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Analysis of Dental Treatment Under General Anesthesia in Special Health Care Needs and Uncooperative Healthy Children

Özel Gereksinimli Çocuklar ve Kooperasyon Sağlanamayan Sağlıklı Çocuklarda Genel Anestezi Altında Diş Tedavisinin İncelenmesi

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Abstract

Objective: This retrospective study aimed to evaluate the characteristics and treatment modalities under general anesthesia (GA) in cases involving children with special health care needs (SHCN) and uncooperative healthy children (UHC).

Materials and Methods: Data regarding children's age, gender, health status, and type of dental treatment were collected, GA and analgesic drugs used, and the results were statistically evaluated. Demographic data were analyzed descriptively, and results were reported as mean ± standard deviation.

Results: Out of 225 cases of scheduled GA, 131 were children who presented with SHCN and 94 were UHC. We found that children with SHCN required more restorative treatments than uncooperative children. There was a statistically significant difference in the American Society of Anesthesiologists scores in children with SHCN.

Conclusion: In general, our study concludes that the medical history of patients can affect dental treatment scenarios and the post-operative approach.

Keywords: Special health care needs, cooperation, dental treatment, general anesthesia

Öz

Amaç: Bu retrospektif çalışma, Özel Bakım Gerektiren Çocuklar (ÖBGÇ) ve dental tedavi için yeterli kooperasyon sağlanamayan sağlıklı çocuklarda genel anestezi altında yapılan dental tedavileri ve genel anestezi prosedürlerini değerlendirmeyi amaçlamıştır.

Gereç ve Yöntemler: Çocukların yaşı, cinsiyeti, sağlık durumu, diş tedavisi türü, genel anestezi ve analjezik için kullanılan ilaçlar ile ilgili veriler toplanmış ve sonuçlar istatistiksel olarak değerlendirilmiştir. Demografik veriler analiz edildi ve sonuçlar; ortalama, ± standart sapma olarak bildirilmiştir.

Bulgular: Toplam 225 planlı genel anestezi olgusundan 131'i ÖBGÇ'den oluşurken, 94'ü yeterli kooperasyon sağlanamamış sağlıklı çocuklardan oluşmaktadır. ÖBGÇ'nin, kooperasyon sağlanamayan çocuklara göre daha fazla restoratif tedaviye ihtiyaç duyduğu gösterilmiştir. ÖBGÇ'de; Amerikan Anestezi Uzmanları Derneği skorlamasında istatistiksel olarak anlamlı bir yükseliş olduğu bulunmuştur.

Sonuç: Genel olarak çalışmamızda; hastaların tıbbi geçmişinin, diş tedavi senaryolarını ve ameliyat sonrası yaklaşımı etkileyebileceği sonucuna varılmıştır.

Anahtar Kelimeler: Özel Bakım Gerektiren Bireyler, kooperasyon, dental tedavi, genel anestezi

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Introduction

Dental treatment under general anesthesia (GA) is often required to complete safe and pain-controlled dental treatment for some patients (1). One of these situations is the population with special needs. Individuals with special health care needs (SHCN) are prone to have inadequate oral hygiene therefore they need comprehensive dental treatments. However, SHCN patients show high anxiety levels and inadequate cooperation (2).

Managing very young children with dental caries in the conventional care setting is extremely difficult (3). Frequently, during the treatment of severe and urgent dental caries in non-cooperative children, GA or sedation is required due to the fact that they fear or cannot endure these procedures in the dental chair.

Even though; GA is a frequent technique for uncooperative and SHCN patients, there are no certain protocols for receiving dental treatment under GA (4). Most of the studies on the provision of dental treatment for SHCN and uncooperative children under GA are retrospective and the majority of them did not clearly address the protocol following dental treatment under GA. All necessary interventions can be created and completed at just one visit under GA (2).

It is necessary to analyse and evaluate the benefits and risks that the patients will receive from GA in preliminary assessment appointments.

It is important to analyse and describe the risks and efficacy, for individuals receiving dental treatments under GA, in preliminary assessment appointments. The primary aim of this study is to compare the different patient categories that come for dental treatment while the secondary aim is to evaluate the characteristics and treatment modalities performed under GA for SHCN and uncooperative healthy children (UHC).

Materials and Methods

The study was reviewed and approved by The Ethics Committee of Marmara University Faculty of Dentistry (protocol no: 169/2018, date: 05.04.2018).

Patients with incomplete records and lack of paperwork information were excluded.

The following data were extracted from patient files

a. Patients demographics

b. Patient's medical condition

- SHCN
- Uncooperative children (5)
- Consultation process

c. Dental treatments under GA

d. American Society of Anesthesiologist's (ASA) Classification (6)

e. Anesthesia duration

- Duration of anesthesia is classified as short (30-60 min), medium (60-180 min), long (>180 min).

f. Information of GA

- Anesthetic drugs
- Analgesic agents
- Types of intubation
- Complications due to anesthesia
- Postoperative pain management

Statistical Analysis

Demographic data were reported as mean ± standard deviation. Mann-Whitney U test was applied and for categorical data, Pearson chi-square test was used to compare in subgroups. Significance level was set at p=0.05.

Results

The database of this study involved 233 records of pediatric dental treatments done under GA. Out of these, 8 records were incomplete and were hence excluded, leaving a total of 225 patient records that were used in the study.

The study group (n=225) was divided into two groups as group SHCN (n=131/58%) and as UHC (n=94/42%). The differences in age and gender between the groups was not statistically significant (p>0.05) (Table 1).

Table 1. General characteristics of the groups: age, gender, weight

		SHCN n=131	UHC n=94	Total n=225	p-value
Mean age		7.25±3.39	6.32±2.78	6.87±3.18	0.079
Mean weight		24.28±10.55	25.41±10.41		0.54
Gender	Girls n=89 (39.5%)	7.08±3.08 n=45 (34%)	6.51±2.72 n=44 (47%)	6.80±2.91	0.072
Total	Boys n=136 (60.5%) 225 (100%)	7.35±3.07 n=86 (66%)	6.16 ± 2.85 n=50 (53%)	6.91±3.36	

SHCN: Special health care needs, UHC: Uncooperative healthy children

Table 2 shows the distribution of treatments in terms of the teeth and jaws. SHCN had significantly more restorative treatments than UHC children ($p<0.05$) (Table 3).

Of all individuals, 13 (UHC=5, SHCN=8) patients received GA twice.

The distribution of ASA scores are summarized in Table 4. A higher frequency 102 (78%) of ASA physical status grade II was observed in children with SHCN. The highest score was in children with DS (50%). Forty patients (17.8%) underwent inhaled induction with sevoflurane, while 2 patients (0.89%) underwent intramuscular induction with ketamine (Table 5). Most of the patients (81.3%) underwent intravenous anesthesia with propofol (2-2.5 mg/kg) (Table 5). Anesthesia team able to access an intravenous line before anesthesia induction because all patients were given oral/intravenous/intramuscular premedication with midazolam. Balanced anesthesia was maintained with sevoflurane and remifentanil under nasal intubation. All patients had smaller sized tracheal tubes and nasotracheal intubation. Opioid analgesics (fentanyl 0.05 mg/kg added to induction drugs and remifentanil 0.125 mcg/kg/hour infusion for balanced anesthesia) were used in addition to local anesthesia for all patients. Systemic analgesia was with conventional pediatric doses of paracetamol (77.8%) and tenoxicam (7.56%). No serious post-operative complications were noted.

Discussion

American Academy of Paediatric Dentistry has listed the indications for GA for children and adolescents who

Table 2. Distribution of treatments across the groups

Total children n=225	SHCN n=131 mean ± SD	UHC n=94 mean ± SD	p-value
Restored teeth	7.15±4.742	5.30±4.80	0.008
Extracted teeth	4.24±4.044	3.80±3.40	0.056
Pulpotomy	1.198±1.9431	1.484±2.0783	0.259
Root canal treatment	0.146±0.5151	0.15±0.51	0.781
Pit and fissure sealants	0.93±2.44	0.28±1.01	0.003

SHCN: Special health care needs, UHC: Uncooperative healthy children, SD: Standard deviation

Table 3. Types of teeth under GA across the groups

	Extraction n=934	Restorations n=1433	Pulpectomy n=300	Root canal n=33
Primary teeth	850	940	289	10
Maxillary molar	164			
Mandibular molar	235			
Permanent teeth	84	483	11	23
Maxillary molar	18			
Mandibular molar	26			

Table 4. GA duration, ASA classification, and analgesic agents used

Duration of GA (min) mean duration of anaesthesia 140.51±68.25 (range: 20-280)		SHCN n=131	UHC n=94	p-value
		146.89±62.89 range: 25-280	132.67±74.48 range: 20-280	0.00
ASA (n=225)	ASA1	9	42	
	ASA2	102	52	
	ASA3	20	0	
	p-value	0.027	0.00	

GA: General anesthesia, ASA: American Society of Anesthesiologist, SHCN: Special health care needs, UHC: Uncooperative healthy children

Table 5. General anesthesia induction			
Anesthesia induction	**SHCN (n=131)**	**UHC (n=94)**	**Total (n=225)**
Inhalational anesthesia with sevoflurane	24 (18.8%)	16 (17.02%)	40 (17.8%)
Ketamine intramuscular	1 (0.76%)	1 (1.06%)	2 (0.88%)
Propofol intravenous	106 (80.9%)	77 (81.9%)	183 (81.3%)
SHCN: Special health care needs, UHC: Uncooperative healthy children			

cannot cooperate due to lack of psychological or emotional maturity and/ or mental, physical, or medical disability (7). A full mouth comprehensive dental treatment under GA is an effective method for treating multiple destructive dental caries of very young children (8) and SHCN (2) both.

According to our retrospective study data, a total of 131 (58%) SHCN patients received dental treatment under GA of which 21 (16%) had autism, 20 (15%) had mental retardation, 16 (12%) had epilepsy, 8 (6%) had cerebral palsy, 7 (5%) had attention deficit hyperactivity disorder, and 6(4%) had Down syndrome (DS). The remaining 53 (40%) patients of SHCN had underlying medical conditions. Özkan et al. (9) observed autism in 6% and mental retardation in 24.4% and DS in 2.4% of the cases that received dental treatment under general anaesthesia. Akpinar (10) reported 620 cases with complicated medical story and 120 DS cases who received dental treatment under GA in the retrospective study. Sevekar et al. (11) showed the distribution of the patients who received GA for dental treatment as flowing 45.46% with cooperation problems and 54.34% with SHCN which included only 1 patient with DS and 2 patients with Autism. The main reason for dental treatment under GA was behaviour problems.

Ethnic differences, genetic variations, and medical conditions could be related of the variability in the composition of selected study populations (12). Although the age range of the patients included in our study varied between 1.5 and 15, the intensity was between 6-11. Özkan et al. (9) reported the mean age and 16.78±12 years, and Baygin et al. (13) reported aged 3 to 15 years in their retrospective data. The gender disproportion was emphasized in many studies, but it has not been clearly stated why men outnumber women (2). Similarly; male patients constituted 60.5% of the population included in our study.

Unfortunately, there is lack of information is available guiding dental treatment protocols to be followed under GA. Dental procedures provided by different teams across the world vary to different degrees and dental treatment under GA has been mainly associated with oral surgery and tooth extraction (14). The modalities of caries management for those children differ in dentitions. In addition, medical restrictions affect the occurrence of differences in treatment indications. Ciftci and Yazicioglu (12) showed that although there were no statistically differences in the total number of restored teeth according to the groups, in group healthy and group SHCN <6-year age categories had received a greater number of restorative treatments compared to the 6-12-year age categories. There was a trend to be extraction and restorative treatments in our study similar to Sari et al. (15), and pit-fissure sealants were performed more in SHCN. Özkan et al. (9) also reported 239 (51.2%) extraction in their study. Sevekar et al. (11) showed a higher number of children with special needs had higher frequency of extraction. In conclusion extraction could be preferred because of faster, cheaper, and often provides a short waiting list for GA procedure (16).

Recurrence of the GA for receiving dental treatment is an important point for some situations regarding side effects coming from GA procedure complications and cost-effectiveness to thehealth insurance system. Landes and Bradnock (17) reported that children who had received GA for baby bottle syndrome; had to undergo repeated GA within one year. Therefore they recommend aggressive dental treatment plans including more extraction (17). In our study, 13 children received GA twice to treat recurrent caries and infections after a while getting the first GA.

An effective anesthetic preoperative examination is the basic procedure to medically optimize the patient (18). Detailed medical, social, and anesthetic history, a physical examination focusing on both general health and dentition, and any further diagnostic tests relevant to either the planned dental procedure or anesthesia should be included in preoperative assessments (19). Thus, the risks of anesthesia are reduced and the quality of care and treatment received from anesthesia is increased. ASA physical status classification system, assess the risk associated with the patients' medical history (6). The use of GA is considered relatively safe, and it has been widely described as a useful modality for the treatment of patients with special needs. Patients with certain syndromes may present with associated underlying disease (20). If necessary, a consultation and agreement with a specialist of the coexisting disease should be received. The consultations have a ratio of 100% in SHCN and 55% in all patients, and ASA score was higher in children with SHCN, this score was statistically significant in children with DS in our results.

After the patient's arrival to the hospital the morning of the operation, the respective pediatric dentist and anesthesiologist did a last preoperative visit to order premedication drugs. All of our patients received premedication via intravenous or oral route. Akpinar (10)

also explained the premedication and GA protocols and post-op follow-up and discharge conditions in his retrospective study on the principles of dental treatment under GA in patients with special needs.

Anesthesia and operative durations are discussed with postoperative complications (21) in which 40% of our patients had operating times that were over 180 min. The United States Food and Drug Administration announced in its December 14, 2016 statement that exposure to certain sedatives and general anesthetics, particularly during procedures longer than 3 hours, may affect the brain development of children under the age of 3 (22).

Propofol was used in 81.3% of the cases for intravenous anesthesia in the study. Akpinar (10) reported in detail that pental sodium and propofol were used 77.4% and 22.6% cases undergoing intravenous induction of GA respectively.

Since our patients were scheduled for outpatient anesthesia previous problem-free anesthesia history meant a lot for unintended airway for intubation problems. Many complications can be occurred in patient with SHCN after receiving GA including extended emergence time from anesthesia, difficult airway management and cardiac problems (20,23). While there were reported complications in a total of 4 patients, Campbell et al. (1) mentioned at a rate of 1.1%. No serious postoperative complications were noted in our study and all cases were discharged with safely.

Postoperative pain control is quite important for SHCN children. Postoperative pain will mimic the epileptiform activity, especially in cases of epilepsy (24,25). Most of the analgesics were applied before the end of dental treatment and anesthesia. Acetaminophen of 1-1.5 mg/kg was mostly preferred, but if the patient's weight was over 30 kg, tenoxicam and pethidine were added in the regimen. SHCN patients cannot properly describe their pain and, younger children sometimes complained about the sensation of anesthesia caused by the local anesthetic agent. Therefore, standard regimen was recommended for all patients. Eventually, post-operative pain was seldom reported. Özkan et al. (9) also reported that 66.6% of cases had paracetamol and 30.2 cases had local anesthesia during the treatment for pain control.

All patients had nasotracheal intubation which is used in mostly of the dental procedures. Özkan et al. (9) reported 82 (17.6 %) cases with classical nasal intubation, on the other hand Akpinar (10) reported that oral intubation mostly preferred. Tracheal tube size is also important in SHCN children. They need smaller sized tubes.

GA is becoming frequently preferred method for patients with SHCN. The medical history and risks should be evaluated in detail. In addition, it is recommended that the dental treatment plan should not cause repetitive treatment needs of the patient.

Conclusion

Special needs may affect the dental treatment provided under GA. Medical history of patients may affect dental treatment scenarios and post op rehabilitation approach.

Ethics

Ethics Committee Approval: The study was reviewed and approved by The Ethics Committee of Marmara University Faculty of Dentistry (protocol no: 169/2018, date: 05.04.2018).

Informed Consent: Retrospective study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: M.A.Y., G.B., B.K., Design: M.A.Y., G.B., B.K., Data Collection or Processing: M.A.Y., G.B., M.Ö., S.B.K., Analysis or Interpretation: M.Ö., B.K., Literature Search: M.A.Y., M.Ö., S.B.K., B.K., Writing: M.A.Y., G.B., M.Ö., B.K.

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Original Article / Özgün Araştırma

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Influence of Sonic Activation Duration on Root Canal Temperature Increase

Sonik Aktivasyon Süresinin Kanal İçi Isı Artışına Etkisi

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Abstract

Objective: This study investigated intracanal heat changes when the EDDY sonic activation system was used for different durations.

Materials and Methods: Sixty (15 per group) maxillary canine teeth were used in this study. Teeth were decoronate and enlarged up to the T-endo must M40 file. Three thermocouples were inserted on the tooth: one at the apical foramen (T1), one at the working length (WL) of -5 mm (T2), and one at the WL of -10 mm (T3). A fixed setup was established, and an irrigation solution inside the root canal was activated for 10 (S10), 20 (S20), 30 (S30), and 60 (S60) seconds per group with an EDDY tip.

Results: The S10 group exhibited less temperature change and the S60 group demonstrated a statistically significant temperature increase.

Conclusion: Prolonged activation durations resulted in a greater temperature increase due to root canal dry out. This temperature is more likely to transmit periodontal tissues than the increasing effect of sodium hypoclorite because of the scattered solution. Shorter activation durations with more sicluses would be more beneficial.

Keywords: Irrigation, EDDY, intracanal temperature

Öz

Amaç: Bu çalışmada sonik irrigasyon aktivasyon sistemi olan EDDY'nin farklı sürelerde kullanıldığında oluşan kanal içi ısı değişimleri incelenmiştir.

Gereç ve Yöntemler: Bu çalışma için 60 adet üst kanin diş (n=15) kullanılmıştır. Dişler dekorone edilip kanallar T-endo must M40 numaralı eğeye kadar genişletilmiştir. Dişlerin üzerine biri apical foramen (T1), biri çalışma boyundan 5 mm kısa (T2) ve diğeri çalışma boyundan 10 mm kısa (T3) olacak şekilde 3 adet termokupl bağlanmış ve sabit bir düzenek oluşturularak kök kanalları içerisindeki irrigasyon solüsyonu gruplara göre 10 (S10), 20 (S20), 30 (S30), ve 60 (S60), saniye süreyle aktive edilmiştir.

Bulgular: S10 grubunda daha az ısı değişimi saptanmış olup S60 grubunda anlamlı düzeyde fazla ısı artışı görülmüştür.

Sonuç: Aktivasyon süresinin uzaması kanal içinde bulunan solüsyonun saçılarak kanalın kurumasına sebep olarak daha yüksek ısı artışına neden olmuştur. Açığa çıkan bu ısının solüsyonun saçılmasından dolayı sodyum hipokloritin etkinliğini artırmaktan ziyade periapikal dokulara iletilmesi muhtemeldir. Kısa aktivasyon sürelerinin daha fazla aktivasyon döngüsü ile birlikte uygulanması daha yararlı olacaktır.

Anahtar Kelimeler: İrigasyon, EDDY, kanal içi ısı

Introduction

Successful endodontic therapy involves removing bacteria and organic remnants from the root canal space (1). Chemomechanic preparation is crucial for this aim. It includes removing debris, bacteria, microorganisms, and endotoxins inside the root canal by root canal preparation and irrigation (2). But even when the performance of chemomechanic preparation follows all protocols, some bacteria can still

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survive (2). Sodium hypochlorite (NaOCl) is the most common irrigation solution because of its high antimicrobial effectiveness (3). The effectiveness of NaOCl is related to temperature, activation as much as contact time, renewal rate, and concentration (3). NaOCl is usually applied with a conventional syringe (4). However, conventional syringes are ineffective in reaching all root canal irregularities (5) and apical thirds due to the vapor lock effect (6). Several studies have shown that the effectiveness of NaOCl can be increased via activation methods (7-9). Therefore, irrigant activation systems have gained popularity.

EDDY (VDW, Munich, Germany) is a recently introduced sonic irrigation activation device that is used with a conventional air scaler with a flexible polyamide irrigation tip (10). Its oscillation power is much higher than that of other equivalent sonic devices (11). Unlike ultrasonic devices, EDDY has a special polymer rod that does not cause dentinal deformations during oscillation (12). It has also demonstrated promising results in terms of canal cleanliness (9,10,12). Different activation durations were performed in several studies (13-15), some of which used 10 seconds for activation (15) while others used 20 (16) seconds or 30 seconds (17). None, however, evaluated temperature changes with different activation durations. The goal of the present study was to determinate the most suitable activation time to protect thermal damage of the periodontal tissue.

Materials and Methods

Sample Size Calculation

Power calculation was performed based on a previous study (18) with the aid of a G-power 3.1.9.4 program. The minimum sample size was determined as 12 for the whole study. However, in order to increase the reliability of the study and in consideration of the number of samples in the reference article, 15 samples per group were chosen.

Sample Preparation

Ethical committee approval was acquired from the Sakarya University Faculty of Medicine Ethical Research Board (approval number: 567, date: 09.12.2021). Forty-five single-rooted maxillary canine teeth (15 per group) with a single root canal and less than a 5° root canal curvature were used based on Schneider (19). Buccal and proximal radiographs were taken to confirm that the teeth had a single root canal. Extracted teeth were examined with an operating microscope (Zumax OMS2350, Zumax Medical Co. Ltd, Jiangsu, China) under x20 magnification to confirm the absence of root cracks and fractures. Teeth with cracks and fractures were replaced with new teeth. Root surfaces were cleaned with curette and ultrasonic scalers to remove calculus and soft tissue remnants and were stored in 0.5% thymol solution until use.

Teeth were decoronated to standardize the working length (WL) at 21 mm (±1 mm) with a slow-speed diamond saw (IsoMet, Buehler, Lake Bluff, IL, USA). To mimic a pulp chamber, the coronal 3 mm of the canal was enlarged using a round bur (no. 23, Dentsply Maillefer) with a diameter of 2.3 mm. Apical patency was controlled with K-files ISO 10. Only teeth with a canal width of approximately ISO 15 near the apical foramen were included. The canals were instrumented with T-endo must reciprocating files (Dentac, İstanbul, Turkey) up to M40 using an Ai endodontic motor (Woodpecker, Guilin, China) in "T-endo must" mode. During root canal enlargement, root canals were irrigated using 2 mL of 3% sodium hypochlorite (NaOCl, Coltene/Whaledent, Altstätten, Switzerland) for 20 seconds with a 30-gauge endodontic needle (NaviTip, Ultradent, UT, USA). Following root canal enlargement, the coronal 3 mm of the samples were enlarged with a round bur (no. 23, Dentsply Maillefer).

After root canal instrumentation, three holes were created to adapt K-type thermocouples connected to a datalogger to measure intracanal temperature from the apical middle and coronal thirds during irrigation activation. Two of the holes were drilled on the buccal side of each root with a diameter of 0.5 mm at a distance of 5 and 10 mm from the apical foramen, and one was drilled at the apical foramen using rose burs (diameter = 0.5 mm; Komet, Lemgo, Germany).

The roots were positioned in modified plastic molds. Three holes were also prepared on the plastic molds and Type K thermocouples were passed through the holes on the plastic mold and inserted at the holes which are on the teeth and positioned just before entering the main root canal (Figure 1). Thermocouple which locates at the apical foramen called T1, middle tip named as T2 and coronally located thermocouple named as T3 (Figure 1). Then, the thermocouples were fixed in their position by a resin composite. The position of the thermocouples was controlled by radiographs. The molds were filled with alginate (Henry Schein, Melville, NY) to stabilize the thermocouples.

To measure the temperatures, the thermocouples were connected to a multi-channel datalogger (Pico Data Logger, TC-08, St Neots, UK), which transmits temperature levels to a computer. To simulate body temperature, the samples were stored at a temperature of 37 °C for 24 hours. All procedures were performed in an incubator.

The next day when thermocouples measured 37±1 °C activation procedure started, a room temperatured 3% NaOCl was delivered inside the root canal, and activation was performed by a sonic activation system (EDDY, VDW, Munich, Germany). Groups are as follows; S10: 10 seconds, S20: 20 seconds, S30: 30 seconds, S60: 60 seconds sonic activation. The sonic tip was placed 3 mm from the thermocouple, which was located at the apex. During irrigation activation process 4 mm vertical strokes were applied with the EDDY tip. Temperature changes were recorded during whole procedure with 5 seconds intervals. Minimum and maximum temperatures were recorded after irrigation and activation for each group and the data analyzed statistically.

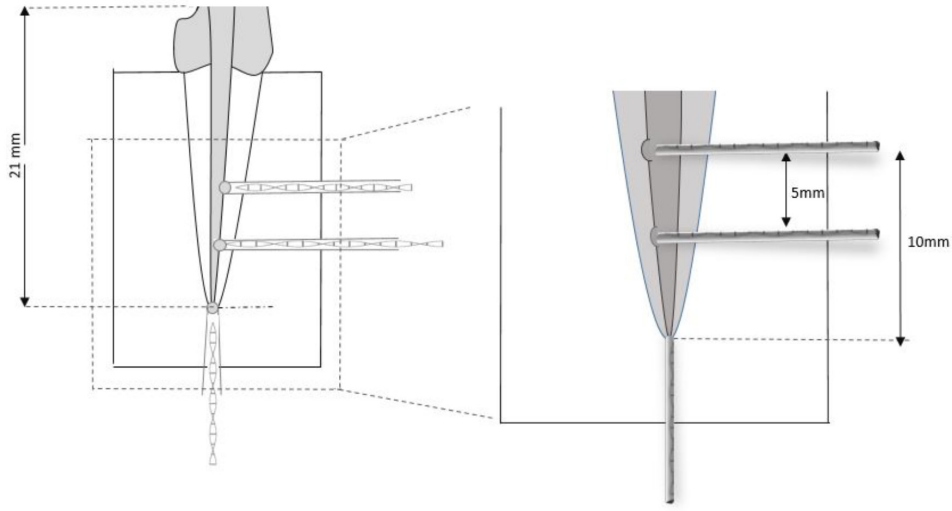

Figure 1. Shematic image of thermocouple positions

Statistical Analysis

Statistical analysis were performed using One-Way ANOVA and a Bonferroni post-hoc test on the IBM SPSS Statistics Version 25 program. Normal distribution control was performed using a Shapiro-Wilk test and Kurtosis-Skewness values ($p>0.05$).

Results

Mean temperature change between onset -irrigation and irrigation- activation can be seen in Table 1. Both changes for all groups were statistically significant. İrrigation significantly reduced, and activation significantly increased the temperature ($p<0.05$).

A One-Way ANOVA test was used to evaluate the difference between activation duration and thermocouple location. Temperature changes between irrigation and activation were found to be statistically significant ($p<0.05$). Post hoc tests were used to determine which periods were statistically significant.

For all activation duration periods, statistically significant difference were seen between T1 and T2 ($p<0.05$). The temperature reduction with irrigation and temperature increase with activation was higher at T2 level. For all activation duration periods, a statistically significant difference was observed between T1 and T3 levels ($p<0.05$). The temperature was reduced more with the irrigation and was increased with the activation at level of T3. The difference between T2 and T3 was statistically insignificant ($p>0.05$).

With the activation; S60 group revealed a statistically significant difference from the S10 group at the T1 level. At the T2 level, there were statistically significant differences between the S10 group and the other groups. The S20 group also differed from the S60 group ($p<0.05$).

A Tamhane test was used to determine the difference between the duration periods. The difference between the T1 and T2 was found to be statistically significant for irrigation and activation ($p<0.05$). The temperature decreased with the irrigation and increased with activation increases at that level. The difference between the T1 and T3 was also found significant for all activation periods ($p<0.05$). The temperature decreased more at the T3 level with irrigation and increased more with activation. There was no statistically significant difference between the T2 and T3 levels ($p>0.05$).

Activation significantly increased the temperature for all groups, and they all differed from the S10 group ($p<0.05$). The S20 group also differed from the S60 group ($p<0.05$).

At the level of T3 mean temperature was found as 49,42±4,54 °C for S60 group. Statistically significant difference was seen at this level between the S60 group and all the others ($p<0.05$), S10 and all the others were significant ($p<0.05$), difference between S20 and S30 groups was found insignificant ($p>0.05$).

Longer activation periods caused higher intracanal temperatures.

Discussion

This study was designed to investigate intracanal temperature increase with different activation durations when a sonic activation system (EDDY) was used. There are some previous studies that evaluated temperature increase caused by different activation devices (18,20,21). But none of them compared the thermal effect of different durations of sonic activation. Although increasing the temperature of the solution is recommended to increase its effectiveness (22), it is known that temperature values higher than 47 °C can cause damage to periodontal tissues (23). For this reason, the most accurate protocol that would respect biological tissue was sought as no prior study has done so.

Table 1. Mean temperature levels for groups

Activation			Onset			Irrigation		
T1	T2	T3	T1	T2	T3	T1	T2	T3
S10	36.59±0.60^{a,A}	36.52±0.76^{a,A}	37.14±0.67^{a,A}	35.23±0.81^{a,A}	29.24±0.51^{a,B}	29.73±0.84^{a,B}	36.46±0.88^{a,A}	38.62±1.76^{a,C}	40.18±2.01^{a,C}
S20	37.73±0.79^{a,A}	37.53±1.15^{a,A}	37.87 ±1.58^{a,A}	36.32±0.86^{a,A}	29.60±0.64^{a}	30.31±0.45^{a,B}	37.38±0.29^{a,A}	41.39±2.19^{b,C}	43.63±2.46^{b,C}
S30	37.13±0.50^{a,A}	36.92±1.16^{a,A}	37.35±0.98^{a,A}	36.05±0.36^{a,A}	29.79±0.75^{a,B}	30.05±0.65^{a,B}	37.43±0.65^{a,A}	43.57±2.42^{b,C}	45.20±2.35^{b,C}
S60	36.95±0.43^{a,A}	37.39±0.56^{a,A}	37.46±0.60^{a,A}	35.40±0.54^{a,A}	29.78±0.89^{a,B}	29.92±0.65^{a,B}	37.81±1.00^{b,A}	47.08±5.05^{c,C}	49.42±4.54^{c,C}

Uppercase letter indicate differences between columns, lowercase letters indicate differences between rows

In the present study, single-rooted maxillary canine teeth were used because they have strong roots and thus helped to establish the study set up (18,24). Samples were decoronated to standardize WL and the amount of solution inside the root canal, and the coronal 3 mm of the canals were enlarged with a 2.3 mm round bur to create a pulp chamber for a solution reservoir (8).

Some of the previous studies placed thermocouples only buccal side of the root (18,20). But in the present study one of the thermocouples was placed at the apical foramen to see temperature change at that point, because of high temperature at the apical foramen might cause post-operative discomfort.

In the current study, samples were fixed with alginate and stored in an incubator for 24 hours before initiation of intracanal heating procedures to imitate intraoral conditions at 37 ˚C and 100% humidity. The study set up was suggested by Donnermeyer et al. (18) for better reflection of intraoral conditions.

In the previous studies, different durations of sonic activation with EDDY was used to compare its effect with other systems (10,25). In the present study 10, 20, 30, and 60 seconds were used as an activation duration, all of which could be used for irrigation activation, were employed to evaluate temperature change.

In a previous study, which studies temperature changes between ultrasonic activation, thermal activation and preheated NaOCl, recordings were done at 10, 20, 40 and 60 seconds (20) in this study recordings were done in every 5 seconds to get more accurate results.

Temperature decrease with irrigation and temperature increase with activation were observed for all study groups. Temperature changes at the T1 point were lowest. This finding is compatible with the other studies (21,24). It could be explained by closed-end study design prevents irrigation solution move towards apically due to vapor lock effect and temperature decrease were lower with the irrigation at this point. Temperature changes between the T1 and T2 and the T1 and T3 were found to be statistically significant for all groups. This can be explained by the fact that the solution was evicted towards the pulp chamber instead of transferring apically during irrigation and activation. This finding is compatible with Donnermeyer et al. (24).

Donnermeyer et al. (24) studied temperature increasing effect of sonic activation with EDDY when used 30s and compared with PIPS, ultrasonic activation, preheated sodium hypoclorite and another sonic activation device. In their study highest temperature measured was around 40 ˚C which is slightly less than our present study. This difference may be due to the fact that their samples contain more NaOCl, which could compansate temperature rise, due to the longer WL.

The S10 group exhibited less temperature increase than the other groups for all levels. S20 group demonstrated significantly less temperature change than the S60 group.

S60 group yielded a significantly higher temperature with activation. This could be due to solution scattering during activation, which leads to root canal dry out and causes temperature increase. Activation increased the temperature more at the level of T3 and less thermal change was seen at the T1 level for all groups. Although there is no study to compare this finding with sonic activation Zeltner et al. (21) showed similar results in their study with ultrasonic activation. Greater temperature increase at the level of T3 may be associated with the solution moving coronally due to a closed-end study design, and cavitation may be more effective at that level because root canal diameter is larger than T1 and T2.

Conclusion

In the current study, it was observed that during irrigation activation most of the solution in the root canals was scattered in the first seconds of activation and moved away from the root canal. In relation to this, findings of the present study showed that prolonged activation time increased temperature beyond biological limits. Therefore the heat caused by irrigation activation is more likely to transmit periodontal tissues than increasing effectiveness of the solution. As a result instead of one long activation period, multiple activation sessions with short durations would be beneficial to control unnecessary temperature increase inside the root canal system. Further studies are needed to understand to what extent temperature increases are transmitted to alveolar bone and if shorter activation periods such as 10 or 20 seconds would be preferred, how many sicluses could provide sufficient antimicrobial and debris removal effect.

Ethics

Ethics Committee Approval: Ethical committee approval was acquired from the Sakarya University Faculty of Medicine Ethical Research Board (approval number: 567, date: 09.12.2021).

Informed Consent: Informed consent is not required.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: A.K.E., Design: A.K.E., F.F., Data Collection or Processing: F.F., Analysis or Interpretation: F.F., Literature Search: A.K.E., Writing: A.K.E.

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

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Impact of Periodontal Disease on Sleep Quality and Oral Health-related Quality of Life

Periodontal Hastalığın Uyku Kalitesi ve Ağız Sağlığı ile ilgili Yaşam Kalitesi Üzerindeki Etkisi

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Abstract

Objective: To investigate the association between periodontitis and sleep quality and oral health-related quality of life.

Materials and Methods: According to the new classification of periodontal diseases (American Academy of Periodontology, 2017), the study comprised 112 patients who were divided into groups. Stage 0-I (periodontally healthy/initial periodontitis) and stage II- IV (periodontitis). Periodontal indicators such as plaque index, gingival index, probing pocket depth, and clinical attachment loss were noted at baseline, and the participants were given Pittsburgh sleep quality index (PSQI) and oral health impact profile (OHIP)-20 questionnaire to observe the mentioned components. After 15 days, data were collected and subjected to statistical analysis.

Results: The PSQI and OHIP-20 global scores were greater in stage II- IV with a mean difference of 2.45 in PSQI scores and 17.467 in OHIP scores between the groups with a highly statistically significant difference.

Conclusion: There is a considerable association between the severity of periodontitis and sleep deprivation, which has an impact on an individual's quality of life.

Keywords: Periodontal disease, sleep quality, oral health related quality of life, wellbeing

Öz

Amaç: Diş eti hastalığı veya periodontitis ile uyku kalitesi ve ağız sağlığına bağlı yaşam kalitesi ile ilişkisini araştırmaktır.

Gereç ve Yöntemler: Bu randomize olmayan, tek kör kesitli bir klinik çalışmadır. Hastalar 2017 Dünya Periodontal ve Peri-İmplant Hastalıkları ve Koşullarının Sınıflandırılması Çalıştayı uyarınca teşhis edildi. Toplam 112 hasta, evre 0-I (periodontal olarak sağlıklı/başlangıç periodontit), evre II-IV (periodontit) gruplarına ayrıldı. Plak indeksi, dişeti indeksi, problama cebi derinliği gibi periodontal göstergeler, başlangıçta klinik bağlanma kaybı not edildi ve katılımcılara belirtilen bileşenleri gözlemlemek için Pittsburg uyku kalitesi endeksi (PSQI) ve ağız sağlığı etki profili (OHIP)-20 anketi verildi. On beş gün sonra veriler toplandı ve istatistiksel analize tabi tutuldu.

Bulgular: PSQI ve OHIP-20 küresel skorları, aşama II-IV'de daha yüksekti ve gruplar arasında PSQI skorlarında ortalama 2,45 ve OHIP skorlarında 17.467 fark vardı. Global PSQI ve OHIP-20 skorları periodontit aşaması ($p<0,001$) ile oldukça istatistiksel anlamlı fark göstermiştir.

Sonuç: Aşama II-IV'teki periodontitisin, bireyin yaşam kalitesini etkileyen uyku yoksunluğu ile önemli ölçüde ilişkili olduğu bulunmuştur.

