoot mineral trioksit agregat'ın (MTA) marjinal adaptasyon yeteneği üzerindeki etkisini değerlendirmektir.

Gereç ve Yöntemler: Seksen tek köklü insan dişi ProTaper Universal F4 eğesine kadar genişletildi. Dişler grup 1'de geleneksel şırınga ile irrigasyon, grup 2'de sonik aktivasyon (EDDY), grup 3'te pasif ultrasonik irrigasyon ve grup 4'te Er:YAG lazer kullanılmak üzere rastgele dört gruba ayrıldı. Sonrasında ProRoot MTA elle kondenzasyon veya ultrasonik kondenzasyon teknikleri ile kondenze edildi ve SEM kullanılarak MTA'nın adaptasyon yeteneği değerlendirildi.

Bulgular: EDDY aktivasyonu ile en düşük boşluk skorları elde edildi ($p<0,001$). Bölgeler ve kondenzasyon teknikleri arasında istatistiksel bir farklılık gözlenmedi ($p>0,05$), ($p>0,05$).

Sonuç: ProRoot MTA'nın kök dentinine marjinal adaptasyonu, EDDY aktivasyonu ile arttı ve materyalin marjinal adaptasyon özelliği kondensasyon tekniğinden etkilenmedi.

Anahtar Kelimeler: Adaptasyon, EDDY, mineral trioksit agregat, PIPS, PUI

Address for Correspondence/Yazışma Adresi: Asst. Prof. Yağız Özbay, Karabük University Faculty of Dentistry, Department of Endodontics, Karabük, Turkey
Phone: +90 370 418 91 06 **E-mail:** yagiz_ozbay@hotmail.com
ORCID ID: orcid.org/0000-0003-2028-8120

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Introduction

Tricalcium silicate cement is aimed to hermetically seal the dentin against leakage and may induce hard tissue formation if in contact with periapical tissues (1). In the past, mineral trioxide aggregate (MTA) was the first used tricalcium silicate cement in many areas: such as retrograde filling, apexification, vital pulp therapy, and perforation repair. MTA still contributes desired results, similar to the recently developed bioceramic-based cements (2). Investigating its marginal adaptation on dentine walls can help to understand sealing ability of MTA (1).

The smear layer hampers penetration of disinfecting agents and blocks sealer penetration in root canal treatment (3). Many devices and technologies, including sonics, ultrasonics, and lasers, have been used for irrigation activation to enhance smear layer removal. Sonic activation is similar to ultrasonic activation, but sonic devices generally use a flexible tip and activate at lower frequencies under 20 kHz.

With the recently developed sonic activation Endo Irrigation Tip (EDDYTM; VDW GmbH, Munich, Germany), which has a non-cutting flexible tip size of 25/0.04, a new standard for safety and efficiency in activation was gained. Three-dimensional movement of the tip supports acoustic streaming in the root canal, contributing to higher cleaning efficiency (4). The frequency parameter of this tip is between 5,000-6,000 Hz (4), and it works with an air-driven handpiece (TA-200-S4H, Micron, Tokyo, Japan). With ultrasonic devices, acoustic microstreaming is produced during their application. As an activation technique, ultrasonic tips can increase the performance of irrigation solutions (5). Small non-cavitating bubbles are created by ultrasonic systems, by which acoustic microstreaming and shear stresses are produced on the root canal surface (4).

In previous studies, Er:YAG laser was indicated to be a valuable tool for removing the smear layer (6). As a technique to be used with Er:YAG laser, photon-induced photoacoustic streaming (PIPS™), which has a stripped tip to be placed into the canal orifice, has been shown to be effective as an irrigation activation technique in debris and smear layer removal. Working principle of the photoacoustic shock waves is explained by inducing 3-dimensional stream of the irrigation solution throughout the root canals (7).

Condensation procedures includes a technique, a well-known simple hand condensation, in which an endodontic condenser, a plugger is used. Other procedure is a modified technique where hand condensation and ultrasonic vibration are combined.

The effects of smear layer removal and adaptation of different root canal materials on dentine walls have been evaluated in many previous studies (2,4). Nonetheless, it is still necessary to evaluate the adaptation of MTA-based cements after using EDDY, a newly developed effective tool. It is also valuable to compare the effect of EDDY with passive ultrasonic irrigation (PUI) and Er:YAG laser (PIPS) activation on the adaptation. Therefore, the aim of this study was to investigate the marginal adaptation of ProRoot MTA (Dentsply Maillefer, Ballaigues, Switzerland) by using two different condensation technique after performing EDDY, PUI or Er:YAG irrigation activation.

Materials and Methods

Ethics approval (approval number: 2021.07.01, date: 08.07.2021) was granted for this study by the Kırıkkale University Non-Interventional Research Ethics Committee. According to a power analysis (G*Power Version 3.0.10, Kiel University, Germany) (F tests, effect size f=0.40, α error probability =0.05, 1-β error probability =0.80), the minimum required sample size was 80 specimens. A total of 80 single-rooted permanent human teeth were selected. More than a single root canal, open apex, resorption, fracture, or dilacerations were discarded. The digital images (Gendex Dental Systems, Hatfield, PA, USA) were taken from the teeth using buccal and proximal aspects to exclude a tooth with a complex root canal anatomy. The teeth not shorter than 14 mm root length and less than 10 degrees of curvature were chosen after obtaining periapical radiographs and were measured using ImageJ software (ImageJ v1.47, National Institutes of Health, USA). The remnants on root surface were removed and the teeth were stored in distilled water during the sample collection. After the preparation of access cavities, the working length was determined by inserting #15 K-file, and the root canals were prepared with ProTaper Universal (Dentsply, Maillefer, Switzerland) up to #F4. The root canals were rinsed with 2 mL 5% NaOCl for 1 min between each instrument. Finally, the root canals were irrigated with distilled water and dried with sterile paper points. Then, the samples were randomly divided into four groups (n=20).

In group 1 [conventional syringe irrigation (CSI)], the root canals were rinsed with 5 mL 5% NaOCl for 60 s. The irrigation solutions were applied to the root canal by the aid of a 30 G endodontic irrigation needle with up and down movement. No further activation was performed. This procedure was repeated using 5 mL 17% EDTA for 60 s. A 2 mL distilled water was used between each solutions. In group 2, with sonic activation tip (EDDY), 5% NaOCl was activated for 10 s at the same time with irrigation. Tip was used with a frequency of 6000 Hz at 1-mm shorter than the working length and in-and-out motion (TA-200-S4H, Micron, Tokyo, Japan). After the activation is completed a 10 second resting period was waited to repeat the procedure. This process was repeated in three-time intervals (10 s for the irrigation and activation and 10 s for the resting period) and completed in 60 s per irrigant. Irrigation and activation procedures were followed using 5 mL 17% EDTA. In group 3 (PUI) the irrigation solutions were applied with an irrigation needle, and activated using a stainless steel ultrasonic tip (NSK, Nakanishi Inc., Tokyo, Japan) with a MiniEndo II Ultrasonic Unit (SybronEndo, Orange, CA) at ½ power setting for 60 s similar to group 1. In group 4, Er:YAG laser activation, the irrigation, and

activation procedures were performed as described in the first group. The irrigation activation was applied with an Er:YAG laser (LightWalker, Fotona, Ljubliana-Slovenia) by inserting a 300 µm fiber tip (PIPS) into the access cavity. The laser parameters were set to 0.3 W, 15 Hz, and 20 mJ in quantum square pulse mode.

Finally, after rinsing the root canals with 5 mL distilled water, apical portion of the root with 3 mm thickness was removed, and two 1 mm thickness dentine discs were obtained using a precision cutting machine (Metkon 151, Bursa, Turkey) under constant water flow at 1st mm (apical) and 7th mm (coronal) of the resected roots. Afterward, each group was divided into two subgroups of 10 samples according to MTA condensation techniques (hand condensation or ultrasonic condensation).

According to the manufacturer's recommendations, ProRoot MTA was applied to dentine discs using a plugger (Dentsply, Ballaigues, Switzerland) condensation with or without ultrasonic vibration (Woodpecker, Guangxi, China). Ultrasonic vibration speed was set to low potency and applied for 5 s for each sample in contact with the plugger tip. When obturation was completed, the remaining material on the dentine surface was cleaned with a cotton pellet. Then, an amount of sponge was moistened using saline solution and placed on the material. All samples were stored at 100% humidity at 37 °C for 24 h to set. Afterwards, eight measuring points were signed, as evenly as possible, around the material under an operation microscope (Zeiss S100/OPMI Pico, Germany) at X10 magnification. The samples were coated with gold-palladium alloy using a coating machine (Balzers-SCd 050, Germany) and evaluated under a scanning electron microscope (SEM) (Jeol JSM-5600, Tokyo, Japan). The gaps between the material and dentin wall were measured linearly in micrometers on clockwise cross-sections (Figure 1a), and the failed samples (Figure 1c) were prepared again.