Anahtar Kelimeler: Periodontal cep, uyku kalitesi, ağız sağlığına bağlı yaşam kalitesi, refah

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Introduction

Oral health is an important aspect of overall wellness and diseases of the oral tissues may have an impact on the general wellbeing of the patients (1,2). Among the varied oral diseases, periodontal disease (gum diseases) is most common with an impact on individual's well-being. Periodontal disease is a chronic infectious entity affecting the tooth supporting structures resulting in tooth loss when left untreated (3). Risk determinants of periodontal disease are common to a number of other chronic diseases accelerating tissue destruction and disease progression (4).

Oral health-related quality of life (OHRQoL) focuses on determining how oral health affects a person's daily life (2). Sleep is one factor that plays a critical part in maintaining physical and mental health. Sleep deprivation is linked to lower immunity and higher inflammatory markers, both of which have a significant impact on the onset and course of a variety of infectious disorders, including periodontal disease. Acute and chronic sleep deprivation can activate inflammatory processes, resulting in increased C-reactive protein concentrations, increased peripheral circulation of leukocytes, and increased levels of interleukin-6 (IL-6) and tumour necrosis factor alpha (TNF-α) (5), all of which can lead to systemic infections and negatively impact an individual's quality of life. Although bacteria invade the tooth surface and gingival sulcus to cause periodontal disease, pro-inflammatory cytokines such as IL-1, 6, TNF-α, and PGE2 generated by the host are thought to have a key role in disrupting tissue homeostasis (6).

Periodontitis-related bleeding, redness, swollen gums, foul breath, tooth movement, and tooth loss have a detrimental impact on self-esteem and quality of life (7). As a result, periodontitis may have an impact on sleep, as inflammation is a common feature of both periodontitis and poor sleep. The goal of this research is to investigate into the relationship between periodontitis and sleep quality and OHRQoL in periodontitis patients.

Materials and Methods

Study Design

This is a cross sectional clinical study in which a total of 135 periodontally healthy and periodontitis patients in age range of 35-55 years were assessed and included during initial examination. The study protocol was approved by The Vishnu Dental Collage Ethics Committee (no: VDC/RP/2020/27, date: 11.2019). All the clinical procedures were followed according to the declaration of Helsinki and good clinical practice guidelines. Written informed consents were obtained from all the participants before the commencement of participation.

Screening and Patient Eligibility

Patients visiting post graduate department of Periodontics, Vishnu Dental College were included in the study. The demographic factors of the patients, such as their age, gender, educational and economic position, as well as the preliminary assessment, which included their medical and dental histories, were all recorded.

Inclusion Criteria

Patient related criteria: Asymptomatic patients.

Teeth related criteria: Minimum number of 20 teeth in the mouth and a minimum of 4 teeth in each quadrant; who had not received non-surgical and surgical periodontal treatment in the past one year.

Exclusion Criteria

Patient related criteria: Smokers, pregnant and lactating woman, patients with uncontrolled systemic diseases like hypertension, diabetes mellitus, salivary gland diseases, patients who had taken antibiotics in the past 3 months, patients with poor dietary intake, students were excluded due to higher prevalence of sleep disorders amongst them.

Teeth related criteria: Presence symptomatic dental diseases (i.e., caries, pulpal disease, trauma, etc.,).

After a thorough clinical examination, periodontal disease activity was recorded using periodontal parameters like plaque index (PI), gingival index (GI), probing pocket depth (PPD) and clinical attachment loss (CAL). Patients were categorized based on the new classification given by AAP 2017 (8). The severity of periodontitis was calculated using the staging approach wherein the stage classifies the severity and complexity of periodontal disease.

A total of 135 patients who satisfied the inclusion and exclusion criteria were enrolled for the study and were categorized into 2 groups in consideration of the low sensitivity of panaromic radiographs for slight crestal bone changes (9,10). Stage 0-I (Periodontally healthy/initial periodontitis)-interproximal attachment loss ≤2 mm at the site of greatest loss and radiographic bone loss extending only upto coronal third i.e., <15% as visible in radiographs. Stage II-IV (periodontitis)-interdental CAL of >2 mm and radiographic bone loss extending beyond the coronal third i.e., >15% as visible in radiographs.

During initial examination all the patients were prescribed chlorhexidine mouth rinse and advised oral hygiene instructions. No periodontal therapy was performed. Awareness and importance of Pittsburg sleep quality index (PSQI) and oral health impact profile (OHIP) questionnaire was explained to all the participants and were made to observe the components mentioned in the questionnaires for 2 weeks after initial examination. However 8 patients in group A and 15 in group B were excluded during follow up due to various reasons such as use of medications for other systemic problems, pain due to pulpal involvement or any other general conditions, underwent dental treatments, failure to report for evaluation after 15 days etc.

PSQI and OHIP assessments were done 15 days after the initial examination. PSQI is a self-reported questionnaire

with 19 self-rated items organised into seven components that is powerful, reliable, and standardized (11). To achieve a global score, the domain scores were added together. The OHIP-20 questionnaire is a modification of OHIP-14 questionnaire to accommodate the regional considerations. After the questionnaire was validated, it was utilised to investigate the impact of periodontitis on OHRQoL (12). The OHIP-20 is divided into 3 basic domains with the highest score indicating poorer quality of life.

Statistical Analysis

Data analysis was done using SPSS software v.20.0 (Armonk, NY, IBM). Comparison of the study groups with means of all the parameters was done by independent t-test. Intragroup comparison of all the clinical parameters was done by One-Way ANOVA and post-hoc Tukey HSD test. Correlation among various parameters was done using Pearson correlation. Data were represented as mean and standard deviation with a statistical significance level of 0.05.

Results

A total of 203 periodontally healthy and periodontitis patients were screened and based on the established selection criteria a total of 135 participants were included. Twenty-three participants were excluded later on, thus data of only 112 participants was analyzed. Stage 0-I included 66 participants with healthy periodontium or initial periodontitis and stage II-IV included 46 subjects with stage II, III and IV periodontitis (Figure 1).

The mean age of all participants was a 35.34±4.5 year ranging from 20 to 55 years. The sample consisted of 58 (51.7%) females and 52 (46.7%) males. There was no statistically significant difference between the groups in any of the above variables.

All the clinical parameters showed significant difference between stage 0-I and stage II-IV with greater PI, GI scores and greater PPDs and attachment loss in Stage II-IV. The mean of the global PSQI score was 7.53±2.07 and OHIP-20 score was 21.13±12.1. The mean PSQI global score was highest in stage II-IV (9.26±1.34) and least in stage 0-I (6.81±1.17) with highly statistically significant difference (Table 1). Stage II-IV showed considerable difference in sleep quality, duration, habitual sleeping and daytime dysfunction as compared to stage 0-I. However there is no significant difference in sleep latency and disturbances between the groups. Amongst all the subjects included in the study only one patient in stage II-IV was under medication for sleep improvement (Table 1).

The mean OHIP-20 global and component scores were assessed and the difference of the scores was found to be highly statistically significant between stage II-IV (32.73±8.58), and stage 0-I (15.27±6.57). All the three components of OHIP score i.e., functional, physical and psychosocial disability showed greater values for stage II-IV compared to stage 0-I. However, the physical disability was more in both Stages compared to functional and psycho-social disability (Table 2).

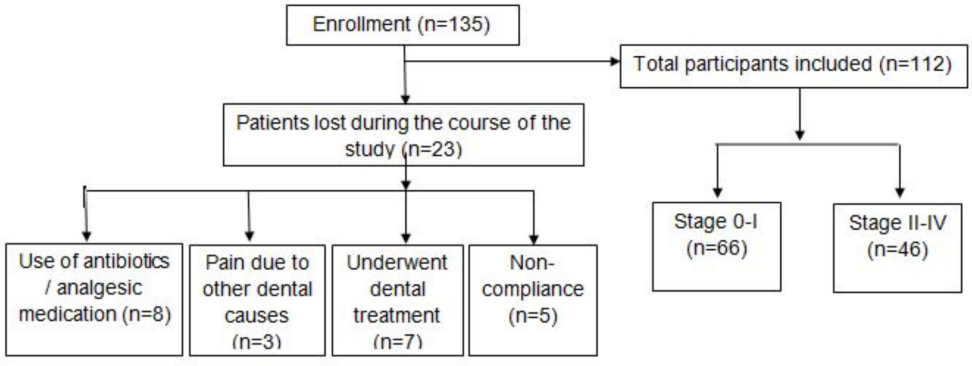

Figure 1. Patient screening chart

Table 1. Distribution of demographic variables in study groups

Variables	Groups	Mean
Age	Stage 0-I	31.02
	Stage II-IV	39.69
Gender (M/F)	Stage 0-I	48.2/51.8% (n=29/28)
	Stage II-IV	46.2/53.8% (n=25/30)

M: Male, F: Female

Table 2. OHIP-20 component scores and stage of periodontitis					
OHIP	Groups	Mean	95% confidence interval for mean		p-value
			Lower limit	Upper limit	
Functional disability	Stage 0-I	3.72±1.574	3.3403	4.1143	0.000**
	Stage II-IV	8.04±1.172	7.6952	8.3918	
Physical disability	Stage 0-I	6.36±3.066	5.6098	7.1175	0.000**
	Stage II-IV	13.95±2.139	13.3213	14.5918	
Psychosocial disability	Stage 0-I	5.15±2.684	4.4915	5.8115	0.000**
	Stage II-IV	11.91±3.437	10.8923	12.9338	
**p<0.001: Highly statistically significant, OHIP: Oral health impact profile					

There was a significant positive correlation between PSQI scores and stage of periodontitis. There was also a significant correlation between the global and all the three domain scores with periodontitis (Table 3).

Discussion

Oral health is all about functional, physical, psychological and social well-being of an individual rather than the surrogate end points measured at the time of treatment procedures. Periodontal disease is a chronic inflammatory disease which influences host immune-inflammatory mechanisms and evidence suggests sleep deprivation also has an increase in the similar inflammatory markers.

Table 3. Correlation between stage and PSQI global, OHIP global, domain scores		
Variables	Stage	
	r-value	p-value
Global PSQI	0.770**	0.000**
Sleep quality	0.337**	0.000**
Sleep latency	0.064	0.487
Sleep duration	0.652**	0.000**
Habitual sleeping	0.658**	0.000**
Sleep disturbances	0.034	0.712
Medication	0.215*	0.019*
Daytime dysfunction	0.578**	0.000**
Correlation between stage and OHIP global, domain scores		
Global OHIP	0.792**	0.000**
Functional disability	0.849**	0.000**
Physical disability	0.837**	0.000**
Psychosocial disability	0.772**	0.000**
r: Pearson correlation coefficient, *Correlation is significant at the 0.05 level (2-tailed), **Correlation is significant at the 0.01 level (2-tailed). OHIP: Oral health impact profile, PSQI: Pittsburg sleep quality index		

Although, improvement in traditional measures i.e., probing depth, clinical attachment level along with various clinical indices are important, little is known about periodontal disease and its influence on other conditions like sleep disturbance to improve ones quality of life (13). Sleep disturbance in patients with periodontal disease can have an impact on the general well-being of the patient and on OHRQoL.

In the present study after eliminating the confounding factors such as systemic risk factors and environmental risk factors, the participants were categorized into stage 0-I and stage II-IV.

Results of the current study elucidated that the mean global PSQI score was greater in stage II-IV periodontitis. A positive correlation between PSQI scores and the stage of periodontitis suggest that sleep quality commensurate with periodontal tissue destruction. Though various other methods for assessing the quality of sleep have been utilized PSQI is found to have 89.6% sensitivity and 86.5% specificity for identifying sleep disturbances. The findings of this study are consistent with earlier research that has suggested a link between sleep and periodontitis (14,15). However all these studies are case control studies which compared the possible association of sleep in periodontitis and non-periodontitis patients. Only one research looked at the relationship between sleep quality and periodontal disease severity i.e., based on the 2017 classification of periodontal disease and the results correlate with the results of the current investigation. Fatigue caused due to sleep deprivation could worsen systemic health in rats and increased gingival inflammation and alveolar bone loss was reported in an experimental periodontitis model (16).

The OHIP 20 results showed that the patients with stage 0-I i.e, periodontally healthy/initial periodontitis had a better OHRQoL scores compared to stage II-IV. The results indicate that the greater the severity of periodontal disease the poorer is the QoL. In particular when all the three domains of OHIP questionnaire were compared, the physical disability was found to be greater when compared to the functional and psychosocial disability. The results of this study are in accordance to a srilankan study which

showed a greater association of periodontitis with physical pain (17). However in a similar study done to assess the association of stage grade of periodontitis to OHRQoL, psychological disability was found to be greater compared to other components (16).

The majority of previous investigations have demonstrated that periodontitis has a detrimental influence on OHRQoL (18-20). In a few studies, however, there is no control for confounding by other oral diseases, and several research only control a few factors in their multivariate analysis. Because OHRQoL is a subjective phenomena that may be influenced by a variety of circumstances, it's critical to account for known confounding factors (such as other clinical disorders that have an impact on people's daily lives) to prevent misinterpreting the results. In this study maximum effort was made either to eliminate or to exclude all confounding factors, and the findings are consistent with a recent study that indicated a strong link between periodontal disease severity and QoL (21). In the present study an attempt was made to measure the dysfunction, discomfort and disability in conjunction with the traditional indicators of clinical disease.

Assessing the individual confounding variables like race/ethnicity, education, and socio-economic status with inclusion of wide range of populations from different geographical areas and their relation or influence on sleep would help in giving more specific result. Further, assessing the impact of periodontal disease and its treatment on functional and social well-being of the patients was also not measured.

Conclusion

Within the scope of the study, there is a probable link between periodontitis severity and sleep deprivation, which might have an influence on quality of life.

There is a need to focus on the prevention of periodontal disease, screening, diagnosis and management of the disease at early stages. Assessing the OHRQoL should become an integral part of examination by health care professionals for the general health and well-being of an individual.

Ethics

Ethics Committee Approval: The study protocol was approved by The Vishnu Dental Collage Ethics Committee (no: VDC/RP/2020/27, date: 11.2019).

Informed Consent: Written informed consents were obtained from all the participants before the commencement of participation.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: R.S.V.K., S.G., A.B., K.V., Concept: R.S.V.K., S.G., Design: R.S.V.K., S.G., M.K.P., Data Collection or Processing: R.S.V.K., S.G., M.K.P., S.P., A.B., G.P., K.V., Analysis or Interpretation: R.S.V.K., S.G., M.K.P., Literature Search: R.S.V.K., S.G., M.K.P., S.P., A.B., G.P., K.V., Writing: R.S.V.K., S.G., S.P.

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Evaluation of Precision and Reliability of Different Bite Registration Materials Using Conventional and Digital Articulator Systems

Konvansiyonel ve Dijital Artikülatör Sistemlerini Kullanarak Farklı Kapanış Kayıt Materyallerinin Hassasiyet ve Güvenilirliğinin Değerlendirilmesi

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Abstract

Objective: Conventional and digital articulators are currently used to associate the lower and upper jaw models. However, there are not enough studies comparing the reliability of different interocclusal registration materials used in conventional and digital articulatory acquisition methods. This study aims to contribute to the literature on this subject.

Materials and Methods: Plastic mandibular and maxillary models were attached to a semi-adjustable articulator adjusted to the mean values in the maximum intercuspal position. Forty interocclusal recordings were obtained using 4 different interocclusal registration materials (n=10). Impressions from the master models and all interocclusal recordings were digitized. For each interocclusal registration sample, measurements were made between the marked points on the correlated models using both digital and conventional methods, and all measurements were compared with the measurements made on the plastic master model. Two-way analysis of variance was used to compare the data obtained from the measurements between the groups, and Dunnett's test was used as a multiple comparison test to compare the measurements of all groups with the initial measurements.

Results: It was concluded that the measurements obtained with the conventional method gave more successful results than those obtained with the digital method, and polyvinyl siloxane materials had more successful results than wax samples in both methods.

Conclusion: According to the method used in this study, the conventional technique has reliable results ($p<0,05$) and continues to be valid. Software development is needed to scan and use interocclusal recordings for digital model articulation.

Keywords: Bite registration, centric relation, digital bite registration, interdental relation

Öz

Amaç: Günümüzde alt ve üst çene modellerini ilişkilendirmek için geleneksel ve dijital artikülatörler kullanılmaktadır. Ancak geleneksel ve dijital artikülatörler ile kapanış kaydı elde etme yöntemlerinde kullanılan farklı interoklüzal kayıt materyallerinin güvenilirliğini karşılaştıran yeterli çalışma bulunmamaktadır. Çalışmanın, bu konudaki literatüre katkı sağlaması amaçlanmaktadır.

Gereç ve Yöntemler: Mandibular ve maksiller fantom modeller, maksimum interkuspal pozisyonda ortalama değerlere ayarlanmış yarı ayarlanabilir artikülatöre bağlandı. Dört farklı interoklüzal kayıt materyali kullanılarak 40 interoklüzal kayıt elde edildi (n=10). Ana modeller ve tüm interoklüzal kayıtlar dijitalleştirildi. Her bir interoklüzal kayıt örneği için, modellerde işaretlenen noktalar arasında hem dijital hem de geleneksel yöntemlerle ölçümler yapıldı ve tüm ölçümler plastik ana modelde yapılan ölçümlerle karşılaştırıldı. Gruplar arası ölçümlerden elde edilen verileri karşılaştırmak için iki yönlü varyans analizi, tüm grupların ölçümlerini ilk ölçümlerle karşılaştırmak için çoklu karşılaştırma testi olarak Dunnett testi kullanıldı.

Bulgular: Konvansiyonel yöntemle elde edilen ölçümlerin dijital yönteme göre istatistiksel olarak daha başarılı sonuçlar verdiği ($p<0,05$) ve polivinil siloksan malzemelerin her iki yöntemde de mum örneklere göre daha başarılı sonuçlar verdiği sonucuna varıldı.

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Sonuç: Bu çalışmada kullanılan yönteme göre geleneksel teknik güvenilir sonuçlara sahiptir ve geçerliliğini sürdürmektedir. Dijital modellerde interoklüzal kayıtları taramak ve kullanmak için yazılımların geliştiirilmesine ihtiyaç vardır.

Anahtar Kelimeler: Kapanış kaydı, sentrik ilişki, dijital kapanış kaydı, dişlerarası ilişki

Introduction

When a tooth needs a restoration due to caries, fractures, wear, or when it is planned to replace missing teeth with fixed or removable dentures, the morphology of the restoration to be made is created in harmony with the patient's existing teeth and occlusion. Once it is decided that occlusal reconstruction is indicated, a correct treatment position for the mandible should be established and this position recorded accurately (1).

In dentistry, the centric relationship is accepted as the most comfortable, reproducible, physiological and stable position for the mandible. For this reason, in cases where dental references are pathological or absent, it is desired that the condyles be in the centric relation position while recording the jaw relation in the desired vertical dimension. Accurately recording the centric relationship position in a predetermined vertical dimension and transferring it to the laboratory environment significantly affects the success of the restoration and plays a major role in reducing the physician's treatment time (2).

With the development of computer-aided design and computer-aided manufacturing systems, the use of digital methods has become widespread in copying teeth and dental surrounding tissues and recording the interocclusal relationship. However, there are very few studies in the literature comparing the reliability of interocclusal relationship recordings obtained with digital and conventional methods and evaluating the suitability of existing interocclusal registration materials with the digital method (3,4).

The aim of this study is to evaluate the compatibility of interocclusal registration materials currently used in the conventional articulatory method with the digital process.

Our null hypothesis is all bite registration materials can register the centric relation and different materials and methods had no effect on the accuracy of interocclusal recording.

Materials and Methods

Ten groups were performed to examine five different interocclusal recording materials using conventional (Occlufast rock, Zhermack SpA., Italy; Variotime Bite, Kulzer, Germany; Futar D, Kettenbach, Germany; Modeling Wax Pinnacle Standard, Dentsply Sirona, Australia) and digital (Dental Wings iSeries Impression Scanner-Dental Wings, Montreal, Canada) methods, and each group included ten samples (Table 1).

Prefabricated acrylic phantom jaws were used in the study (AD-J 01, Arma Dental, Turkey). After the upper jaw model was fixed to the adjusted articulator according to the mean values, the lower jaw was brought to the maximum intercuspal position and fixed to the articulator with type 2 plaster. The pin of the articulator was fixed by compression in the vertical dimension at the maximum intercuspal position. All maxillary teeth were prepared to represent a situation where the vertical stop points resulting from natural tooth contacts were completely lost (Figure 1). After the preparations, the locations of the markings to be measured were determined to be in the cervical gingival regions of the lower and upper first molars and canines. Triangular shaped reference points were created with the bases facing the teeth and the apical apex of 1 mm in depth. Impressions were taken by applying putty-wash technique with pubovaginal sling (PVS) impression material (Elite HD+, Zhermack SpA, Italy) using prefabricated impression trays.

All PVS-based bite registration materials were applied directly to the occlusal surfaces of the mandibular teeth, using the automatic mixing and application tips included in their packages, with a compatible disperser (3M garant dispenser, 3M, USA) in accordance with the manufacturer's instructions. The upper part of the articulator, which

Table 1. Test groups

Materials	Digital	Conventional
Futar D	FD	FC
Modelling wax	WD	WC
Occlufast rock	OD	OC
Variotime bite	VD	VC

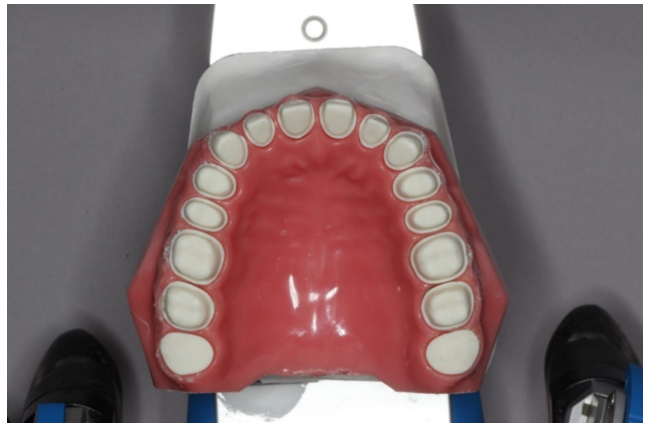

Figure 1. The prepared teeth

was fixed with a pin, was closed and the maxillary teeth were embedded in the material. The materials whose polymerization was completed were separated from the model and homogeneously softened wax interocclusal recording material was placed on the mandibular teeth by giving an arc form and the upper jaw was closed on the material in vertical dimension fixed with pins.

Dental Wings iSeries impression scanner (Dental Wings, Montreal, Canada) was used as an indirect digital scanner for transferring the obtained impressions and interocclusal recordings to digital media.

Mandibular impression, maxillary impression and interocclusal recording material were scanned with an indirect digital scanner and the scan data was transferred to the device compatible software (DWOS 9.1, Dental Wings, Montreal, Canada). Models and bite registrations were associated with precise positioning by the software. The distances between the predetermined reference points on the main models were measured with the digital ruler included in the software, and the obtained values were recorded (Figure 2).

Type 4 plaster was used for the plaster model (Elite rock, Zhermack SpA, Italy). The plaster maxillary model was fixed to the articulator adjusted according to the mean values using type 2 plaster, and the intermaxillary recording was placed on the maxillary occlusal surface. The occlusal surface of the mandibular model was placed on the interocclusal recording, and the mandible was fixed to the articulator with type 2 plaster in the position where maxillo-mandibular relationship was achieved. The mean values were recorded by measuring the distances between the previously marked reference points using a digital caliper. For all subsequent specimens, only the mandibular model was detached from the articulator and all steps were repeated after the maxillary model was fixed to the articulator.

Statistical Analysis

Two-way analysis of variance was used to compare the data obtained from the measurements between the groups, and Dunnett's test was used as a multiple comparison test to compare the measurements of all groups with the initial measurements (Table 2).

Results

When Table 2 (which shows the average differences from the gold standard) is examined, it is seen that the conventional method gives superior results than the digital method. In the conventional method, the largest mean difference was recorded in the WK group with 0.2 mm, while in the digital method, the smallest mean difference was recorded in the VD group with 0.26 mm. It is seen that the results recorded in the PVS-containing materials in the conventional method are very close to each other, while the results obtained with the wax-containing material are far from the PVS group. When the results recorded in the digital method are examined, it is seen that the differences between the groups are more pronounced.

In order to make the comparison between the materials examined with two different methods more understandable, the values recorded in 4 regions of ten samples in each group were recorded by calculating the difference in mm from the gold standard value of the relevant region. During the collection of the differences obtained, the distances away from the gold standard value were added without taking into account the + and - signs indicating the burial and elevation status, in front of the values, and the negative and positive values obtained in this way were prevented from zeroing each other. The distance from the gold standard value of the relevant region for a total of 40 measurements of 10 samples in each group in 4 different regions was calculated and summed in this way, the obtained value was divided by 40 and the deviation distance from the mean gold standard in a measurement for each group was obtained in mm (Table 3).

When Table 3 is examined, it is seen that the average difference of all materials from the gold standard is higher in the digital method than in the conventional method. The smallest mean difference recorded in the conventional method was 0.1 mm in the FK group, and the largest mean difference was recorded in the WK group with 0.2 mm. In the conventional method, the order of the mean difference between the materials from the gold standard is as follows, from smallest to largest, Futar D, Variotime Bite, Occlufast Rock, Modeling Wax.

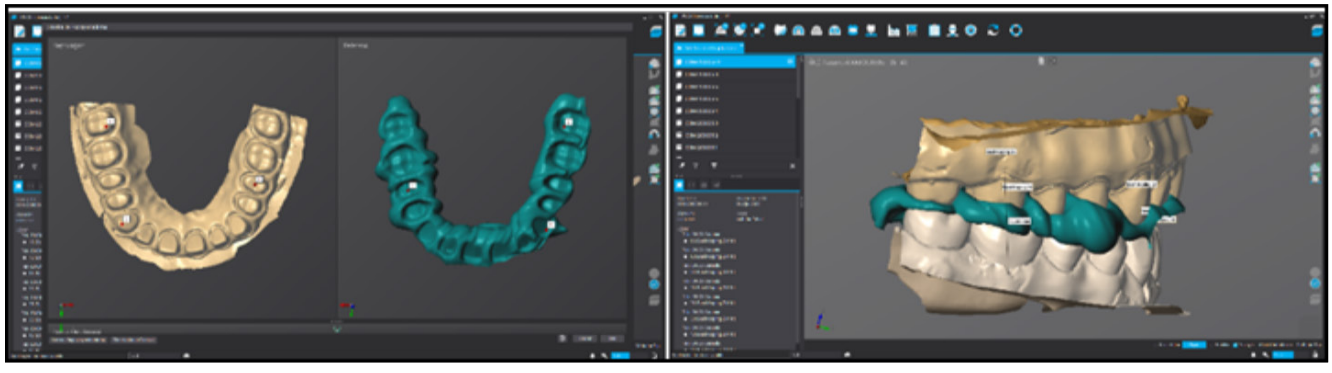

Figure 2. Interocclusal digital registration

Table 2. ANOVA test results

	26-36		p-value from GS	23-33		p-value from GS	13-43		p-value from GS	16-46		p-value from GS
	Mean	SD		Mean	SD		Mean	SD		Mean	SD	
GS	16.96	0.03		20.87	0.02		20.91	0.04		15.42	0.03	
FD	16.46	0.19	0.000	20.60	0.31	0.130	21.19	0.27	0.087	15.34	0.27	0.884
FC	16.99	0.04	1.000	20.70	0.08	0.579	20.86	0.08	1.000	15.35	0.07	0.940
WD	16.66	0.26	0.004	20.65	0.66	0.309	20.85	0.62	0.999	15.24	0.21	0.067
WC	17.28	0.22	0.002	20.76	0.14	0.942	20.84	0.16	0.999	15.62	0.09	0.022
OD	16.46	0.18	0.000	20.69	0.23	0.519	20.90	0.25	1.000	15.25	0.17	0.089
OC	16.93	0.11	1.000	20.71	0.10	0.644	20.86	0.12	1.000	15.50	0.07	0.743
VD	16.54	0.23	0.000	20.41	0.17	0.001	20.93	0.24	1.000	15.37	0.22	0.986
VC	17.02	0.12	0.995	20.68	0.09	0.460	20.83	0.06	0.995	15.37	0.09	0.992
ANOVA p-value	0.000			0.014			0.088			0.000		

SD: Standard deviation, FD: Futar digital, FC: Futar conventional, WD: Modelling wax digital, WC: Modelling wax conventional, OD: Occlufast rock digital, OC: Occlufast rock conventional, VD: Variotime bite digital, VC: Variotime bite conventional

Table 3. Total and mean distances of the groups from the gold standard

Materials	Total difference	Mean
FC	4,114	0.102
VC	4,502	0.112
OC	4,625	0.115
WC	8,176	0.204
VD	10,567	0.264
OD	10,974	0.274
FD	13,491	0.337
WD	16,043	0.401

FC: Futar conventional, VC: Variotime bite conventional, OC: Occlufast rock conventional, WC: Modelling wax conventional, VD: Variotime bite digital, OD: Occlufast rock digital, FD: Futar digital, WD: Modelling wax digital

Discussion

All additional silicone bite registration materials examined in the study gave results close to the gold standard value in the conventional method. The wax bite registration material, on the other hand, gave higher results in the posterior regions compared to the gold standard values when compared with additional silicone materials. In the conventional articulatory method, similar results are found in studies comparing the bite registration materials containing wax and PVS. Ockert-Eriksson et al. (5) investigated the effects of bite registration materials containing two waxes, three PVS and an irreversible hydrocolloid on the precision of interocclusal recordings in fixed, HBP and full dentures. In their study, in which the position changes of the lower and upper jaw models were examined in 3D, it was reported that materials containing PVS in the vertical direction gave significantly more successful results compared to materials containing wax (5). The results of this study show parallel data we obtained from the WC group in our study.

When conventional groups were analyzed by regions, no such pattern was encountered. This suggests that this situation is not due to the materials, but to the sensitivity of the method. In the study conducted by Porter et al. (6), the effect of intraoral and extraoral scanners on closing sensitivity was examined, and it was reported that values outside the confidence interval and farther from the gold standard value were recorded in the left molar region, in two extraoral and one intraoral scanner groups.

The extraoral scanner used in the study scans the interocclusal relationship starting from the right side of the arch. Although it is known that the probability of distortion in full-arch scans performed using extraoral scanners is lower than in intraoral scanners, the scanning sensitivity of measurements and models has been evaluated in studies (6-9). While no problems were encountered in the impression scans we obtained in our study, it was observed that the surface detail of the right half of the quadrant was lower than the left half of the interocclusal recording scans. It is thought that this may be due to the fact that the closing sensitivity gives much more successful results on the right side than on the left side.

However, even when the results recorded in the right quadrant were examined, it was seen that the conventional method gave more sensitive results compared to the digital method. It is not possible to explain this situation only with the difference in scanning sensitivity between regions. In the conventional method, the practitioner can feel the fit of the plaster models on the interocclusal recording material

and the material creates a physical barrier between the models. The compressibility of the material and its resistance to plastic deformation determine the maximum distance that the applicator can compress the models. On the other hand, in the digital method, there is no physical limit that can prevent the models from approaching each other, and the correct articulation completely depends on the scanning sensitivity of the scanner and the success of the best fit algorithm used in matching the surfaces.

Sweeney et al. (3) investigated the effect of different interocclusal recording materials on the sensitivity of model articulation and reported that all values recorded with the digital method, regardless of the material, were significantly far from the gold standard value. Although only the digital method was included in their studies, the results recorded in this area are similar (3).

While Futar D material was the most successful material in the conventional method in order of mean difference from the gold standard, it could not show the same success in the digital method. The success of the material in the conventional method can be explained by its superior physical properties such as high hardness and compression resistance. The inability to show the same success in the digital method can be explained by the inadequacy of the scanning sensitivity of the device. However, it is thought that the color of the material and the color of the laser beam of the device may be incompatible with each other (10).

The Variotime Bite bite registration material is the closest to the gold standard in the digital group. Variotime Bite is an interocclusal registration material produced for laser scanning. The material has a matte appearance after polymerization, and this feature allows the laser beams reflected from the surface to be easily detected by the device camera during scanning. It is thought that the success of the material in the digital method can be explained by this feature. However, the material gave similar and successful results with other polyvinyl siloxane materials in the conventional method. It was observed that the material was more fragile than other polyvinyl siloxane interocclusal recording materials used in the study. Due to this feature, difficulties were experienced when separating the polymerized recordings from the teeth, and two of the obtained recordings were renewed because they were broken during the separation from the teeth (11).

Occlufast Rock is the material with the highest hardness level after polymerization among the materials used in the study. It was observed that the material gave successful results in both methods (12).

Modeling Wax was the material in which the most distant mean values to the gold standard value were recorded in both digital and conventional methods. Similar results were encountered in many studies comparing wax-containing interocclusal recording materials and polyvinyl siloxane interocclusal recording materials. Physical properties such as insufficient dimensional stability of the material, distortion as a result of heat exchange, and low compression resistance have been held responsible for this situation.

When the results of the study were evaluated clinically, it was seen that the conventional articulatory method gave more reliable results than the digital articulatory method. When the data obtained in the study were evaluated statistically, it was seen that the effect of different methods and materials on the accuracy of interocclusal relationship recording was statistically highly significant, and the null hypothesis that different materials and methods had no effect on the accuracy of interocclusal recording was rejected.

Conclusion

The results obtained within the limits of this study, in which the sensitivity and reliability of different closing recording materials were evaluated using digital and conventional articulatory methods, are as follows:

1- Significantly different results were obtained in digital and conventional articulatory acquisition methods for the same interocclusal recording materials. More successful results were obtained in the conventional method compared to the digital method.

2- It was observed that there was a significant difference between the regions measured in the digital method, and the amount of error in closing increased as it progressed from the right end of the arc to the left.

Based on the results of our *in vitro* study under laboratory conditions, it can be said that the conventional method continues to be valid until the full digital method reaches the desired point with software and hardware developments. It is thought that the results obtained from the study will contribute to the clinicians in the choice of interocclusal recording materials. We believe that this issue should be supported by in-vivo studies in order to obtain more reliable clinical information in material comparison.

Ethics

Ethics Committee Approval: Ethics committee approval is not required.

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Authorship Contributions

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Evaluation of the Effectiveness of Polyether Silicone-based and Polyvinyl Siloxane Dental Impression Materials for Shielding Scattered Radiation During Radiotherapy

Radyoterapi Sırasında Saçılan Radyasyona Karşı Koruyucu Olarak Polieter Silikon Bazlı ve Polivinil Siloksan Diş Ölçü Malzemelerinin Etkinliğinin Değerlendirilmesi

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Abstract

Objective: Radiation-induced oral mucositis is a major problem associated with radiotherapy. This study aimed to investigate the effectiveness of polyether silicone-based (PE) and polyvinyl siloxane (PVS) impression materials in protecting adjacent tissues from radiation scattered from dental materials.

Materials and Methods: Amalgam, zirconium, and titanium dental material specimens were covered with 5 mm PE and PVS in the study group. The dental materials were placed in a linear accelerator device at a distance of 100 cm from the radiation source and coincided with a field size of 15x15 mm. Samples placed perpendicular to the central beam were irradiated with 6 MV photons at a fractional daily therapeutic radiation dose of 2 Gy. Thermoluminescent dosimeters (TLD-100) placed 90 degrees lateral to the specimens were used to record the scattered dose data. In the control group, uncovered dental materials were irradiated, and scattered doses were measured by TLD. The TLD data of the study and control groups were compared by independent t-test to analyze the shielding effect of PE and PVS. In addition, the photon stopper properties of PE and PVS were compared. The photon interaction parameters and effective atomic numbers of dental materials were calculated.

Results: It was calculated that the PE and PVS significantly prevent the dose enhancement caused by dental materials ($p<0.05$). There was no difference between impression materials in the photon-stopping properties ($p>0.05$).

Conclusion: PE and PVS can be used as scatter dose shields for the 2 Gy daily fractional dose. This study demonstrates the radiation-shielding properties of PE for the first time.

Keywords: Amalgam, dental materials, dental impression materials, X-rays, zirconium

Öz

Amaç: Radyasyona bağlı oral mukozit, radyoterapinin önemli bir sorunudur. Bu çalışmanın amacı, polieter silikon bazlı (PE) ve polivinil siloksan ölçü materyallerinin (PVS) komşu dokuları dental materyallerden saçılan radyasyondan korumadaki etkinliğini araştırmaktır.

Gereç ve Yöntemler: Çalışma grubundaki amalgam, zirkonyum ve titanyum dental materyal örnekleri 5 mm PE ve PVS ile kaplandı. Lineer bir hızlandırıcı cihaza dental materyaller radyasyon kaynağından 100 cm uzakta ve 15x15 mm alan boyutuna denk gelicek şekilde yerleştirildi. Merkezi ışına dik olarak yerleştirilen örnekler 2 Gy fraksiyonel günlük terapötik radyasyon dozu ile 6 MV fotonla ışınlandı. Saçılan doz verilerinin kaydedilmesi için numunelerin 90 derece lateraline yerleştirilen termolüminesan dozimetreler (TLD-100) kullanıldı. Kontrol grubunda ise üzeri kaplanmamış dental materyaller ışınlandı ve TLD ile saçılan doz ölçüldü. Çalışma ve kontrol

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gruplarının TLD verileri, PE ve PVS'nin koruyucu etkisini analiz etmek için bağımsız t-testi ile karşılaştırıldı. Ayrıca PE ve PVS'nin foton durdurucu özellikleri karşılaştırıldı. Dental materyallerin foton etkileşim parametreleri ve etkin atom numaraları hesaplandı.

Bulgular: PE ve PVS'nin dental materyallerin neden olduğu doz artışını önemli ölçüde önlediği hesaplandı ($p<0,05$). Foton durdurma özelliklerinde ölçü materyalleri arasında fark yoktu ($p>0,05$).

Sonuç: PE ve PVS, 2 Gy günlük fraksiyonel doz için bir saçılan radyasyon kalkanı olarak kullanılabilir. Bu çalışma, PE'nin radyasyon koruma özelliğini ilk kez göstermektedir.

Anahtar Kelimeler: Amalgam, dental materyal, dental ölçü malzemeleri, X-ışınları, zirkonyum

Introduction

Radiation-induced oral mucositis is a tissue damage that starts as acute inflammation in the oral mucosa, tongue, and pharynx after exposure between 7 and 98 days (1). As a result of these, oral pain was reported in 69% and dysphagia in 56% of patients with radiation-induced oral mucositis. In addition, it was shown that 53% of the patients had a history of opioid use, 15% of them had a feeding tube inserted, and 11-16% of them had a history of changing or discontinuing treatment due to oral mucositis. It can progress to an acute life-threatening stage because of reduced food and water intake (2,3).

In this context, dental materials including metals (e.g., gold and silver/mercury alloys) can increase the radiation dose up to 2 times in the region adjacent to the dental restoration (4). Since dental materials have higher atomic numbers than soft tissues, they cause the reflection of electrons during radiotherapy (RT). Regard, the radiation dose enhancement often leads to severe mucositis or osteoradionecrosis, especially in patients suffering from oral tumors (5).

There are studies on the oral stents and plates used to protect surrounding tissues to reduce or delay complications arising from that backscatter radiation (6-11). In reported studies, it has been stated that the use of intraoral stents reduces the RT side effects such as mucositis, osteoradionecrosis, and xerostomia (6-9). Methyl methacrylate and hydro-plastic materials (7,8) new polymer-based lightweight, non-toxic composite materials such as polypropylene, polystyrene, and polyethylene are widely used in the production of stents for radiation protection (10,11). However, the effectiveness of the polyether (PE) impressions materials protecting healthy oral tissues from undesirable radiation effects has not been presented yet.

The present study aimed to investigate the use of PE and polyvinyl siloxane (PVS) in different thicknesses as radiation shielding that have advantages such as being biocompatible, non-irritant, easy to apply, accessible, inexpensive, and reproducible before each fraction of the RT. The null hypothesis of the study was that PE and PVS did not prevent scattered radiation caused by DMs.

Materials and Methods

Preparing of the Impression Material Samples (IMs)

The PE silicone-based IM (PE: Impregum Penta H-DuoSoft Quick, 3M ESPE) and PVS IM (PVS: Betasil Vario Implant, Muller-Omicron Gmbh & Co KG) were used in the study as a radiation shield. Firstly, cylindrical plastic templates were prepared to provide 5 mm thick IMs all over the DM. Another cylindrical plastic block of 5 mm diameter, and 5 mm height was placed in the center of the template base to obtain a cavity for use in placing the DM samples (amalgam, zirconium, and titanium).

IMs were obtained by mixing base and catalyst. The chemical composition of the PE (Impregum Penta H-DuoSoft Quick, 3M ESPE) includes PE macromer, fillers, triglycerides, and plasticizers for the base, and initiator, fillers, plasticizers for the catalyst. The base material of the PVS (Betasil Vario Implant, Muller-Omicron Gmbh & Co KG) contains a polymethyl hydrogen siloxane copolymer and amorphous silica. Additionally, the catalyst of the PVS includes the vinyl-terminated polydimethylsiloxane and chloroplatinic acid. Both IMs were mixed by an automatic mixing unit (Pentamix II; 3M ESPE) in offered mixture ratios due to the recommendations of the manufacturer. The mixed IMs were molded into these templates and kept in the templates until they hardened. Then, hardened IMs were removed from the templates. Every IM covered each point of the DM except the top surface of the hardened IMs because, the central beam was directed to the upper surface of the DM (Figure 1).

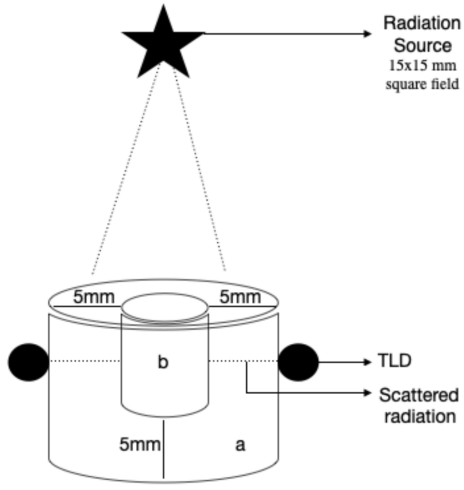

Figure 1. Presents the set-ups included dental materials covered dental impression materials. a: is the IM covering the dental materials; b: shows the dental material in 5 mm diameter, and 5 mm height
TLD: Thermoluminescent dosimeters

Scatter radiation was measured in the lateral area by TLD. Therefore, 120 IMs [PE (n=60), PVS (n=60)] were produced for the study group in total.

Preparing of the Dental Samples

A total of 180 DMs in a cylindrical form in 5 mm diameter and 5 mm height were prepared for PE and PVS groups. Sixty dental material samples [amalgam (n=20), zirconium (n=20), and implant (n=20)] were produced for PE, and 60 DM specimens for the PVS group. In addition, 60 DMs were prepared for the control group.

The amalgam (Southern Dental Industries Ltd., SDI, Australia) and zirconium blanks (Nacera Pearl 1, Doceram GmbH, Dortmund, Germany) in a cylindrical shape with a diameter of 5 mm and a height of 5 mm were prepared. To prepare amalgam samples, the powdered alloy encapsulated amalgam with liquid mercury was mixed with an amalgamator to form an amalgam putty. This softened amalgam putty was placed and shaped into the previously prepared cylindrical mold (5 mm diameter and 5 mm height), where it quickly hardened into a solid filling. For obtaining zirconium samples, single-brand monolithic zirconium blocs (Nacera Pearl 1, Doceram Medical Ceramics, Germany) were prepared with computer-aided design/computer-aided manufacturing (Amanngirbach, Ceramill Motion, Germany) and sintered due to the instructions of the manufacturer. The final dimensions of the samples were controlled with a digital compass (Mitutoyo, Japan). For titanium samples, 5 mm diameter titanium implants (Astra Tech Implant System) were used.

Control Group

To calculate the shielding effect of the IMs, uncovered amalgam (n=20), zirconium (n=20), and titanium (n=20) samples were irradiated by 6 MV, and the scattered radiation was recorded the same as other set-ups. The percentage dose increase (PDI) caused by DMs was calculated. For this, tissue equivalent bolus (without DM and IMs) was irradiated, and the base dose calculated by TLD was placed in the same place as the study groups. The dose increase caused by DMs was calculated by the PDI formula.

The PDI according to TLD data were calculated by using the following formula:

$$PDI = \frac{\text{Control group (DM without IMs)} - \text{Base dose (Bolus without IMs and DMs)}}{\text{Base dose}} X\ 100$$

Radiation Dose Measurement

Thermoluminescent dosimeters (TLD-100; Harshaw Chemical Company) having 3.2×3.2×0.89 mm^3 sizes, and 2 mm spatial resolution was used for recording the scattered dose data. The TLD-100s were calibrated by Win-TLD software before every irradiation process. TLDs were placed in the bolus at the lateral side of the IMs. Three TLDs were placed around each setup. After every irradiation process TLD chips were read by TLD reader RE 2000A (Mirion), and Win-TLD Software.

XCOM Program

The theoretical mass attenuation coefficients (μ/ρ) for the DMs (amalgam, zirconium, and implant) were obtained from the XCOM computer program (Version 3.1., National Instute of Standards and Technology, America). The program can determine to get information about the interaction between DMs and photons (12). This program can determine the μ/ρ of an element, compound, and mixture at different energy levels (0.001 up to 105 MeV). In this study μ/ρ of the DMs was calculated according to their prescriptions given by the manufacturer at 0.2, 0.4, 0.6, 0.8, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, and 6 MeV photon energies.

Irradiation Process

The set-ups were embedded in tissue-equivalent bolus material (Superflab Bolus 30×30×1.0 cm) simulating the soft tissue. Besides, a 15-mm-thick layer of bolus materials were placed on the superior and inferior surface of the specimens. Under the bolus material, a 10 cm RW3 solid water phantom (30 cm x 30 cm x 1 cm, Slab Phantom, Sun Nuclear Corporation, Melbourne, Florida) was used to prevent the backscattering factor. The samples irradiated in a linear accelerator device (Clinac iX, RapidARC, Varian Medical Systems, USA) operated at 6 MV photons. Specimens were placed perpendicular to the beam collimated to a 15×15 mm square field. The surfaces of the DMs were located 100 cm from the radiation source. Diagram of the experimental design is shown in Figure 2.

Defining the Theoretical Properties of DMs

The Phy-X/ZeXTRa software program was used to calculate the effective atomic numbers for photon energy absorption (ZPEAeff) of DMs according to the 6 MV photon energy and elemental compound of the materials (13).

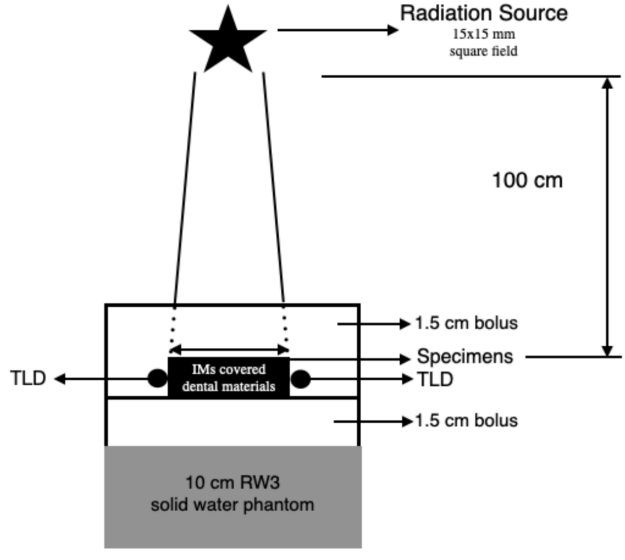

Figure 2. Diagram of experimental design
TLD: Thermoluminescent dosimeters, IM: Impression material sample

Table 1. Presents the recorded data on TLDs and PDI caused by dental materials and, ZPEAeff values due to dental materials. The TLD data are compatible with the calculated ZPEAeff values which describe the absorbsion of the encountered photon energy

Control group	Mean (mSv)	Std. deviation	Std. Error	Min.	Max.	PDI%	ZPEAeff
Base dose	9.042	0.295	0.170	8.77	9.36	-	-
Amalgam	9.486	0.053	0.031	9.43	9.53	4.910	41.77
Zirconium	10.725	0.643	0.371	10.17	11.43	18.613	29.36
Titanium	11.898	2.431	1.403	9.42	14.28	31.585	21.54

TLD: Thermoluminescent dosimeters, PDI: Percentage dose increase, Std.: Standard, min: Minimum, max: Maximum

Statistical Analysis

Statistical analysis was performed using SPSS (IBM Corp., Windows, version 22.0). Independent t-test was used to compare the TLD data of the control group and study group. Besides, independent t-test was used to compare different IMs effectiveness. P-values less than 0.05 were considered statistically significant.

Results

Value of Scattered Radiation from the DM Surface and Data of the XCOM

The maximum PDI was presented for the titanium calculated with a value of 31.585% compared to the dose of the control group. It is shown that the TLD values, and ZPEAeff data are in a harmony (Table 1). As the ZPEAeff value indicating photon-energy absorption calculated by Phy-X/ZeXTRa diminished, the reported data in TLD increased. These results support the accuracy of the values measured in TLDs.

As seen in Table 2, the μ/ρ of the amalgam was found as higher than the other materials in each photon energy line. The mass absorption coefficients for amalgam, zirconium, and titanium in descending order.

Evaluation of the Protective Properties of IMs

It was calculated that 5 mm IMs significantly prevent the dose enhancement caused by all three DMs ($p<0.05$). Table 3 shows the dose enhancements caused by three DMs. No difference was measured between the photon-stopping properties of the two IMs ($p>0.05$) (Table 3).

Table 2. Presents the μ/ρ values calculated by XCOM. It is shown that μ/ρ values are higher in amalgam group that other material groups in between fraction of 0.2-6.0 MeV photon energy

Energy (MeV)	Amalgam (XCOM) (cm^2/g)	Zirconium (XCOM) (cm^2/g)	Titanium (XCOM) (cm^2/g)
0.2	0.58800	0.19820	0.13090
0.4	0.16240	0.10020	0.09089
0.6	0.09938	0.07831	0.07541
0.8	0.07632	0.06698	0.06584
1.0	0.06406	0.05949	0.05902
1.5	0.04944	0.04821	0.04810
2.0	0.04366	0.04224	0.04186
2.5	0.04085	0.03863	0.03790
3.0	0.03934	0.03631	0.03512
3.5	0.03857	0.03473	0.03314
4.0	0.03823	0.03362	0.03168
4.5	0.03817	0.03285	0.03058
5.0	0.03829	0.03230	0.02972
5.5	0.03852	0.03192	0.02906
6.0	0.03885	0.03167	0.02855

Table 3. Shows the comparison the TLD values capturing the transmissed radiation amount from PE and PVS in the each of dental material groups. Table presents that 5 mm PE and PVS increased the scattered dose when compared TLD data of control group. PE*PVS column indicate that there is not any difference between impression materials about shielding of the scattered radiation dose result from the dental materials in 6 MeV

	Control	5 mm PE	p-value	5 mm PVS	p-value	p-value (PE*PVS)
Amalgam	9.48±0.05	5.86±0.30	0.010	5.51±0.60	0.004	1.000
Zirconium	10.72±0.64	5.96±1.30	0.008	6.57±1.24	0.003	0.719
Titanium	11.89±2.43	6.75±1.38	0.033	6.07±1.36	0.032	1.000

TLD: Thermoluminescent dosimeters, PE: Polyether silicone-based, PVS: Polyvinyl siloxane

Discussions

The X-ray and gamma rays (in a wide energy range) interact with matter through the photoelectric effect, coherent scattering, incoherent scattering, and pair production (14,15). In this way, DMs interact with the X-rays and cause to secondary electrons detached from the atomic shield of the materials. These seconder electrons may cause dose enhancement especially in the tissues adjacent to dental alloys which is results in mucositis.

The interaction parameters of the radiations (X-ray, gamma, neutron, etc) with materials depends on the effective atomic number (Zeff), and μ/ρ considered as a type of absorption cross-section (15). The Zeff depends on the energy, type of the incident radiation, and the density of the material, that plays an important role to determine the effects of X-rays in the matter (16). The μ/ρ is a value that gives the average number of interactions between incident photons and material. As a result of these interactions, amount of average photon energy transferred in kinetic energy can be measured by μ/ρ. The μ/ρ has an essential role in estimating absorbed dose in medical physics and other fields used irradiation technologies such as industrial and agricultural studies (17).

The value of the ZPEAeff obtained using μ/ρ s may be attributed to the pair production, photoelectric effect, coherent scattering, and incoherent scattering (15). Regard, the ZPEAeff values of the DMs were calculated in this study. The results were compatible with the Phy-X/ZeXTRa database and recorded TLD values. It should be noted that the percentage of components in the same DMs may differ between commercial products.

In a previous study conducted by an H&N anthropomorphic and IMRT technic, it was reported that the backscatter dose of the dental amalgam measured by TLD, causes dose increase in the parotid glands (up to 24.38%) and a reduction in the mean dose (up to -6.25%). The closest position of the parotid gland to the tooth was 6.1 cm, while the farthest position was 10.9 cm from the tooth with amalgam in the H&N phantom (18). But in this current study, it was observed that the dose enhancement at a distance of 5 mm from amalgam was 5% (18). Although both studies showed a dose enhancement due to the amalgam, the differences in PDI values may be caused by the different RT methods.

Regard, the intraoral stents have been used to decrease the potentially adverse effect of the irradiation in the normal tissues outside the planned target volume by increasing the distance between the maxilla and mandible or depressing to the healthy tissues such as the tongue (19). This method can set apart the healthy tissues from the irradiation field. Additionally, it leads to the stabilization of the mandible in each fraction of RT (6). Besides oral stents, other devices which easily adapt to anatomical structures can be used to separate the tumor area by covering healthy tissues. Due to fact that their easy adaptable properties, the effectiveness of the IMs as an X-ray stopper was evaluated. Besides the IMs are considered as a useful material because they are practical for manipulation, soft and non-irritating for tissues, low cost, easy and rapid. Additionally, they don't need any laboratory process for creating.

For the protection of the oral mucosa from the radiation-induced radicals, we offer using a new prepared IM for every fractional irradiation. Additionally, to avoid the radioactivation of IMs, Kawamura et al. (8) offered the taking out the irradiated PVS from the patient's mouth within 1 minute. Moreover, researchers reported that the radioactivation of the PVS was less than 1 μSv/h after 2 Gy-proton irradiation and decreased to the background level after 30 minutes (8). One of the potential criticisms of this current study would be using the only TLDs for dose measurements. If another dose calculation method had been used additionally and compared with the results of TLDs, more confident results would have been obtained. Regard, to minimize uncertainties of all values, dose measurements were made with TLDs three times from three different regions for each set-up.

Conclusions

According to the knowledge of the authors, no reports have been published confirming in advance PE material safety and impact thoroughly on preventing the backscatter radiation dose caused by the interaction of the X-ray with DMs. PE and PVS could be used in patients with oral cancer to reduce the occurrence of oral complications such as radiation mucositis. But it should be noted that we believe it is very important to examine the use of the material in a comprehensive approach before bringing the technique to the clinic application.

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Ethics

Ethics Committee Approval: Ethics committee approval was not obtained since the study was carried out in an experimental environment on materials that did not belong to any living organism.

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Surgical and Medical Practices: Y.D., Ç.A., E.I.A., Concept: Y.D., Ç.A., E.I.A., Design: Y.D., Ç.A., E.I.A., Data Collection or Processing: Y.D., Ç.A., E.I.A., Analysis or Interpretation:

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Relationship between Newly Diagnosed Hypertensive Patients' Dipping Status and Mean Neutrophil Volume

Yeni Tanı Almış Hipertansif Hastaların Dipping Durumu ile Ortalama Nötrofil Hacmi Arasındaki İlişki

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Abstract

Objective: Little is known about the pathogenesis of essential hypertension (HT) despite the research conducted in this field. However, similar to other chronic diseases, an association has been shown between HT and inflammation. Thus, this study aimed to explore the association between an indicator of inflammatory response and mean neutrophil volume (MNV) in newly diagnosed hypertensive patients.

Materials and Methods: The medical records of patients newly diagnosed with HT were retrospectively reviewed. The control group comprised healthy persons with normal ambulatory blood pressure records. In accordance with their immersion status, newly diagnosed hypertensive patients were divided into two groups.

Results: This study included 222 patients: One hundred and forty four patients had HT, and one hundred and eighty-eight were normotensives. HT patients had significantly higher MNV than normotensive patients [144.1 (range: 136.1-152.1 vs.) 140.3 (range: 135.1-145.5), p=0.001], respectively. There were 51 patients with dipper HT and 53 patients with non-dipper HT in the hypertensive group. MNV was significantly higher in the non-dipper HT group [145.7 (range: 138.1-153.46) vs. 142.3 (range: 134.3-150.3), p=0.022], respectively. Multivariate regression analysis demonstrated that MNV [95% confidence interval (CI): 1,006-1,122, p=0.032] and pulse wave velocity (95% CI: 1,203-2,655, p=0.004) were independently correlated with the non-dipping status in newly diagnosed hypertensive patients.

Conclusion: Patients with newly diagnosed HT had higher MNV. In addition, increased MNV measurements were associated with non-dipper HT.

Keywords: Hypertension, mean neutrophil volume, pulse wave velocity, non-dipper hypertension, MNV

Öz

Amaç: Hipertansiyon (HT) alanında yapılan araştırmalara rağmen esansiyel HT'nin patogenezi hakkında çok az şey bilinmektedir. Bununla birlikte, diğer kronik hastalıklara benzer şekilde, HT ve enflamasyon arasında bir ilişki gösterilmiştir. Bu nedenle, bu çalışma yeni tanı konmuş hipertansif hastalarda bir inflamatuar yanıt göstergesi ile ortalama nötrofil hacmi (MNV) arasındaki ilişkiyi incelemeyi amaçlamaktadır.

Gereç ve Yöntemler: Yeni HT tanısı almış hastaların tıbbi kayıtları retrospektif olarak incelendi. Kontrol grubu, kan basıncı kayıtları normal olan sağlıklı hastalardan oluşturuldu. Yeni tanı almış hipertansif hastalar, dipping durumlarına göre iki gruba ayrıldı.

Bulgular: Bu çalışmaya 222 hasta dahil edildi; 144 hasta yeni HT tanılı, 188 hastada ise normotansif olarak değerlendirildi. Hipertansif hastalar, normotansif hastalardan istatistiki olarak daha yüksek MNV'ye sahipti (sırasıyla 144,1, 140,3, p=0,001). Hipertansif grupta dipper HT'si olan 51 hasta ve non-dipper HT olan 53 hasta vardı. MNV, non-dipper HT grubunda anlamlı olarak daha yüksekti (sırasıyla 145,7, 142,3, p=0,022). Yeni tanı konmuş hipertansif hastaların multivariate regresyon analizinde, MNV'nin [%95 güven aralığı (GA): 1,006-1,122, p=0,032] ve nabız dalga hızının (%95 CI: 1.203-2.655, p=0,004) non-dipper HT ile ilişkili olduğunu göstermiştir.

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Sonuç: Yeni tanı konmuş hipertansif hastalarda MNV normotansif hastalara gore daha yüksekti. Ek olarak, artan MNV ölçümleri non-dipper HT ile ilişkiliydi.

Anahtar Kelimeler: Hipertansiyon, ortalama nötrofil hacmi, nabız dalga hızı, non-dipper hipertansiyon, MNV

Introduction

Hypertension (HT) is a major public health concern worldwide, and is considered to be one of the primary causes of preventable deaths. HT has become a disease with high morbidity and mortality due to damage it causes to the peripheral arteries, heart, kidney, brain, and eyes, leading to a heavy burden on healthcare resources (1-4).

Despite numerous previous studies, the pathogenetic mechanisms underlying HT are unclear. However, it is known that, as in many other chronic diseases, inflammatory dysregulation is a common culprit in HT (5). An independent association between C-reactive protein, interleukin-6, or adhesion molecules, indicators of chronic low-grade inflammation and responsible for vascular changes in essential HT patients, has been demonstrated. Inflammation increases the proliferation of smooth muscle cells in vascular structures and, as a consequence, contributes to high blood pressure (6-9). At the same time, this inflammation causes increased adhesion molecule expression, immune cell activation, cytokine release, and oxidative stress leading to end-organ damage and progression (9).

Mean neutrophil volume (MNV) refers to the average size of circulating neutrophils and can be easily detected by automatic hematological cell analyzers (10,11). The presence of differences in MNV has been linked to an increased inflammatory response, and MNV is a marker of illness severity in various infectious illnesses, acute myocardial infarction, and trauma, regardless of neutrophil count (10,12-18).

There is insufficient knowledge about the possible relationship between MNV and BP variation in hypertensive and normotensive subjects. Thus, the purpose of this study was to compare MNV in normotensive and newly diagnosed hypertensive patients. Additionally, we examined the relationship between dipping status and MNV in patients who have recently been diagnosed with HT.

Materials and Methods

Study Population

Patients equipped with a 24 h ambulatory BP monitoring device with a pulse wave velocity (PWV) measurement feature for evaluating HT in the cardiology clinic between January 2013 and December 2018 were retrospectively involved in the study. This study was approved by the Ethics Committee of Selçuk University of Medical Sciences (decision no: 2018/407, date: 21.11.2018). After obtaining the ethics committee's approval, the patient's digital records, including demographic and clinical data, were collected. A patient was excluded if he or she had any of the following conditions in the past: diabetes mellitus, chronic renal disease (serum creatinine ≥1.5 mg/dL in men and ≥ 1.4 mg/dL in women), coronary artery disease, congenital heart disease, left ventricular systolic dysfunction (on echocardiography <50% ejection fraction), local or systemic infection, moderate or severe valvular disease, atrial fibrillation, obstructive sleep apnea, hyperthyroidism, known malignancy, anemia, hematological disorders, and taking regular medication for any reason (including antihypertensive drugs). Moreover, patients who had a recent history of infection or another acute inflammatory condition were excluded from this study.

Blood Pressure Measurement Using Ambulatory BP Monitoring and Diagnosis of Hypertension

Ambulatory blood pressure was measured using the Mobil-O-Graph Arteriograph (I.E.M. GmbH, Stolberg, Germany) and was recorded on the computer. The arm cuff was placed on the non-dominant arm, and recordings were taken for 24 hours. During the study, blood pressure measurements were taken every 15 minutes throughout the day (from 7 a.m. to 10 p.m.) and every 30 minutes during the night (from 10 p.m. to 7 a.m.). The patients were instructed to continue their daily routine and remain calm when they felt the cuff swell. Mean systolic blood pressure (SBP), mean blood pressure and mean diastolic blood pressure (DBP) values were calculated for each patient during the daytime, nighttime, and 24 hours. In this study, HT was defined as having a 24-hour mean SBP greater than 130 mmHg and/or DBP greater than 80 mmHg, as well as a mean daytime SBP greater than 135 mmHg and/or DBP greater than 85 mmHg, according to the diagnostic criteria, and mean nighttime systolic BP greater than 125 and/or diastolic BP greater than 70 mmHg (19). Dipper HT was defined as an average decrease of more than 10% in the diastolic and SBP measurements taken at night. The ambulatory blood pressure measuring device automatically measured PWV (20).

Blood Samples

Complete blood count and serum biochemistry values were recorded from blood samples taken during cardiology outpatient clinic admission. Hemoglobin, white blood cells, neutrophils, platelets, lymphocytes, and MNV were evaluated in the whole blood count. In addition, total cholesterol, high-density lipoproteins, low-density lipoproteins, serum creatinine, fasting blood glucose, and triglycerides were recorded in serum biochemistry.

The Beckman Coulter DXH 800 analyzer (Beckman Coulter, Fullerton, CA, USA) was used to measure MNV. The VCS technology of the Coulter DXH 800 analyzer measures the

volume conductivity and light scatter. The Coulter analyzer can assess the morphological changes seen in reactive neutrophils, including volume, conductivity, and scatter changes.

Statistical Analysis

A statistical evaluation was conducted using SPSS 21.0 (SPSS Inc., Chicago, IL, USA). To test the compatibility of numerical variables with a normal distribution, the Kolmogorov-Smirnov test was employed. Descriptive statistics were presented as the arithmetic mean ± standard deviation and median (25-75%) for numerical variables, and as numbers and percentages for categorical data. In cases where the parametric test assumptions were met, the significance test of the difference between the two means was used to compare the two groups with respect to numerical variables. The Mann-Whitney U test was used if not. Differences between groups concerning categorical variables were analyzed using chi-square and Fisher's exact chi-square tests. Binary logistic regression analysis was used to detect factors associated with non-dipper HT. A value of $p<0.05$ was accepted as significant for all evaluations.

Results

A total of two hundred and twenty-two patients were included in this retrospective study. HT was found in 104 patients (51.20±12.33 years, 57% male), while 118 patients (49.36±13.32 years, 55% male) were normotensive. The demographic, clinical, and laboratory parameters distribution was similar between the normotensive and hypertensive groups (Table 1). The hypertensive group exhibited higher 24-hour systolic and DBP compared to the normotensive

Table 1. Comparison of demographic, clinical and laboratory characteristics of normotensive and hypertensive groups

Variable	Normotensive (n=118)	Hypertensive (n=104)	p-value
Age, year	49.36±13.32	51.20±12.33	0.08
Gender (male), n (%)	55 (46.6%)	57 (54.8%)	0.223
Body mass index (kg/m^2)	28.70±3.71	28.42±2.43	0.972
LDL, mg/dL	125.06±38.41	134.21±31.89	0.271
HDL, mg/dL	43.62±9.41	45.64±9.13	0.176
Triglyceride, mg/dL	183.01±122	209±149.97	0.321
Hgb, g/dL	13.88±1.75	13.65±1.95	0.376
White blood cell, 10^3/µL	8.09±218	8.01±2.38	0.574
Platelet, 10^3/µL	246.75±69.45	261.07±66.04	0.191
Glucose, mg/dL	107.60±40.57	125.46±84.87	0.096
Creatinine, mg/dL	0.75±0.22	0.79±0.16	0.064
BUN, mg/dL	28.37±13.54	29.21±9.03	0.219
Na^+, mEq/L	137.91±2.56	138.17±2.63	0.482
K^+, mEq/L	4.32±0.40	4.40±0.45	0.296
24-h SBP, mmHg	110.61±8.74	135.87±12.48	<0.001
24-h DBP, mmHg	69.00±7.79	83.39±9.52	<0.001
Daytime SBP, mmHg	112.51±9.27	138.40±17.58	<0.001
Daytime DBP, mmHg	70.12±8.39	85.14±10.29	<0.001
Nighttime SBP, mmHg	107.15±8.39	130.89±10.18	<0.001
Nighttime DBP, mmHg	66.89±8.11	81.57±8.87	<0.001
Pulse wave velocity, m/s	6.77±1.69	8.62±1.49	<0.001
MNV	140.3±5.21	144.07±7.99	0.001
Neutrophil count	5.13±219	5.56±6.03	0.659

LDL: Low-density lipoprotein, HDL: High-density lipoprotein, Hgb: Hemoglobin, BUN: Blood urea nitrogen, Na^+: Sodium, K^+: Potassium, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, MNV: Mean neutrophil volume

group (p<0.001). Furthermore, the hypertensive group demonstrated higher levels of PWV and MNV compared to the normotensive group in the study (p<0.001).

When 104 patients with HT were grouped as dipper and non-dipper, 53 patients (52.62±11.27 years, 56.6% male) were grouped as non-dipper HT and 51 patients (49.73±13.30 years, 52.9% male) as dipper HT. At baseline, the dipper and non-dipper groups demonstrated similar clinical and demographic characteristics (Table 2). Markers, such as hemogram, neutrophil, and thrombocyte, obtained from the complete blood count of the non-dipper and dipper HT groups were similar between the two groups (p>0.05). However, daytime and 24-hour diastolic and SBP were higher in the dipper group (p<0.05). In addition, PWV and MNV measurements were higher in the non-dipper group than in the dipper group (p=0.042, p=0.022, respectively).

In multivariate binary logistic regression analysis including Hb, night SBP, body mass index, MNV, and PWV, only MNV and PWV were related to non-dipper HT (p=0.032, p=0.004) (Table 3).

Discussion

Previous studies have investigated multiple parameters of neutrophil activity in HT patients. The study's findings indicate that neutrophils could potentially modify the microenvironment in blood vessels by heightening oxidative stress, which in turn favors endothelial dysfunction. MNV has emerged as a new marker of activated neutrophils. To our knowledge, this is the first study to demonstrate that MNV levels are higher in hypertensive subjects than in normotensive subjects. Also, we found that MNV levels were significantly associated with dipper status in HT

Table 2. Comparison of demographic, clinical and laboratory characteristics of dipper and non-dipper groups

Variable	Non-dipper (n=53)	Dipper (n=51)	p-value
Age, year	52.62±11.27	49.73±13.30	0.174
Gender (male), n (%)	30 (56.6%)	27 (52.9%)	0.708
Body mass index (kg/m^2)	28.61±2.16	28.21±2.7	0.564
LDL, mg/dL	141.11±35.86	127.75±26.64	0.207
HDL, mg/dL	44.90±9.85	46.30±8.51	0.539
Triglyceride, mg/dL	194.43±153.51	221.13±144.48	0.151
Hgb, g/dL	13.73±1.82	13.56±2.10	0.887
White blood cell, 10^3/µL	7.65±2.13	8.38±2.58	0.101
Platelet, 10^3/µL	262.43±68.92	258.68±63.69	0.682
Glucose, mg/dL	142.84±113.19	109.52±4.65	0.374
Creatinine, mg/dL	0.88±0.28	0.79±0.19	0.127
BUN, mg/dL	36.44±19.27	30.41±11.60	0.163
Na^+, mEq/L	138.02±2.97	138.32±2.29	0.730
K^+, mEq/L	4.38±0.41	4.41±0.48	0.768
24-h SBP, mmHg	133.03±11.85	138.82±12.55	0.007
24-h DBP, mmHg	81.58±8.60	85.27±10.14	0.021
Daytime SBP, mmHg	133.03±19.06	144.19±13.86	<0.001
Daytime DBP, mmHg	81.58±8.77	88.84±10.53	<0.001
Nighttime SBP, mmHg	129.83±9.97	132.00±10.38	0.268
Nighttime DBP, mmHg	81.92±8.65	81.21±9.19	0.995
Pulse wave velocity, m/s	8.91±1.17	8.31±1.72	0.011
MNV	145.76±7.70	142.31±7.99	0.022
Neutrophil count	5.85±8.27	5.27±2.05	0.057

LDL: Low-density lipoprotein, HDL: High-density lipoprotein, Hgb: Hemoglobin, BUN: Blood urea nitrogen, Na^+: Sodium, K^+: Potassium, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, MNV: Mean neutrophil volume

Table 3. Binary logistic regression analysis to determine independent variables associated with non-dipper hypertension

	Univariate analysis			Multivariate analysis		
Variable	OR	95% CI	p-value	OR	95% CI	p-value
PWV	1,334	1,008-1,766	0.044	1,787	1,203-2,655	0.004
MNV	1,058	1,005-1,114	0.03	1,067	1,006-1,122	0.032
Nighttime SBP	0.979	0.942-1,017	0.279	0.953	0.904-1,005	0.077
BMI	1,071	0.9713-1,257	0.401	1,220	0.978-1,510	0.069
Hgb	1,045	0.850-1,286	0.676	1,268	0.978-1,644	0.073

PWV: Pulse wave velocity, MNV: Mean neutrophil volume, SBP: Systolic blood pressure, BMI: Body mass index, Hgb: Hemoglobin, CI: Confidence interval, OR: Odds ratio

patients.

It has been recognized that low-grade inflammation plays a very significant pathophysiological role in cardiovascular disease and HT (6,10). Inflammation is involved in many processes that may contribute to developing high blood pressure. Vascular inflammation plays a role in vascular remodeling by increasing the proliferation of smooth muscle cells. Neutrophils are essential cells in inflammation (12,13,15,21). MNV represents the average size of the circulating neutrophil population. Studies showed that changes in MNV values are related to increased inflammatory response and indicate disease severity in many early trauma and infectious diseases, regardless of neutrophil counts (10,12,14,16,18).

In previous studies, a significant increase in MNV in cardiovascular diseases and sepsis conditions has been shown. van Hout et al. (13) carried out a study including 373 patients with ST-elevation myocardial infarction (STEMI), stable coronary artery disease, and coronary artery disease, which showed that MNV was significantly increased in patients with myocardial damage. In the study by Buyukterzi et al., (22) 121 patients without ACS, stable coronary artery disease, and coronary artery disease were, and their findings showed that MNV increased significantly in ACS patients. In our study, MNV was statistically significantly higher in patients with HT (22).

It has been shown in previous studies that non-dipper HT is related to a worse cardiovascular prognosis than dipper HT (23). In a study involving 42,947 patients, non-dipper HT has been associated with age, obesity, diabetes mellitus, and overt cardiovascular or kidney disease (24). In addition, Cuspidi et al.'s (25) study showed that non-dipper HT is more frequently associated with target organ damage than dipper HT. A statistically significant difference was observed between non-dipper HT patients and dipper HT patients in our study, and increased MNV values were associated with non-dipper HT.

Although the strict definition of the patient population in our study and the exclusion of patient groups that may be associated with high MNV other than HT constitute the strength of this study, there are some limitations to our study. The main limitations of our study are the relatively small number of patients enrolled in this study and the lack of evaluation of their response to antihypertensive treatment. However, we should note that MNV is a marker that can be easily measured and is a significant inflammatory indicator that may be a guide for further studies.

Conclusion

MNV was higher in hypertensive patients than normotensive patients. In addition, increased MNV measurements were associated with non-dipper HT. Our study results need to be supported by large-scale prospective studies showing long-term prognosis.

Ethics

Ethics Committee Approval: This study was approved by the Ethics Committee of Selçuk University of Medical Sciences (decision no: 2018/407, date: 21.11.2018)

Informed Consent: Informed consent is not required.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: M.S.A., M.U.Y., Design: M.S.A., M.U.Y., Data Collection or Processing: M.S.A., A.T., K.D., N.A., Analysis or Interpretation: M.S.A., M.U.Y., A.T., K.D., N.A., Literature Search: M.S.A., M.U.Y., B.B.A., Writing: M.S.A., M.U.Y., B.B.A.