Statistical Analysis

The SPSS 22.0 software (IBM, Armonk, NY) was used for statistical analysis, and p-values were established at 0.05. Due to a lack of normal distributions, log 10 transformations were performed to homogenize the variances and decrease the number of outliners. Thus, two-way ANOVA, Tukey tests, and Independent-Samples t-tests were used for statistical analysis.

Results

Gaps between the MTA and dentin were observable in all groups at different magnifications (Figure 2, 3). The mean and standard deviation of the values for the gap measurements obtained on the SEM images are shown in Table 1. There was a significant difference in the adaptation of MTA between the groups ($p<0.001$). The lowest gap values were observed in group 2 with the sonic activation. There was no significant difference between the coronal and apical thirds in MTA adaptation ability ($p=0.096$). Moreover, no significant difference was found among the MTA condensation methods ($p=0.118$).

Discussion

Adaptation ability is an expected feature of MTA-based repair materials and root canal sealers. Most of the samples in this study had a complete adaptation between the material and the dentine wall interfaces. On the other hand, if data could not be obtained from the sample due to adaptation failure, the sample was re-prepared and evaluated.

When comparing CSI with EDDY, sonic activation had significant differences regarding dentine and smear removal from curved canals (8). On the other hand the efficacy of sonic activation in terms of the adaptation of MTA and bioceramic-based cements has not been examined in previous studies. In this study, MTA adaptation after sonic activation had the lowest gap measurement and the highest marginal adaptation. As shown in an earlier study, number of the viable microorganisms decreased significantly with EDDY tips compared to passive ultrasonic irrigation (Irrisafe, Acteon, France) and manual irrigation (9). EDDY was more effective than XP-endo Finisher (FKG-Dentaire, La-Chaux-de-Fonds, Switzerland) in removing calcium hydroxide from artificial grooves, having a similar effect to PUI (10). Sonic activation with EDDY also enhances removal of residual filling materials during retreatment, as reported in a previous study with micro-CT (11).

It was concluded in several studies that ultrasonics failed to enhance the smear layer removal efficiency of EDTA or a combination of NaOCl and EDTA (12,13). In this study, the PUI procedure had a lower performance than EDDY. Its performance was similar to that of PIPS. It was also reported that the sonic activation was significantly more efficient than PUI and laser-activated irrigation in smear layer removal at different levels of the root canal (14). However, another study showed that PUI and EDDY had similar performances in smear layer removal (4). Different frequencies for sonic and ultrasonic systems, different lasers at various wavelengths, and the study methods may affect the results.

When the crown is maintained as a reservoir for irrigant, the constant movement of the solution into root canals is possible without depletion of the irrigant (15). Therefore, the coronal portion of the teeth was not removed, and access cavities were used in this study. PIPS is activated in the pulp chamber, and EDDY is activated in the root canal. This difference between the application techniques resulted in a more remarkable adaptation ability in the EDDY group because of closer performance to the apical region and more mechanical agitation to the root dentine surface.

In the present study, there was no statistically significant difference in the adaptation ability of MTA after CSI, PUI, and PIPS activation there is no other study comparing the adaptation of MTA after using irrigation activation systems

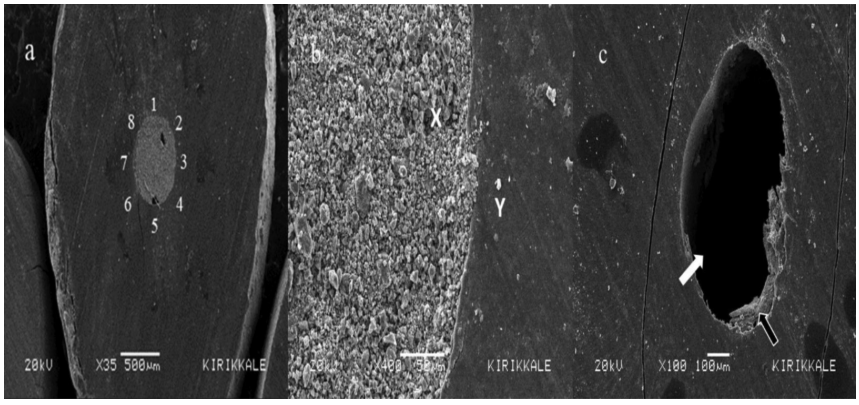

Figure 1. a. The numbers show the sites selected for evaluation in each sample at x500 magnification. **b.** Showing a tight adaptation between MTA (X) and dentine wall (Y). **c.** Adaptation failure. Black arrow shows remaining material and white arrow shows root canal space

MTA: Mineral trioxide aggregate

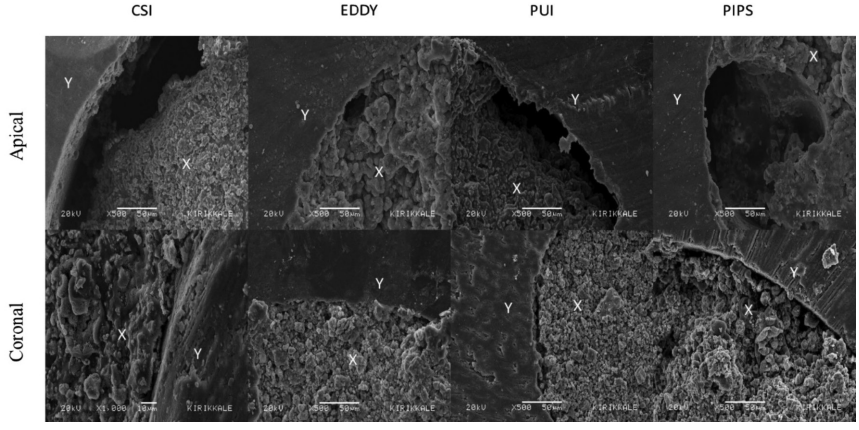

Figure 2. SEM micrograph of hand condensation groups. Gaps are showing between MTA (X) and root dentine (Y)

SEM: Scanning electron microscope, MTA: Mineral trioxide aggregate, CSI: Conventional syringe irrigation, EDDY: Passive sonic activation, PUI: Passive ultrasonic irrigation, PIPS: Photoacoustic activation

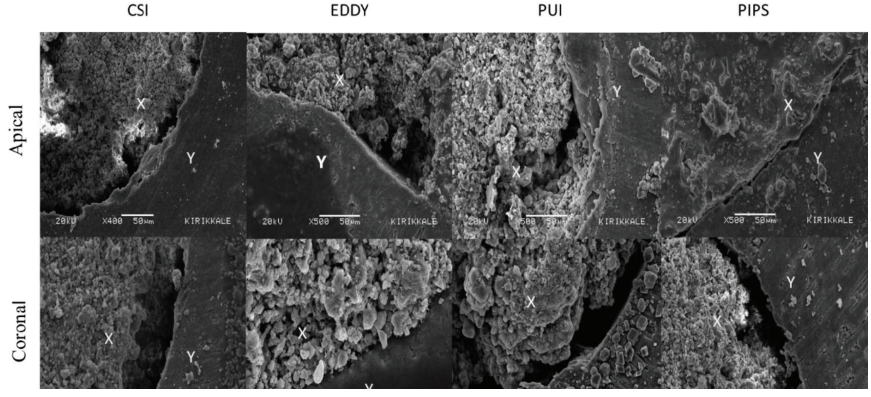

Figure 3. SEM micrograph of ultrasonic condensation groups. Gaps are shown between MTA (X) and root dentine (Y)

SEM: Scanning electron microscope, MTA: Mineral trioxide aggregate

Table 1. The intergroup comparison between the irrigation methods and in intra-group comparisons between condensation techniques and regions. The data were shown as log 10 transformation

					Region	
					Coronal	Apical
Irrigation Method	Mean ± SD (µm)	Condensation Technique	Mean ± SD (µm)	Irrigation Method	Mean ± SD (µm)	Mean ± SD (µm)
CSI	1.27±0.54[a]	HC	1.05±0.62[a]	CSI	1.00±0.44[A]	1.15±0.80[A]
				EDDY	0.56±0.60[A]	0.38±0.53[A]
EDDY	0.62±0.52[b]			PUI	1.43±0.30[A]	1.45±0.36[A]
				PIPS	1.02±0.62[A]	1.44±0.23[A]
PUI	1.40±0.45[a]	UC	1.20±0.55[a]	CSI	1.36±0.23[A]	1.56±0.39[A]
				EDDY	0.65±0.45[A]	0.87±0.45[A]
PIPS	1.23±0.56[a]			PUI	1.19±0.64[A]	1.52±0.41[A]
				PIPS	1.25±0.18[A]	1.20±0.88[A]
p-value	<0.001		0.118[#]		0.096*	

CSI: Conventional syringe irrigation, EDDY: Passive sonic activation, PUI: Passive ultrasonic irrigation, PIPS: Photoacoustic activation, HC: Hand condensation, UC: Ultrasonic condensatio.n

The same superscript lowercase letters in each column is not statistically significant (p>0.05). The same superscript uppercase letters in each row is not statistically significant (p>0.05), #HC-UC comparison *Coronal-apical comparison

in the literature. On the other hand, it was reported in a study that MI, PUI, and PIPS had the same performance regarding apical debris extrusion (16).