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A Cross-sectional View of Rational Antibiotic Use

Akılcı Antibiyotik Kullanımına Kesitsel Bir Bakış

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Abstract

Objective: Antimicrobial resistance is an issue that threatens human health worldwide. One of the main factors responsible for the formation of resistance is the irrational use of antibiotics. The inappropriate use of antibiotics has many direct and indirect effects. Ceftriaxone and quinolones are the leading antibiotics associated with this condition, which is known as collateral damage. In this study, we aimed to evaluate the consultations requested regarding the use of these antibiotics in a cross-sectional manner.

Materials and Methods: Ceftriaxone and parenteral quinolone consultations from adult patients hospitalized in our hospital between 01.01.2022 and 30.06.2022 were included in the study. Demographic data and consultation results of the patients were retrospectively evaluated.

Results: A total of 560 consultations from 538 patients were evaluated, of which 40.7% were women. Three hundred forty-seven patients (64.5%) were followed in internal clinics. The most requested antibiotic was ceftriaxone (73.6%). There was no diagnosis of infection in 82 (15.2%), and antibiotics were continued postoperatively in 75 (13.9%) patients. The rate of patients who were not cultured before treatment was 45.4%, while the rate of patients who were diagnosed with infection but were not cultured was 36.6%. Pre-treatment culture rate was lower and antibiotic withdrawal rate was higher in surgical units than in internal units.

Conclusion: Resistance can be slowed down by the sensitivity of not only infectious disease specialists but also all physicians prescribing antibiotics, the use of antibiotic stewardship programs and local guides, and education. Scientific studies should be continued to generate local and national data.

Keywords: Antimicrobial resistance, infectious diseases consultation, third generation cephalosporin, fluoroquinolone

Öz

Amaç: Antimikrobiyal direnç tüm dünyada insan sağlığını tehdit eden bir konudur. Direnç oluşumunda başlıca sorumlu faktörlerden biri irrasyonel antibiyotik kullanımıdır. Antibiyotiğin uygunsuz kullanımı doğrudan ve dolaylı olarak birçok etkiye neden olmaktadır. Kollateral hasar olarak adlandırılan bu durumla ilişkili antibiyotiklerin başında seftriakson ve kinolonlar gelmektedir. Bu çalışmada bu antibiyotiklerin kullanımı ile ilgili istenen konsültasyonların kesitsel olarak değerlendirilmesi amaçlanmıştır.

Gereç ve Yöntemler: Hastanemizde 01.01.2022-30.06.2022 tarihleri arasında yatan erişkin hastalardan gelen seftriakson ve parenteral kinolon konsültasyonları çalışmaya dahil edildi. Hastaların demografik verileri ve konsültasyon sonuçları retrospektif olarak değerlendirildi.

Bulgular: Beş yüz otuz sekiz hastadan toplam 560 konsültasyon değerlendirildi. Hastaların %40,7'si kadındı. Üç yüz kırk yedi hasta (%64,5) dahili kliniklerde takip edilmekteydi. En çok talep edilen antibiyotik seftriaksondu (%73,6). Seksen iki (%15,2) hastada enfeksiyon tanısı yoktu, 75 (%13,9) hastada ise postoperatif antibiyotik tedavisi devam ediyordu. Tedavi öncesinde kültür alınmayan hastaların oranı %45,4, enfeksiyon tanısı olup kültür yapılmayan hastaların oranı ise %36,6 olarak belirlendi. Cerrahi birimlerde dahili birimlere göre tedavi öncesi kültür alma oranı düşük, antibiyotiğin kesilme oranı daha yüksekti.

Sonuç: Sadece enfeksiyon hastalıkları uzmanları değil antibiyotik reçete eden tüm hekimlerin direnç konusunda hassas olması, antibiyotik yönetişim programlarının ve lokal rehberlerin kullanılması ve eğitim ile direnç yavaşlatılabilir. Yerel ve ulusal verilerin oluşturulması için bilimsel çalışmalara devam edilmelidir.

Anahtar Kelimeler: Antimikrobiyal direnç, enfeksiyon hastalıkları konsültasyonu, üçüncü kuşak sefalosporin, florokinolon

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Introduction

Antimicrobial resistance (AMR) is defined as the ability of all microorganisms to resist the action of antimicrobial agents and to survive and grow even in the presence of drugs that previously affected them (1).

The increasing consumption of antibiotics in the healthcare and agriculture sectors has led to the emergence of microorganisms resistant to antibiotics all over the world. This trend is manifested in a high prevalence in a wide variety of microorganisms. This problem has become one of the biggest public health threats today, and World Health Organization estimates that 10 million deaths could occur by 2050 due to infections with resistant microorganisms (2). AMR causes to serious illness, treatment failures, need for treatment with second-generation drugs, prolonged hospital stays and, higher healthcare costs (3).

Inaccuracies in the use of antibiotics in general, difficulties in developing new antibiotics, easy travel routes and bacterial biological factors are contributing factors to AMR (1). Undesirable effects detected in bacterial ecology such as selection of resistant bacteria, colonization/infection with multi-resistant bacteria as a result of inappropriate or excessive use of antibiotics are defined as "collateral damage". The antibiotics most commonly associated with collateral damage are third-generation cephalosporins, fluoroquinolones, and carbapenems (4).

One of the rational antibiotic use methods in the fight against AMR is the policies to restrict the use of antibiotics. In our country, antibiotic prescribing rules were determined with the budget implementation instruction that came into force in 2003 (5). Infectious diseases specialists (IDS) were authorized to prescribe broad-spectrum antibacterials, antifungals and antivirals used in the hospital. Some group antibiotics including third-generation cephalosporins, fluoroquinolones can be prescribed by any specialist at the beginning of the treatment, but requiring IDS approval if it will be used more than 72 hours.

In this study, it was aimed to draw attention to the rational use of antibiotics evaluating the consultations in a period of six months requested from the internal and surgical clinics of Aydın Adnan Menderes University Hospital regarding the use of this group antibiotic.

Materials and Methods

Hospital Setting

The hospital where the study was conducted is a 997-bed tertiary hospital. There are nine internal medicine services and nine surgical services at the hospital. There are four internal, four surgical, one general and three pediatric intensive care units.

Antibiotic Prescription Policy

Within the IDS approval definitions;

1. Non-restriction antibiotics (e.g., ampicillin, cefazolin).

2. Antibiotics that can be prescribed by specialist physicians in outpatient treatment or by all physicians including general practitioners depending on the specialist physician's report, and by all physicians in inpatient treatment (e.g., ampicillin sulbactam, cefuroxime).

3. Antibiotics for which IDS is not required for prescription but if the same drug will be used for longer than 72 hours, IDS approval must be obtained within the first 72 hours at the latest (*the group of antibiotics evaluated in this study) (e.g., piperacillin, cefoperazone, cefotaxime, ceftriaxone, parenteral ciprofloxacin- levofloxacin- moxifloxacin).

4. Antibiotics that only IDS can prescribe. If there is no IDS, they can be prescribed by an internist or pediatrician. (e.g., piperacillin tazobactam, ceftazidime)

Data Collection

In the study, consultations requested for third group antibiotics at Aydın Adnan Menderes University Hospital between 01.01.2022 and 30.06.2022 were evaluated retrospectively. Cefotaxime, ceftriaxone, parenteral ciprofloxacin, levofloxacin and moxifloxacin are available in our hospital as third group antibiotics.

Of all patients' who used these group antibiotics for an average of 72 hours, demographic features, departments in which they were hospitalized (internal/surgical, clinic/intensive care), antibiotics used, infection diagnoses, doses and dose range, and whether or not microbiological culture was performed were recorded in the study form.

As a result of the consultations; the decision to continue antibiotics is made in patients with a diagnosis of infection for the initiation of antibiotics, appropriate cultures were taken at the beginning, and the antibiotic was administered at the correct dose and dose range. Treatment is discontinued in patients without a diagnosis of infection and no indication for antibiotic therapy and receiving unnecessary or prolonged prophylaxis. A decision can be made to change the treatment (escalation or deescalation) in patients who do not improve clinically or according to the susceptibility of microorganism grown in cultures taken before treatment. In some patients, additional tests, especially microbiologic evaluation may be recommended.

The consultation decision was recorded in the study form. Consultations requested again from the same patient were recorded as re-consultations.

Patients with incomplete examination and follow-up processes, whose data could not be reached, and patients in the pediatric age group were excluded from the study. IDS approval consultations requested for antibiotics other than the specified group were not included in the study.

Statistical Analysis

Data was analyzed using IBM SPSS Statistics 22 (SPSS Inc, ABD). Chi-square tests were used for comparing the two groups. $P<0.05$ value was accepted as significant.

The study was approved by Aydın Adnan Menderes University Faculty of Medicine Non-interventional Clinical Research Ethics Committee (decision no: 31, date: 04.05.2023).

Results

During the 6-month study period, a total of 7,962 consultations, including repeated consultations from the same patient, were scanned from the hospital information system.

A total of 560 consultations, including 538 consultations and 22 re-consultations, which met the study criteria were included in the study. Of the 538 patients for whom consultation was requested, 219 were female (40.7%) and 319 were male (59.3%). The mean age was 68.81±16.4.

Three hundred and fourty-seven patients were followed in internal clinics and 191 patients were followed in surgical clinics. It was observed that the most requested antibiotic was ceftriaxone (73.6%). According to the consultation request, the patients were divided into four groups. While 371 (69%) patients had a diagnosis of infection, 82 (15.2%) patients did not have. There was a request to continue antibiotherapy for prophylactic purposes in 10 (1.9%) patients and postoperative antibiotic therapy in 75 (13.9%) patients. Among the patients who received postoperative treatment (n=75), it was decided to continue or revise the treatment due to infection (perforation, etc.) in 19 (25.4%) patients, and to discontinue the treatment in 56 (74.6%) patients.

No culture was sent before the start of antibiotic therapy in 244 of 538 patients (45.4%), while the rate of patients who were diagnosed with infection but were not cultured was 136/371 (36.6%). Cultures sent from other patients and pre-diagnosis/diagnoses of infection are shown in Table 1. As a result of the consultation, it was decided to discontinue the treatment in 202 (37.5%) patients, to continue in 283 (52.6%), and to revise antibiotics in 53 (9.9%) patients. Additional tests were requested from 46 (8.6%) patients. The numbers and percentages according to the units are given in Table 2. It was observed that antibiotics were used in the correct dose and dose range in all patients.

When the re-consultations were evaluated, antibiotics were not started in 11 patients because there was no infective focus. It was decided to continue antibiotics in 6 patients and to revise treatment in 5 patients.

Discussion

In this study, consultation data on the use of certain groups of antibiotics in a 6-month period were evaluated with a cross-sectional view. When the literature is reviewed, most of the studies on rational antibiotic use have been conducted using the one-day point prevalence method (6-8). It is reported that the rates of antibiotic use in hospitalized patients vary between 36.2% and 63.2% in studies reported from our country (9). In the point prevalence study evaluating the use of antibiotics in Latin American countries, the rate of antibiotic use was found to be 54.6% (10). In various studies from other countries, antibiotic use rates were reported as 46%, 32% and 14.1% (8,11,12).

In a multicenter study conducted in Korea, when a total of 10,948 therapeutic, surgical and medical prophylactic treatments were evaluated, the rate of inappropriateness was found to be 27.7% (12). In a systemic review and meta-analysis containing data from Turkey, the mean rate of inappropriate antibiotic prescribing was reported as 36%. The reasons for inappropriateness were listed as, use of similar antimicrobials together, failure to use antibiotics in the correct indication, administration of antibiotics at improper dose and dose range, inaccurate prophylaxis and, unnecessarily prolonged treatment cure (13). Accordingly, we can characterize antibiotherapy as inappropriate or suboptimal for our study in patients without a diagnosis of infection (15.2%) and in patients who started therapy as prophylaxis and continued unnecessarily postoperatively (74.6%).

In addition, in studies evaluating the use of antibiotics according to clinics, it is noteworthy that surgical departments use inappropriate antibiotics at a higher rate than internal departments. The most important reason for this is seen as inappropriate surgical prophylaxis of surgical

Table 1. Clinic, culture samples and infection diagnosis of the patients

Clinic	n (%)
Internal medicine service	286 (53.2)
Medical intensive care unit	61 (11.3)
Surgical service	161 (29.9)
Surgical intensive care unit	30 (5.6)
Culture sample	
Blood	75 (13.9)
Sputum	66 (12.3)
Urine	40 (7.4)
Wound/body fluid	32 (5.9)
Feces	6 (1.1)
More than one culture	73 (13.6)
Infection diagnosis	
Respiratory	187 (34.8)
Biliary tract	58 (10.8)
Gastrointestinal system	44 (8.2)
Urinary system	34 (6.3)
Soft tissue, surgical field	20 (3.7)
Sepsis	16 (3)
Other	12 (2.2)

departments. In our study, the rate of culture taking before treatment in patients with infection was lower (p>0.05) and the rate of discontinuation of the current treatment was higher (p<0.001) in surgical units compared to internal clinics (Table 2).

Kömür et al. (14) in their study to determine the appropriateness of surgical prophylaxis reported that they evaluated the prophylaxis in 49.1% of patients as inappropriate in various aspects. Starting time of the antibiotics, given prophylaxis at clean surgery, continuing prophylaxis at a prolonged time, type and dosage of the antibiotic, not giving prophylaxis when it's indicated, were reasons of incompliance (14).

In a multicenter point prevalence study in Ghana in which only surgical units were evaluated, the rate of antibiotic use in 540 patients was found to be as high as 70.7%. It was stated that surgical prophylaxis was used longer than recommended in 88.4% of the patients who received antibiotics (15). Although first generation cephalosporins, which are frequently used prophylactically, were not in the scope of our study, it was recommended to discontinue antibiotics in 56 patients (74.6%) whose postoperative treatment was continued. In our study, while the rate of culture in the appropriate indication was 56.4% in surgical units, it was emphasized that microbiological analysis was performed in only 3.7% of the patients in this study (15).

In our study, respiratory tract infections, primarily pneumonia, were found to be the most common diagnosis in patients with an infection diagnosis. In the meta-analysis containing data from our country, it was reported that third generation cephalosporins (36%) were the most commonly prescribed antibiotics, and respiratory tract infection (88%) was the most common infection diagnosis (13). In a study conducted in Kenya, it was reported that antibiotics were given most frequently with the diagnoses of soft tissue infection (18%), sepsis (17%) and pneumonia (15%), respectively (11). In another study conducted in internal medical wards, the most common possible infectious disease was pneumonia, following meningitis. Ceftriaxone was the most commonly used agent in patients receiving single or combined antibiotics (16).

In our study, the rate of patients who were not cultured before treatment was found 45.4% while the rate of patients who were diagnosed with infection but were not cultured was 36.6%. Taking the necessary cultures before antibiotic therapy significantly reduces inappropriate antibiotic use. In the cross-sectional study of Oğuz et al., (17) it was stated that 67% of 126 patients were asked to culture before treatment, and the rate of inappropriate antibiotic use was 24% in general. In another study, it was reported that culture samples were taken from 57.8% of the patients who were given antibiotics for treatment (7). Additional tests including culture examinations were recommended to 8.6% of the patients in our study. However, since our study group consisted of patients who had been taking antibiotics for an average of 3 days, it was thought that the sensitivity of the cultures taken afterwards might be low.

Conclusions

Although the rates of antibiotic use are variable, inappropriate use of antibiotics is a problem all over the world. Although not addressed in this study, the negative

Table 2. Antibiotics requested, pretreatment culture in infectious patients and consultation results according to the unit

Hospital unit	Surgical clinics (n=191)	%	Internal clinics (n=347)	%	p-value
Age	58.27±16.4	-	66.71±15.9	-	<0.001
Sex (female/male)	75/116	-	144/203	-	-
Requested antibiotic					<0.001
Ceftriaxone	174	91	222	63.9	
Ciprofloxacin	12	6.3	36	10.4	
Moxifloxacin	4	2.1	49	14.2	
Levofloxacin	1	0.6	40	11.5	
Pretreatment culture					0.146
No	38	43.6	98	34.5	
Yes	49	56.4	186	65.5	
Consultation decision					<0.001
Continuation of the antibiotic	73	38.2	210	60.5	
Discontinuation of the antibiotic	100	52.3	102	29.4	
Revision (escalation/de-escalation)	18	9.5	35	10.1	

effects of irrational antibiotic use on cost and resistance cannot be ignored. The size of the problem should be revealed by obtaining hospital and country data. The sensitivity of all physicians ordering antibiotics, especially in the use of unrestricted antibiotics, can only be achieved by knowing the extent of the problem. Antibiotic stewardship programs, education, local antibiotic guidelines and their active use are effective methods in the fight against resistance.

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Ethics

Ethics Committee Approval: The study was approved by Aydın Adnan Menderes University Faculty of Medicine Non-Interventional Clinical Research Ethics Committee (decision no: 31, date: 04.05.2023).

Informed Consent: Retrospective study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

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

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Antioxidant Effects of Styrax Liquidus on DMBA-exposed Rat Tongue Tissues

Styrax Liquidus'un DMBA Uygulanmış Rat Dil Dokuları Üzerindeki Antioksidan Etkileri

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Abstract

Objective: Natural products with antioxidant components are believed to have a strong potential for the prevention of cancer and some degenerative diseases. Liquidambar orientalis Miller (Styrax Liquidus) has strong *in vitro* antioxidant activity. The purpose of this study was to evaluate the effects of Styrax Liquidus on antioxidant defense mechanisms in 7,12-dimethylbenz(a)anthracene (DMBA)-applied rat tongue tissue.

Materials and Methods: Wistar rats (n=30) were randomly divided into control, DMBA, DMBA + SL, and SL groups. The control group was treated with liquid paraffin only, the DMBA group was treated with 0.5% DMBA, DMBA and Styrax Liquidus were applied to the DMBA + SL group, and only Styrax Liquidus was applied to the SL group. All applications were made to the oral mucosa. Sixteen weeks later, the tongue tissue of all animals were removed. Superoxide dismutase, catalase, glutathione peroxidase enzyme activities, and malondialdehyde and total antioxidant status levels were measured.

Results: All parameters were significantly lower in the SL + DMBA group. Antioxidant enzyme activities and oxidative stress parameters were lowered in the SL + DMBA and SL groups. SL + DMBA application is believed to have an inhibitory effect on the antioxidant enzymes measured in this study; however, the decrease in malondialdehyde levels (lipid peroxidation marker) highlights the antioxidant effect of Styrax Liquidus in 7,12-dimethylbenz(a)anthracene-exposed rat tongue tissues.

Conclusion: Styrax Liquidus exhibited *in vivo* antioxidant activity in an oral cancer model. Further research may be useful in understanding the exact mechanisms underlying this effect.

Keywords: Antioxidant, 7,12-Dimethylbenzanthracene, Liquidambar, oral cancer, oxidant, Styrax

Öz

Amaç: Antioksidan içerikli doğal ürünlerin kanser ve bazı dejeneratif hastalıkların önlenmesinde güçlü bir potansiyele sahip olduğuna inanılmaktadır. Liquidambar orientalis Miller'ın (Styrax Liquidus) güçlü *in vitro* antioksidan aktiviteye sahip olduğu bilinmektedir. Bu çalışmada, Styrax Liquidus'un 7,12-dimetilbenz(a)antrasen (DMBA) uygulanan rat dil dokularında antioksidan savunma mekanizmaları üzerindeki etkilerinin değerlendirilmesi amaçlanmıştır.

Gereç ve Yöntemler: Çalışmaya dahil edilen otuz adet Wistar türü rat rastgele kontrol, DMBA, DMBA + SL ve SL gruplarına ayrılmıştır. Kontrol grubuna sıvı parafin, DMBA grubuna %0,5'lik DMBA, DMBA + SL grubuna DMBA ve Styrax Liquidus, SL grubuna ise yalnızca Styrax Liquidus uygulanmıştır. Tüm uygulamalar oral mukozaya yapılmış olup, deney süresinin sonunda tüm hayvanların dil dokuları alınmıştır. Dokulardaki süperoksit dismutaz, katalaz, glutatyon peroksidaz enzim aktiviteleri ve malondialdehit ve total antioksidan durum düzeyleri ölçülmüştür.

Bulgular: SL + DMBA grubunda tüm parametreler anlamlı derecede düşük bulunmuştur. SL + DMBA ve SL gruplarında antioksidan enzim aktiviteleri ve oksidatif stres parametreleri diğer gruplara kıyasla daha az olarak bulunmuştur. Styrax Liquidus ve DMBA uygulamasının bu çalışmada ölçülen antioksidan enzimler üzerinde inhibitör etkisi olduğu düşünülmüştür; ancak, lipid peroksidasyon

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belirteci olan malondialdehit düzeylerindeki azalma, Styrax Liquidus'un DMBA'ya maruz kalan rat dil dokuları üzerindeki antioksidan etkisinin altını çizmektedir.

Sonuç: Styrax Liquidus bu çalışmada oral kanser modelinde *in vivo* antioksidan aktivite sergilemiştir. Bu etkinin mekanizmasının anlaşılması için başka çalışmalara da ihtiyaç vardır.

Anahtar Kelimeler: Antioksidan, 7,12-Dimetilbenzanthrasen, Liquidambar, oral kanser, oksidan, Styrax

Introduction

Reactive oxygen species (ROS) are a highly reactive group of molecules produced mostly in the mitochondria (electron transport chain) during the respiratory functions of a cell. These molecules include hydroxyl and superoxide radicals and stable molecules such as H_2O_2 (1). In healthy cells, ROS levels are balanced through various mechanisms and detoxification processes. However; if the redox balance is somehow disrupted, this causes oxidative stress and may have pathological consequences such as diabetes mellitus, atherosclerosis and cancer (2).

Cancer is serious disease that has very high mortality rates around the world (3). Oral cancer, including lip and oral cavity, salivary gland, oropharynx, nasopharynx and hypopharynx cancers, is the 7th most commonly encountered type of cancer worldwide (4). Oral squamous cell carcinomas (OSCC) include more than 90% of oral cancers (5). Oral cancers have high mortality and morbidity rates, treatment is costly, and it does not always have such good prognosis (6,7).

Chemoprevention is a novel and promising method that has been subject to numerous studies investigating the anticancer effects of plants and plant-derived chemicals (8). Many plants used in chemoprevention studies have a variety of pharmacological activities such as antioxidant, anticancer, antitumor and/or cytotoxic properties (9-12). Chemopreventive plants or plant-derived chemicals may inhibit the initiation phase or revert the promotion phase of the carcinogenesis process. They also have the potential to inhibit the progression of premalignant lesions to malignant stages (13).

L. orientalis Mill. is an herbaceous plant mostly found in several regions of Southeast Asia and the Mediterranean region. L. orientalis Mill. trees exudate a resinous balsam from the wounded parts of their trunk called Styrax Liquidus. It has been reported to possess antiseptic (14), antimicrobial (15), antibacterial (16,17), antiulcerogenic (18), antiviral (19), antifungal (20), antihypertensive (21), anticonvulsant (22), antioxidant (23) and antimutagenic (24) properties. Moreover, it has been used in different cultures as a phytotherapeutic agent for skin (wounds, cuts, burns, psoriasis and other skin diseases), stomach (ulcers, stomach ache) and respiratory diseases (cough, asthma and bronchitis) (25). Also, it has been shown to have neuroprotective effects in cerebrovascular diseases (26). The major components of Styrax Liquidus are cinnamic esters (especially cinnamic acid), styrene and vanillin. Cinnamic acid is known for its antimicrobial and antioxidant properties (20,22). The aim of this study was to evaluate the effects of Styrax Liquidus on antioxidant defense mechanisms in tongue tissues of rats that have been locally exposed to 7,12-dimethylbenz(a)anthracene (DMBA).

Materials and Methods

This study was approved by the Gazi University Animal Experiments Local Ethics Committee (approval number: G.Ü.ET-17.018, date of approval: 02.03.2017).

Plant Material

The crude balsam of L. orientalis Mill. was obtained from the provincial directorate of Ministry of Agriculture and Forestry in Muğla province of Turkey. 100 mg pure Styrax Liquidus was dissolved in 1 mL ethanol (99.9%) (100 mg/mL). The solution was diluted with ethanol to procure 50 mg/mL concentration. Then it was mixed with glycerin with a 1:1 (v/v) ethanol-glycerin ratio to achieve 10 mg/mL concentration. The solution was kept in dark-colored bottles at +4 ˚C.

Carcinogenic Material

DMBA (Sigma-Aldrich, Milwaukee, WI, USA) is a potent carcinogen used in oral cancer animal models, which induces oxidative stress and eventually results in precancerous and cancerous lesions histopathologically and morphologically similar to human oral precancerous and cancerous lesions (10,27). The DMBA used in this study was prepared with liquid paraffin (0.5%, w/v), according to previous research protocols (28,29). The solution was kept in opaque bottles at 27 ˚C.

Animals and Experimental Design

Male Wistar rats (n=30) were randomly divided into four groups (Control, DMBA, SL + DMBA, SL). The control group consisted of 6 rats, while 8 rats were assigned to other groups. Liquid paraffin, 0.5% DMBA dissolved in liquid paraffin and L. orientalis Mill. extract was applied to the oral mucosa of the animals with a no 4 paint brush. Control group was treated with liquid paraffin thrice a week (Monday, Wednesday, and Friday). DMBA group was painted with 0.5% DMBA frequently as the control group. SL + DMBA group received 10 mg/mL L. orientalis Mill. extract application twice (Tuesday, Thursday), and was treated with 0.5% DMBA thrice a week. SL group was applied L. orientalis Mill. extract twice a week (Tuesday, Thursday). Animals were kept under controlled conditions. Animals

were provided with rat chow and water. After 16 weeks of applications, all animals were sacrificed and tongue tissues of all animals were excised as a whole. The samples were kept at -80 ˚C until homogenization.

Biochemical Analysis

Tongue tissue samples were prepared and analyzed using the same methods as previously by the authors (30). Catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) enzyme activities and malondialdehyde (MDA) and total antioxidant status (TAS) levels measured in all samples.

Statistical Analysis

SPSS 11.5 software was used for statistics. Mean ± standard deviation and median (minimum-maximum) were used to express normally and not normally distributed values, respectively. ANOVA was used if the values were normally distributed and Kruskall-Wallis test, if not. Post-hoc Tukey test was used for binary comparisons after ANOVA and Bonferroni adjusted Mann-Whitney U test was used after Kruskall-Wallis test. P<0.05 were considered as statistically significant.

Results

All enzyme activities were significantly lower in Styrax Liquidus applied groups in comparison to the DMBA group (p<0.05) (Table 1, Figures 1-3). SL+DMBA and SL groups also revealed significantly lower values compared to the Control group considering CAT and SOD levels (p<0.05) (Table 1, Figures 1, 2). MDA levels were statistically lower with regard to the DMBA group, in SL + DMBA and SL groups (p<0.05) (Table 1, Figure 4). TAS levels were significantly decreased in the SL + DMBA group with regard to control and DMBA groups (p<0.05) (Table 1, Figure 5).

Discussion

This study was conducted to evaluate the impact of Styrax Liquidus on the oxidant/antioxidant system in tongue tissues of DMBA-exposed rats. DMBA is a potent carcinogen that exhibits its effects mainly through chronic inflammation, ROS production and oxidative DNA damage (9,27,31). It is widely used in experimental OSCC models because it induces lesions very similar to human OSCC regarding histological, morphological and invasive properties (11). Carcinogenesis is a multi-stage process with initiation, promotion and progression phases.

Table 1. CAT (IU/mg), SOD (U/mg), GSH-Px (mIU/mg) enzyme activities and MDA (nmol/mg) and TAS (µmol Trolox eq/L) values of the tongue tissue samples, descriptive statistics and multiple comparisons

	CAT Median (min-max)	SOD (mean ± SD)	GSH-Px (mean ± SD)	MDA (mean ± SD)	TAS (mean ± SD)
Control (n=6)	12.17 (11.04-23.04)	3.96±0.84	36.83±12.91	1.42±0.53	0.27±0.04
DMBA (n=8)	12.47 (10.77-16.89)	3.30±0.30	32.13±2.10	1.18±0.15	0.23±0.03
SL + DMBA (n=8)	7.17 (5.40-9.18)	2.27±0.14	23.50±5.95	0.88±0.10	0.17±0.02
SL (n=8)	7.13 (6.09-8.77)	2.47±0.20	28.87±2.10	0.97±0.04	0.23±0.07
Kruskall-Wallis test/ANOVA					
Multiple comparison (p-values)	0.000	0.000	0.007	0.002	0.002
Bonferroni adjusted Mann-Whitney U test/Tukey test (p-values)					
Control vs. DMBA	1.0	0.338	0.813	0.717	0.342
Control vs. SL + DMBA	0.010	0.015	0.180	0.173	0.001
Control vs. SL	0.006	0.025	0.501	0.270	0.265
DMBA vs. SL + DMBA	0.007	0.000	0.017	0.002	0.042
DMBA vs. SL	0.004	0.000	0.035	0.020	0.998
SL + DMBA vs. SL	1.0	0.151	0.147	0.173	0.062

CAT: Catalase, SOD: Superoxide dismutase, GSH-Px: Glutathione peroxidase, MDA: Malondialdehyde, TAS: Total antioxidant status, min-max: Minimum-maximum, DMBA: 7,12-dimethylbenz(a)anthracene, SD: Standard deviation

There is no single element associated with cancer formation. However, DNA oxidation is considered the most important factor due to involvement of oxidative stress in all stages of carcinogenesis (6).

L. orientalis Mill. has numerous pharmacological properties including antimutagenic, genotoxic, cytotoxic and antioxidant effects (23,24,32). Recently, in other studies its biological effects on cancer cell lines through cytotoxicity, apoptosis and autophagy has been investigated (32-34). Atmaca et al. (32) has shown that Styrax Liquidus inhibits viability of prostate cancer cell lines through induction of autophagy by inhibition of various signaling pathways. Cetinkaya et al. (34) used the aerial parts of the plant to obtain an extract and found that the extract showed anticancer activity on colorectal cancer cell lines through apoptotic pathways, Lastly, Baloglu et al. (33) used L. orientalis oil on breast, lung and prostate cell lines which revealed antitumor effect on all cancer lines but that it had the most cytotoxic effect on the breast cancer cell lines. All studies were done in vitro on cancer cell lines; however, the present study was carried out *in vivo*. In this study, the in vivo antioxidant effect of Styrax Liquidus was investigated on DMBA-exposed rat tongue tissues.

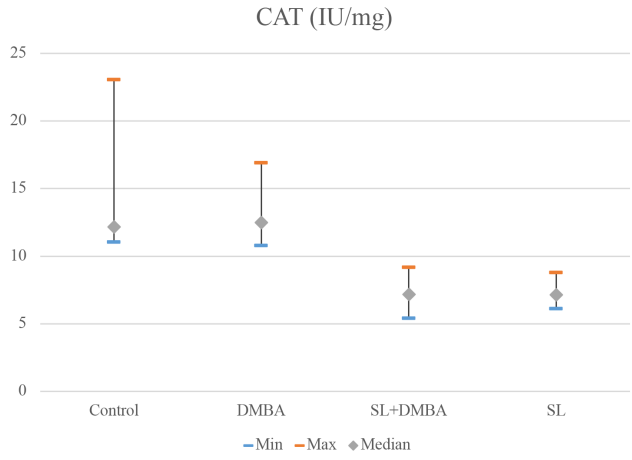

Figure 1. Descriptive statistics of the CAT (IU/mg) values
CAT: Catalase, DMBA: 7,12-dimethylbenz(a)anthracene

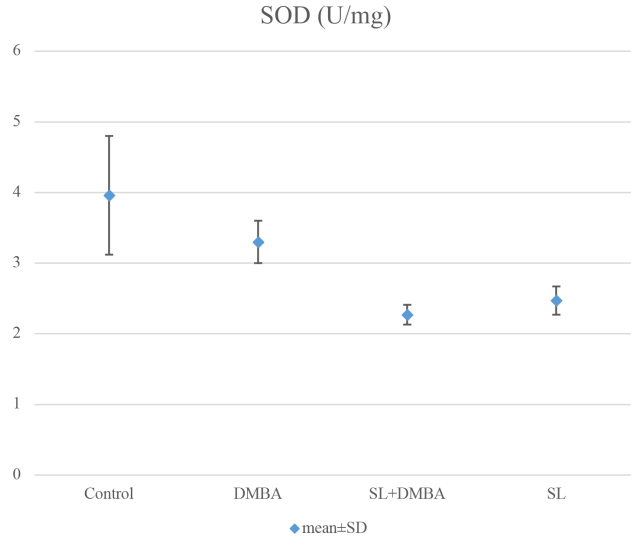

Figure 2. Descriptive statistics of the SOD (U/mg) values
SOD: Superoxide dismutase, DMBA: 7,12-dimethylbenz(a) anthracene, SD: Standard deviation

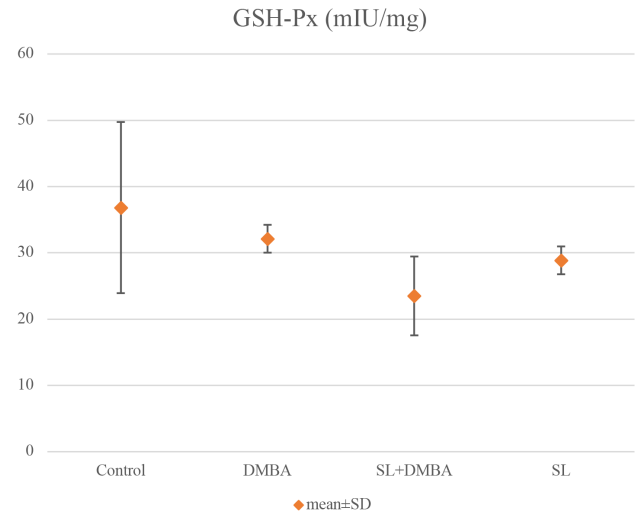

Figure 3. Descriptive statistics of the GSH-Px (mIU/mg) values
GSH-Px: Glutathione peroxidase, DMBA: 7,12-dimethylbenz(a) anthracene

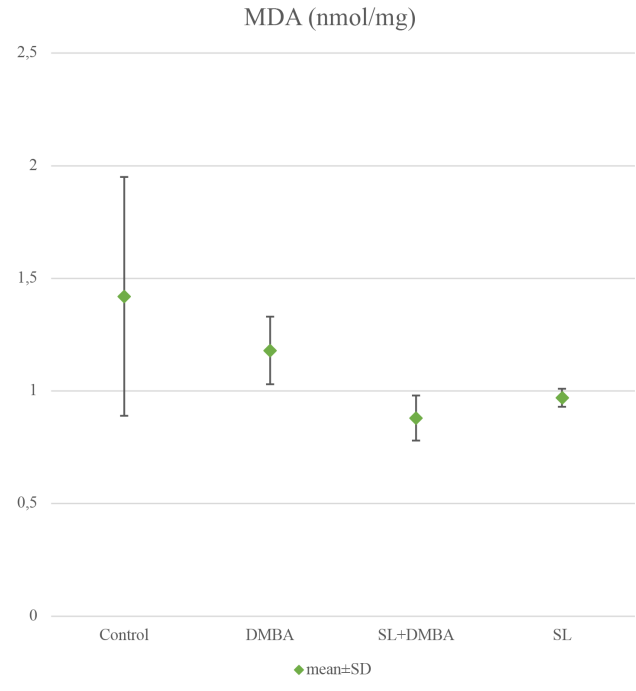

Figure 4. Descriptive statistics of the MDA (nmol/mg) values
MDA: Malondialdehyde, DMBA: 7,12-dimethylbenz(a)anthracene, SD: Standard deviation

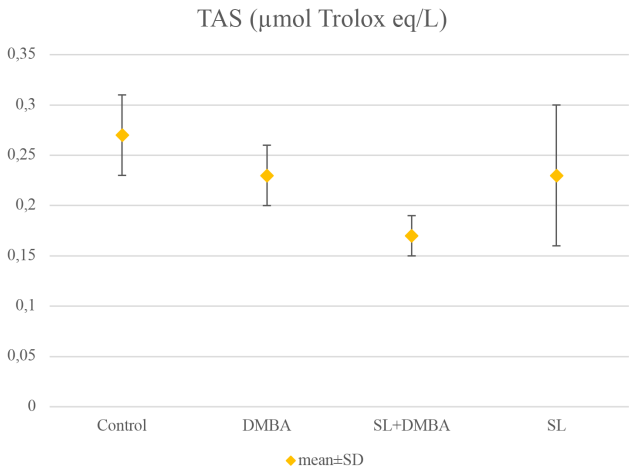

Figure 5. Descriptive statistics of the TAS (µmol Trolox eq/L) values
TAS: Total antioxidant status, DMBA: 7,12-dimethylbenz(a) anthracene

CAT, SOD and GSH-Px enzyme activities were lowest in SL + DMBA group. This is believed to have happened because of an interaction between Styrax Liquidus and DMBA, which may have produced a by-product that has the potential to disrupt these enzymes' activities. More studies are needed to further enlighten this mechanism. TAS levels were also significantly decreased in the corresponding group. This decrease was interpreted to be the result of lowered antioxidant enzyme activities (CAT, SOD, and GSH-Px). TAS levels express the residual free radical scavenging capacity after ROS neutralization (35). The decrease in TAS levels is supportive of the assumption that the antioxidant enzyme activities were disrupted in the SL + DMBA group.

In the SL group, all enzyme activities were significantly lower compared to the DMBA and control groups. This decrease was believed to be a result of the antioxidant effect of Styrax Liquidus. The antioxidant effect of Styrax Liquidus as an exogenous antioxidant might have prevented oxidative stress formation; thus, suppressing the need for endogenous antioxidant enzyme production and activity.

MDA is a marker of lipid peroxidation that is considered indicative of oxidation or oxidative stress. As lipid peroxidation in a tissue is increased consequently MDA levels increase (6,36). In the present study, MDA levels were significantly lower in Styrax Liquidus-applied groups compared to other groups. In the SL + DMBA group, the MDA levels were thought to have decreased as a consequence of the radical scavenging activity of Styrax Liquidus. Lower MDA levels in the S group show Styrax Liquidus does not cause any oxidative damage to healthy tissues; on the contrary, it acts as an exogenous antioxidant and lowers the oxidative stress.

Conclusion

Considering the results obtained in this study, Styrax Liquidus has revealed *in vivo* antioxidant efficacy in DMBA-exposed rat tongue tissues. Especially the decreased MDA levels in the SL + DMBA group is an important data pointing out the antioxidant effect. The fact that a decrease in MDA level was achieved regardless of the suppressed antioxidant enzyme activities is believed to be caused by the antioxidant components of Styrax Liquidus. Styrax Liquidus may be a promising candidate for further research regarding its mechanisms of action against oxidative stress and cancer.

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We acknowledge Prof. Aysel Uğur and Assoc. Prof. Nurdan Saraç for their contributions about extract preparation.

Ethics

Ethics Committee Approval: This study was approved by the Gazi University Animal Experiments Local Ethics Committee (approval number: G.Ü.ET-17.018, date of approval: 02.03.2017).

Informed Consent: Informed consent is not required.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: D.N.Ş., Concept: İ.R.K., H.S.Ö., Design: İ.R.K., H.S.Ö., Data Collection or Processing: D.N.Ş., Analysis or Interpretation: H.S.Ö., Literature Search: D.N.Ş., Writing: D.N.Ş., İ.R.K., H.S.Ö.

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

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Evaluation of Somatic *PIK3CA* Mutations Detected by Next-generation Sequencing in Breast Cancer Cases

Meme Kanserli Olgularda Next-generation Sequencing ile Saptanan Somatik *PIK3CA* Mutasyonlarının Değerlendirilmesi

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Abstract

Objective: There have been new developments in determining the development, treatment, and prognosis of breast cancer. In particular, determining the characteristics of breast cancer at the molecular level has become crucial in the initiation of new therapies. In recent years, the detection of *PIK3CA* mutations in breast cancer, as in many types of cancers, and especially in cases that have become resistant to treatment, is guiding the use of new targeted drugs. Therefore, the aim of this study was to evaluate *PIK3CA* mutations in patients with breast cancer.

Materials and Methods: In this study, *PIK3CA* mutations were detected using next-generation sequencing technology applied to paraffin-fixed, paraffin-embedded samples of primary tumor tissue from 110 breast cancer patients who did not receive neoadjuvant treatment previously. The relationship between *PIK3CA* mutation and tumor molecular subtypes, immunohistochemical estrogen receptor (ER), progesterone receptor (PR), c-erbB2, Ki-67 staining, and human epidermal growth factor 2 (HER2)Neu status detected by fluorescence *in situ* hybridization were investigated.

Results: *The PIK3CA* mutation was found in 21 (19.1%) cases. A significant positive correlation was detected between ER, PR, and luminal A type and *PIK3CA* mutations ($p<0.05$). *PIK3CA* mutation was not observed in any case with triple negative type. No statistically significant correlation was found with other clinicopathological parameters. The most common *PIK3CA* mutation subtypes were H1047R and E542K.

Conclusion: The results of our study showed that *PIK3CA* mutations were observed at significantly higher rates in hormone receptor-positive patients, but *PIK3CA* mutations may be less frequently observed in HER2+ patients.

Keywords: Breast cancer, hormone receptors, HER2Neu, NGS, *PIK3CA* mutations

Öz

Amaç: Meme kanseri gelişimi, tedavisi ve prognozunu belirlemede günümüzde yeni gelişmeler yaşanmaktadır. Özellikle moleküler düzeyde meme kanserinin özelliklerini belirlemek yeni tedavilerin kullanımında çok önemli hale gelmiştir. Son yıllarda *PIK3CA* mutasyonlarının tespiti, birçok kanser tipinde olduğu gibi meme kanserlerinde ve özellikle tedaviye direnç kazanan olgularda hedefe yönelik yeni ilaçların kullanımında yol göstericidir. Bu yüzden bu çalışmada meme kanserli olgularda *PIK3CA* mutasyonları değerlendirilmesi amaçlanmıştır.

Gereç ve Yöntemler: Çalışmada meme kanseri tanısı alan ve neoadjuvan tedavisi bulunmayan 110 olgunun primer tümör dokusuna ait "paraffin-fixed, paraffin-embedded" örneklerinde *PIK3CA* mutasyonları next-generation sequencing ile saptanmıştır. Olgularda *PIK3CA* mutasyonu ile tümör moleküler alt tipleri, immunohistokimyasal östrojen reseptörü (ER), progesteron reseptörü (PR), c-erbB2, Ki-67 boyamaları ile FISH ile saptanan epidermal büyüme faktörü reseptörü 2 (HER2)Neu durumu arasındaki ilişki araştırılmıştır.

Bulgular: Toplam 21 olguda *PIK3CA* mutasyonu (%19,1) bulunmuştur. ER, PR ve Luminal A tip ile *PIK3CA* mutasyonları arasında pozitif anlamlı ilişki saptanmıştır ($p<0,05$). Triple negatif tipte hiçbir olguda *PIK3CA* mutasyonu görülmemiştir. Diğer klinikopatolojik parametreler ile istatistiksel olarak anlamlı ilişki bulunmamıştır. En sık *PIK3CA* mutasyon alt tipleri H1047R, E542K'dir.

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Sonuç: Çalışmamız sonuçları hormon reseptörleri pozitif olgularda anlamlı derecede yüksek *PIK3CA* mutasyonu bulunduğunu ancak HER2+ olgularda da *PIK3CA* mutasyonlarının daha az sıklıkta görülebileceğini göstermiştir.

Anahtar Kelimeler: Meme kanseri, hormon reseptörleri, HER2Neu, NGS, *PIK3CA* mutasyonları

Introduction

Nowadays, classification and management of breast cancer are guided by histopathological grade, stage, metastasis status of the tumor, as well as molecular subtypes that can be determined by overexpression/amplification of immunohistochemical (IHC) histopathological parameters which provide prognostic information and predict response to treatment including hormone receptor profile, estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor 2 (HER2) and their molecular subtypes which can be determined by Ki-67 Proliferation index (1,2) The evolution of novel escape mechanisms in advanced breast cancer patients and the growing utilization of targeted therapies have altered the course of hormone receptor positive (HR+) breast cancers (3). Although hormone therapy has revolutionized the treatment of breast cancer and outcomes have significantly improved in these patients, optimal management remains a significant challenge (4). A subset of HR+ cases either exhibit resistance to endocrine therapy or develop endocrine-resistant disease (5). In the treatment-resistant setting, it is important to identify molecular somatic alterations. It has become a clinical necessity to identify new predictive biomarkers for current standard therapies and also to find new therapies (4). The present emphasis on molecular pathological testing has become a valuable supplementary prognostic tool, guiding the incorporation of chemotherapy alongside endocrine therapy. The treatment landscape for HR+ breast cancer is experiencing a significant transformation with a variety of targeted individualized therapies, such as phosphoinositide 3-kinase (PI3K) inhibitors. These therapies are now approved for use in combination with endocrine therapies, thanks to the progress made in molecular identification (3). PI3K initiates the activation of AKT and mTOR downstream, creating the PI3K/AKT/mTOR signaling pathway. This pathway plays a crucial role in regulating various cellular activities, often leading to cellular growth, metabolism, proliferation, and survival (6,7). The PI3K/AKT/mTOR pathway is activated in various tumor types, and its therapeutic targeting has garnered significant interest from the research community. The gene encoding the catalytic alpha (α) subunit of PI3K, i.e., *PIK3CA* (OMIM#17184), is prevalently detected activating mutations in breast cancer and *PIK3CA* mutations have been reported in a total of 40% of patients with ER+ breast cancer (8). The prognostic effect of *PIK3CA* mutations may differ between breast cancer subtypes (3). *PIK3CA* has been proposed as a favorable prognostic biomarker and is associated with positive survival outcomes in patients diagnosed with early-stage HR+/HER2- breast cancer (6,9,10). While the frequency of *PIK3CA* mutations may not significantly differ between early-stage and metastatic HR+/HER2- breast cancer, studies have demonstrated that metastatic breast cancer patients with *PIK3CA* mutations experience worse prognosis and show resistance to chemotherapy (11). In this study, we investigated *PIK3CA* status and its association with some known clinicopathologic features in breast cancer patients using next generation sequencing (NGS) technology.

Materials and Methods

The study included 110 patients who were diagnosed with breast cancer, without a history of neoadjuvant treatment in whom NGS was performed at the Department of Pathology, Aydın Adnan Menderes Unıversity Faculty of Medicine, between 2017-2018 were screened. ER, PR, c-erbB2, Ki-67 IHC staining results and also Her2-Neu results determined by fluorescence in situ hybridization (FISH) method, age, TNM stage, Modified Bloom Richardson grade of all cases were recorded.

Histopathologic examination; hematoxylin eosin (HE) stained 4-µm thick sections prepared from tissues embedded in paraffin blocks after routine tissue follow-up, and fixed in 10% neutral buffered formalin were examined and evaluated under a light microscope (BX51, Olympus, Tokyo, Japan) at x10, x20 and x40 magnifications. From the blocks containing invasive tumoral areas, 4-µm thick sections were placed on positively charged slides for IHC studies. The study protocol was approved by the Aydın Adnan Menderes University Local Ethics Committee (protocol no: 2023/172, date: 07.09.2023).

IHC Staining and Evaluation

In IHC staining performed using ER, PR, c-erbB-2, Ki-67 stains, DAKO Autostainer Universal Staining System (Autostainer Link 48 DAKO, Glostrup, Denmark) was used. After staining, the sections were observed using a light microscope (Olympus BX51, Tokyo, Japan) at magnifications of 4X, 10X, 20X, and 40X by the same pathologist. The immunostaining scoring was determined based on both the intensity of staining and the percentage of cells that exhibited staining. Staining intensity in the invasive tumoral area was scored ranging from 0 to +3 (no staining: 0, mild: +1, moderate: +2, intense: +3). Percentage of stained cells in the invasive tumor area was scored similarly.

HER2 FISH Application and Evaluation

HER2 amplification was evaluated by FISH method with immunofluorescence microscope using probe sets [HER2 FISH pharmDx (Dako)].

HER2 FISH was performed on an Olympus BX50 binuclear fluorescence microscope under triple (dogi/red/green) and

dual (red/green) filters and at least 20 tumor cell nuclei were evaluated in each tissue, taking care not to count non-tumor cell nuclei. Of the 20 interphase nuclei analyzed, if the sum of the number of red signals divided by the sum of the number of green signals was less than two, it was considered as "no amplification", and if it was greater than or equal to two, it was considered as "amplification".

Next Generation Sequencing

DNA Isolation from Formalin-Fixed Paraffin Embedded (FFPE) Tissues

Tumor areas were marked by the pathologist and 10 µm-thick DNA was isolated using a Qiagen FFPE DNA tissue extraction kit according to the manufacturer's instructions.

Pre- and Sequencing Stages for NGS

This step was conducted using the MiniSEQ NGS platform (MiniSEQ, MN00676, Illumina, Singapore) using a manufacturer protocol optimized with the QIAseq-targeted Breast Cancer Panel (DHS-005Z, Qiagen, Strasse, Hilden, Germany) (Table 1). The FFPE DNA fragment obtained after isolation, which ranged from 100-150 ng, underwent an end repair process. Target enrichment process and libraries were amplified by polymerase chain reaction (PCR) using the Labcycler from Sensoquest GmbH (Göttinger, Germany). Subsequently, barcoding and library preparation were carried out. The libraries were amplified using PCR on the Labcycler from Sensoquest GmbH and then purified for target enrichment. At the clonal amplification step, the target-enriched library was sequenced on MiniSEQ NGS platforms utilizing a MiniSEQ High Output Reagent Cartridge (Illumina, San Diego, CA, USA).

Data Analysis

The NGS results were subjected to data analysis and quality control using the Qiagen Clinical Insight analysis universal commercial software. After data quality control, variants were imported into the Qiagen Clinical Insight interpretation web interface, which enables data interpretation for predefined variants. The selected variants were analyzed by expert physicians experienced in molecular medicine to demonstrate, and evaluate the impact of these variants on validation of diagnosis, clinical effects, and treatment protocols using bioinformatics software tools [(CADD(v1.3), Allele Frequency Ensemble, EVS (ESP6500SIV2), RefSeq gene model, JASPAR, Vista Rviewer hg18 or hg19 builds) and clinical trials were analyzed in correlation with the disease phenotype using (Stepford 181112. 001)], PolyPhen-2, 1,000 genome frequency (phase3v5b) softwares. Genomic variations within patients were identified on the Qiagen reporter and QIAGEN Clinical Insights Browser platforms.

Table 1. QIAseq-targeted breast cancer panel list

PIK3CA, AR, APC, MLH1, ACVR1B, AXIN2, BAP1, BARD1, BLM, BMPR1A, BRIP1, CASP8, CBFB, PALLD, CHEK2, HERC1, CDH1, ATR, CCND1, CDK4, CTNNB1, BRCA1, IRAK4, BRCA2, GATA3, MSH2, EGFR, PTEN, EP300, ERBB3, ERCC4, EXOC2, EXT2, FAM175A, ANCC, FBXO32, FGFR1, FGFR2, PBRM1, HOXB13, ESR1, ERBB2, KRAS, MDM2, MED12, MEN1, MRE11A, MUC16, MUTYH, DIRAS3, MYC, NBN, NCOR1, NEK2, NF1, PALB2, PCGF2, RB1, PPM1L, ITCH, RET, SMAD4, RAD51D, RAD50, CSMD1, TP53, PMS2, ATM, SEPT9, MAP2K4, SMAD4, SMARCA4, STK11, SYNE1, TGFB1, TRAF5, ZBED4, VHL, WEE1, PDGFR, EPCAM, MAP3K1, PMS1, XRCC2, XRCC3, CDK6, AKT1, CDKN2A, RAD51C, MSH6, PIK3R1, GEN1, RAD51, TGFB2

Statistical Analysis

Mean and percentage were used for descriptive statistics. Statistical Analysis was performed using SPSS version 22.0 (SPSS Inc., Chicago, IL, USA). Chi-square test was used for categorical variables and Student's t-test was used to compare independent data. Results were considered significant at $p<0.05$.

Results

Our patients with an average age of 46 years (range: 26-78 years) were either over (n=87; 79.09%) or under 40 (n=23; 20.90%) years old. Clinicopathologic features of the cases are given in Table 2.

ER was positive in 91 (82.7%) and negative in 19 (17.3%) patients. PR was positive in 84 (76.4%) and negative in 26 (23.6%) patients. In the IHC and *HER2* FISH study, HER2Neu positivity, and negativity were detected in 33 (30%) and 77 (70%) cases, respectively. Ki-67 proliferation index was above, and below 15% in 56 (50.9%) and 54 (49.1%) patients, respectively. According to these results, respective number of patients were in the Luminal A (n=63; 57.3%), Luminal B (n=28; 25.5%), triple negative (n=14; 12.7%), and Her2Neu positive (n=5; 4.5%) groups.

In the *PIK3CA* mutation analysis evaluated in the NGS study, pathogenic *PIK3CA* mutation was observed in 21 (19.1%) of 110 cases, while in 89 (80.9%) cases pathogenic mutation was not detected. The most common *PIK3CA* mutation was detected in Luminal A type cases. The most commonly observed hotspot mutations were p. H1047R (Exon 20) in 12 (57.2%) and p.E542K (Exon 9) in 4 cases (19%). In addition, E726K (Exon 13) mutation was found in 2 (9.5%), p.Q546 (Exon 9) mutation in 2 (9.5%) and p.M1043V (Exon 20) mutation in 1 (4.8%) case.

The distribution of different *PIK3CA* mutations found in the cases according to molecular subtypes is shown in Table 3.

In our study, a statistically and significantly positive correlation was detected between *PIK3CA*, ER, PR ($p=0.028$ and $p=0.041$) and Luminal A type ($p=0.039$), in cases where pathogenic *PIK3CA* mutation was observed, while the correlations between different mutation types in cases with mutations and other molecular types, age, tumor size, grade, stage, metastasis, metastasis site, Ki-67, Her2Neu were not statistically significant ($p>0.05$).

Discussion

Great advances have been made in the development, prognosis and treatment of breast cancer in recent years. The molecular classification of breast cancers has brought different treatment options to the agenda, but some cases of these cancers are still treatment-refractory or show disease progression (1). In such cases, the search for new markers that will make hormone therapy and targeted therapies more predictable continues. Today, with the development and widespread use of NGS technologies, new steps are being taken to predict the response to the treatment outcomes and prognosis for patients with breast cancer (12). Disclosing somatic mutations in cancer cells reveals important results in approaching cases before and after treatment. The use of PI3K inhibitors, which are among targeted therapies, is becoming widespread in breast cancer, the most common cancer observed in women worldwide. PI3K/Akt/mTOR pathway gains importance especially in HR+, HER2-negative metastatic patients refractory to hormone therapy that occurs with various pathogenetic mechanisms. In this case, the use of *PIK3CA* inhibitors such as alpelisib has been approved and the detection of *PIK3CA* mutations in various specimens using various techniques has gained importance (3). There are various publications in the literature on breast cancer and *PIK3CA* mutations (3). These publications focus especially on technical methods and mutation types. There are also studies evaluating *PIK3CA* mutations in breast cancer subtypes. PI3K plays a role in many cellular processes including protein synthesis, cell proliferation and DNA repair (13,14). *PIK3CA*, one of the PI3K enzyme isoforms, has been shown to be associated with cancer development, progression and drug resistance. This relationship has also been shown in breast cancers, especially in HR+/HER2- tumors. Although there are conflicting results in the literature on the use of fresh tumor tissue samples, FFPE tissue samples as well as liquid biopsies in the detection of *PIK3CA* mutation, it is seen that use of all these samples yielded comparably successful rates of mutation detection. There are also comparative studies in the literature on the use of methods such as PCR, Sanger Sequencing and NGS in mutation detection (15). In some studies, mutations were found at slightly higher rates especially when Sanger Sequencing and NGS technologies were used. In our study, FFPE tissue samples were used with NGS method and *PIK3CA* somatic mutation was found in 19.1% of the cases. This rate is similar to the 20-30% *PIK3CA* mutation rates reported in the literature in all breast cancers (16). However, some differences in the methodologies used in the studies cited in the literature, especially those employed in the study of samples after neoadjuvant treatment or in the evaluation of metastatic tissue samples may cause differences in reported *PIK3CA* somatic mutation rates. The results of our study should be considered important in terms of showing

Table 2. Clinopathological characteristics of the cases included in the study

Age (years)	Median (minimum-maximum) 46 (26-78)
Length (cm)	4.5 (1.3-10.5)
Tumor grades (modified Bloom Richardson grading system)	n (%)
Grade 1	2 (2.1%)
Grade 2	60 (63.8%)
Grade 3	32 (34.1%)
Stage (TNM)	
pT1 and pT2	80 (72.7%)
pT3 and pT4	30 (27.3%)
Metastasis	
Yes	44 (40%)
No	66 (60%)
Metastatic region	
Lymph node	35 (79.5%)
Distant organs (bone, lungs, liver etc.)	9 (20.5%)
Molecular subtype	
Luminal A	63 (57.3%)
Luminal B	28 (25.4%)
HER2 positive	5 (4.6%)
Triple negative	14 (12.7%)

TNM: Tumor, node, and metastasis

Table 3. Distribution of 21 different *PIK3CA* mutations according to molecular subtypes

	p.E542K	p.H1047R	p.Q546	p.E726K	p.M1043V	Total (n=21)
Luminal A (n=63)	2	9	2	2	1	16 (76.2%)
Luminal B (n=28)	1	3				4 (19.1%)
HER2 positive (n=5)	1					1 (4.70%)
Triple negative (n=14)						0
Total	4 (19%)	12 (57.2%)	2 (9.5%)	2 (9.5%)	1 (4.8%)	21

the *PIK3CA* somatic mutation rate on a regional basis in samples retrieved from the patients that did not receive neoadjuvant treatment and studied with NGS technology using primary tumor tissues. According to the molecular classification of breast cancer cases, significant differences emerge between subtypes. The high *PIK3CA* mutation rate in HR+/HER2- tumors, which has been reported in many studies in the literature, was also indicated by the results of our study. According to the results of our study, 76.2% of all cases with *PIK3CA* mutation were in the HR+/HER2- group classified as Luminal A and *PIK3CA* mutation was detected in 25% of Luminal A cases. A significant positive correlation was found between Luminal A type and *PIK3CA* mutation in breast cancer patients. In addition, the results of our study have shown that there is a significant positive correlation between ER and PR positivity in tumor cells and *PIK3CA* mutation. These findings support the relationship between *PIK3CA* mutation and HR+ cases reported in the literature. In the Luminal B group, where *PIK3CA* was detected with the second frequency, the *PIK3CA* mutation rate was 19.1%. Considering that HER2- positive cases were found in this group, apparently *PIK3CA* mutations may be detected at a considerable rate in HR+/HER2+ breast carcinoma cases. Although similar results have been reported in the literature, studies on *PIK3CA* mutation have been mostly focused on HR+/HER2- cases and HR+/HER2+ breast carcinoma cases may be overlooked. It is thought that evaluation of *PIK3CA* mutation should be performed independently of *HER2*, especially in cases refractory to hormone therapy. In our study, although *PIK3CA* mutation was not found in 14 triple-negative cases, mutation was found in 1 out of 5 cases in the HER2+ group, which supports our assessment. In our study, the most frequently observed somatic mutations in *PIK3CA* were p.H1047R and p.E542K. In addition, p.Q546, p.E726K, p.M1043V mutations were also found in *PIK3CA*. These results are similar to the literature. The results of the studies performed hitherto have shown that p.H1047R mutation is the most common mutation, followed by p. E545K mutation (16,8). There are also studies suggesting that these mutation types are differently associated with breast cancer molecular subtypes (3). In our study, although p.H1047R was the most common mutation observed in Luminal A and B subtypes, this relationship was not found to be statistically significant. In recent years, the detection of effective *PIK3CA* mutations has become increasingly important. Therefore, the evaluation of different *PIK3CA* mutations, which can be detected less frequently in a large number of cases may lead to the creation of new and effective treatment options in cases resistant to hormone therapy (4).

Our study shows the importance of *PIK3CA* mutations in hormone receptor-positive cases in breast cancers and draws attention to *PIK3CA* mutations in targeted drug therapies. The limitation of our study is that more patients can be included in our study in order to obtain more meaningful results.

Conclusion

In our study, we have observed that 19.1% of 110 breast cancer patients had *PIK3CA* mutations regardless of subtypes, and 25% of these mutations were in Luminal A type. However, *PIK3CA* mutations were not detected in any patient with triple negative type breast cancer. In addition, considerable rates of *PIK3CA* mutations were detected in HER2+ cases. In our study, similar results with the literature were obtained in the evaluation of *PIK3CA* somatic mutations in FFPE tumor tissue samples using NGS method in a relatively small number of breast cancer patients.

Ethics

Ethics Committee Approval: The study protocol was approved by the Aydın Adnan Menderes University Local Ethics Committee (protocol no: 2023/172, date: 07.09.2023).

Informed Consent: Informed consent is not required.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: İ.H.E., Concept: İ.H.E., D.G., Design: İ.H.E., D.G., Data Collection or Processing: İ.H.E., Analysis or Interpretation: İ.H.E., Literature Search: İ.H.E., D.G., Writing: İ.H.E.

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Flexural Strength of Monolithic Zirconia After Zirconia-specific Grinding Procedures and Hydrothermal Aging

Zirkonyaya Özgü Aşındırma Prosedürleri ve Hidrotermal Yaşlandırma Sonrası Monolitik Zirkonyanın Eğilme Dayanımı

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Abstract

Objective: To evaluate the effect of grinding with different diamond burs on the surface roughness (Ra) and flexural strength (FS) of hydrothermally aged zirconia.

Materials and Methods: Ninety-eight bar-shaped monolithic zirconia specimens were prepared and divided into 7 subgroups according to grinding procedures: control, grinding with diamond burs (F; fine, M; medium, C; coarse); and grinding with zirconia-specific diamond burs (ZF; fine, ZM; medium, ZC; coarse). All ground specimens were polished using a two-step zirconia polishing system. All specimens were subjected to autoclave aging. Ra was measured using a profilometer. One specimen per group was examined by scanning electron microscopy and X-ray diffractometry. A 3-point FS test was performed using a universal testing machine.

Results: The lowest and highest Ra values were obtained in the control and C groups, respectively. The ZC group showed higher Ra values than the ZF and ZM groups. There was no difference between the FS values of the ZF and control groups. However, other grinding procedures led to decreased FS.

Conclusion: Zirconia-specific fine diamond burs are recommended to maintain the mechanical strength of zirconia when clinical adjustments are needed.

Keywords: Flexural strength, grinding, monolithic zirconia, surface roughness, zirconia-specific diamond bur

Öz

Amaç: Farklı elmas frezler ile yapılan aşındırmanın hidrotermal olarak yaşlandırılmış zirkonyanın yüzey pürüzlülüğü (Ra) ve eğilme dayanımına (FS) etkisini değerlendirmektir.

Gereç ve Yöntemler: Doksan sekiz adet bar şeklinde monolitik zirkonya örnek hazırlandı ve aşındırma prosedürlerine göre 7 gruba ayrıldı: kontrol, elmas frezlerle aşındırma (F; ince, M; orta, C; kalın), zirkonyaya özgü elmas frezlerle aşındırma (ZF; ince, ZM; orta, ZC; kalın). Aşındırma yapılan tüm örneklere 2 aşamalı zirkonya polisaj sistemi kullanılarak polisaj işlemi uygulandı. Tüm örnekler otoklavda yaşlandırıldı. Ra değerleri profilometre kullanılarak ölçüldü. Her gruptan bir örnek taramalı elektron mikroskobu ve X-ışını difraktometresi kullanılarak incelendi. Evrensel bir test cihazı kullanılarak 3 nokta eğme testi yapıldı.

Bulgular: En düşük ve en yüksek Ra değerleri sırasıyla kontrol ve C gruplarında elde edildi. ZC grubu ZF ve ZM gruplarından daha yüksek Ra değerleri gösterdi. ZF ve kontrol gruplarının FS değerleri arasında farklılık gözlenmedi. Ancak diğer aşındırma prosedürleri daha düşük FS değerlerine yol açtı.

Sonuç: Klinik düzenlemeler gerekli olduğunda zirkonyaya özel ince grenli elmas frezlerin kullanımı zirkonyanın mekanik dayanıklılığını korumak için önerilmektedir.

Anahtar Kelimeler: Eğilme dayanımı, aşındırma, monolitik zirkonya, yüzey pürüzlülüğü, zirkonyaya özgü elmas frez

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Introduction

Zirconium oxide has gained considerable popularity for prosthetic restorations due to its esthetic potential, mechanical properties, and biocompatibility. It is well-documented that one common clinical failure for zirconia-based restorations is the chipping of veneering porcelain (1,2). Monolithic zirconia is widely used to overcome this complication. These materials not only eliminate the need for veneering porcelain owing to their improved optical properties but also offer high flexural strength (FS), preservation of tooth structure during preparation, and reduced clinical and laboratory time (3).

Polycrystalline zirconia has three crystallographic phases: monoclinic (*m*), tetragonal (*t*), and cubic (*c*). It exists in *m*-phase at room temperature and *t*-phase between 1,170 °C and 2,370 °C. To retain the *t*-phase at room temperature, metal oxides, such as MgO, CaO, or Y_2O_3, are added to zirconium oxide, with yttria (Y_2O_3)-stabilized tetragonal zirconia polycrystal being the most commonly used type (4). However, surface treatments such as airborne particle abrasion and clinical adjustments using burs can trigger $t \rightarrow m$ phase transformation (5). This transformation also occurs when zirconia is exposed to the moist environment of the oral cavity. This phenomenon is referred to as low-temperature degradation, which may adversely affect the mechanical properties of zirconia ceramic (6-8).

Intraoral adjustments may be necessary for monolithic zirconia restorations (9,10). However, such adjustments with discs or burs can cause surface damage (5). Besides, chairside adjustments can increase surface roughness (Ra) on the monolithic zirconia restoration, resulting in undesirable conditions such as plaque accumulation and wear on opposing teeth (9,10). Intraoral polishing systems offer advantages over re-glazing, including reduced office visits and avoidance of multiple firing cycles. Moreover, zirconia polishing systems can reduce surface flaws and enhance the FS of restorations, thereby contributing to their longevity (10).

The influence of grinding and polishing procedures on the FS of zirconia is widely investigated in the literature (4,11). However, the effects of grinding with zirconia-specific diamond burs followed by manual polishing on the FS of monolithic zirconia are still unclear. Therefore, this study investigated the impact of grinding with either zirconia-specific or conventional diamond burs of varying grain sizes and polishing with zirconia-specific polishing systems on monolithic zirconia. The null hypotheses were that Ra and FS would remain unaffected by applying different bur types.

Materials and Methods

Specimen Preparation

The sample size was determined using a power analysis conducted with G*power software (v.3.1.9.2, Dusseldorf, Germany). With an effect size of 0.4, a significance level of 0.05, and a power of 80%, 14 specimens per group were determined sufficient. Ninety-eight bar-shaped specimens were obtained from a monolithic zirconia blank (CopraSmile, Whitepeaks Dental Solutions, Essen, Germany) using a diamond saw (Metcon 19-150, Metkon Instruments, Bursa, Turkey) mounted to a cutting device (MOD Dental, Esetron Smart Robotechnologies, Ankara, Turkey) under running water. The specimens were finished using 600, 1,000, and 1,200 grit silicon-carbide abrasive papers. The long edges of the bar-shaped specimens were chamfered using the final abrasive paper. All samples underwent ultrasonic cleaning in distilled water for 10 min. Specimens were sintered at 1,500 °C (Programat S1, Ivoclar Vivadent, Schaan, Liechtenstein) according to the manufacturer's instructions. The thickness of the samples for the grinding groups was considered 1.25 mm, according to the material to be removed during the process; sample thickness in the control group was adjusted to 1.2 mm. The width and length of sintered specimens were 4 mm and 20 mm, respectively. The specimens were randomly divided into 7 subgroups;

Control: No grinding and polishing

F: Grinding with fine diamond bur + polishing

M: Grinding with medium diamond bur + polishing

C: Grinding with coarse diamond bur + polishing

ZF: Grinding with zirconia-specific fine diamond bur + polishing

ZM: Grinding with zirconia-specific medium diamond bur + polishing

ZC: Grinding with zirconia-specific coarse diamond bur + polishing

Ethics committee approval was not obtained since the study was carried out in an experimental environment on materials that did not belong to any living organism.

Grinding and Polishing Procedures

Grinding was done using diamond burs and zirconia-specific diamond burs (Meisinger, Hager & Meisinger, Neuss, Germany) with a sweeping motion, removing 0.05 mm material from one entire surface of the specimen. A digital caliper was used to verify the final thickness of the specimens. Subsequently, manual polishing was performed on ground surfaces with a 2-step zirconia polishing system (Drendel + Zweiling Diamant, Kalletal, Germany) using each tool for 20 s. Grinding and polishing procedures were performed under water cooling. All procedures were conducted by a single experienced operator (H.Ş.). After polishing, all specimens were ultrasonically cleaned again in distilled water for 10 min. The burs used for grinding and the 2-step polishing system are shown in Figure 1.

Hydrothermal Aging

All specimens were subjected to an accelerated aging procedure using a steam autoclave (Yeson YS-22L-E, Ningbo Haishu Yeson Medical Device, Zhejiang, China).

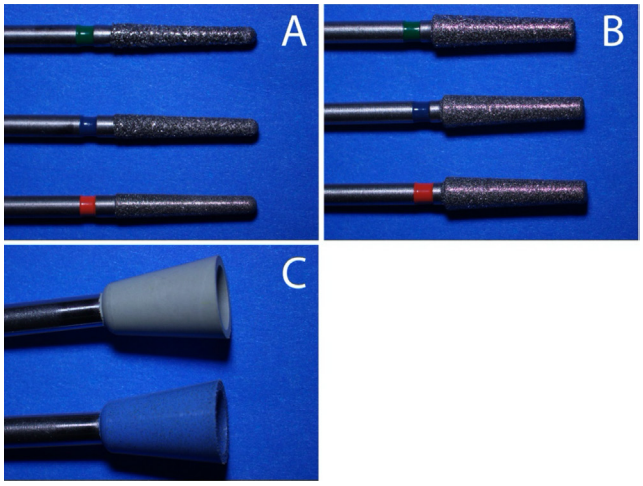

Figure 1. Grinding burs and polishing system A) Diamond grinding burs; B) Zirconia-specific diamond grinding burs; C) Zirconia-specific polishing system

The specimens were positioned in autoclave-safe trays and exposed to 5 sequential cycles, each lasting 60 min. Thus, the total exposure time amounted to 5 hours, maintaining a temperature of 134 ˚C and a pressure of 2 bars. Five-hour autoclave aging corresponds to approximately 15-20 years of actual aging (6,12).

Surface Roughness Evaluation

The average Ra values were obtained using a profilometer (Surftest SJ-210, Mitutoyo, Kanagawa, Japan). Measurements were taken at a constant speed of 0.5 mm/s and a cut-off value of λc=0.25 mm. The arithmetic mean of 5 perpendicular readings was accepted as the final Ra score for each specimen. The surface profilometer was recalibrated after measuring every 5 specimens.

Scanning Electron Microscopy

Additional samples from each group were analyzed using scanning electron microscopy (SEM). The samples were subjected to gold sputter-coating (Quorum Q150R ES, Quorum Technologies, East Grinstead, UK). Subsequently, SEM images were captured using the scanning electron microscope (EVO LS-10, Carl Zeiss Microscopy, Cambridge, UK) at magnifications of ×1000 and ×5000, operating at 25 kV.

X-ray Diffraction (XRD) Analysis

Crystal structure analysis was performed using an X-ray diffractometer (Bruker D8 Advance, Bruker AXS, Karlsruhe, Germany). Operating current and voltage conditions were set at 40 mA and 40 kV, respectively. Surface scans were performed within the range of 20 to 40 2θ degrees, using a step size of 0.019. The relative amount of *m*-phase (Xm) was determined by applying equation 1 (13), while the volumetric fraction (Fm) was calculated using equation 2 (14):

[1] $Xm=(Im(-111) + Im(111))/(Im(-111) + Im(111) + It(101))$

[2] $Fm=(1.311Xm)/(1+0.311Xm)$

where Im (–111) and Im (111) represent *m*-peak intensities at approximately 28 and 31 2θ˚, respectively, and It (101) denotes *t*-peak intensity at approximately 30 2θ˚.

Flexural Strength

FS data were obtained by implementing a three-point bending test using a universal testing machine (Marestek, Mares Engineering, İstanbul, Turkey). The specimens were positioned on metal supports with the treated surfaces under tension, and the force was applied to the center of samples with a constant cross-head speed of 1 mm/min until failure occurred. The radii of the 2 metal supports and loading piston were 0.8 mm, and the distance between the centers of the supports was 14 mm. FS values were calculated in MPa using the following equation based on ISO 6872:

$\sigma = 3Fd/2wh^2$

where σ is the FS; F is the fracture load (N); d is the span (distance between the center of the supports) (mm); w is the width of the specimen (mm); h is the thickness of the specimen (mm).

Statistical Analysis

Statistical analyses were performed at a significance level of α=0.05 (SPSS/PC Version 24.0; SPSS Inc., Chicago, IL, USA). The normality of the data was assessed using the Shapiro-Wilk test, while the homogeneity of variances was evaluated using the Levene test. To analyze both Ra and FS data, One-Way analysis of variance (ANOVA) and Tamhane's T2 tests were performed. Pearson correlation analysis was performed to determine the relationship between Ra and FS.