In this study, sonic activation EDDY was the most effective method increasing MTA adaptation on the root dentine surface. Challenges in MTA application may be considered one of its drawbacks; different carriers including pluggers have been used for placement of MTA.

In recent studies, the ultrasonic vibration technique has been used during condensation to increase adaptation ability (17,18). It has been reported that the adaptation ability of MTA condensed with this technique increased in teeth with open apex (18). Contrarily, it was also shown that when MTA was condensed with ultrasonic tips, its sealing ability did not differ significantly compared to that of MTA condensed with paper points or pluggers (19).

Yeung et al. (20) showed that indirect ultrasonic vibration, after hand condensation, resulted in significantly denser MTA filling than the MTA filling applied by hand condensation alone. It was also concluded that ultrasonic vibration might be more beneficial at MTA placement for open apices than hand condensation and results in better MTA condensation (21). Sisli and Ozbas (22) reported no significant differences when comparing hand condensation and ultrasonic activation on MTA placement. However, another study (23) assessed MTA adaptability to the walls of polyethylene tubes using different application methods was reported that MTA placement by hand condensation led to better adaptation with fewer gaps than direct ultrasonic technique. Similarly, results of the present study indicated that MTA condensation was not significantly different in these two techniques.

Conclusions

MTA adaptation to the root dentine after sonic activation was more effective than MTA adaptation after the manual irrigation and ultrasonic activation techniques. The adaptation ability was also similar in the apical and coronal regions. Moreover, the use of ultrasonic vibration in MTA placement did not affect the adaptation of the material. Further studies are required to elucidate the correlation of different irrigation activation techniques and the adaptation of the MTA or bioceramic-based materials.

Ethics

Ethics Committee Approval: Ethics approval (approval number: 2021.07.01, date: 08.07.2021) was granted for this study by the Kırıkkale University Non-Interventional Research Ethics Committee.

Informed Consent: Informed consent is not required.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Concept: A.T., A.E., Design: A.T., A.E., Data Collection or Processing: A.T., Y.Ö., G.A.D., Analysis or Interpretation: A.T., Literature Search: A.T., Y.Ö., G.A.D., Writing: A.T., Y.Ö., A.E.

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Invasive *Candida* Infections in Children: Species Distribution, Antifungal Susceptibility, and Risk Factors Associated with Mortality

Çocuklarda İnvaziv *Candida* Enfeksiyonları: Tür Dağılımı, Antifungal Duyarlılık ve Mortalite ile İlişkili Risk Faktörleri

Zeynep Güleç Köksal[1], Nurşen Belet[2], Mahmut Cem Ergon[3], Ahmet Naci Emecen[4], Mine Doluca Dereli[3]

[1]Dokuz Eylül University Faculty of Medicine, Department of Child Health and Diseases, İzmir, Turkey
[2]Dokuz Eylül University Faculty of Medicine, Department of Pediatric Infectious Diseases, İzmir, Turkey
[3]Dokuz Eylül University Faculty of Medicine, Department of Medical Microbiology, İzmir, Turkey
[4]Dokuz Eylül University Faculty of Medicine, Department of Public Health, İzmir, Turkey

Abstract

Objective: This study determined the distribution and antifungal susceptibility of *Candida* species, risk factors, and mortality in invasive candidiasis (IC).

Materials and Methods: The medical data of the pediatric patients with IC were analyzed retrospectively between September 2014 and September 2018. The first IC episodes were included, and the susceptibility was determined by the microdilution method performed according to The Clinical and Laboratory Standards Institute M27-A3 standards. Kaplan-Meier curves were prepared for survival on the 7th and 30th day after the first positive culture and the curves were compared with the log-rank test.

Results: Forty-eight *Candida* isolates were detected in 45 IC episodes. *C. albicans* and *C. parapsilosis* were the most common species (both 41.7%). Fluconazole, caspofungin, and amphotericin B resistance were 38.2%, 3.1%, and 2.9%, respectively. Fluconazole resistance was 73.3% among *C. parapsilosis*. The most common risk factors were underlying diseases (100%), previous antibiotic use (95.6%), and central venous catheter (73.3%). Six (13.3%) patients were deceased within the 30 days. Patients with neutropenia and dialysis had a higher rate of mortality and lower mean survival times for 7-day and 30-day mortality. Mean survival times for 7-day mortality were lower for the patients who had abdominal surgery (p=0.04).

Conclusions: There was high fluconazole resistance in *C. parapsilosis*, which was 73.3%. Neutropenia, dialysis, and abdominal surgery were associated with a significant increase in mortality. These data will help us identify patients who are at risk for IC and will guide us in the selection of empirical treatment.

Keywords: Antifungal susceptibility, amphotericin B, candidaemia, *Candida* spp., fluconazole resistance, pediatric

Öz

Amaç: Bu çalışma, invaziv kandidiyazise (İK) neden olan *Candida* türlerinin dağılımını, antifungal duyarlılığını, risk faktörlerini ve mortaliteyi belirlemek amacıyla yapılmıştır.

Gereç ve Yöntemler: Eylül 2014-Eylül 2018 tarihleri arasında İK'lı pediatrik hastaların tıbbi verileri retrospektif olarak incelendi. Çalışmaya hastaların ilk İK epizotları dahil edildi. *Candida* izolatlarının duyarlılığı, Klinik Laboratuvar Standartları Enstitüsü M27-A3 standartlarına göre yapılan mikrodilüsyon yöntemi ile belirlenmiştir. İlk pozitif kültürden sonraki 7. ve 30. gündeki sağkalım için Kaplan-Meier eğrileri oluşturulmuştur ve eğriler log-rank testi ile karşılaştırılmıştır.

Bulgular: Kırk beş IC atağında 48 *Candida* izolatı tespit edildi. *C. albicans* ve *C. parapsilosis* en yaygın türlerdi (her ikisi de %41,7). Flukonazol, kaspofungin ve amfoterisin B direnci sırasıyla %38,2, %3,1 ve %2,9 idi. *C. parapsilosis*'te flukonazol direnci %73,3 idi. En sık görülen risk faktörleri altta yatan hastalık (%100), önceden antibiyotik kullanımı (%95,6), santral venöz kateter (%73,3) idi. Altı (%13,3) hasta 30 gün içinde öldü. Nötropeni ve diyaliz hastalarında daha yüksek mortalite oranı ve 7-günlük ve 30-günlük mortalite

Address for Correspondence/Yazışma Adresi: Zeynep Güleç Köksal MD, Dokuz Eylül University Faculty of Medicine, Department of Child Health and Diseases, İzmir, Turkey
Phone: +90 538 624 08 30 **E-mail:** zynp.glc@hotmail.com
ORCID ID: orcid.org/0000-0001-9464-4605

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için daha düşük ortalama sağkalım süreleri vardı. Abdominal cerrahi geçiren hastalarda 7 günlük mortalite için ortalama sağkalım süreleri daha düşüktü (p=0,04).

Sonuç: *C. parapsilosis*'te %73,3 oranında yüksek flukonazol direnci vardı. Nötropeni, diyaliz ve abdominal cerrahi, mortalitede önemli bir artışla ilişkilendirildi. Bu veriler, invaziv kandidiyazis riski taşıyan hastaları belirlememize yardımcı olacak ve ampirik tedavi seçiminde bize yol gösterecektir.

Anahtar Kelimeler: Antifungal duyarlılık, amfoterisin B, kandidemi, *Candida* türleri, flukonazol direnci, pediatrik

Introduction

Candidiasis is the most common fungal infection and usually affects children with chronic illness, prematurity, immunodeficiency, and critical diseases (1). In the United States, *Candida* spp. is the widespread cause of invasive fungal disease and is the second most common problem of central line-associated bloodstream infections (CLABSI) in pediatric patients (2).

Candidiasis leads to increased mortality, morbidity, prolonged hospitalization time, and health care costs in children (3). Regional distribution of *Candida* species, antifungal susceptibility and risk factors for invasive candidiasis (IC) are important for effective empirical treatment and prevention strategies.

We aimed to establish the distribution of *Candida* species, antifungal susceptibility, risk factors and mortality in hospitalized children with IC.

Materials and Methods

Patient Data and Definitions

This cross-sectional study was performed at a tertiary university hospital. Pediatric patients with culture-proven invasive *Candida* spp. infection during hospitalization during a four year (from September 2014 to September 2018). The demographic data, clinic features, and microbiological results of the pediatric patients with IC were analyzed retrospectively.