Results

Surface Roughness

One-way ANOVA revealed significant differences among subgroups ($F=256.581$, $p<0.001$). All treated groups showed significantly higher Ra values than the control group ($p<0.001$) (Table 1). For the diamond bur groups (F, M, and C), Ra increased statistically as the grain size increased. ZC showed statistically higher Ra values than both ZM and ZF ($p<0.001$). Although the mean Ra of ZM was higher than that of ZF, this difference was statistically insignificant ($p=1,000$). While there was no significant difference between F and ZF groups ($p=0.448$), medium and coarse zirconia-specific diamond burs led to decreased Ra compared to diamond burs with the same grain sizes ($p<0.001$).

SEM Analysis

In line with the Ra results, SEM images of the control group showed the smoothest surface (Figure 2). In contrast, the C group exhibited deep surface grooves and microcracks. The F group exhibited smoother surfaces than both C and M. The ZC group showed more irregular surfaces than both ZM and ZF.

Phase Transformation

No distinct *m*-peaks were observed in the control group (Figure 3), while ground specimens exhibited similar diffraction patterns with minimal *m*-peaks (Fm values= F: 2.9 %; M: 2.9%; C: 3.3%; ZF: 3%; ZM: 2.7%; ZC: 2.9%).

Flexural Strength

According to One-Way ANOVA, there were significant differences among test groups (F=191.126, p<0.001). All the ground groups, except ZF, showed significantly lower FS values than the control group (p<0.001) (Table 2). There was no significant difference between ZF and control groups (p=0.996). Zirconia-specific diamond bur groups showed statistically higher FS values than diamond bur groups with the same grain sizes (p<0.001). FS values decreased significantly with the increase in grain size for both diamond bur groups and zirconia-specific diamond bur groups (p<0.001).

Pearson correlation analysis revealed a significant negative correlation (r=-0.851, p<0.001) between Ra and FS.

Discussion

Based on the results obtained from this study, all null hypotheses were rejected due to significant differences among the test groups for the dependent variables.

Table 1. Results of the statistical analysis of surface roughness (Ra; µm)

	Mean ± SD*	Minimum	Maximum	95% CI
Control	0.384±0.047^{A}	0.29	0.46	0.357-0.411
ZF	0.697±0.051^{B}	0.58	0.78	0.667-0.726
ZM	0.716±0.051^{B}	0.66	0.85	0.687-0.746
F	0.768±0.099^{B}	0.59	0.89	0.710-0.825
ZC	0.934±0.049^{C}	0.87	1.02	0.906-0.962
M	0.982±0.079^{C}	0.85	1.14	0.936-1.028
C	1.254±0.050^{D}	1.12	1.32	1.225-1.283

SD: Standard deviation, CI: Confidence interval, *The groups with the same superscript letters are not statistically different (p>0.05)

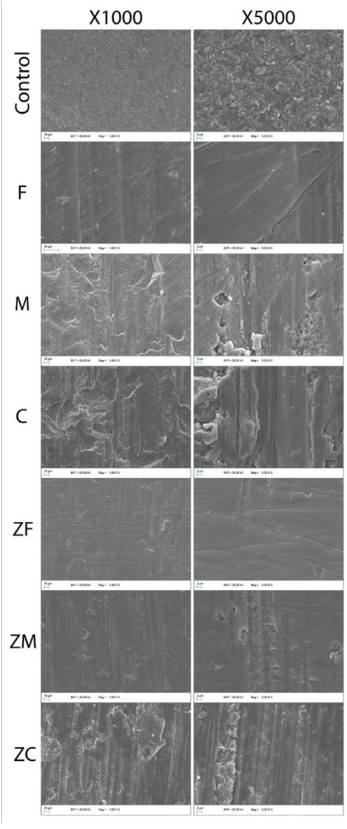

Figure 2. Scanning electron microscopy images

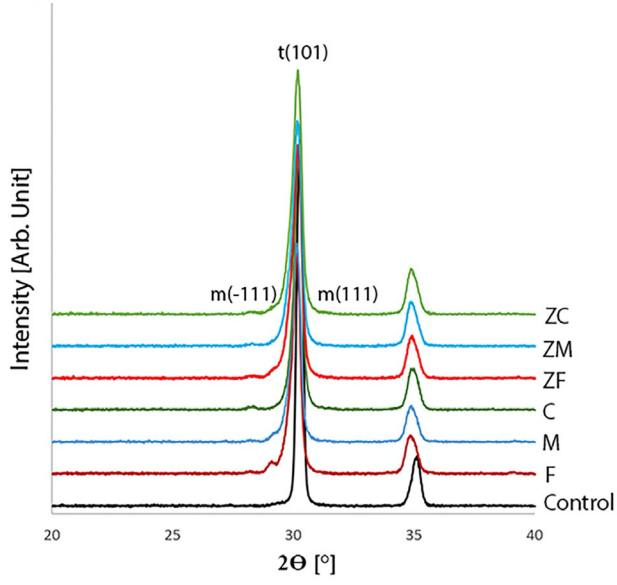

Figure 3. X-ray diffraction patterns

Table 2. Results of the statistical analysis of flexural strength (FS; MPa)

	Mean ± SD*	Minimum	Maximum	95% CI
C	310.19±11.32^{A}	294.71	329.47	303.65-316.73
M	357.96±21.10^{B}	327.24	386.17	345.78-370.15
ZC	372.20±22.20^{B}	327.21	420.49	359.39-385.02
F	401.48±22.95^{C}	358.77	445.02	388.23-414.73
ZM	440.58±32.34^{D}	387.91	493.36	421.91-459.26
ZF	533.57±34.90^{E}	473.75	590.41	513.42-553.72
Control	546.13±14.81^{E}	526.01	575.46	537.59-554.68

SD: Standard deviation, CI: Confidence interval, *The groups with the same superscript letters are not statistically different (p>0.05)

After chairside adjustments, the primary objective is to achieve a smooth surface comparable to the glazed surface, promoting oral tissue compatibility and resistance to plaque accumulation (15,16). Although glazed restorations display smooth surfaces, their wear behavior is not significantly superior to that of polished restorations. Glazed surfaces tend to result in more wear of the opposing teeth than polished surfaces (17,18). In a recent study, Badarneh et al. (19) preferred polishing over glazing as the surface finishing procedure for monolithic zirconia as it significantly reduced the wear of enamel antagonists.

In this study, Ra values were statistically higher in all grinding-applied groups than in the control group, even if the specimens were polished after grinding. Besides, Ra values significantly increased as the grain size increased in diamond bur and zirconia-specific diamond bur groups, except for the similarity between ZF and ZM groups. Corroborating this result, Hmaidouch et al. (1) concluded that coarse grinding is closely related to high roughness values. In line with the Ra results, SEM analyses of the current study revealed that coarse grinding caused more distinct grooves than grinding with medium and fine burs.

Various factors, such as phase change, crack formation, and surface flaws, can determine the mechanical strength of zirconia (20). In earlier research, Kosmac et al. (21) concluded that the relation between the depth of grinding-induced surface compressive layer resulting from phase transformation and the length of surface flaws was critical. According to the same authors, when the length of surface flaws exceeded the thickness of the surface compressive layer, the mechanical strength of zirconia tended to decrease. Microcracks or flaws due to surface grinding act as sites of stress concentration, which may cause a reduction in the FS of zirconia (22). In the present study, the mean FS of the grinding-applied groups, except ZF, was lower than the control group. However, no significant difference was observed between the FS of ZF and control groups, probably due to the low Ra values of ZF and few surface defects seen in SEM images. Therefore, grinding with zirconia-specific fine diamond burs followed by polishing may be a promising protocol if clinical adjustments are needed. Conversely, the C group showed the lowest mean FS, which may be strongly related to microcracks, as seen in SEM images. Moreover, FS values significantly decreased as the grain size increased in diamond bur and zirconia-specific diamond bur groups. Similarly, some studies highlighted that excessive grinding could lead to deep surface flaws (20,23). Therefore, clinicians should avoid coarse grinding of monolithic zirconia restorations.

To simulate long-term intraoral conditions, accelerated hydrothermal aging was applied to all zirconia specimens, which can be also effective on mechanical properties (24). However, XRD analysis showed no distinct *m*-peaks for the control group. This result indicated that hydrothermal aging did not trigger $t \rightarrow m$ phase transformation, possibly due to the high yttria content of the monolithic zirconia used. Moreover, each type of diamond bur led to similar XRD patterns. Fm values of ground specimens ranged between 2.7% and 3.3%. Thus, the FS of samples may not have been adversely affected by grinding-induced minimal $t \rightarrow m$ phase transformation.

In a recent study, Kheur et al. (16) used diamond and modified diamond burs (zirconia specified) for zirconia cutting. The results showed no relationship between mean Ra and FS. Lee et al. (4) reported that coarse grinding without subsequent polishing resulted in higher Ra and lower FS than fine grinding. The current study showed a meaningful negative correlation between Ra and FS. Moreover, grinding with diamond burs, except fine grinding, resulted in higher Ra values than grinding with zirconia-specific diamond burs with the same grain size; FS values were statistically lower in diamond bur groups compared to zirconia-specific bur groups.

In this study, chairside adjustment procedures were simulated by a single operator. Despite efforts to standardize the grinding and polishing procedures and maintain consistent pressure, the pressure applied was possibly not as precisely controlled as in a controlled experimental setup. Another limitation of the study was that crucial factors, such as dynamic occlusal load, neuromuscular forces, and parafunctional habits, were excluded. The present study focused on the FS of a single brand of zirconia that consists

of approximately 9% yttria. Therefore, additional studies evaluating different types of zirconia are needed.

Conclusion

From a clinical perspective, chairside adjustments of monolithic zirconia restorations should be avoided. However, we recommend zirconia-specific diamond burs with smaller grain sizes when occlusal adjustments are necessary for achieving optimal occlusal harmony. This approach may ensure long-term durability without jeopardizing the mechanical properties of monolithic zirconia.

Ethics

Ethics Committee Approval: Ethics committee approval was not obtained since the study was carried out in an experimental environment on materials that did not belong to any living organism.

Informed Consent: Informed consent is not required.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

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

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Original Article / Özgün Araştırma

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Determination of *Trichomonas vaginalis* Frequency Among Symptomatic Cases in Muğla Sıtkı Koçman Hospital Using Different Methods (Direct Microscopy, Culture, PCR and Immunochromatographic Method)

Muğla Sıtkı Koçman Üniversitesi Hastanesi'ndeki Semptomatik Olgularda *Trichomonas vaginalis* Görülme Sıklığının Farklı Yöntemler (Direkt Mikroskobi, Kültür, PCR ve İmmünokromatografik Yöntem) ile Araştırılması

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Abstract

Objective: *Trichomonas vaginalis* (*T. vaginalis*) is a sexually transmitted protozoan parasite that colonizes the urogenital system. Because of the various health risks it poses, the diagnosis of the parasite is crucial. Therefore, the current study investigated the incidence of *T. vaginalis* in cases with vaginal discharge complaints.

Materials and Methods: A total of 150 cases presenting with vaginal discharge complaints were included in the study conducted at Muğla Sıtkı Koçman Hospital's Gynaecology and Obstetrics Clinics. The demographic characteristics and clinical findings of the cases were recorded. Swab samples obtained from the cases were evaluated for the detection of *T. vaginalis* using direct microscopy (DM), culture, polymerase chain reaction (PCR), and immunochromatographic rapid diagnostic test (RDT).

Results: Among the 150 women studied, two cases (1.3%) were positive for *T. vaginalis* with DM, whereas three cases (2%) were positive using other methods, including culture, PCR, and RDT. There was no statistically significant difference between the methods ($p>0.05$). In addition to vaginal discharge, the most common symptoms observed were itching (45%) and abdominal pain (41%).

Conclusion: We reported the frequency of *T. vaginalis* in Muğla province. In addition, RDT was preferable for routine *T. vaginalis* diagnosis because of its ease of use, lack of equipment requirements, and rapid results.

Keywords: *Trichomonas vaginalis*, direct microscopy, culture, PCR, immunochromatographic technique

Öz

Amaç: *Trichomonas vaginalis* (*T. vaginalis*), cinsel yolla bulaşan bir protozoon olup ürogenital sisteme yerleşmektedir. Neden olduğu çeşitli sağlık risklerinden dolayı parazitin tanısı çok önemlidir. Bu nedenle mevcut çalışmada vajinal akıntı şikayeti olan hastalarda *T. vaginalis* varlığının araştırılması amaçlanmıştır.

Gereç ve Yöntemler: Çalışmaya Muğla Sıtkı Koçman Üniversitesi Hastanesi Kadın Hastalıkları ve Doğum Poliklinikleri'ne vajinal akıntı şikayeti ile başvuran 150 olgu dahil edilmiştir. Olguların demografik özellikleri ve klinik bulguları kayıt altına alınmıştır. *T. vaginalis* saptanması için hastalardan alınan sürüntü örnekleri direkt mikroskopi (DM), kültür, polimeraz zincir reaksiyonu (PZR) ve immünokromotografik hızlı tanı testi (RDT) ile değerlendirilmiştir.

Bulgular: Olgularda saptanan akıntı semptomunun yanında en sık kaşıntı (%45) ve batın ağrısı (%41) olduğu belirlenmiştir. İki (%1,3) olgu DM yöntemi ile, üç (%2) olguda ise kültür, PZR ve RDT ile *T. vaginalis* pozitifliği saptanmıştır. Kullanılan tanı yöntemleri arasında istatistiksel olarak anlamlı fark saptanmadı ($p>0,05$).

Sonuç: Bu çalışmada Muğla ilinde *T. vaginalis* görülme sıklığı araştırılmıştır. Ayrıca, RDT yöntemi uygulamasının kolay olması, ekipman gerektirmemesi ve hızlı sonuç vermesi nedenleriyle *T. vaginalis*'in rutin tanısında kullanılabilceği düşünülmüştür.

Anahtar Kelimeler: *Trichomonas vaginalis*, direkt mikroskobi, kültür, PZR, immünokromatografik yöntem

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Introduction

Trichomoniasis, caused by *Trichomonas vaginalis* (*T. vaginalis*), is a prevalent sexually transmitted infection worldwide and infects only humans (1). It is an anaerobic protozoan, pear-shaped, measuring 10-20 µm in size, and possesses flagella (2). According to the World Health Organization, 156 million new cases of trichomoniasis were reported worldwide in 2016 (3).

Trichomonas vaginalis infection can manifest as asymptomatic or symptomatic, with a range of symptoms observed. In women, these symptoms may include vaginitis, urethritis, cervicitis, and endometritis, while in men, prostatitis and epididymitis can occur. Furthermore, trichomoniasis in women is characterized by yellow-green vaginal discharge, dysuria, genital itching, and burning sensations (4). Apart from sexual transmission, it has been reported that transmission of *T. vaginalis* can also occur through the use of shared bathwater and equipment, as well as during passage through the birth canal from an infected mother (5).

The prevalence of *T. vaginalis* varies depending on features such as sexual activity, age, presence of other sexually transmitted infections, socioeconomic factors, and diagnostic methods used (6). The prevalence of *T. vaginalis* infection has been reported as 5.3% in women and 0.6% in men (3). Studies conducted in Turkey have reported *T. vaginalis* prevalence rates ranging from 1.9% to 72.3% (7,8).

Direct microscopy (DM), culture methods, staining techniques, serology-based methods, molecular methods, such as polymerase chain reaction (PCR), immunochromatographic rapid diagnostic tests (RDT), and immunocytochemical methods are used in the diagnosis of *T. vaginalis*. Each of these methods has been reported to have unique advantages and disadvantages (9,10).

Our study aimed to investigate *T. vaginalis* using DM, culture, PCR, and immunochromatographic RDT in cases presenting with vaginal discharge complaints at the gynaecology and obstetrics outpatient clinic.

Materials and Methods

This study has been approved by the Muğla Sıtkı Koçman Universtiy Scientific Research Ethics Committee (decision no: 125, date: 21.08.2015). After obtaining ethics committee permission, cases who accepted the informed consent form were included in the study. A total of 150 cases between the ages of 18 and 45, presenting with vaginal discharge complaints, were included in the study at Muğla Sıtkı Koçman Universtiy Hospital Gynaecology and Obstetrics Clinics. For each included case, a patient information form was completed, which included personal information, complaints, and findings. Three vaginal swab samples were collected from the posterior fornix by a gynaecologist. The presence of *T. vaginalis* was investigated in the swab samples using DM, culture, PCR, and RDT.

An examination for *T. vaginalis* was performed at the patient's bedside using the RDT method from the swab sample obtained with a Dacron swab. The other swab samples obtained with a cotton swab were processed as follows: one sample was placed in tubes containing 8 mL of trypticase-yeast extract-maltose (TYM) with 10 µg/mL gentamycin, 100 U/mL penicillin-streptomycin, and another sample was placed in tubes containing 1 mL of physiological saline for DM and PCR. All samples were rapidly transported to the microbiology laboratory.

A drop of the vaginal swab sample, obtained in a tube containing 1 mL of physiological saline, was examined under a microscope using 20x and 40x objectives for DM examination. Samples in which *T. vaginalis* trophozoites were detected were considered positive. After the swab sample was inoculated onto TYM culture medium, it was incubated at 37 °C, and the presence of growth was microscopically examined daily for a week. The swab samples were collected in tubes containing 1 mL of physiological saline, centrifuged at 2,000 g, and stored at -20 °C until DNA isolation. DNA isolation was performed using the Nucleospin Tissue DNA Isolation Kit® according to the manufacturer's recommendations.

Trichomonas vaginalis specific primers TV3 (5' TTG TCG AAC ATT GGT CTTA CCC TC 3') and TV7 (5' TCT GTG CCG TCT TCA AGT ATG C 3') were used for PCR amplification. The reaction was set in a 30-µL volume containing 0.4 pmol of each of the primers, 0.3U of Taq DNA polymerase, 0.2 mM of each deoxynucleotide triphosphate (dNTP), 2 mM magnesium chloride (MgCl2), and 1× Taq buffer with ammonium sulfate $(NH_4)_2SO_4$. The amplification was performed using the following protocol: initial denaturation step at 96 °C for 2 min and 30 cycles (1 min at 90 °C, 30 s at 60 °C, and 2 min at 72 °C) with a final extension step at 72 °C for 7 min. The PCR product was run on a 1.5% agarose gel and the gels were visualized using a gel documentation system (Vilber Lourmat, Basel, Switzerland).

The commercial RDT (OSOM) method (Sekisui Diagnostics, LLC, Lexington, PMA) was performed according to the manufacturer's recommendations. The swab obtained from the posterior fornix using a Dacron swab was placed in plastic tubes provided in the kit containing 0.5 mL of sample buffer. The swab was rapidly mixed in the sample buffer and left to stand for approximately one minute. Then, using the plastic tube, the swab was squeezed thoroughly and discarded. After, test strips were inserted into the plastic tubes and left for 10 minutes. At the end of this time, strips viewing only a red control line were measured negative, strips showing both a red control line and a blue test line were considered positive, and strips with no visible lines for both were considered invalid.

Statistical Analysis

The results of the methods were compared with the non-parametric McNemar test in SPSS statistics version 22.

Results

Vaginal swab samples from 150 different cases presenting with vaginal discharge at the Muğla Sıtkı Koçman Universtiy Hospital Obstetrics and Gynaecology Clinics were evaluated. Patient information forms containing personal details, complaints, and examination findings of the cases were reviewed. The age range of the cases was 18-45 years, with a mean age of 34.8±6.7. Of the cases, 91.4% were married, and 49% were working. The measurement data of the education level of the cases, the history of sexually transmitted disease in themselves or their partners, and the level of knowledge about *T. vaginalis* are given in Table 1. Evaluating the cases in terms of symptoms, the most common complaint following vaginal discharge was itching (45%). The characteristics of the discharge were determined as follows: yellow-green discharge (37%), curd-like discharge (33%), grey discharge (21%), and foul-smelling discharge (9%). The overall clinical findings of 150 women are given in Table 2.

In the current study, out of a total of 150 vaginal swab samples, two (1.3%) samples were positive for *T. vaginalis* in DM, while three (2%) samples were positive in culture, PCR, and RDT methods. The results of DM, culture, PCR and RDT diagnostic methods used in the detection of *T. vaginalis* are presented in Table 3. There was no statistically significant

Table 1. The analysis of demographic characteristics, clinical features and knowledge about trichomoniasis

		n	%
Job status	Working	73	49
	Housewife	77	51
Educational status	Primary school	44	29.4
	Middle school	20	13.3
	High school	50	33.3
	University	36	24
History of sexually transmitted disease	Exist	1	0.6
	None	149	99.4
History of sexually transmitted disease in partner	Exist	1	0.6
	None	149	99.4
T. vaginalis knowledge level	Exist	6	4
	None	144	96
Preterm labor	Exist	1	0.6
	None	149	99.4
Total		150	100
	Minimum-maximum	Mean ± SD	
Age (years)	18-45	34.8±6.7	

SD: Standard deviation

Table 2. The clinical findings of study population (n=150)

Symptoms	Number	%
Vaginal discharge	150	100
Vaginal itching	67	44.6
Lower abdomen pain	61	40.6
Genital pain	48	32
Vulval erythema	41	27.3
Dysuria	35	23.3
Punctate hemorrhagic spots (strawberry cervix)	29	19.3

Table 3. Evaluation of diagnostic methods in the detection of *T. vaginalis

	DM	OSOM	Culture	PCR
Positive	2 (1.3%)	3 (2%)	3 (2%)	3 (2%)
Negative	148 (98.7%)	147 (98%)	147 (98%)	147 (98%)
Total	150 (100%)	150 (100%)	150 (100%)	150 (100%)

p>0.05. DM: Direct microscopy, PCR: Polymerase chain reaction

difference between the methods (Mcnemar test, p>0.05). The ages of these cases were 35, 45, and 45, respectively. All positive cases presented with yellow-green discharge, along with symptoms and findings such as itching, lower abdominal pain, and vulvar erythema. The presentation of clinical findings of *T. vaginalis*-positive three cases were given in Table 4.

Discussion

Trichomoniasis represents a considerable global public health concern due to its associated health risks. Studies have indicated that the presence of the parasite is linked to infertility, cervical neoplasia, premature membrane rupture, and preterm labour (11). Also, trichomoniasis infection increases the risk of HIV-1 transmission by approximately 1.5-3 times, highlighting the importance of the infection (12). The use of more advanced diagnostic techniques, early diagnosis and appropriate behavioural approaches in developed countries has led to a decrease in the prevalence of *T. vaginalis* in the general population. However, in developing countries, it is still detected at high rates (13). Due to the asymptomatic nature and challenging detection of *T. vaginalis* infection, many diagnostic methods such as microscopy, molecular techniques, culture, and RDT are employed for the detection of the parasite in today's medical practice. However, these methods have disadvantages. Particularly, although the culture method is considered the gold standard for diagnosing the parasite, it has drawbacks such as susceptibility to contamination and a turnaround time of 2-7 days (14,15). Therefore, in recent years, the use of rapid point-of-care diagnostic tests has been increased because of easy handling and implementation for *T. vaginalis* diagnosis (16). One of these commercial RDT methods is OSOM that is FDA-approved and can be used at the point of care.

Multiple methods have been utilized in global studies for the diagnosis of *T. vaginalis*. However, there are only a limited number of studies that employ immunochromatographic-based rapid tests. In India, *T. vaginalis* investigation was conducted on 418 cases using DM, the rapid test, and culture. While the culture method detected 68 (16.3%) positive samples, wet mount microscopy identified 56 of the culture-positive samples along with four false positive samples. The OSOM test identified 60 of the culture-positive samples and two false-positive cases. This straightforward test has the potential to enhance the screening and diagnosis of *T. vaginalis* infection in settings where microscopy and culture are not available and resources are limited (17). In our study, one DM negative sample was positive with the OSOM test. In another study, vaginal swabs were collected from 835 female patients. The rates of positivity were determined as follows: 5.4% with DM, 8.1% with culture, 7.5% with the same rapid test, and 6.3% with acridine orange staining. The study concluded that due to its superior performance compared to other methods, the *Trichomonas* Rapid Test could be favoured in laboratory settings (18). In the United States, the prevalence of *T. vaginalis* among 449 cases with vaginal symptoms was determined to be 23.4%. The study highlighted the potential use of the RDT, especially in situations where microscopy and culture are not available (19). There are few studies in Turkey regarding the methods used for the detection of *T. vaginalis*, and no study comparing RDT has been identified. In the gynaecology clinic of Hatay province, among 104 symptomatic patients, 12 (11.53%) tested positive through DM, 14 (13.4%) through the culture method, 12 (11.53%) through Giemsa staining, and 5 (4.8%) through Papanicolaou staining. The sensitivities of direct microscopic examination, Giemsa staining, and cytological diagnosis were reported as 85.7%, 85.7%, and 35.7 % respectively, with specificities of 100% for all three methods (20). In a study conducted in Sivas, positivity was found in 258 cases with a pre-diagnosis of vaginitis at a rate of 1,9% with DM and 1,5% with culture method (7). Vaginal discharge samples were collected from 233 women with symptoms of vaginal discharge and vulvar itching in Manisa, as well as from 100 women in a control group who sought routine gynaecological examination without presenting vaginal discharge or vulvar itching. The samples were examined using the TYM medium, DM, and culture methods. Through both direct examination and culture, *T. vaginalis* was detected in 11 out of 233 (4.7%) patients with vaginitis. None of the 100 women in the control group showed evidence of the parasites in either method (21).

In the present study, vaginal swab samples from 150 cases presenting with vaginal discharge at Muğla Sıtkı Koçman Universtiy Hospital Gynaecology and Obstetrics Clinics were investigated for *T. vaginalis* using DM, TYM, PCR, and RDT methods. *T. vaginalis* was detected in two (1.3%) samples by DM and three (2%) samples by the other three methods. Limited studies are comparing multiple diagnostic methods for *T. vaginalis* in our country. Ertabaklar et al. (22) reported in their study in 2011 that PCR was used for the first time in our country for the diagnosis of *T. vaginalis*. In the current study, *T. vaginalis* positivity rates were found 2.9% by DM, and 4.9% by culture and PCR. Moreover, our

Table 4. Clinical presentation of *T. vaginalis* positive cases (n=3)

	Case 1	Case 2	Case 3
Vaginal discharge	+	+	+
Vaginal itching	+	+	-
Lower abdomen pain	-	+	-
Genital pain	+	+	-
Vulval erythema	-	+	+
Dysuria	-	+	+
Punctate hemorrhagic spots (strawberry cervix)	+	+	-

+: Present, -: Absent

study is the first study in Turkey to use the commercial RDT (OSOM) method for the diagnosis of *T. vaginalis.*

Many studies in the literature use more than one method for the diagnosis of *T. vaginalis*. However, limited studies are using immunochromatographic tests. The sensitivity of the rapid test has been reported to range from 83.3% to 100% (23,24), while the specificity ranges from 96% to 99.6% (18,24). In our study, although we did not find statistical differences between the methods. DM caused misleading of a *T. vaginalis* case, giving false negative results.

Conclusion

In conclusion, OSOM, which was used for RDT in this study, offers several advantages in antigen detection. These include being a point-of-care test, which means it is not affected by delays during transportation, and not requiring experienced personnel or specialized equipment. Additionally, it provides rapid results, and its high sensitivity, as reported in other studies, was also confirmed in our study. It can be concluded that the RDT test may a better option than DM for the diagnosis of *T. vaginalis*. However, further studies are needed to compare it with the reference methods, culture, and molecular methods.

Ethics

Ethics Committee Approval: This study has been approved by the Muğla Sıtkı Koçman Universtiy Scientific Research Ethics Committee (decision no: 125, date: 21.08.2015).

Informed Consent: After obtaining ethics committee permission, cases who accepted the informed consent form were included in the study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

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

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Glycated Albumin and Hba1c for the Diagnosis of Prediabetes in Obese and Non-obese Individuals

Obez ve Obez Olmayan Bireylerde Prediyabet Tanısında Glikozile Albümin ve Hba1c

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Abstract

Objective: In our study, we examined whether glycated albumin (GA) had any superiority over the HbA1c test in detecting individuals with insulin resistance, prediabetes, and diabetes in obese and non-obese groups. This study is the first to examine the diagnostic power of HbA1c and GA tests alone or together for prediabetes, diabetes, and insulin resistance in non-obese and obese individuals.

Materials and Methods: The study was conducted at Ankara Yıldırım Beyazıt University Faculty of Medicine and Atatürk Training and Research Hospital. Individuals were divided into three groups: diabetes, prediabetes, and insulin resistance, which were further sub-grouped as obese and non-obese according to their body mass index values.

Results: When we examined the HbA1c and GA values in the diabetes, prediabetes, and insulin resistance groups, we found significantly higher rates of correct detection of prediabetes and diabetes (sensitivity) for GA than for HbA1c in non-obese individuals. The specificity of GA was lower than HbA1c in these non-obese individuals, whereas the specificity of GA was similar to HbA1c in obese individuals. Our data show that in non-obese individuals, GA measurement is a more sensitive but less specific tool compared with the measurement of HbA1c. Therefore, we suggest that, while HbA1c and GA were in agreement with oral glucose tolerance test and fasting glucose levels in the diagnosis of diabetes in obese individuals ($p<0.05$), GA alone or together with HbA1c may be a valuable tool in the diagnosis of prediabetes and diabetes in non-obese individuals.

Conclusion: This study shows that GA levels have higher sensitivity and lower specificity than HbA1c in the diagnosis of type 2 diabetes in non-obese individuals.

Keywords: Prediabetes, diabetes mellitus, glycated albumin, HbA1c

Öz

Amaç: Çalışmamızda obez ve obez olmayan gruplarda insülin direnci, prediyabet ve diyabeti tespit etmede glikozillenmiş albüminin (GA) HbA1c testine üstünlüğü olup olmadığını inceledik. Bu çalışma, obez olmayan ve obez bireylerde prediyabet, diyabet ve insülin direnci için tek başına veya birlikte HbA1c ve GA testlerinin tanısal gücünü inceleyen ilk çalışmadır.

Gereç ve Yöntemler: Çalışma Ankara Yıldırım Beyazıt Üniversitesi Tıp Fakültesi ve Atatürk Eğitim ve Araştırma Hastanesi'nde gerçekleştirilmiştir. Bireyler önce diyabet, prediyabet, insülin direnci ve daha sonra vücut kitle indeksi değerlerine göre obez ve obez olmayanlar alt gruplara ayrıldı.

Bulgular: Diyabet, prediyabet ve insülin direnci gruplarında HbA1c ve GA değerlerini incelediğimizde; obez olmayan bireylerde HbA1c'ye kıyasla GA için prediyabet ve diyabetin (hassasiyet) doğru saptanma oranlarının önemli ölçüde daha yüksek olduğunu bulduk. Obez olmayan bu bireylerde GA'nın özgüllüğü HbA1c'den düşükken, obezlerde GA'nın özgüllüğü HbA1c'ye benzerdi. Verilerimiz obez olmayan bireylerde GA ölçümünün HbA1c ölçümüne kıyasla daha duyarlı ancak daha az spesifik bir araç olduğunu göstermektedir. Bu nedenle şunları öneriyoruz; obez bireylerde diyabet tanısında HbA1c ve GA, OGTT ve açlık glukoz düzeyleri ile uyumlu iken ($p<0,05$), GA tek başına veya HbA1c ile birlikte obez olmayan bireylerde prediyabet ve diyabet tanısında değerli bir araç olabilir.

Sonuç: Bu çalışma, obez olmayan bireylerde tip 2 diyabet tanısında GA düzeylerinin HbA1c'den daha yüksek duyarlılığa ve daha düşük özgüllüğe sahip olduğunu göstermektedir.

Anahtar Kelimeler: Prediyabet, diyabet, glikozile albumin, HbA1c

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Introduction

Prediabetes term is used for individuals whose glucose levels do not meet the criteria for diabetes but are too high to be considered normal (1). Fasting plasma glucose level (FPG) values between 100-125 mg/dL are defined as IFG, and values with a plasma glucose level of 140-199 mg/dL after 75 grams of oral glucose in the second hour are defined as impaired glucose tolerance (IGT). Patients with these values are included in the prediabetic group. Besides, oral glucose tolerance test (OGTT) and HbA1c are equally valuable in the diagnosis of prediabetes. Patients with an HbA1c level of 5.7-6.4% are also considered prediabetic. Other glycated proteins such as glycated albumin (GA) as measures of average glycemia are also available, however, their diagnostic and prognostic significance are not as clear as for HbA1c. HbA1c predicts glycemia over the past (2-3) months, whereas glycated albumin (GA), an early Amadori-type glycation protein of the non-enzymatic glycation reaction between glucose and serum albumin, indicate glycemic status for the past (2-3) weeks (2,3). Glycated albumin, reflecting short term glycaemia and is not affected by many conditions that alter HbA1c. The GA level is calculated as a percentage by dividing the amount of glycated albumin by total albumin (4). Obesity, an important risk factor in diabetes development, has been shown to affect the test results used in the diagnosis and follow-up of prediabetes. Body mass index (BMI) is the most practical step to assess the degree of overweight and obesity. Effective interventions for weight loss favourably increase insulin sensitivity (5,6). In our study, assessed the diagnostic power of HbA1c and GA tests alone or together for prediabetes, insulin resistance and diabetes in non-obese and obese individuals.

Materials and Methods

The study was conducted at Atatürk Taining and Research Hospital and Dr. Rıdvan Ege Hospital. This research was approved by the Ethical Committee at Yıldırım Beyazıt University (protocol no: 2017/23, date: 10.08.2017). We obtained signed informed consent from all participants.

Study Population and Design

Patients (n=126) diagnosed either with diabetes, prediabetes and insulin resistance were selected among the patients who attended the Internal Diseases and Endocrinology outpatient clinics, considering the indications for OGTT. Patients with severe hepatic and renal dysfunction, pregnancy, malabsorption syndrome, steroid or alpha-glucosidase inhibitor use, patients with a history of gastrectomy, and other endocrine and metabolic disorders such as thyroid disease and metabolic syndrome were excluded. The demographic information and anthropometric measurements were recorded. The waist circumference [midway between the lowest rib and the iliac crest in a standing position, as recommended by the World Health Organization (WHO)] measured. The BMI of patients participating in the study were evaluated using a bioimpedance device TANITA (7). BMI ≥ 30 kg/m^2 was accepted as obese according to the WHO classification (8).

Sample Collection and Storage

Blood samples from individuals for analyses of routine and specific parameters were collected into yellow top blood tubes without anticoagulant (BD Vacutainer®) or lavender top blood tubes containing 5.4 mg K2-EDTA (BD Vacutainer®). Fasting blood samples were drawn between 8.00 to 10.00 am. after overnight fasting of 8-12 h. For postprandial glucose measurement, blood sample were taken two hours after the meal. All serum samples were processed within two hours of blood collection and one aliquot for glycated albumin was stored at -80 °C until the day of analysis.

Glucose Tolerance Testing

In cases with insulin resistance and prediabetes clinic, OGTT was performed as a follow-up. Individuals were given 75 grams of glucose, and OGTT and simultaneous insulin measurements were performed in blood samples taken at 0, 30, 60, 90 and 120th minutes, respectively. OGTT results of the cases were evaluated according to American Diabetes Association criteria (1). Individuals were then divided into 3 groups; diabetes, prediabetes and insulin resistance which further sub-grouped obese and non-obese according to their OGTT results and BMI values. These groups are shown below:

Group 1: Obese, prediabetes

Group 2: Non-obese, prediabetes

Group 3: Obese, insulin resistant

Group 4: Non-obese, insulin resistant

Group 5: Obese, Diabetes

Group 6: Non-Obese, diabetes

Laboratory Analyses

Blood glucose levels were measured using Roche Cobas 501 device by spectrophotometric method. Serum albumin levels were measured using Roche Cobas 501 device by immune-turbidimetric method. Haemoglobin was measured by the impedance and flow-cell method in the Sysmex 2100 device. Insulin levels were measured with Roche Cobas 600 ECLIA method simultaneously with OGTT (at 0, 30, 60, 90, and 120 minutes). Insulin resistance of the individuals was evaluated using the formula (HOMA-IR = FPG (mg/dL) x fasting plasma insulin (microunit/mL)/405) and HOMA-IR >2.5 accepted as insulin resistance (6-9). HbA1c was measured in the same whole blood samples by ion exchange chromatographic HPLC method using Agilent 1100 Series device (NGSP-certified). The results were given as % Hb. Serum glycated albumin (GA) was measured with a commercially available kit, DIAZYME Glycated Serum Protein Assay by enyzmatic method, using Cobas 501 device. % GA was calculated using the equation; glycated

albumin (%) =2.9 + {[glycated albumin concentration (g/dL): total albumin concentration (g/dL)]: 1.4} * 100. The inter-assay CVs for GA was 1.7% at 0.58 g/L and 4.5% at 1.67g/L.

Gycated albumin (%) =2.9 + {[glycated albumin concentration (g/dL): total albumin concentration (g/dL)]: 1.4} * 100.

Statistical Analysis

IBM-SPSS Statistics 21.0 for Windows program was used for statistical analysis. P<0.05 was accepted as statistically significant. The compliance of numerical variables examined in the study to normal distribution was examined with the Shapiro-Wilks normality test and normality plots. Mean ± standard deviation, median, minimum, and maximum values were used to display the descriptive statistics of the variables. Frequencies and percentages were given in categorical variables. The correlation between BMI and HbA1c and GA was analysed with the Spearman rho correlation coefficient. Mann-Whitney U and Kruskal-Wallis tests were used for comparing independent groups. Pearson chi-square test result was given for categorical variables. The "HUM" package in the R program was used to determine the cut-off points for discriminating the class with GA (9). Correct classification probabilities (CCP) and 95% confidence intervals were obtained for the groups from 3x3 cross tables according to the cut-off points determined for GA and specified for HbA1c. The calculated CCP1, CCP2, and CCP3 values are the probability of determining those with insulin resistance (specificity), prediabetes (intermediate fraction), and diabetes (sensitivity). In the case of using GA and HbA1c together, an ordinal logistic model was used to determine the class determination ability. CCP and 95% confidence limits were calculated according to the classes determined as a result of the model. The comparison of the CCP determined by the variables was Performed with the McNemar-Bowker test.

Results

Descriptive Properties of Patients and Blood Chemistry Values

Among patients involved in the study, 42.9 % were male (n=54) and 57.1 % were female (n=72). Median age was 49 (18; 76). Descriptive properties and blood chemistry values of individuals, grouped according to diabetes status, were summarized in Table 1. Diagnoses as insulin resistance, prediabetes and diabetes were defined by the endocrinology specialist, according to the fasting glucose levels and OGTT results of the individuals (Table 1). Gender distribution in and among insulin resistance, prediabetes and diabetes groups were similar (p=0.131). HbA1c (%) and GA (%) levels were highest in diabetes and lowest in insulin resistance groups (p<0.001). Pairwise comparison showed that GA values in diabetic patients were significantly higher than those with insulin resistance and prediabetes (p<0.05).

Test results of individuals were also grouped according to their BMI categories (Table 2). Obesity was more prominent in females, compared to males and the gender distributions of the BMI categories were not similar between the two groups (p<0.001). Glucose and postprandial blood glucose levels did not differ in obese and non-obese patients (p>0.05).

Predictive Value of HbA1c and GA Alone or Together in Patients

HbA1c levels <5.7% were classified as "insulin resistant", between 5.7-6.4% as "prediabetes" and ≥6.5% as "diabetes" groups (10). Patients with GA values <12.81% were accepted as "insulin resistant", between 12.81-16.06% as "prediabetes" and ≥16.07% as "diabetes" groups (14). Groups for HbA1c and GA were determined using an ordinal logistic regression model and the correct classification rates using HbA1c (%) and GA (%) were given in Table 3. We observed that an agreement with the clinician's diagnosis

Table 1. Biochemical tests according to diabetes categories

Variable	Insulin resistance	Prediabetes	Diabetes	Z, χ^2	p-value
BMI (kg/m²)	33.95 (24.3; 45)	30.4 (21.9; 47.7)	30.2 (19.6; 50.8)	3.876	0.144
Glucose (mg/dL)	89 (65; 116)[a]	103 (80; 137)[b]	138 (89; 322)[c]	70.189	**<0.001**
Postprandial glucose (mg/dL)	100 (68; 151)[a]	117 (60; 200)[a]	215 (75; 521)[b]	63.350	**<0.001**
HbA1c (%)	5.34 (3.96; 6.66)[a]	5.39 (4.46; 7)[a]	6.87 (4.49; 11.45)[b]	55.094	**<0.001**
Albumin (g/dL)	4.69 (4.16; 5.36)	4.63 (3.51; 5.32)	4.63 (4.12; 5.41)	0.454	0.797
GA (mmol/L)	237.25 (180.8; 304.2)[a]	270.6 (174.5; 373.1)[a]	366.3 (244.1; 1116)[b]	71.388	**<0.001**
GA (%)	12.36 (10.01; 15.28)[a]	13.86 (11.03; 17.83)[a]	18.08 (12.73; 48.07)[b]	71.478	**<0.001**
HGB (g/dL)	14.55 (12.4; 17.5)	14.7 (11.4; 17)	14.5 (9.2; 17.2)	0.045	0.978

Data were summarized as frequency (percentage) and or median (minimum; maximum). The Kruskal-Wallis test (χ^2) for more than two groups, Mann-Whitney U test (Z) for two groups and Pearson chi-square test (χ^2) results were reported for quantitative and qualitative variables, respectively. Bold type p-values were lower than 0.05. a,b,c Values were similar in groups denoted by the same letter. BMI: Body mass index, GA: Glycated albumin, HGB: Hemoglobin

was obtained with laboratory results, when HbA1c + GA is used together. In non-obese group, while HbA1c had higher specificity compared to GA in diagnosis, it was GA, alone or in combination with HbA1c, was found to be the more sensitive test in diagnosis (Table 3).

When HbA1c (%) and GA (%) measurements were applied for diabetes classification and compared with the diagnosis made by the clinician, an agreement in diagnosis was achieved only in the obese group (p=0.064) (Table 4). On the other hand, in the non-obese group, the diabetes classifications were found to be in agreement with the clinician's diagnosis, only when GA values [alone or combined with HbA1c (%) values] were applied (p=0.317) (Table 4).

In our study, in the prediabetes group, 16 out of 31 individuals were classified as prediabetic by GA measurements alone

Table 2. Biochemical tests of individuals according to BMI categories categories

Variable	Total	Non-obese	Obese	χ^2; Z	p-value
Diabetes n (%)					
Insulin resistance	32 (25.4)	13 (23.6)	19 (26.8)	0.164	0.921
Prediabetes	31 (24.6)	14 (25.5)	17 (23.9)		
Diabetes	63 (50.0)	28 (50.9)	35 (49.3)		
BMI (kg/m^2)	30.5 (19.6; 50.8)	27.4 (19.6; 29.8)	35.1 (30.0; 50.8)	-9.605	**<0.001**
Glucose (mg/dL)	110 (65; 322)	109 (65; 322)	112 (73; 320)	-1.279	0.201
Postprandial blood glucose (mg/dL)	135 (60; 521)	141 (71; 521)	133 (60; 414)	-0.620	0.535
HbA1c (%)	5.88 (3.96; 11.45)	5.76 (4.27; 10.35)	6.01 (3.96; 11.45)	-1.552	0.121
Albumin (g/dL)	4.65 (3.51; 5.41)	4.68 (4.12; 5.41)	4.63 (3.51; 5.36)	-1.065	0.287
Serum GA (mmol/L)	302.4 (174.5; 1116.0)	302.4 (187.3; 1116)	302.4 (174.5; 856)	-0.497	0.619
GA (%)	14.74 (10.01; 48.07)	15.03 (10.35; 48.07)	14.61 (10.01; 38.43)	-0.401	0.688
HGB (g/dL)	14.6 (9.2; 17.5)	15.2 (11.4; 17.2)	14.1 (9.2; 17.5)	-3.752	**<0.001**

Data were summarized as frequency (percentage) or median (minimum; maximum). The Mann-Whitney U test (Z) and Pearson chi-square test (χ^2) results were reported for quantitative and qualitative variables, respectively. Bold type p-values were lower than 0.05. BMI: Body mass index, GA: Glycated albumin, HGB: Hemoglobin

Table 3. Correct classification probabilities for HbA1c (%), GA (%) and HbA1c (%) + GA (%)

		Insulin resistance	Prediabetes	Diabetes	
		Specificity CCP1 (min. and max. limits of 95% CI)	Intermediate fraction CCP2 (min. and max. limits of 95% CI)	Sensitivity CCP3 (min. and max. limits of 95% CI)	p-value*
Total	**HbA1c**	81.3 (67.7; 94.8)	29.0 (13.1; 45.0)	58.7 (46.6; 70.9)	**<0.001**
	GA	62.5 (45.7; 79.3)	74.2 (58.8; 89.6)	74.6 (63.9; 85.4)	**0.010**
	HbA1c + GA	62.5 (45.7; 79.3)	71.0 (55.0; 86.9)	76.2 (65.7; 86.7)	0.057
Non-obese	**HbA1c**	84.6 (65.0; 99.9)	28.6 (4.9; 52.2)	42.9 (24.5; 61.2)	**0.002**
	GA	38.5 (12.0; 64.9)	64.3 (39.2; 89.4)	67.9 (50.6; 85.2)	**0.037**
	HbA1c + GA	38.5 (12.0; 64.9)	64.3 (39.2; 89.4)	60.7 (42.6; 78.8)	**0.017**
Obese	**HbA1c**	78.9 (60.6; 97.3)	29.4 (7.8; 51.1)	71.4 (56.5; 86.4)	0.053
	GA	78.9 (60.6; 97.3)	82.4 (64.2; 99.9)	80.0 (66.8; 93.3)	0.155
	HbA1c + GA	78.9 (60.6; 97.3)	76.5 (56.3; 96.6)	88.6 (78.0; 99.1)	0.940

CCP: Correct classification probability, CI: Confidence interval, GA: Glycated albumin. *In McNemar-Bowker test results, when the p-value was higher than 0.05, it means that there was an agreement between the classification based on the related variable (only HbA1c, only GA or HbA1c + GA) and the actual classes. P-value lower than 0.05 means no agreement

and 9 by HbA1c test with/without GA test. On the other hand, in the diabetes group, 14 out of 63 individuals classified as diabetic by GA test alone and 37 individuals by HbA1c with/without GA test. The diabetic group defined by using GA also had lower BMI (Table 5).

Discussion

HbA1c and fasting blood glucose are equally effective screening tools to detect type 2 diabetes. In the diagnosis of prediabetes, OGTT and HbA1c levels are also regularly used. In recent years, the superiority of different glycosylated proteins such as GA over HbA1c values in detecting individuals with prediabetes has been in discussion. Obesity, an important risk factor in diabetes development, has been shown to affect the test results used in the diagnosis and follow-up of prediabetes (6,7).

In our study, we examined whether GA had any superiority over HbA1c test in detecting individuals with insulin resistance, prediabetes and diabetes in obese and non-obese groups. To the best of our knowledge, this study is the first to examine the diagnostic power of HbA1c and GA tests alone or together for prediabetes, diabetes and insulin resistance in non-obese and obese individuals. Koga et al. (11) showed that HbA1c was not always an ideal glycemic control index and does not accurately reflect the status of plasma glucose control in various pathological conditions. HbA1c levels may be falsely high in hemolytic anemia, blood loss, splenomegaly, iron deficiency anemia, vitamin B12 deficiency, severe hypertriglyceridemia, and uremia (12). Therefore, measurements may need to be validated by different methods or to be evaluated using different diabetes biomarkers.

In this study, we also examined the HbA1c and GA values of individuals in the prediabetes and diabetes groups, which were grouped according to fasting blood glucose and OGTT values, alone or together. When GA and HbA1c values were evaluated without considering BMI, GA values were

Table 4. Comparison of correct classification probabilities obtained by only HbA1c (%), only GA (%) and HbA1c (%) + GA (%) in BMI groups

	HbA1c - GA	HbA1c - HbA1c + GA	GA - HbA1c + GA
	p-value	p-value	p-value
Total	**<0.001**	**<0.001**	0.317
Non-obese	**<0.001**	**<0.001**	0.317
Obese	0.064	**0.003**	**0.025**

McNemar-Bowker test results. When the p-value was higher than 0.05, it means that there was an agreement between the classifications based on the related variables. p-value lower than 0.05 means no agreement. BMI: Body mass index, GA: Glycated albumin

Table 5. Comparison of demographic characteristics patients and biochemical parameters determined as prediabetic and diabetic using HbA1c and GA levels alone

Diagnosed by clinician (n)	Prediabetes (31)		p-value	Diabetes (63)		p-value
Classified by HbA1c and/or GA (n)	HbA1c or HbA1c and GA (9)	GA (15)		HbA1c or HbA1c and GA (37)	GA (14)	
BMI (kg/m²)	29.9 (23.1; 47.7)	31.9 (25.4; 37.4)	0.640	32.4 (19.6; 50.8)	27.6 (22.0; 39.3)	**0.023**
Glucose (mg/dL)	109 (86; 121)	98 (80; 117)	0.108	168 (89; 322)	117 (100; 189)	**0.001**
Postrandial glucose (mg/dL)	124 (94; 194)	114 (60; 182)	0.290	250 (83; 521)	189 (94; 311)	**0.014**
HbA1c (%)	5.9 (5.7; 6.4)	5.1 (4.5; 5.6)	**<0.001**	7.60 (6.6; 11.4)	6.11 (4.53; 6.47)	**<0.001**
GA (%)	13.9 (12.5; 16.9)	13.7(12.9; 15.0)	0.519	19.92 (13.9;48.1)	17.27 (16.07; 22.14)	**0.020**

Data were summarized as frequency (percentage) and or median (minimum; maximum). The Mann-Whitney U test (Z) and Pearson chi-square test (χ^2) results were ported for quantitative and qualitative variables, respectively. Bold type p-values were lower than 0.05. BMI: Body mass index, GA: Glycated albumin

higher in the diabetes group compared to the other groups (p<0.001). In line with our study, a study conducted by Hsu et al. (13) in which GA and HbA1c were compared for diabetes screening, reported a significant positive correlation among FPG, GA, and HbA1c levels. Other researchers reported that GA better reflects glycemic control and is a better marker in diabetes screening compared to the gold standard HbA1c in some patient groups (14). A study by Kengne et al. (15) examining the OGTT, HbA1c, GA and fructosamine values in prediabetes and diabetes patients in an African population where sickle cell anemia, human immunodefiency virus and chronic kidney diseases are common, showed that IGT detected by OGTT test is more compatible with GA values than HbA1c. Shima et al. (16) reported that a single random measurement of GA is more useful than HbA1c for screening for diabetes in the population, but neither of these two parameters is sensitive enough to detect individuals with IGT.

Studies have proven a strong relationship between BMI and diabetes and insulin resistance (17,18). Non-obese type 2 diabetes phenotype is characterized by disproportionately reduced insulin secretion and less insulin resistance, compared to obese patients with type 2 diabetes. We detected significantly lower fasting blood glucose levels (p=0.001), postprandial blood sugar levels (p=0.014), BMI (p=0.023) and waist circumference (p=0.046) values in the group with diabetes classified by GA against the group classified as diabetes by HbA1c test. Supporting our data, Sumner et al. (19) found that the BMI values of the prediabetes group based on GA values were lower than the group based on HbA1c values. On the other hand, Koga et al. (11,20) showed a negative correlation between obesity and serum glycated albumin and a positive correlation between BMI and HbA1c, whilst there was a negative correlation between BMI and GA in non-diabetic subjects. The same group revealed by multivariate regression analyses that BMI was the strongest negative variable for GA. A study by Nishimura et al. (21) reported that when the relationship between BMI and HbA1c and GA is examined, a significant positive correlation between BMI and HbA1c, and a significant negative correlation were observed between BMI and GA. Similar to the literature, in our study, GA levels were slightly lower in obese individuals than in non-obese individuals, however, this value was not statistically significant (p=0.688). We did not observe any statistical difference between obese and non-obese individuals in terms of HbA1c values. On the other hand, we found significantly higher rates of correct detection of prediabetes and diabetes (sensitivity) for GA compared to HbA1c in non-obese individuals. The specificity of GA was lower than HbA1c in these non-obese individuals whereas the specificity of GA was similar to HbA1c in obese ones.

Our data show that in non-obese individuals, GA measurement is a more sensitive but less specific tool compared to measurement of HbA1c and is not affected by many conditions that alter HbA1c. Therefore, we suggest that; while HbA1c and GA were in agreement with OGTT and fasting glucose levels in diagnosis of diabetes in obese individuals (p<0.05), GA alone or together with HbA1c may be a valuable tool in diagnosis of prediabetes and diabetes in non-obese individuals.

Conclusion

This study shows that GA levels have higher sensitivity but lower specificity than HbA1c in the diagnosis of type 2 diabetes in non-obese individuals. There are some limitations in our study. First, our study was a cross-sectional study and the number of subjects in this study is relatively small. Secondly, since our study was conducted in a single centre. Nevertheless, this study provides important information about the tests of choice for diagnosing prediabetes and diabetes in obese and non-obese individuals. Improving diagnostic sensitivity with the combined use of HbA1c and GA may be useful in detecting diabetes earlier in non-obese individuals and taking preventive measures.

Ethics

Ethics Committee Approval: This research was approved by the Ethical Committee at Yıldırım Beyazıt University (protocol no: 2017/23, date: 10.08.2017).

Informed Consent: We obtained signed informed consent from all participants.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: R.A., A.Ş., L.D.K., Concept: R.A., A.Ş., T.Ç., R.E., L.D.K., Design: R.A., T.Ç., L.D.K., Data Collection or Processing: R.A., P.D., R.E., L.D.K., Analysis or Interpretation: R.A., P.D., A.Ş., T.Ç., L.D.K., Literature Search: R.A., P.D., T.Ç., L.D.K., Writing: R.A., L.D.K.

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Differences in the Differential Expression of MicroRNAs Between Patients with Familial Multiple Sclerosis and Those with Sporadic Multiple Sclerosis

Ailesel Multipl Skleroz ve Sporadik Multipl Skleroz Hastaları Arasında MikroRNA'ların Diferansiyel Ekspresyon Farklılıkları

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Abstract

Objective: Multiple sclerosis (MS) is a heterogeneous disease with clinical and immunological features. Most MS cases occur sporadically, but a considerable proportion of patients have a family history of MS. The etiology and pathophysiology of MS remain unclear. Recent epidemiological and gene expression studies have indicated that dysregulation of microRNAs (miRNAs) may play a role in MS pathogenesis. This study aimed to evaluate the differential expression of miRNAs in sporadic MS (sMS) and familial MS (FMS) patients.

Materials and Methods: This cross-section, single-center study was conducted in 20 FMS and 10 sMS patients and 8 healthy controls. The patients were in the remission. In total, 2,549 miRNA genes were screened in the blood mononuclear cells from the whole blood samples of MS patients depending on miRBase 21. Differential expression of miRNAs in MS patients was identified compared with the control group, and miRNAs with a fold change ≥2 were validated using reverse transcription-polymerase chain reaction. Differentially expressed miRNAs were then compared between FMS and sMS patients.

Results: Initial findings showed that miR-5100 and hsa-miR-16-2-3p were increased and miR-432-3p was decreased in FMS compared with sMS, whereas miR-548-aa, hsa-miR-142-3p, and miR-451-b were increased in both sMS and FMS, but miR-548-v was increased only in sMS. Some miRNAs showed the same expression patterns in both groups.

Conclusion: Differential expression of certain miRNAs may be a useful biomarker in the diagnosis of MS. This study showed that miRNAs may discriminate between FMS and sMS cases and MS subtypes, as indicated in earlier studies.

Keywords: Multiple sclerosis, familial MS, sporadic MS, miRNA expression

Öz

Amaç: Multipl skleroz (MS), klinik ve immünolojik özellikler açısından heterojen bir hastalıktır. MS olgularının çoğu sporadik meydana gelir, ancak hastaların önemli bir kısmında ailede MS öyküsü vardır. MS hastalığının etiyolojisi ve patofizyolojisi hala net değildir. Son epidemiyolojik ve gen ekspresyonu çalışmaları, mikroRNA'ların (miRNA'lar) disregülasyonunun MS'in patogenezinde rol oynayabileceğini göstermektedir. Bu çalışmanın amacı, hem sporadik MS (sMS) hem de ailesel MS (FMS) hastalarında miRNA'ların diferansiyel ekspresyonlarını değerlendirmektir.

Gereç ve Yöntemler: Bu kesitsel tek merkezli çalışma 20 FMS ve 10 sMS hastası ve 8 sağlıklı kontrolle gerçekleştirilmiştir. Hastaların remisyon döneminde olma şartı aranmıştır. Toplamda 2.549 miRNA geni, MS hastalarının tam kan örneklerinden izole edilen

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mononükleer kan hücrelerinde miRbase 21'e dayalı olarak taranmıştır. MS hastalarında miRNA'lerin diferansiyel ekspresyonları kontrol grubuyla karşılaştırılarak belirlenmiş ve ≥2 kat değişim olan miRNA'ların RT-PCR ile validasyonu yapılmıştır. Daha sonra, diferansiyel eksprese olan miRNA'lar FMS ve sMS hastaları arasında karşılaştırılmıştır.

Bulgular: İlk bulgular, sMS'ye kıyasla FMS'de miR-5100 ve hsa-miR-16-2-3p ekspresyonlarının arttığını ve miR-432-3p ekspresyonun azaldığını gösterirken, miR-548-aa, hsa-miR-142-3p ve miR-451-b'nin ekspresyonunun hem sMS hem de FMS'de arttığını, ancak miR-548-v'nin ekspresyonunun yalnızca sMS'de arttığını göstermiştir. Bazı miRNA'lar her iki grupta da aynı ekspresyon paternini göstermiştir.

Sonuç: Belli miRNA'ların diferansiyel ekspresyonları MS teşhisinde yararlı bir biyobelirteç olabilir. Bu çalışma, miRNA'ların FMS ve sMS olguları arasında olduğu kadar daha önceki çalışmalarda belirtildiği gibi MS alt tipleri arasında da ayırt edici olabileceğini göstermiştir.

Anahtar Kelimeler: Multipl skleroz, ailevi MS, sporadik MS, miRNA ekspresyonu

Introduction

Multiple sclerosis (MS) is an autoimmune disease of the central nervous system (CNS) with a life-time risk of 1/400 and affecting primarily young females (1).

MS has four subtypes first defined in 1996 by the US National Multiple Sclerosis Society Advisory Committee on Clinical Trials in MS and was then revised in 2013 (2): relapsing remitting MS (RRMS), primary progressive MS (PPMS), secondary progressive MS (SPMS), and progressive relapsing MS. The majority of MS cases presents with RRMS and most of RRMS cases occur sporadically. However, a considerable proportion of cases have family history. In a meta-analysis, the prevalence of familial MS (FMS) was reported as 12.6% with monozygotic twins having the highest risk as 27%. The incidence of developing MS for the first-degree relatives ranges from 2% to 5% (3).

Although MS was discovered more than a century ago, the etiology and pathophysiology of the disease remain unclear. The most significant genetic association was found with human leukocyte antigen (HLA) region. The incidence of MS is higher in patients with HLA-DR2 (DR1501) serotype (4). Nevertheless, recent epidemiological and gene expression studies indicated that dysregulation of microRNAs (miRNAs) may also have a role in MS pathogenesis (5).

miRNAs are small, single-stranded, non-coding RNA molecules consisting approximately of 22 nucleotides (range, 19-25 nucleotides) that mediate mRNA translation repression or mRNA degradation. They control the expression of more than 2/3 of protein-coding genes in mammals and play a role in various biological process in the immune system and in neuroinflammation. They function as immune regulators by repressing target genes at the posttranscriptional level, which is essential for immune homeostasis and preventing autoimmune diseases (6).

Aberrant miRNA expression is responsible for the pathogenesis of various diseases such as neurodegeneration, autoimmunity, and cancer (7). MS is also associated with aberrant miRNA expressions. Up-regulated or down-regulated miRNAs have been reported in MS patients compared to healthy controls (8-11).

Identifying the miRNA expressions specific to MS subtypes help clinicians in establishing diagnosis and treatment planning. It is important to identify whether FMS differs from sporadic MS (sMS) regarding demographic, clinical, genetic, and radiological characteristics; accordingly, FMS cases can be identified and other family members prone to MS are diagnosed early. This study aimed to evaluate differential miRNA expressions in the peripheral blood mononuclear cells (PBMC) of FMS patients comparing with sMS patients.

Materials and Methods

This cross-sectional, single-center study included 32 MS patients, who visited Neurology Department of Dokuz Eylül University Medical Faculty between December 2014 and November 2016. The control group included eight healthy individuals from the hospital staff. The patients were required to be in remission period and analyzed in two groups: patients with FMS (n=20) and patients with sMS (n=12). Patients who had comorbidities such as demyelinating diseases other than MS and cancer were excluded. The study was approved by the Non-interventional Clinical Research Ethics Committee of Dokuz Eylül University (approval no: 2014/34-21, date: 06.11.2014) and Scientific Research and Publishing Ethics Committee of Dokuz Eylül University (decision no: 1, date: 08.11.2022) and conducted in accordance with the Helsinki Declaration as revised in 2013. Informed consents of the participants were obtained.

The participants were investigated in detail regarding demographic (age, sex), clinical (MS sub-type, disease duration), laboratory [oligoclonal band (OCB), cerebrospinal-fluid (CSF)-serum total protein, albumin, immunoglobulin G (IgG) index], and radiological characteristics [cranial and spinal magnetic resonance imaging (MRI)]. Lesions on MRI were evaluated separately as cortical-subcortical, corpus callosum, periventricular, cerebellar, mesencephalon, pons, and bulbus on axial, coronal and sagittal sections, T2 (spin-spin/transverse relaxation time)/Flair, T1 (spin-lattice/longitudinal relaxation time) and Gd (gadolinium) series.

RNAs were isolated from PBMC in blood samples of MS patients taken into tubes containing EDTA for routine

blood analysis. RNA concentrations and their purity were determined by spectrophotometric method. Thereafter, the mRNA samples were stored at -80 ºC until the time of analysis.

Differential miRNA expressions (up-regulated and down-regulated) were first identified by microarray analysis. For this purpose, totally 2,549 miRNA genes in the MiRBase 21 (http://www.mirbase.org/) were screened and then miRNAs with a fold change ≥2 were validated using real-time polymerase chain reaction (PCR) after reverse transcription of miRNAs to complementary DNA (cDNA).

The cDNAs were first diluted by 80x (5 ul cDNA +395 ul water) and housekeeping gene and cDNA were checked using spike-in primers. SNORD48 and U6 were used as housekeeping genes. The mixture containing Snord48 and U6 primers were used to evaluate the expression of miRNAs in RT-PCR. The expression of miRNAs were then normalized to the SNORD48 and U6 housekeeping genes. Regarding CT values for SNORD48 and spike-in outcomes of the cDNAs, those with a CT value between 15 and 29 (CT15-29) were analyzed for miRNAs using miRNA LNA™ primer sets (EXIQON). Relative quantitation of the outcomes was performed and calculated using Δ/Δ CT approach (12).

Statistical Analysis

Data were analyzed using the IBM SPSS Statistics for Windows, Version 22.0. (IBM Corp., Armonk, NY, USA). Kolmogorov-Smirnov test was used to test the parametric test assumptions. The Mann-Whitney U test and chi-square test were used to determine the differences of miRNA expression between FMS and sMS patients. A p-value <0.05 was considered statistically significant.

Results

Of 32 MS patients included, 20 had FMS (mean age 37.6±14.2 years, 16 females) and 12 had sMS (mean age, 35.5±9.3 years, 6 females). Patients' demographic and clinical characteristics are demonstrated in Table 1. The FMS and sMS groups were comparable for age (p>0.05). The mean disease duration was 95.8±106.1 months and 134.9±113.1 months in the patients with sMS and FMS, respectively, with no significant difference between the groups (p>0.05). All patients in the FMS group were first-degree relatives from 10 families (three couples of mother-daughter, three couples of sister-sister, three couples of sister-brother, and a couple of father-daughter). Patients in both groups were in remission period. RRMS was the most common type of MS in both groups (100% in sMS and 80% in FMS group). There was no significant difference between the groups regarding subtypes of MS (p>0.05). The mean expanded disability status scale (EDSS) score was higher in FMS cases than in sMS cases (2.9±0.4 vs. 1.6±0.2), but did not reach the level of statistical significance (p>0.05). The OCB was positive in 88.9% and 72.7% of the FMS and sMS groups, respectively (p>0.05).

Table 1. Demographic and clinical characteristics of the patients with sMS and FMS

	Patients with		p-value
Demographic and clinical features	**sMS**	**FMS**	
Age, year, mean ± SD	35.5±9.3	37.6±14.2	>0.05
Disease duration, month, mean ± SD	95.8±106.1	134.9±113.1	>0.05
Clinical phenotype, n (%)			>0.05
CIS	0 (0)	1 (5)	
RRMS	12 (100)	16 (80)	
SPMS	0 (0)	3 (15)	
EDSS score, mean ± SD	1.6±0.2	2.9±0.4	>0.05
Laboratory findings			
OCB, n (%)	8 (72.7)	16 (88.9)	>0.05
IgG index	1.1±0.6	0.6±0.3	>0.05
MRI findings, n (%)			
Pons T2 lesion	4 (40)	3 (16.7)	**0.023**
Cervical T2 lesion	8 (80)	9 (52.9)	**0.017**
Upper thoracic T2 lesion	4 (40)	2 (12.5)	**0.038**
Lower thoracic T2 lesion	6 (60)	5 (31.3)	**0.019**

sMS: Sporadic multiple sclerosis, FMS: Familial multiple sclerosis, SD: Standard deviation, CIS: Clinically isolated syndrome, RRMS: Relapsing remitting multiple sclerosis, SPMS: Secondary progressive multiple sclerosis, EDSS: Expanded Disability Status Scale, OCB: Oligoclonal band, IgG: Immunoglobulin G, MRI: Magnetic resonance imaging, T2: Spin-spin/transverse relaxation time

With regard to the MRI findings, sMS cases had significantly more lesions in the pons (p=0.023), cervical spinal cord (p=0.017), and thoracic spinal cord (p=0.038 for upper thoracic and p=0.019 for lower thoracic).

Up-regulated miRNAs

Analysis of miRNA expression revealed that 12 miRNAs were up-regulated only in the FMS cases and 65 miRNAs were up-regulated only in the sMS cases (Table 2). Genetic analysis revealed that miR-5100 was significantly up-regulated in the FMS cases comparing with the sMS cases. While hsa-miR-16-2-3p was up-regulated only in FMS cases, miR-548-U was up-regulated only in sMS cases. There were 53 miRNAs up-regulated in both FMS and sMS groups (Table 3). Gene analysis revealed up-regulated miR-451-b, miR-142-3p and miR-548aa in both groups.

Table 2. Up-regulated miRNAs only in the patients with FMS and only in the patients with sMS

FMS		sMS					
miRNA	Fold increment	miRNA	Fold increment	miRNA	Fold increment	miRNA	Fold increment
hsa-miR-5100	2.18, 3.2	hsa-miR-6500-5p	2.96	hsa-let-7f-1-3p	2.34, 2.5, 2.42	hsa-miR-629-3p	2.13
hsa-miR-4633-5p	2.04	hsa-miR-466	8.49, 8.53, 8.07, 6.37	hsa-miR-4290	3.22, 3.59	hsa-let-7b-3p	2, 2.01
hsa-miR-125a-3p	2.08	hsa-miR-3190-5p	2.65	hsa-miR-574-5p	3.24, 2.49	hsa-miR-3656	2.19, 2.18
hsa-miR-486-3p	2.4	hsa-miR-4713-5p	2.82	hsa-miR-933	2.43, 2.58	hsa-miR-381-3p	2.33
hsa-miR-30e-5p	2.09	hsa-miR-5704	2.55, 2.36	hsa-miR-300	4.06	hsa-miR-485-3p	2,76, 2.11
hsa-miR-125 a-3p	2.14, 2.08	hsa-miR-654-3p	2.39	hsa-miR-2278	3.79	Has-miR-4646-3p	2.19
hsa-miR-4286	2.55	hsa-miR-4725-5p	2.61, 2.65	hsa-miR-6072	3.29	hsa-miR-3160-5p	2.19
hsa-miR-335-3p	2.01	hsa-miR-3935	3.52, 2.66	hsa-miR-328-3p	2.09	hsa-miR-640	2.5
hsa-miR-139-3p	3.25	hsa-miR-551a	2.36	hsa-miR-1273c	2.39	hsa-miR-483-3p	2.13
hsa-miR-3907	2.33	hsa-miR-764	2.26	hsa-miR-5194	2.21, 2.49	hsa-miR-299-5p	2.46, 2.51
hsa-miR-6126	2.27	hsa-miR-6127	2.07, 2,06	hsa-miR-3940-5p	2.48	hsa-miR-595	2.63
hsa-miR-16-2-3p	4.86	hsa-miR-3191-5p	2.14	hsa-miR-3665	2.17	hsa-miR-3646	2.65, 2.69
		hsa-miR-449c-3p	2.24, 3.48, 2.98	hsa-miR-3150b-5p	2.52	hsa-miR-3940-3p	2.49, 2.78
		hsa-miR-1275	2.43	hsa-miR-766-3p	2.1	hsa-miR-605-5p	2.46, 2.05
		hsa-miR-4281	2.01	hsa-miR-4493	2.6, 2.28	hsa-miR-320a	2.3
		hsa-miR-5195-3p	-	hsa-miR-5787	2.6, 2.88	hsa-miR-3162-3p	2.62, 2.2
		hsa-miR-135a-3p	5.06	hsa-miR-328-3p	2.09	hsa-miR-574-3p	2.31, 2.59
		hsa-miR-1273c	2.39	hsa-miR-4728-3p	2.29	hsa-miR-6508-5p	2.56
		hsa-miR-4254	3.77, 3.92	hsa-miR-5001-5p	2.25	hsa-miR-4312	2.34
		hsa-miR-1255b-2-3p	3.22, 2.35	hsa-miR-3927-5p	2.14	hsa-miR-609	2.02
		hsa-miR-149-5p	2.62, 2.72	hsa-miR-4649-3p	2.01, 2.37	hsa-miR-584v	4.59
		hsa-miR-647	4.13, 4.17, 4.26, 3.04	hsa-miR-4695-3p	2.1, 2.31	-	

miRNA: MicroRNA, FMS: Familial multiple sclerosis, sMS: Sporadic multiple sclerosis

Down-regulated miRNAs

There were 10 miRNAs down-regulated only in FMS patients and 19 miRNAs down-regulated only in sMS patients (Table 4). MiRNA analysis revealed that 14 miRNAs were down-regulated both in FMS and sMS cases (Table 5). Gene analysis demonstrated that miR-432-3p was down-regulated in FMS vs. sMS cases.

Discussion

In the present study, genetic analysis revealed that miR-5100 was significantly up-regulated but miR-432-3p was significantly down-regulated in FMS cases comparing with sMS cases, suggesting that these two miRNAs can be used as biomarkers in discriminating between FMS and sMS. Additionally, hsa-miR-16-2-3p was up-regulated only in FMS, whereas miR 548-U was up-regulated only in sMS. However, miR-451-b, miR-548aa and hsa-miR-142-3p were up-regulated in both groups.

MS is a chronic neurodegenerative disease of the CNS and develops due to the combination of genetic and environmental factors. Although not being a hereditary disorder, it can be seen among family members with FMS accounting for

Table 3. Up-regulated miRNAs both in the patients with FMS and sMS

miRNA	Fold increment		miRNA	Fold increment	
	FMS	sMS		FMS	sMS
hsa-miR-3688-3p	10.42	2.37; 9.96	hsa-miR-33b-3p	2.51	2.87, 4.25
hsa-miR-371b-5p	9.41, 8.11	11.25, 12.7	hsa-miR-449b-3p	2.45, 2.2	5.38, 4.92
miRNABrightCorner30	4.62	5.62	hsa-miR-6085	2.34; 2.92	2.95, 2.56
hsa-miR-135b-5p	4.57, 3.44	5.27; 7.47	hsa-miR-1238-5p	2.03	4.14, 3.29
hsa-miR-491-3p	6.65, 6.24, 6.24; 6.97	12.12, 11.13, 13.07, 10.86	hsa-miR-197-3p	2.09, 2.14	4.14, 4.16
hsa-miR-4428	4.08	3.94	hsa-miR-4455	2.2; 2.42	4.77, 4.92
hsa-miR-3129-3p	3.36	3.34, 2.82	hsa-miR-491-5p	3.54	8.64
hsa-miR-142-3p	7.17, 3.42	3	hsa-miR-494-3p	2.32	2.69
hsa-miR-4694-5p	4.27, 3.75	7.48, 6.26	hsa-miR-665	3.65	3.68
hsa-miR-423-5p	3.02	2.67	hsa-miR-4310	2.14	3.81
hsa-miR-4668-5p	5.57	4.58	hsa-miR-30d-5p	2.29, 2.19	2.12
hsa-let-7d-3p	3.7, 2.11	4.51, 2.78	hsa-miR-2116-3p	2.07	3.3, 2.35
hsa-miR-1260b	2.6	3.56, 3.59	hsa-miR-1296-5p	2.25, 2.11	4.47, 4.49
hsa-miR-4787-5p	3.05	2.31, 3.7	hsa-miR-4275	2.26	2.78
hsa-miR-1260a	3.15	3.41, 3.63	hsa-miR-1181	3.88	3.75
hsa-miR-6125	2.58, 2.55	3.61, 3.64	hsa-miR-4485-3p	3.73, 2.27	3.98
hsa-miR-4515	2.07, 2.27	2.37, 2.13	hsa-miR-1915-3p	2.48, 2.59	3.64, 3.46
hsa-miR-4446-5p	3.76, 4.05	8.48, 9.31	hsa-miR-211-3p	2.36, 2.38	2.22
hsa-miR-937-3p	2.64, 2.21, 2.04	4.18, 4.55, 4.44, 4.29	hsa-miR-3149	5.8	15.71
hsa-miR-6514-3p	3.92, 2.21, 2.2, 2.04, 2.64	4.44, 4.29, 4.18, 4.55	hsa-miR-487b-3p	2.86	6.04
hsa-miR-32-3p	4.71, 4.71, 3.37, 2.69	12.56, 10.12, 7.17, 8.68	hsa-miR-885-5p	2.74, 2.25	4.12, 5.17
hsa-miR-4465	3.74, 4.15	3.57, 3.29	hsa-miR-6504-3p	4.12	9.94
hsa-miR-2861	2.02, 2.16	2.87, 2.85	hsa-miR-3681-3p	2.86, 3.76	7.49, 9.99
hsa-miR-6499-3p	2.52, 2.57	5.31, 5.46	hsa-miR-548aa	3.17	5.84
hsa-miR-5011-5p	5.1	17.87	hsa-miR-136-5p	3.6	6.04
hsa-miR-4685-5p	3.53, 4.02	4.28, 4.93	hsa-miR-6514-3p	3.49, 3.92	9.61, 10.61
hsa-miR-5010-3p	2.45, 2.56	5.84, 5.87		-	-

miRNA: MicroRNA, FMS: Familial multiple sclerosis, sMS: Sporadic multiple sclerosis

12.5% of the MS cases (3). A biomarker that distinguishes MS from other demyelinating diseases is critically valuable. Biomarkers may shed light to the diagnosis of MS subtypes, prediction of disease period and course, treatment choice and success, as well as considering new therapies, and prediction of prognosis. Identification of FMS cases is also important to determine family members who are prone to developing MS. Detection of biomarkers associated with the natural history of MS including inflammation, demyelination, oxidative stress and axonal injury is of great importance. Gene expression profiling is a beneficial tool to provide information about molecular pathways involved in MS pathogenesis.