IC was characterized as the isolation of *Candida* spp. from sterile body fluids. Candidemia was described as the isolation of *Candida* spp. in blood culture from a peripheral vessel or a central venous catheter (CVC). The isolation of *Candida* spp. from any blood culture in a patient with a CVC or *Candida* spp. growth from a catheter-tip culture was defined as catheter-associated candidemia.

The previous hospitalization (history hospitalization) was defined as hospitalization history within three months before the IC (4). The presence of mechanical ventilation was recorded within two days before IC. Arterial catheter, CVC, urinary catheter, and use of total parenteral nutrition (TPN) were recorded within a week before infection. In the two weeks before infection, surgical intervention (abdominal and non-abdominal), broad-spectrum antibiotic use, immunosuppressive drug use, presence of neutropenia, and previous use of antifungal drugs were recorded. Initial therapy was deemed to be delayed when the time elapsed between taking the culture sample and the start of antifungal therapy was more than 72 hours. The time of first culture positivity was always considered as a benchmark to evaluate time to infection and mortality.

Identification of Organism and Susceptibility Testing

The isolates were cultured from several clinical samples (blood, CVC, and peritoneal fluid). Blood and sterile body fluid cultures were processed by the BACTEC FX 200 system (Becton Dickinson, United States of America). Growth was determined from the culture of specimens on blood and "Eosine Methylene Blue" agar plates. The colonies identified as yeast growth were transferred to the mycology laboratory for identification and antifungal susceptibility tests. The colonies were subcultured to "sabouraud dextrose agar" (Oxoid, England), and identification was performed after the strains were determined to be pure. Yeasts were identified by germ tube test, microscopic morphology on Cornmeal tween 80 agar (Oxoid, England) and CHROMagar *Candida* (CHROMagar, France), and API 20C AUX (BioMérieux, France).

The susceptibility of *Candida* isolates to fluconazole, amphotericin B (AmB), and caspofungin was determined by microdilution method performed based on The Clinical and Laboratory Standards Institute (CLSI) M27-A3 standards (5). Minimal inhibitory concentrations (MICs) for fluconazole, AmB, and caspofungin were evaluated according to the CLSI species specific clinical breakpoints. These were as follows: for fluconazole *C. albicans, C. tropicalis* and *C. parapsilosis* susceptible (MIC ≤2 µg/mL), susceptible-dose dependent (MIC 4 µg/mL), resistant (MIC ≥8 µg/mL); for fluconazole *C. glabrata* susceptible-dose dependent (MIC ≤ 32 µg/mL), resistant (MIC ≥64 µg/mL); for AmB susceptible (MIC ≤1 µg/mL), resistant (MIC >1 µg/mL); for caspofungin *C. albicans, C. krusei, C. tropicalis,* and *C. glabrata* resistant (MIC >0,5 µg/mL), *C. parapsilosis* resistant (MIC >4 µg/mL). *C. krusei* was considered as naturally resistant to fluconazole regardless of MIC (6).

Statistical Analysis

Statistical analyses were performed using SPSS version 20 (SPSS, Inc, Chicago, IL) software. Categorical factors were shown as numbers and percentages. Shapiro-Wilk normality test was used for test normality. Non-normal distributed continuous variables were presented as medians and interquartile ranges unless stated otherwise. Qualitative data were compared using the chi-square test, while quantitative variables were compared between groups using the Mann-

Whitney U test or Fisher's exact test. The response variable of this study was mortality. Patients were right-censored at the 7th and 30th days after the initial positive culture. Kaplan-Meier survival curves were prepared, and the log-rank test was used to assess the differences between survival curves. Statistical analyses were applied in R (7) version 3.4.3, using the packages "survival" and "survminer". Significance was defined at the double-sided p-value of <0.05.

This study was approved by the Dokuz Eylül University Non-Invasive Research Ethics Committee (decision no: 2016/13-23, date: 12.5.2016).

Results

Demographics, Characteristics and Risk Factors

There were 45 IC patients during the four years. Four patients had one more recurrent episode, but only the first episodes were included. Two patients had more than one *Candida* spp. Two candida strains (*C. albicans, C. tropicalis*) were isolated in one patient, and three Candida strains (*C. parapsilosis, C. albicans, C. krusei*) in another patient.

Demographics and characteristics of the patients with IC are shown in Table 1. The most prominent risk factors were having underlying diseases (100%), a previous antibiotic use (95.6%), CVC (73.3%), and previous hospitalization (55.6%). *Candida* endocarditis and peritonitis developed in 2 (4.4%) and 1 (2.2%) patients, respectively.

Distribution and Antifungal Susceptibility of the *Candida* Species

Forty-eight *Candida* strains and seven different *Candida* species were isolated. The most common species were *C. albicans* and *C. parapsilosis,* both 20 (41.7%). Antifungal susceptibility testing was performed for 34 *Candida* isolates. The susceptibility of the isolates to the antifungal agents is shown in Table 2.

Therapy

Antifungal therapy was given in 42 episodes (93.3%), monotherapy in 17 (37.8%), and sequential treatment in 25 (55.6%) episodes. Twelve (26%) patients were receiving antifungal agents before IC. Initial therapy was delayed in seven patients (15.5%).

Table 1. Demographics of the patients with invasive candidiasis

	n (%)
Age, [Months, median (IQR)]	11.1 (3.88-53.02)
Minimum-maximum age, months	0-200.97
Length of stay [days, median (IQR)]	48 (32-95.5)
Time to infection [days, median (IQR)][a]	19 (10-30.5)
Length of stay to after onset of IC [days, median (IQR)]	26 (14.5-49.5)
Nosocomial infection	41 (91.1)
Catheter-related candidemia	32 (71)

[a]The time from admission to the date of the first positive culture for nosocomial acquired infection only. IC: Invasive candidiasis, IQR: Interquartile range

Table 2. *Candida* spp. isolates and antifungal susceptibility testing results

			Fluconazole		Caspofungin		Amphotericin B	
	n (%)	AST, n	MIC, Min-max	R, n	MIC, Min-max	R, n	MIC, Min-max	R, n
C. albicans	20 (41.7)	13	0.25-1	0	0.015-3	1	0.25-1	0
C. parapsilosis	20 (41.7)	15	0.5-32	11	0.25-1	0	0.25-1	0
C. tropicalis	3 (6.3)	2	1-32	1	0.015-0.06	0	0.5-1	0
C. krusei	2 (4.2)	1	NRF	NRF	0.13	0	2	1
C. glabrata	1 (2.1)	1	8	0	-	-	0.5	0
C. lusitaniae	1 (2.1)	1	2	0	-	-	0.5	0
C. pelliculosa	1 (2.1)	1	0.5	0	0.015	0	0.25	0

AST: Antifungal susceptibility testing, MIC: Minimum inhibitory concentration (Mg/Ml), R: Resistant, NRF: Naturally resistant to fluconazole, Min-max: Minimum-maximum

Outcome

Six of the patients died within 30 days following a positive culture, and the overall mortality rate was 13.3%. The demographic and clinical risk factors for 7-day and 30-day mortality were presented in Table 3. Patients with neutropenia and dialysis had a higher rate of mortality in 7-day (both p=0.02) and 30-day (p=0.04 and p=0.003, respectively). Mean survival times for 7-day mortality were lower for the patients with neutropenia (Mean ± SE: 5.86±0.97 vs 6.97±0.04), dialysis (Mean ± SE: 6.14±0.97 vs 6.92±0.06), and abdominal surgery (5.67±0.90 vs 6.97±0.03) when compared to patients who had not. Also, mean survival times for 30-day mortality were lower for the patients with neutropenia (Mean ± SE: 19.00±4.84 vs 28.29±0.97), dialysis (Mean ± SE: 15.86±4.36 vs 28.66±0.92) when compared to patients who had not (Figure 1).

Of the total of 32 patients with CLABSI, 29 had a catheter removed following positive culture, and 16 of them were removed within 72 hours. There was no difference for mortality (both p=0.33) between catheter withdrawals within 72 hours and those not withdrawn within 72 hours.

Discussion

In our study, 58.3% and 41.7% of IC episodes were related to non-albicans *Candida* species and *C. albicans*, respectively. *C. albicans* and *C. parapsilosis* were the most common isolated species (both 41.7%). Fluconazole, caspofungin, and AmB resistance rates were 38.2%, 3.1%, and 2.9%, respectively. 73.3% of *C. parapsilosis* isolates were resistant to fluconazole. Risk factors associated with mortality were neutropenia, dialysis, and abdominal surgery. Also, most of the IC cases were catheter-related candidemia and nosocomial candidiasis.

According to recent studies, non-albicans *Candida* species cause more than half of IC cases in children (8). Neu et al. (9), 74% of 203 episodes of pediatric candidiasis were found to be related to non-*albicans* species (43% *C. parapsilosis*). Our study confirmed that non-*albicans Candida* species have been increasing in recent years in IC as in previous studies, and it was remarkable that the frequency of *C. parapsilosis* was at least as frequent as *C. albicans*.

Various risk factors have been identified in candidiasis, and these are the presence of immunodeficiency, underlying

Table 3. Comparison of demographic and clinical features for 7-day and 30-day mortality among survivors and deceased

	7-day mortality			30-day mortality		
	Survivors (n=40)	Deceased (n=5)	p-value	Survivors (n=39)	Deceased (n=6)	p-value
Gender, boys	22 (88)	3 (12)	>0.99	21 (84)	4 (16)	0.68
Age [months, median (IQR)]	11.15 (4.17-56.89)	1.43 (1.07-104.78)	0.43	11.2 (4.27-54.77)	1.37 (0.83-62.53)	0.19
Lenght of stay [days, median (IQR)]	52 (37.25-110.50)	27 (6.5-39.5)	0.02	53 (37-112)	29 (7.75-43.5)	0.02
Prematurity	12 (85.7)	2 (14.3)	0.64	11 (78.6)	3 (21.4)	0.36
Previous hospitalization	23 (92)	2 (8)	0.64	23 (92)	2 (8)	0.38
Abdominal surgery	4 (66.7)	2 (33.3)	0.13	4 (66.7)	2 (33.3)	0.18
Immunosupression	9 (81.8)	2 (18.2)	0.58	9 (81.8)	2 (18.2)	0.62
Neutropenia	4 (57.1)	3 (42.9)	0.02	4 (57.1)	3 (42.9)	0.04
Mechanical ventilation	19 (82.6)	4 (17.4)	0.35	18 (78.3)	5 (21.7)	0.19
Dialysis	4 (57.1)	3 (42.9)	0.02	3 (42.9)	4 (57.1)	0.003
Total parenteral nutrition	17 (81)	4 (19)	0.17	16 (76.2)	5 (23.8)	0.08
Nosocomial infection	37 (90.2)	4 (9.8)	0.39	36 (87.8)	5 (12.2)	0.45
Central venous catheter	28 (84.8)	5 (15.2)	0.30	27 (81.8)	6 (18.2)	0.17
Urinary catheter	15 (83.3)	3 (16.7)	0.36	15 (83.3)	3 (16.7)	0.67
Arterial catheter	8 (72.7)	3 (27.3)	0.09	8 (72.7)	3 (27.3)	0.15
Catheter removal after 72 h[a]	11 (84.6)	2 (15.4)	0.33	11 (84.6)	2 (15.4)	0.33

[a]Catheter removal were evaluated among the patients with central line-associated bloodstream infections (n=32), IQR: Interquartile range

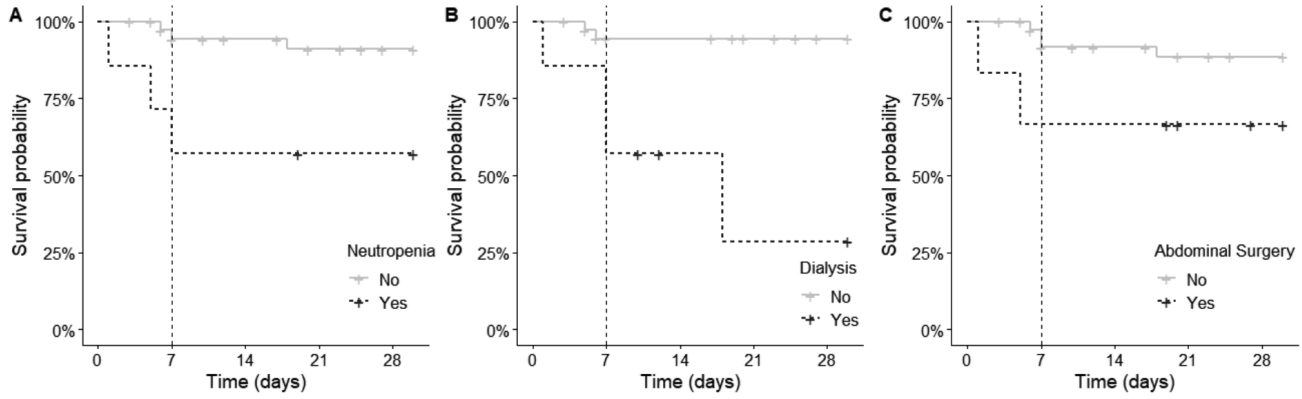

Figure 1. Kaplan-Meier survival curves of the neutropenia, dialysis, and abdominal surgery. Mean survival times for 7-day mortality were lower for the patients with neutropenia, dialysis, and abdominal surgery when compared to patients who had not

disease, neutropenia, prematurity, transplantation, parenteral nutrition, surgery (especially gastrointestinal surgery), bacterial infections, malignancy, colonization with *Candida* spp., broad-spectrum antibiotics, corticosteroids and chemotherapeutic agents, CVCs, dialysis, endotracheal intubation, and stay in the ICU (10-12). Risk factors for IC in this study were similar to those reported in previous studies, and all patients had an underlying disease. Prematurity, congenital heart disease, and solid organ tumor were the most common underlying diseases. Most of our patients had CVCs and received broad-spectrum antibiotic treatment. Particularly, candidemia was catheter-related in 97% of patients with CVC. Also, 41 of 45 IC episodes (91.1%) were considered nosocomial. The high incidence of catheter-related infections and nosocomial candidiasis emphasized the importance of infection control measures and careful care.

Candida species are reported to have differences in clinical features and outcomes. Celebi et al. (12) reported that neutropenia and pre-infection hospitalization were more frequent in non-*albicans* candidemia. Mortality and disseminated candidiasis were higher in patients with *C. albicans*. Similarly, in a study with pediatric 248 candidiasis cases, the mortality rate for *C. albicans* was 34.1% contrast to a rate of 22.4% with non-*albicans* species (13). Dotis et al. (14) showed that using a mechanical ventilator in the two days before *Candida* infection is a significant risk factor for *C. parapsilosis* infection. In a retrospective case-control study at Texas Children's Hospital in 276 episodes of candidemia, there was no difference between *C. albicans* and non-*albicans* candidemia in terms of demographics, underlying diagnosis, risk factors, clinical features, dissemination, or 30-day mortality (15). There was no difference between the invasive infections due to *C. albicans* versus non-*albicans Candida* species and *C. parapsilosis* versus non-*parapsilosis*, in our study. Our sample size might be not enough to determine a small difference.

With the increase of infections caused by non-*albicans Candida* spp., there are problems in the choice of empirical treatment of patients with IC. Studies show that antifungal resistance to non-*albicans Candida* species is higher (9). The sensitivity of *Candida* spp. to antifungal agents can usually be predicted if the species of infectious isolation are known.

However, some *Candida* spp. are not in parallel with the general sensitivity pattern similar to our study. We found that 61.8% of all *Candida* isolates were fluconazole sensitive, and 38.2% were fluconazole-resistant. The most prominent feature of our study was high fluconazole resistance in *C. parapsilosis* strains, which was 73.3%. However, fluconazole is the first recommended antifungal agent for *C. parapsilosis* infections (16). A prospective study which includes 1,218 episodes of *Candida* BSI conducted by using species-specific CLSI reference broth microdilution method for the sensitivity of antifungal agents established that 2.9% of *C. parapsilosis* was non-susceptible to fluconazole (17). Devrim et al. (18) reported that fluconazole resistance was 58.4% in 12 pediatric hematology and oncology patients with catheter-associated *C. parapsilosis* blood supply infection. In our country, revised susceptibility of 453 *Candida* strains isolated from adult and pediatric patients, according to CLSI M27-A3 criteria was evaluated. Fluconazole resistance was 1.4% in *C. albicans*, 18.2% in *C. parapsilosis*, 2.6% in *C. tropicalis,* and %14.3 in *C. glabrata*. There were no AmB-resistant isolates in this study. The highest resistance to fluconazole was in *C. parapsilosis* species (19). Therefore, antifungal susceptibility testing is beneficial for effective antifungal therapy. Considering the current dominance of non-*albicans* strains and fluconazole resistance rates in our hospital, the treatment of echinocandin or AmB as initial treatment is seen as a good option.

It is difficult to assess the death attributed to *Candida*, and the published attributable mortality rates vary depending on the type of study. The overall mortality rate in children with candidiasis ranges from 10% to 26% (8,20,21) In most studies, the presence of an arterial catheter, neutropenia, steroid treatment, insufficiency of antifungal

therapy, prolonged antibiotic therapy, immunosuppressive conditions, disseminated candidiasis, use of TPN and mechanical ventilation, intensive care, and *C. albicans* isolation was established to be associated with mortality due to candidemia (12,22). In our study, the overall mortality rate was 13.3%, in *C. albicans* isolated patients 10%, in non-*albicans Candida* spp. isolated 14.2% and in *C. parapsilosis* isolated 15%. This mortality rate was within the ranges reported in the literature. Although there are studies analyzing the risk factors affecting mortality in the first 30 days, there are not enough studies investigating the risk factors affecting mortality in the first 7 days. In our study, dialysis, neutropenia were risk factors that increased mortality on the 7th and 30th days. Additionally, abdominal surgery is also a risk factor in the first 7 days of mortality. So in the first 7 days of follow-up of patients undergoing abdominal surgery, dialysis, and neutropenia with IC, caution should be exercised for IC and may be evaluated for prophylactic antifungal therapy.

The principal limitations of this study are relatively small sample size and it may not be possible to evaluate multivariate analyzes. Another limitation is that our studies are single-centered and cannot be generalized to other centers.

Conclusions

In summary, we found that *C. parapsilosis* and *C. albicans* are seen more frequently. When considering that the fluconazole resistance of *C. parapsilosis* is 73.3%, the most effective treatment seems to be AmB and caspofungin. Dialysis, neutropenia, and abdominal surgery were risk factors that increased mortality. These data will help us to identify patients who are at risk for IC and will guide us in the selection of empirical treatment.

Ethics

Ethics Committee Approval: This study was approved by the Dokuz Eylül University Non-Invasive Research Ethics Committee (decision no: 2016/13-23, date: 12.5.2016).

Informed Consent: Retrospective study.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Z.G.K., N.B., Concept: Z.G.K., N.B., M.C.E., M.D.D., Design: Z.G.K., N.B., M.C.E., A.N.E., M.D.D., Data Collection or Processing: Z.G.K., N.B., M.C.E., M.D.D., Analysis or Interpretation: Z.G.K., N.B., A.N.E., Literature Search: Z.G.K., N.B., A.N.E., Writing: Z.G.K., N.B., M.C.E., A.N.E., M.D.D.

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Autism Spectrum Disorder Knowledge Among Family Medicine Residents in Izmir, Turkey

İzmir İlinde Aile Hekimliği Uzmanlık Öğrencilerinin Otizm Spektrum Bozukluğu Hakkında Bilgi Düzeyi

Adli Başar Gürbüz[1], Özge Tuncer[2], Börte Gürbüz Özgür[3]

[1]İzmir Karabağlar Bahçelievler Family Health Center No. 13, İzmir, Turkey
[2]University of Health Sciences Turkey, İzmir Bozyaka Research and Training Hospital, Clinic of Family Medicine, İzmir, Turkey
[3]Aydın Adnan Menderes University Faculty of Medicine, Department of Child and Adolescent Psychiatry, Aydın, Turkey

Abstract

Objective: Today, there are initiatives worldwide for the early diagnosis of autism spectrum disorder (ASD). Family physicians encounter children with autism risk from their infancy. The aim of this study was to determine the knowledge and factors affecting the knowledge about ASD of family medicine residents.

Materials and Methods: Knowledge about Childhood Autism among Health Workers (KCAHW) questionnaire and the sociodemographic data form were applied to 210 family medicine residents in İzmir, Turkey.

Results: One hundred thirty-three (63.3%) participants were female. The mean KCAHW score was 12.3±3.15. The mean scores for domains 1, 2, 3 and 4 were; 5.8±1.56, 0.82±0.38, 2.51±1.06 and 3.16±1.45, respectively. Variables that increase the level of knowledge were determined as prior follow-up and/or participation in the evaluation process of a child with ASD, ASD training during residency, and having medical specialty residency experience in another branch before.

Conclusion: It was determined that family medicine residents had deficiencies in knowledge about ASD. Besides, the most important factors that increase knowledge about ASD were previous training about ASD and having previously followed-up patients with ASD. We think that adding a theoretical course on autism and following up a child with ASD with a child psychiatrist during the residency training process will contribute to the level of knowledge.

Keywords: Family practice, knowledge, autism spectrum disorder, awareness, medical residency

Öz

Amaç: Günümüzde tüm dünyada otizm spektrum bozukluğunun (OSB) erken teşhisi için girişimler vardır. Aile hekimleri, otizm riski olan çocuklarla bebeklik döneminden itibaren karşılaşmaktadır. Bu çalışmanın amacı, aile hekimliği asistanlarının OSB hakkındaki bilgi düzeyini ve bilgilerini etkileyen faktörleri belirlemektir.

Gereç ve Yöntemler: İzmir'de 210 aile hekimliği asistanına Sağlık Çalışanlarının Çocukluk Çağı Otizmi Hakkında Bilgi Anketi (SÇ-OBA) ve sosyodemografik veri formu uygulanmıştır.

Bulgular: Katılımcıların 133'ü (%63,3) kadındı. Ortalama SÇ-OBA puanı 12,3±3,15 idi. SÇ-OBA anketinin alan 1, 2, 3 ve 4 için ortalama puanları sırasıyla 5,8±1,56, 0,82±0,38, 2,51±1,06 ve 3,16±1,45 idi. Bilgi düzeyini artıran değişkenler OSB'li hastaları daha önce takip etmek ve/veya değerlendirme sürecinde olmak, asistanlığı sırasında OSB eğitimi almak ve daha önce başka bir branşta uzmanlık asistanlığı deneyimine sahip olmak olarak belirlendi.

Sonuç: Aile hekimliği asistanlarının OSB hakkında bilgi eksiklikleri olduğu belirlendi. Ayrıca OSB hakkında bilgi birikimini artıran en önemli faktörlerin daha önce OSB ile ilgili eğitim almış olmak ve daha önce OSB olan bir hastayı takip etmiş olmak olduğu belirlendi. Asistanlık eğitimi sürecinde otizme yönelik teorik dersin işlenmesinin ve çocuk psikiyatristi ile otizm hastası takip etmenin bilgi düzeyine katkı sağlayacağını düşünmekteyiz.

Anahtar Kelimeler: Aile hekimliği, bilgi düzeyi, otizm spektrum bozukluğu, farkındalık, tıp asistanlığı

Address for Correspondence/Yazışma Adresi: Assoc. Prof. Börte Gürbüz Özgür, Aydın Adnan Menderes University Faculty of Medicine, Department of Child and Adolescent Psychiatry, Aydın, Turkey
Phone: +90 533 620 00 48 **E-mail:** borte.gurbuz@adu.edu.tr
ORCID ID: orcid.org/0000-0002-9176-7359

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Introduction

Autism spectrum disorder (ASD) is a life-long neurodevelopmental disorder whose symptoms begin at the early stages of life, characterized by limitations in social and emotional relationships, insufficiency in communication skills, stereotypical odd behaviors and limited interest areas (1). ASD is an important public health issue due to its prevalence. Although there is no autism-specific treatment today, there is an increasing number of studies which show that early diagnosis and beginning special education and behavioral interventions as early as possible are the most important factors which positively influence ASD prognosis and allow some patients to be excluded from the diagnosis (2,3).

It has been reported that children with ASD who are diagnosed and begin a special education program before the age of four have better cognitive functions, more developed adaptation behaviors and functionality, less ASD related symptoms and more language development (4,5). However, since there is 10 times more risk of having ASD for younger siblings of children diagnosed with ASD, early diagnosis of ASD is important in terms of genetics consultancy as well (6). The most important stage for diagnosis of ASD in a child is to recognize the symptoms and suspect ASD. Therefore, health workers such as family physicians, pediatricians, otorhinolaryngology specialists and nurses having a high level of knowledge and awareness about ASD may have significant contributions to early diagnosis.

In a recent meta-analysis involving the data of a total of 40 different countries in which 56 studies were included, the average diagnosis age was reported as 60.48 months. As a result of this data, it was underlined that efforts to lower the diagnosis age needs to be increased (7).

Diagnosis is generally put as parents observe that their child has different characteristics from other children, when there is speech delay in the child or when the child does not respond when spoken to and parents take the child to a physician with the thought that there might be hearing loss and the physician suspects ASD (8). In the light of these data, the need to carry out studies on health workers' awareness and knowledge about onset of autism. Many studies report that the number of health workers who have a high chance of coming across children under risk for ASD is not at a sufficient level in terms of knowledge on autism (9-12).

It was aimed in this study to determine the knowledge of family medicine residency students about ASD and the parameters that may be related to the level of ASD knowledge by using an objective measuring scale.

Materials and Methods

The study was carried out in line with the Helsinki Declaration. The ethics committee approval of the study was given by University of Health Science Turkey, Bozyaka Training and Research Hospital Clinical Research ethics committee (decision number: 01, date: 12.06.2019). The study and the procedure was explained in detail to all of the volunteers who participated in the study and their consent was taken.

This study, which was designed as a cross-sectional type, was carried out in the city of Izmir, in universities and training and research hospitals which provide family medicine residency education, between the dates 1.4.2019-1.10.2019. According to data from December 2018, a total of 241 individuals undergoing Family Medicine specialization training within the province of İzmir were identified. The study included all individuals within this population, and 87.5% of the participants, a total of 210 individuals, were reached and surveyed. The data form which included sociodemographic data and variables which can influence knowledge level on autism prepared by the researcher and the Knowledge about Childhood Autism among Health Workers (KCAHW) questionnaire were applied to the participants face to face.

KCAHW questionnaire developed by Bakare et al. (13) consists of 19 questions. The survey which is used to measure the knowledge of health workers on autism evaluates 4 domains: (1) impairments in social interaction, (2) development of communication and language, (3) repetitive and stereotypical behaviors, (4) comorbid situations and emergence age period. The total score received from the survey varies between 0 and 19. The answers are graded as I do not know, yes and no. Turkish validity and reliability study was conducted by Gürbüz Özgür et al. (14).

Statistical Analysis

The data of the subjects were evaluated with the SPSS 17.0 (Chicago, IL, USA) software for Windows. The descriptive data were indicated as average, standard deviation, number, percentage, median and 25-75 percentile. The suitability for normal distribution was evaluated with the Shapiro-Wilk test. Non-parametric tests, including Mann-Whitney U for comparing two groups and Kruskal-Wallis tests for comparing three or more groups, were utilized. The relationship between two variables was determined with the Spearman correlation test. For statistical significance, p-value was accepted as lower than 0.05.

Results

Out of the 210 participants, 133 (63.3%) were female and 77 (36.7%) were male. The mean age of all participants was 31.65±7.36 years. The data related to the variables on the participants' sociodemographic characteristics and knowledge level on autism are given in Table 1.

The mean KCAHW total score of all participants was 12.3±3.15. The mean scores received from the domains were successively: domain 1: 5.8±1.56; domain 2: 0.82±0.38; domain 3: 2.51±1.06; domain 4: 3.16±1.45. The comparison of the domains and total scores between the groups in terms of various variables are shown in Table 2. The distribution of

Table 1. Sociodemographic characteristics of the participants		
Variables		**n (%)**
Gender	Female	133 (63.3)
	Male	77 (36.7)
Marital status	Married/with a partner	111 (54.4)
	Single/lives alone	87 (42.6)
	Divorced/widowed	6 (2.9)
Previous training of specialization in medicine	Yes	17 (8.1)
	No	193 (91.9)
Income-expense status	My income is much below my expenses	23 (11.2)
	My income is a little below my expenses	33 (16.1)
	My income and expenses are equal	89 (43.4)
	My income is a little higher than my expenses	57 (27.8)
	My income is much higher than my expenses	3 (1.5)
Work experience in a mental health clinic	Yes	24 (11.5)
	No	184 (88.5)
Completion of a child psychiatry internship during medical school	Yes	97 (46.2)
	No	113 (53.8)
Completion of a child psychiatry internship during family medicine residency	Yes	7 (3.3)
	No	203 (96.7)
Participation in the evaluation process of a child diagnosed with autism	Yes	36 (17.1)
	No	173 (82.4)
	Non-response	1 (0.5)
Parenting a child with autism diagnosis	Yes	1 (0.5)
	No	210 (99.5)
Parenting a child with a chronic illness or special needs	Yes	3 (1.4)
	No	206 (98.6)
Close association with an individual diagnosed with autism	Yes	36 (17.1)
	No	173 (82.8)
Prior follow-up of a patient with autism diagnosis	Yes	37 (17.6)
	No	173 (82.4)
Completion of autism training program	Yes	65 (31)
	No	145 (69)
Familiarity with the autism screening and follow-up program implemented by the Ministry of Health	Yes	91 (43.3)
	No	117 (55.7)
	Non-response	2 (1)

Table 2. Comparison results of KCAHW domains and total scores in terms of variables[†]

		Domain 1 score		Domain 2 score		Domain 3 score		Domain 4 score		Total score	
		Median (25-75 p)	p-value	Median (25-75 p)	p-value	Median (25-75 p)	p-value	Median (25-75 p)	p-value	Median (25-75 p)	p-value
Gender	Female	6 (5-7)	0.43	1 (1-1)	0.87	3 (2-3)	0.72	3 (2-4)	0.1	13 (10-14)	0.47
	Male	7 (5-7)		1 (1-1)		3 (2-3)		3 (2-4)		13 (11-14)	
Previous training on autism	Yes	7 (6-7)	<0.01	1 (1-1)	0.01	3 (2-3)	0.18	3 (3-4)	0.27	14 (12-15)	<0.01
	No	6 (5-7)		1 (1-1)		2 (2-3)		3 (2-4)		12 (10-14)	
Autism training during residency	Yes	7 (6-7)	0.03	1 (1-1)	0.73	3 (2-3)	0.42	4 (2-5)	0.14	15 (12-16)	0.02
	No	6 (5-7)		1 (1-1)		3 (2-3)		3 (2-4)		13 (11-14)	
Prior follow-up of a patient diagnosed with autism	Yes	7 (6-7)	0.001	1 (1-1)	0.03	3 (2-3)	0.06	4 (3-5)	0.01	14 (13-15)	<0.001
	No	6 (5-7)		1 (1-1)		2 (2-3)		3 (2-4)		13 (10-14)	
Participation in the evaluation process of a child diagnosed with autism	Yes	7 (6-7)	<0.01	1 (1-1)	0.1	3 (2-4)	<0.01	4 (3-4.75)	<0.01	14 (13-15.75)	<0.001
	No	6 (5-7)		1 (1-1)		3 (2-3)		3 (2-4)		13 (10-14)	
Having knowledge about autism screening program	Yes	7 (6-7)	0.29	1 (1-1)	0.66	3 (2-3)	0.22	3 (2-4)	0.87	13 (11-15)	0.23
	No	6 (5-7)		1 (1-1)		3 (2-3)		3 (2-4)		13 (10-14)	
Close association with an individual diagnosed with autism	Yes	6 (6-7)	0.32	1 (1-1)	0.25	2 (2-3)	0.64	3.5 (3-4)	0.1	13 (12-14)	0.18
	No	6 (5-7)		1 (1-1)		3 (2-3)		3 (2-4)		13 (10-14)	
Completion of a child psychiatry internship during family medicine residency	Yes	6 (5-7)	0.81	1 (1-1)	0.29	3 (2-3)	0.9	3 (2-4)	0.27	13 (10.5-14)	0.9
	No	6 (5-7)		1 (1-1)		3 (2-3)		3 (2-4)		13 (11-15)	
Work experience in a mental health clinic	Yes	6 (6-7)	0.73	1 (1-1)	0.5	2.5 (2-3)	0.43	3 (2-4)	0.96	13 (11-14)	0.89
	No	6 (5-7)		1 (1-1)		3 (2-3)		3 (2-4)		13 (10-14)	
Previous medical residency experience	Yes	6 (5.5-7)	0.66	1 (1-1)	0.18	3 (2.5-4)	0.02	4 (3-5)	0.04	14 (12.5-15.5)	0.01
	No	6 (5-7)		1 (1-1)		3 (2-3)		3 (2-4)		13 (10-14)	

[†]Mann-Whitney U test, KCAHW: Knowledge About Childhood Autism among Health Workers

the answers given by family medicine residents to KCAHW questions are given in Table 3.

While there is a positive weak relationship between KCAHW total score and duration in the occupation (p=0.02, r=0.158), there was no relationship between family medicine residency duration (p=0.39) and duration of working in a mental health clinic (p=0.39). A positive weak relationship was determined between age and KCAHW total score (p=0.005, r=0.193).

In the question answered by the participants as self-evaluation of their knowledge level on autism (Figure 1), KCAHW total score was significant between the groups and

Table 3. Distribution of the answers to the KCAHW questions

Domain/question	Yes n (%)	No n (%)	I do not know n (%)	Correct n (%)	Incorrect n (%)
Domain 1					
Marked impairment in use of multiple non-verbal behaviors such as eye to eye contact, facial expression, body postures and gestures during social interaction?	192 (91.9)	13 (6.2)	4 (1.9)	192 (91.4)	18 (8.6)
Failure to develop peer relationship appropriate for developmental age?	183 (87.6)	13 (6.7)	12 (5.7)	183 (87.1)	27 (12.9)
Lack of spontaneous will to share enjoyment, interest or activities with other people?	155 (74.5)	25 (12)	28 (13.5)	155 (73.8)	55 (26.2)
Lack of social or emotional reciprocity?	165 (79.3)	14 (6.7)	29 (13.9)	165 (78.6)	45 (21.4)
Staring into open space and not focusing on any thing specific?	172 (82.7)	17 (8.2)	19 (9.1)	172 (81.9)	38 (18.1)
The child can appear as if deaf or dumb?	172 (82.7)	22 (10.6)	14 (6.7)	172 (81.9)	38 (18.1)
Loss of interest in the environment and surroundings?	180 (86.1)	17 (8.1)	12 (5.7)	180 (85.7)	30 (14.3)
Social smile is usually absent in a child with autism?	153 (73.2)	33 (15.8)	23 (11)	153 (72.9)	57 (27.1)
Domain 2					
Delay or total lack of development of spoken language?	173 (82.8)	17 (8.1)	19 (9.1)	173 (82.4)	37 (17.6)
Domain 3					
Stereotyped and repetitive movement (e.g. Hand or finger flapping or twisting)?	165 (78.9)	18 (8.6)	26 (12.4)	165 (78.6)	45 (21.4)
May be associated with abnormal eating habit?	98 (47.1)	41 (19.7)	69 (33.2)	98 (46.7)	112 (53.3)
Persistent preoccupation with parts of objects?	162 (77.5)	16 (7.6)	31 (14.8)	162 (77.1)	48 (22.9)
Love for regimented routine activities?	103 (49.5)	72 (34.6)	33 (15.7)	103 (49)	107 (51)
Domain 4					
Autism is childhood schizophrenia?	23 (11)	144 (68.9)	42 (20.1)	144 (68.6)	66 (31.4)
Autism is an auto-immune condition?	29 (13.9)	129 (61.7)	51 (24.4)	129 (61.4)	81 (38.6)
Autism is a neuro-developmental disorder?	140 (67)	44 (21.1)	25 (12)	140 (66.7)	70 (33.3)
Autism could be associated with mental retardation?	77 (36.8)	103 (49.3)	29 (13.9)	77 (36.7)	133 (63.3)
Autism could be associated with epilepsy?	72 (34.4)	103 (49.3)	34 (16.3)	72 (34.3)	138 (65.7)
Onset of autism is usually in	Neonatal age 19 (9.2)	Infancy 85 (41.3)	Childhood 102 (49.5)	102 (48.6)	108 (51.4)

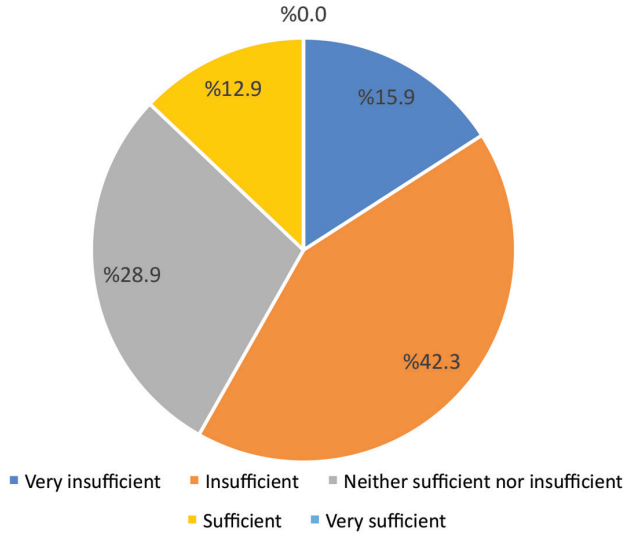

Figure 1. The participants' perception of knowledge about autism

as perception of sufficiency increased, KCAHW total score also increased ($p<0.001$, Kruskal-Wallis test).

Discussion

We investigated the level of knowledge of family medicine residents about autism using KCAHW. The validity and reliability studies of this survey for different languages have been performed and it is widely used. According to the results of the review in which the studies using KCAHW were analyzed, the authors reported that although there are no strong evidences about the global usability of the survey, it can be used in evaluating knowledge level on ASD and educational needs (15).

In our study, the mean KCAHW total score of all participants was 12.3±3.15. In a study involving senior students of medicine, nursing and psychology, the mean score was 12.24±3.24, 10.76±3.5 and 9.01±3.76 respectively (16). When the literature was reviewed in terms of studies in which the knowledge levels of family medicine resident students on autism, it was seen that there is a limited number of studies (9,17,18). None of these studies were conducted by using reliable and valid questionnaire to evaluate the knowledge about autism.

The mean score of KCAHW domain 1 which evaluates knowledge level on impairment in social interaction was 5.8±1.56 in our study. It was observed that the first question in this domain received the highest amount of correct answers (91.4%). In Citil et al.'s (19) study on pediatricians and pediatric residents, it was similarly reported that awareness and knowledge level about symptoms of ASD in terms of social interaction was the highest.

The question in domain 2 on language development and delay in speech received 82.4% accurate answers. One of the reasons for high rate of correct answer may be that parents with children who have ASD most frequently consult physicians is speech delay in their children (8). Therefore, it is a positive result that a majority of family medicine residents who will be coming across these children in primary care know that there might be speech delay in ASD. A striking finding in our study was the answer given to ASD's time of emergence. More than half of the participants gave the incorrect answer to this question. This result indicates the lack of knowledge of family medicine residents about the age period in which ASD's initial symptoms are observed.

Our study revealed that residents who had received previous training on autism, had undergone autism training during residency, had prior experience in the follow-up of a patient diagnosed with autism, had participated in the evaluation process of a child diagnosed with autism, and had previous medical residency experience obtained statistically significantly higher total scores. It has been reported that those who have taken part in the diagnosis and follow-up process of at least one patient diagnosed with ASD had statistically significant scores in all domains (20). In Pirincci et al.'s (21) study, according to their report, there was a significant increase in the knowledge levels of medical students who participated in the follow-up of patients with ASD during their child psychiatry internship period. It has been reported in other studies as well that the ASD knowledge levels of health workers who have done the follow-up of children diagnosed with ASD are higher (14,22-24). Similarly, it was determined in our study that prior follow-up of patients diagnosed with ASD in the past leads to statistically significantly higher scores in all domains and the total score with the exception of domain 2.

Knowledge about autism is statistically more significant in students who have had training (seminars, conferences, etc.) on autism in the past ($p<0.01$) and have had courses on autism during residency ($p=0.02$). Igwe et al. (16) determined in their study that KCAHW scores had a positive relationship with the number of weeks spent both in pediatrics and psychiatry and number of course hours on psychiatry/abnormal psychology. In another study, it was reported that only 10% of 277 family medicine residents accurately answered 50% or more of the survey questions and only 10.1% attended a workshop or conference on autism in the past (18). In a study carried out in England, it was reported that about two thirds of practicing physicians have not received any training on autism during their faculty of medicine or family medicine specialty education (25). It was determined in our study that 31% of the residents received some kind of training on autism in the past.

There is a limited number of studies which analyze the knowledge about autism of family medicine specialty students. In Turkey, there is a study by Sabuncuoglu et al. (17) reported that gender, age and the duration after graduation from the faculty of medicine are significantly

influential on scores received from ASD and attention deficit hyperactivity disorder scales. Another study carried out in Turkey by Hidiroglu et al. (9) reported that the knowledge level of residents receiving education in branches other than neuropsychiatry including family medicine residents on autism is medium. Alshammari et al. (18) reported that the perception of knowledge level on autism in family medicine residents is low and this might be related to lack of courses on autism in their residency education program. Since early diagnosis is important in the prognosis of ASD and family physicians contact children in early ages, we believe that it is necessary to include a theoretical course about autism in the residency education program to be able to train competent physicians, besides the rule to participate in the evaluation process of a child diagnosed with ASD.

The strong aspects of our study are being the first study which evaluates autism knowledge with an objective measurement tool among the family medicine residency students and the sample of the study embodying all family medicine residents in the city of İzmir. Since the sample of this study does not include the family medicine residents in the other cities in Turkey, the results cannot be generalized. Another limitation of this study is that it was not designed as a longitudinal study to evaluate the relationship between the level of knowledge and the family medicine education process over time.

Conclusion

One of the most important factors in the prognosis of ASD is the early diagnosis of children with ASD and the early initiation of treatment interventions. As a result of this study, it was determined that family medicine residents' knowledge level on autism is not sufficient. Prior follow-up of patients with ASD and/or involvement in the evaluation process of patients with ASD and having had ASD training during residency are variables which increase knowledge level.

In the light of these findings, we believe that it is important in terms of increasing awareness and knowledge level on autism that training is given in certain intervals both prior to and after graduation about autism; courses on neurodevelopmental disorders are added to family medicine residency education curriculum; rotation in child and adolescent psychiatry is made possible for residents to acquire clinical competency; education is provided on autism screening and follow-up programs and children with risk of autism are referred to child and adolescent psychiatrists.

Ethics

Ethics Committee Approval: The ethics committee approval of the study was given by University of Health Science Turkey, İzmir Bozyaka Training and Research Hospital Clinical Research ethics committee (decision number: 01, date: 12.06.2019).

Informed Consent: The study and the procedure was explained in detail to all of the volunteers who participated in the study and their consent was taken.

Peer-review: Externally peer-reviewed.

Authorship Contributions

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

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

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