Table 4. Down-regulated miRNAs only in the patients with FMS and only in the patients with sMS

FMS		sMS	
miRNA	Fold decrement	miRNA	Fold decrement
hsa-miR-877-3p	-2.04	hsa-miR-133b	-2.77
hsa-miR-675-3p	-2.77	hsa-miR-23a-3p	-4.76
hsa-miR-320d	-2.32	hsa-let-7a-5p	-9.09
hsa-miR-432-3p	-2.5	hsa-miR-107	-6.66, -0.09
hsa-miR-320e	2.22	hsa-miR-5096	-2.08
hsa-miR-1180-3p	-2.32	hsa-miR-17-5p	-7.14
hsa-miR-4707-5p	-2.32	hsa-miR-185-5p	-4.76
hsa-miR-642b-3p	-2.08	hsa-miR-4646-5p	-2.04
hsa-miR-1236-5p	-2.43	hsa-miR-4639-3p	-2.43
hsa-miR-1224-5p	-2, -2.04	dmr_316	-3.33
		hsa-miR-521	-2.38
		hsa-miR-96-5p	-8.33
		hsa-miR-4650-5p	-2.38
		hsa-miR-211-5p	-2.38
		hsa-miR-342-3p	-8.33
		hsa-miR-566	-2.08
		hsa-miR-193b-3p	-3.03
		dmr_3	-4.34, -7.69
		hsa-miR-4307	-3.57

miRNA: MicroRNA, FMS: Familial multiple sclerosis; sMS: Sporadic multiple sclerosis

Table 5. Down-regulated miRNAs both in the patients with FMS and sMS

miRNA	Fold decrement		miRNA	Fold decrement	
	FMS	sMS		FMS	sMS
has-miR-623	-4.16, -2.56, -3.70	-4, -3.12, -2.70	has-miR-5196-5p	-4.76	-5.55
has-miR-181d-5p	-4, -2.77	-5, -3.44	has-miR-550a-3-5p	-3.57	-4
has-miR-4710	-2.5, -3.03	-2.38, -2.63	has-miR-4306	-2.63, -2.38	-3.03, -2.7
has-miR-331-3p	-3.7	-6.25	has-miR-660-5p	-5.88	-8.33
has-miR-4271	-2.08	-2.04	has-miR-4700-3p	-2.38	-2.85
has-miR-4632-5p	-3.125	-2.77	has-miR-3676-5p_v19.0	-2.85	-2.32
has-miR-4728-5p	-2.22	-3.03	has-miR-1343-3p	-4	-4.76

miRNA: MicroRNA, FMS: Familial multiple sclerosis; sMS: Sporadic multiple sclerosis

Studies have demonstrated that dysregulation of miRNAs play a role in MS pathogenesis (5). Circulating miRNAs are good candidates to be diagnostic biomarkers for MS as they stay stable for a long time and are non-invasive, cheap and time-saving for disease monitoring (13). Moreover, they can be used to assess disease severity, to monitor progression, and to assess therapeutic responses. Besides, unique miRNA signatures may allow discrimination between MS subtypes.

Most studies have focused on miRNA expression in MS patients compared with healthy controls. Otaegui et al. (8) demonstrated that miRNA expression is significantly different between RRMS patients and healthy controls. In a recent review, miR-18b, miR-493, miR-599, miR-145, miRNA-26a, miR-149-5pand miR-708-5p were up-regulated in PBMC of MS patients (9). Using PCR, Nuzziello et al. (10) found up-regulated miR-652-3p, miR-125a-5p, miR-185-5p, miR-320a, miR-942-5p, and miR-25-3p in MS patients vs. healthy controls. Another study have revealed decreased expression of miR-10 (p=0.0002), miR-21 (p=0.0014) and miR-124 (p=0.0091) in RRMS patients vs. healthy controls and concluded that miR-10, miR-124 and miR-21 are promising diagnostic tools for MS (11).

Recently, numerous studies have also been performed to distinguish MS subtypes. A study investigating miRNA expression in CSF of patients with different types of MS found dysregulated miR-20a-5p and miR-320b expression in serum samples of PPMS patients comparing with RRMS and other neurological diseases. Additionally, it was found that miR-26a-5p and miR-485-3p were down-regulated in PPMS vs. RRMS, whereas miR-142-5p was up-regulated in RRMS patients vs. patients with other neurological diseases. They also demonstrated that let-7b-5p and miR-143-3p were down-regulated in CSF samples of patients with PPMS vs. patients with other neurological diseases (14). In our study, we also found that miR-142-3p was up-regulated in both FMS and sMS cases. These findings suggest that FMS and/or sMS may be associated with different dysregulated miRNAs than in other types of MS.

Discrimination between FMS and sMS cases is also important because identification of miRNAs specific to FMS may provide early diagnosis of family members likely to develop MS via screening for signature miRNAs for familial cases before the onset of symptoms or, probably, occurrence of neurodegeneration.

In our study, of 173 dysregulated miRNAs identified, 12 were up-regulated in only FMS cases. Although not significant, miR-16-2-3p was up-regulated in only FMS cases, as was previously demonstrated by Keller et al. (15). The genetic analysis revealed that miR-5100 was increased only in FMS cases. Since miR-5100 was associated with various types of cancer (16,17), dysregulation of miR-5100 in MS needs to be investigated in further studies with larger patient populations.

Similar to the findings of the study by Gandhi et al. (7), in which increased miR-30e expression was shown in RRMS cases, we identified up-regulated miR-30e only in FMS cases, which was not significant in genetic analysis. Likewise, up-regulation of miR-125a only in FMS cases was not significant in the genetic analysis. Consistent with the results of our study, studies have reported increased miR-125a expression in MS patients (18).

It was determined that some miRNAs have been up-regulated only in sMS cases. Up-regulation of none of these miR-RNAs, except for miR-548-U, was found significant in the genetic analysis. Other miRNAs up-regulated only in sMS cases in our study included miR-584 and miR-1275, which were found to be up-regulated in the blood cells of RRMS patients in earlier studies (19,20). Additionally, Gandhi et al. (7) reported up-regulated miR-449b, let-7f, miR-574-3p, let-7b, let-7d and miR-135a in the plasma of RRMS patients, which is consistent with the findings of our study; however, they did not compare these miRNAs between FMS and sMS cases. Although the present study failed to find a significant difference, earlier studies have suggested that miR-135a and miR-574-3p can be considered potential biomarkers in distinguishing RRMS cases from both controls and SPMS cases; moreover, a correlation was demonstrated between these miRNAs and EDSS, disease duration and frequency of relapses (6), which were not analyzed in the present study.

Dysregulation of let-7d found in the present study was also confirmed by Piket et al. (13) in RRMS vs. SPMS cases. In our study, miR-629 and miR-328 were also up-regulated in sMS patients. Although up-regulation of miR-629 was confirmed in Chinese population (18), miR-328 was found to be down-regulated in the PBMC of MS patients as compared to healthy controls (21).

In the present study, we identified up-regulated let-7b-3p in sMS cases. However, a study found significant reduction in -5p form (let-7b-5p) in the CSF of patients with progressive MS vs RRMS. In the non-progressive phase, they demonstrated that let-7b-5p was inversely associated with inflammation; whereas, it was negatively correlated with clinical disability in progressive MS and the authors concluded that let-7b-5p can be used as a biomarker for disease course (22). Nevertheless, two other studies failed to demonstrate a difference in the expression of let-7b between MS patients and healthy controls (23).

In addition to these miRNAs up-regulated in either FMS or sMS cases, we also found that some miRNAs were up-regulated in both FMS and sMS cases (Table 3). Studies have shown that miR-142-3p was up-regulated in the PBMCs of MS patients (19-21). In the present study, we also identified significantly up-regulated miR-142-3p in both FMS and sMS cases. Likewise, miR-211-3p was up-regulated in both groups in the present study; however, different from the present study, Cox et al. (24) demonstrated down-regulation of -5p form of miR-142 (miR-142-5p) in all MS subtypes. Given that they also used microarray analysis,

this difference may be attributed to small sample size in the present study or to the ethnic differences between the cohorts, which warrants further analysis.

Keller et al. (19) detected up-regulated mir-491-5p in the PBMCs of MS patients. miR-32 and miR-197 were among the miRNAs up-regulated in both FMS and sMS cases in the present study. While up-regulation of miR-32 in MS was supported by Yang et al. (18), they detected significantly down-regulated miR-197 in the PBMCs of MS cases vs. healthy controls. We also identified significant up-regulation of miR-548aa in both groups, warranting further investigation about the role of miR548 in MS.

There were 14 miRNAs down-regulated in all RRMS cases (familial and sporadic) in the present study. Likewise, Cox et al. (24) found that miR-623 was down-regulated in all MS types. In the same study, miR-17 was also down-regulated in all MS cases, whereas we determined up-regulation of miR-17 in only sMS cases suggesting that this can be used as a marker to distinguish sporadic cases from familial cases. While we determined that miR-660 was down-regulated in both groups, a study reported up-regulation of miR-660 in the plasma of patients with RRMS (23). Although miR-211-3p was up-regulated in both groups in the present study, -5p form (miR-211-5p) was down-regulated in sMS cases. Likewise, down-regulated miR-211-5p in all subtypes of MS as compared to healthy controls was also reported (24).

Ebrahimkhani et al. (25) investigated exosomal miRNAs and reported differently expressed nine miRNAs including miR-342-3p and miR-432-5p in RRMS and in progressive MS; in the present study, however, -3p form (miR-342-3p) was down-regulated in sMS cases. Likewise, -3p form of miR-432 (miR-432-3p) was also significantly down-regulated in FMS cases, making it a candidate biomarker to discriminate between FMS and sMS cases.

We identified decreased levels of miR-96 in the sMS cases, which was shown to be involved in the remission phase of MS (8). Different from the present study, another study reported increased plasma levels of miR-96 in RRMS cases (7). In the present study, miR-1180 was also down-regulated in the FMS patients, whereas it was found to be increased in the PBMC of Chinese MS patients (18), which can be attributed to the ethnic differences. Moreover, we identified down-regulated miR-23a in sMS cases. Gandhi et al. (7) reported that miR-23a detected in body fluids can discriminate between MS subtypes and might be associated with certain parameters including EDSS score and disease duration. In the present study, however, we did not analyze the correlation between miRNAs and EDSS score and disease duration, which can be considered a limitation of the study.

Although it was reported in a study that miR-155 is the one of the most consistently dysregulated miRNA in MS (20), we failed to identify any in the present patient population. Differences between studies regarding differentially expressed miRNAs might be resulted from the differences in sample sizes and cohorts, and body fluids and methods used to detect miRNAs.

Our study has some limitations including small sample size and absence of correlation analysis between significantly different miRNA expressions and disease parameters such as EDSS score and disease duration. Nevertheless, this study is one of the limited studies investigating the differences between FMS and sMS regarding miRNA expression and contributes to the discrimination of FMS from sMS. Showing that miR-5100, miR-548aa and miR-548-U may be associated with MS pathogenesis and that miR-5100 and miR-432-3p are potential biomarkers to differentiate FMS from sMS makes the present study valuable.

Conclusion

In this study, both different and similar expressions of miRNAs were observed in familial and sporadic cases, some of which were supported by earlier studies. We showed that miR-5100 and hsa-miR-16-2-3p expressions were increased but miR-432-3p expression was decreased in FMS, whereas miR-548-v was increased in sMS. These data suggest that expression of certain miRNAs may be a useful biomarker for MS diagnosis and may discriminate between not only MS subtypes, as was indicated in earlier studies, but also between FMS and sMS cases.

The clinical relevance and significance of these miRNA genes identified in this study should be investigated in different clinical samples in further studies with larger patient cohorts.

Ethics

Ethics Committee Approval: The study was approved by the Non-interventional Clinical Research Ethics Committee of Dokuz Eylül University (approval no: 2014/34-21) and Scientific Research and Publishing Ethics Committee of Dokuz Eylül University (decision no: 1, date: 8.11.2022) and conducted in accordance with the Helsinki Declaration as revised in 2013.

Informed Consent: Informed consents of the participants were obtained.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: H.G., H.A.U., T.P., Z.A., D.K., P.Ö., E.İ., Concept: H.G., H.A.U., T.P., Z.A., D.K., Design: H.G., H.A.U., T.P., Z.A., D.K., Data Collection or Processing: H.G., H.A.U., T.P., Z.A., D.K., Analysis or Interpretation: H.G., H.A.U., T.P., Z.A., D.K., Literature Search: H.G., H.A.U., T.P., Z.A., D.K., Writing: H.G., H.A.U., T.P., Z.A., D.K.

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Original Article / Özgün Araştırma

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Fractal Analysis of Nuclear Architecture in Oral Squamous Cell Carcinoma by Using Transmission Electron Microscopy: An Original Research

Oral Skuamöz Hücreli Karsinomda Nükleer Mimarinin Transmisyon Elektron Mikroskobu Kullanılarak Fraktal Analizi: Orijinal Bir Araştırma

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Abstract

Objective: A tumor's histological traits can be digitally assessed and quantified using fractal dimension (FD), a mathematical measure of a shape's irregularity and complexity. The goal of this study was to examine and contrast the nuclear FD between normal and oral squamous cell carcinoma (OSCC).

Materials and Methods: The present study comprised 15 OSCC patients and 15 patients in an age- and sex-matched control group. Fresh biopsy was taken from both groups reported over a period of one year at St. Joseph Dental College, Duggirala, Italy. Half of the tissue sample was used for transmission electron microscopy and the remaining half for H&E staining for grading. Fractal box counting on the ImageJ program serves for the analysis of images in the assessment of nuclear architecture. The FD of the nucleus was calculated in accordance with the Sarkar fractal box counting method.

Results: Results showed a statistically significant increase in the mean nuclear FD value of OSCC compared with normal mucosa. The minimum FD value of the nucleus obtained for normal mucosa is 0.7516 and the maximum value is 1.7982, whereas for OSCC, the minimum FD value of the nucleus is 1.8230 and maximum value of 1.9587. Mean ± standard deviation value of FD of the nucleus in normal mucosa is 1.5806±0.2928 and in OSCC is 1.9244±0.0414.

Conclusion: Significant differences in FD values were obtained compared with normal oral mucosa, thus crediting this as a novel and interesting tool in the diagnosis of cancer. Because the fractal analysis technique is non-invasive and cost-effective, it can be used in developing countries.

Keywords: Fractal dimension, nuclear architecture, oral squamous cell carcinoma, transmission electron microscopy

Öz

Amaç: Fraktal boyut (FD), bir şeklin düzensizliği ve karmaşıklığının matematiksel bir ölçüsüdür ve tümördeki histolojik özelliklerin dijital değerlendirmesi ve miktarının belirlenmesi için kullanılabilir. Çalışmanın amacı OSCC'deki ve normaldeki nükleer FD'yi analiz etmek ve karşılaştırmaktır.

Gereç ve Yöntemler: Bu çalışma 15 oral skuamöz hücreli karsinom (OSCC) hastası ve yaş ve cinsiyet açısından eşleştirilmiş 15 kontrol grubundan oluşmaktadır. Duggirala'daki St. Joseph Dental College'da bir yıllık bir süre boyunca bildirilen her iki gruptan da taze biyopsi alındı. Doku örneğinin yarısı TEM için, geri kalan yarısı ise sınıflandırma amacıyla H&E boyama için kullanıldı. Nükleer mimarinin değerlendirilmesi, ImageJ yazılımında fraktal kutu sayımı kullanılarak görüntü analizi yoluyla yapılır. Çekirdeğin FD'si sarkar fraktal kutu sayma yöntemine göre hesaplanır.

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Bulgular: Sonuçlar normal mukoza ile karşılaştırıldığında OSCC'nin ortalama nükleer fraktal boyut değerinde istatistiksel olarak anlamlı artış olduğunu gösterdi. Normal mukoza için elde edilen çekirdeğin minimum FD değeri 0,7516 ve maksimum değeri 1,7982 iken, OSCC için çekirdeğin minimum FD değeri 1,8230 ve maksimum değeri 1,9587'dir. Normal mukozadaki çekirdeğin FD'sinin ortalama ± standart sapma değeri; 1,5806±0,2928 ve OSCC'de 1,9244±0,0414'tür.

Sonuç: Normal ağız mukozası ile karşılaştırıldığında FD değerlerinde önemli farklılıklar elde edildi, bu da bunun kanser tanısında yeni ve ilginç bir araç olduğunu gösteriyor. Fraktal analiz tekniği non-invaziv ve uygun maliyetli olduğundan gelişmekte olan ülkelerde kullanılabilir.

Anahtar Kelimeler: Fraktal boyut, nükleer mimari, oral skuamöz hücreli karsinom, transmisyon elektron mikroskobu

Introduction

With an incidence of over 3 lakh cases annually, 62% of which occur in poor nations, oral cancer is the sixth most frequent type of cancer. With an incidence rate of 12.8 and 7.5 for men and women, respectively, per 100,000 persons, it is the second most frequent cancer in India among men and the third most common cancer among women. The primary risk factors are drinking alcohol and using tobacco products, such as chewing and smoking. Up to 40% of all malignancies in India are oral cancers, making them a serious health concern (1).

The combined incidence and mortality of oral squamous cell carcinoma (OSCC) are increasing despite a number of diagnostic and treatment advancements; age-standardized incidence and death estimates for men and women respectively are 1.6/100,000 and 3.1/100,000. Hence, the use of screening and detection aids and the development of molecular markers may improve the early diagnosis of this dreadful disease and may help in reducing mortality rate. Chutta smoking is widespread in coastal areas of Andhra Pradesh. Chuttas are coarsely prepared cheroots (2).

The "reverse chutta smoking" is a special variety of cheroot which is smoked with the burning end inside the mouth due to which local effects of heat and smoke cause chronic stomatitis, leukoplakia and OSCC. In cancer cell undergoes many changes in shape of nucleus, nuclear membrane or margin, chromatin pattern, nucleoli and organization of nuclear chromatin (3). An important factor in determining the malignancy and alterations of tumors is the nuclei of neoplastic cells. Fractal geometry has recently been applied to histomorphometrical techniques, quantifying the morphological traits that pathologists often utilize to characterize and describe cancers. Fractal dimensions (FD) are a perfect tool to describe a naturally occurring item with an irregular shape (1,3).

Recent researches on OSCC have highlighted various diagnostic techniques to detect cancer at initial its stages. This study is the first of its kind to assess FD in OSCC. While clinical and histological variables have been helpful in predicting OSCC, more specialized diagnostic techniques are needed to detect cancer. So, in the present study, interest has been cultivated to use FD as a diagnostic tool. By calculating FD of OSCC and normal mucosa in transmission electron microscopy (TEM) images having higher resolving power to visualize even small changes occurring at ultrastructural level in great detail can felicitate early diagnosis (4).

Materials and Methods

The present study comprises of age and sex matched 30 patients which were divided into two study groups. Group 1: Comprises of 15 histologically diagnosed cases of well differentiated squamous cell carcinoma according to Broder's classification. Group 2: Comprises of 15 normal oral mucosa tissues from patients undergoing minor dental surgery. Explanation of study design was done to both the study groups, written consent and detailed case history was recorded from each individual and Institutional ethical clearance was requested and granted. Fresh biopsy was taken from the clinically diagnosed cases of OSCC and half of the tissue sample was used for TEM and remaining for H&E staining for grading.

The St. Joseph Dental College's Institutional Ethics Committee in Duggirala, Eluru, granted ethics approval for this study (approval no: CEC/10/2015-16, date: 22.12.2015). We got informed consent from each and every study participant.

Specimen Preparation Protocol Followed

Fixation: Fixation was the first and most important step in any electron microscope study. Tissues can be fixed by immersion or perfusion. In present study 2.5% glutaraldehyde was diluted in 100 mM phosphate buffer at pH 7.0 for 4 hours. Fixation was done at room temperature and after 15-30 minutes fixed at 4 *°C*. Fixation at 4 °C slows down autolytic processes and reduces tissue shrinkage.

Post fixation: After post-fixing the tissue for two hours with 1% aqueous osmium tetroxide, the tissue was cleaned four times every 45 minutes with deionized distilled water.

Dehydration: Dehydrated by means of a progression of acetones.

Resin embedding (epon mix): Embedding was done using Araldite 6005 resin or spur resin (Spurr 1969) next Propylene oxide used for 2 changes, each of 15 minutes.

Sectioning: Sections of one micron were taken, and copper grids were used to hold these incredibly thin sections. Glass knife and ultra microtome (Leica Ultra cut UCT-GA-D/E-1/00) were used to cut ultra thin (60 nm) sections,

which were then examined under a TEM (Model: Hitachi, H-7500 from JAPAN) at the necessary magnifications.

Fractal Analysis

Image preprocessing was the initial step in the analysis of nFD, which was then followed by segmentation and feature extraction. For fractal analysis, color images were simply thresholded to create binary versions. By utilizing the sarkar fractal box counting method in ImageJ software 1.43u with the FracLac plugin Java 1.6 (Wayne Rasband, National Institutes of Health, Bethesda, USA), nFD of each image was calculated (Figure 1, 2).

In ImageJ, choosing the nucleus required manual intervention. The box count approach was straightforward. This technique superimposes a grid of several small boxes, each with a specific pixel length, over the digital image. The software then automatically "boxed" irregular image profiles into the proper number of boxes based on the size of the image in pixels. As an outcome, a regression line graph was generated. The object's FD was equivalent to the slope of the regression line. The box counting technique that implements the formula is used by the software to automatically estimate the fractal dimension: DF=log N/log ε (where DF=Fractal dimension, N=number of pieces which the line can be broken into when using pieces of scale ε).

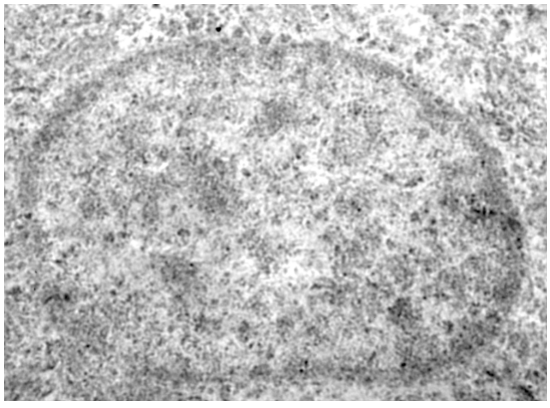


Figure 1. Nucleus of normal mucosa in magnification of 15440X19 bar in TEM
TEM: Transmission electron microscopy

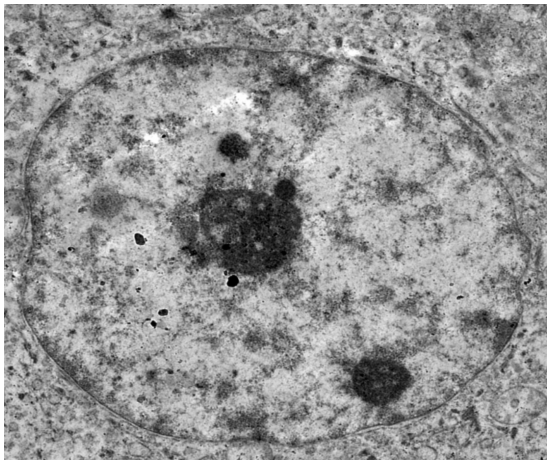


Figure 2. Nucleus of OSCC in magnification of 15440X19 bar in TEM
OSCC: Oral squamous cell carcinoma, TEM: Transmission electron microscopy

Statistical Analysis

The obtained FD values were compared with those of OSCC and normal mucosa. The independent simple t-test was used to compare the mean FD values for the normal mucosa and OSCC. A significance level of $p<0.05$ was set, and SPSS statistical software version 22 was utilized for the statistical analysis.

Results

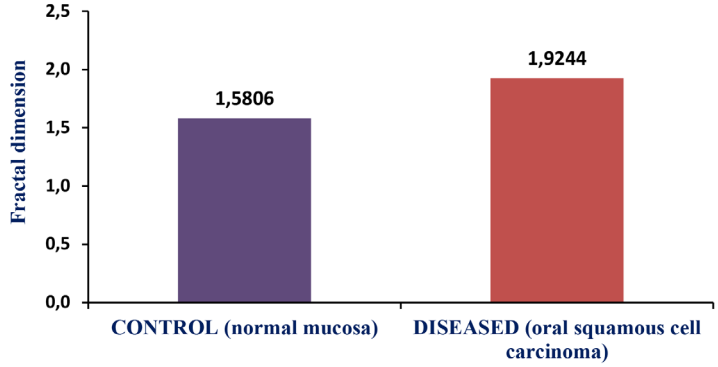
The present study comprised of 15 cases of OSCC and 15 cases of normal mucosa. FDs were calculated for all the cases using box counting fractal analysis method. The most typical clinical characteristics of an OSCC were elevated rolled borders surrounding an ulcerated lesion with a necrotic central area. Also associated with symptoms of pain, difficult in swallowing and tooth mobility. The minimum FD value of nucleus obtained for normal mucosa is 0.7516 and maximum value is 1.7982, whereas for OSCC minimum FD value of nucleus is 1.8230 and maximum value of 1.9587 (Table 1). Mean ± standard deviation value of FD of nucleus in normal mucosa is 1.5806±0.2928 and in OSCC is 1.9244±0.0414. Table 1 summarizes the fracture dimension values in relation to various clinic-pathological features of patients with oral squamous carcinoma.

Results showed increased mean nFD value of OSCC when compared with normal mucosa Graphic 1-3. Fractal values were determined using the independent simple t-test, and FD values between the two groups were found to be significantly different ($p<0.05$).

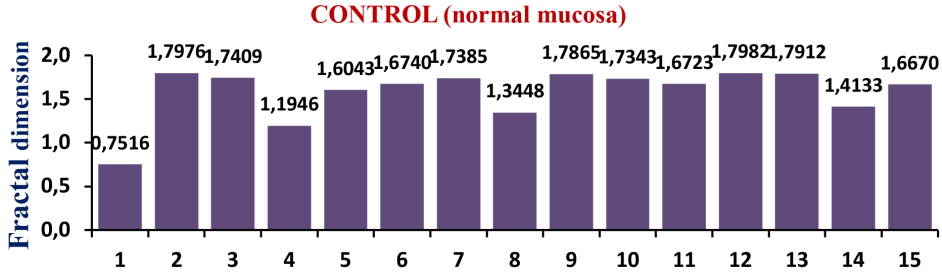
Table 1. Fractal dimension values respect to different clinicopathological characteristics of patients with oral squamous carcinoma

Groups	Mean ± SD	Std. error mean	p-value
Gender			
Male	1.9271±0.04725	0.01494	0.736
Female	1.9190±0.03054	0.01366	
Topography			
Tongue	1.9500±0.00756	0.00252	0.001
Mucosal tissue	1.8861±0.04221	0.01723	
Grade			
I	1.8773±0.04063	0.01817	0.000
II	1.9480±0.00954	0.00302	

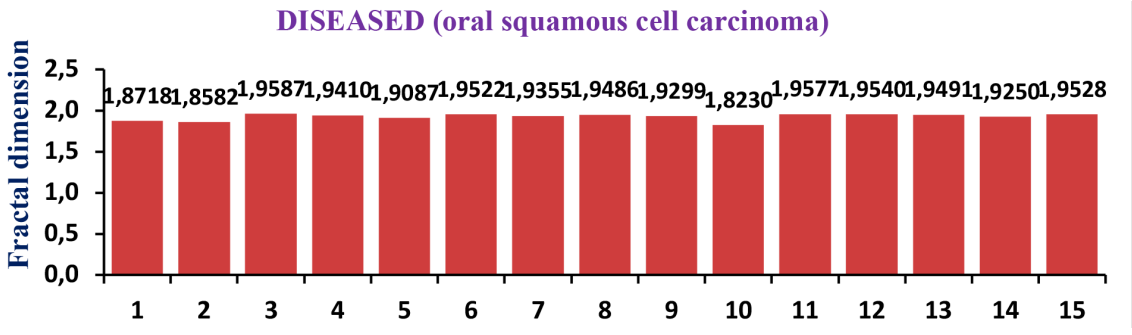
SD: Standard deviation, Std.: Standard



Graphic 1. Graph showing comparison of FD values between normal mucosa and OSCC. The mean ± SD value in normal mucosa was 1.5806±0.2928 and mean ± SD value of OSCC was 1.9244±0.0414. The graph showing significant statistical difference between both the groups
FD: Fractal dimension, OSCC: Oral squamous cell carcinoma, SD: Standard deviation



Graphic 2. Graph illustrating the FD values of normal mucosa. The highest FD value obtained for normal mucosa is 1.7982 and lowest is 0.7516
FD: Fractal dimension



Graphic 3. Graph illustrating the FD values of OSCC. The highest FD value obtained for OSCC is 1.9587 and lowest is 1.8718, but when comparison of FD values done it suggested that there was an increase in FD value of OSCC
FD: Fractal dimension, OSCC: Oral squamous cell carcinoma

Discussion

A cell's biological potential and activity are reflected in its nucleus. A normal cell typically has a single, round or oval-shaped nucleus with uniform chromatin distribution, a regular nuclear membrane or border, one or two small nucleoli, and normal mitotic figures. During carcinogenesis, changes occur in the size, shape, number, nuclear membrane, margin, nucleoli, chromatin pattern, and organization. These changes result in modifications to nuclear architecture, which in turn affect FD in OSCC (5,6).

Variations in the degree of chromatin condensation as well as chromatin condensation or de condensation are likely the causes of chromatin texture changes, which are commonly seen in cancer cells. Because of increased protein synthesis, OSCC higher grades display coarse clumped chromatin and heterogeneous chromatin pattern, while lower grades display delicate chromatin strands and homogenous chromatin pattern (7,8).

Tumor cell chromatin causes chromatin configurations such as open chromatin and chromatin coarsening, which correlate to an increase or decrease in heterochromatin

aggregates and are highly diagnostically significant. As a result, the nuclear architectures of cancer cells differ characteristically from those of normal cells. It's also critical to recognize that certain tumor types are linked to distinctive changes in the course of cancer. Newer diagnostic techniques thus serve as the foundation for cancer treatments (9,10).

The pathologist typically uses the qualitative and empirical characteristics of the cells in biopsy sections or cytological preparations to make the final diagnosis of neoplasia (11). In order to improve the examination of the internal components, such as the nucleus, number of nucleoli, amount of chromatin, and abnormalities in the nuclear membrane, these approaches have been aided by morphometric methods, such as surface area determination, volume, axes ratios, and population density estimation (12,13).

The need for efficient treatment protocols and the recognition of oral cancer as a serious public health issue have led to the development of new diagnostic systems like FD, which use clinical and histopathological criteria to help with early diagnosis. Over the past 20 years, medicine has used fractal geometry-first presented by Mandelbrot in 1982-to describe unique patterns found in a variety of organs and normal tissues (14,15).

It is unclear if nuclear fractals will eventually play a significant role in oncologists' toolkits as society shifts away from traditional methods and toward a reliance on technology in all areas. Further comparisons between fractal-related studies and traditional pathological procedures are necessary to achieve this radical shift (16,17).

The nuclear FD (nFD) in OSCC was assessed in a study by Yinti et al. (17) in 2015 utilizing computer-aided image analysis. According to the study's findings, when compared to buccal mucosa that was normal, nuclear FD gradually rose towards the worst tumour stage. These findings are consistent with the current research, which suggests that nuclear FD can be used to quantify nuclear architectural changes as a prognostic indication in OSCC (17).

Normal mucosa had a mean nFD of 1.5806, while well-differentiated squamous cell carcinoma had a mean nFD of 1.9244. This suggests a significant rise in the mean, indicating a continued use of fractal geometry to histological investigation. However, because only the well-differentiated OSCC carcinoma/Grade I tumour according to the Broders classification was included in the study, the maximum and lowest FD values for normal mucosa and OSCC were near. These findings were in line with those reported by Phulari et al. (18), who found that normal mucosa had a mean nFD of 1.7578, epithelial dysplasia had a mean nFD of 1.8363, and squamous cell carcinoma had a mean nFD of 1.9621.

A study by Goutzanis et al. (19) investigated the potential prognostic value of the nFD in tissue samples from patients with oral cavity carcinomas. The present inquiry and the research conducted by Goutzanis et al. (19) examined the connections between FD and additional variables, including clinicopathologic characteristics. Both studies showed that in OSCCs, there are several clinically and statistically significant associations between FD and other morphometric or clinicopathologic variables (19).

Mincione et al. (20) conducted a study in 2015 to evaluate the FD in tissue samples from patients with oral squamous cell cancer (OSCC). Studies looked into relationships between patient survival and clinicopathological factors, as well as FD values at different stages of OSCC. The study's conclusions, which agreed with the results of the current investigation, suggested that fractal geometry might shed light on tumor morphology and be a useful tool for looking at abnormal tumor growth patterns. Furthermore, the current study found statistically significant differences in survival between patients with lower FD values and patients with higher FD values. Thus, one could consider FD to be a predictor of OSCC (20).

The present study adopted the use of TEM images to evaluate the nFD of OSCC and normal mucosa. Significant differences in FD values were obtained when compared with normal oral mucosa thus crediting FDs as a novel, interesting tool, independent diagnostic factor with promising significance in detection of cancer. nFD may be able to assist in classifying a tumor as a low- or high-grade lesion by determining an appropriate cutoff, which would allow us to determine the necessary course of treatment (21).

Despite having a small sample size, the current study has produced encouraging findings that pave the way for more investigation. The techniques described here are quantitative, repeatable, and low in subjectivity and error. They could be modified for use in automated diagnostic systems and expedited screening of numerous histopathological sections (22).

Present study has emphasized the diagnostic importance thereby carrying out many studies in this sector by improving sample size may help in determining more reliable, accurate methods to diagnose OSCC. Further research should be carried out to use fractal analysis in the field of diagnostic pathology.

Conclusion

Scientists around the world are taking part in oral and oro-pharyngeal cancer research in many universities, institutes and medical centers. This research focuses more on what causes the disease, newer strategies for its prevention and how to improve treatment aspects. As of right now, the best approach to manage oral cancer is to combine prompt, appropriate treatment with an early diagnosis. To get a conclusive diagnosis, patients with complaints lasting longer than two to four weeks should be referred to the appropriate specialist.

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Ethics

Ethics Committee Approval: The St. Joseph Dental College's Institutional Ethics Committee in Duggirala, Eluru, granted ethics approval for this study (approval no: CEC/10/2015-16, date: 22.12.2015).

Informed Consent: Informed consent was obtained.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: A.A., P.H.K., B.B., M.K.P., V.S.G., Concept: S.S., A.A., V.S.G., Design: S.S., A.A., P.H.K., B.B., V.S.G., Data Collection or Processing: S.S., M.K.P., Analysis or Interpretation: A.A., V.S.G., Literature Search: P.H.K., B.B., M.K.P., Writing: S.S., M.K.P., V.S.G.

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Awareness of Oral and Medical Healthcare Professionals in the Prevention, Diagnosis, and Management of Bisphosphonate-related Osteonecrosis of the Jaw

Bifosfonatlarla İlişkili Çene Kemiği Nekrozunun Önlenmesi, Tanı ve Tedavisinde Diş ve Tıp Hekimlerinin Farkındalığı

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Abstract

Objective: The aim of this study was to evaluate the awareness and knowledge of oral and medical healthcare professionals regarding the prevention, diagnosis, and management of bisphosphonate-related osteonecrosis of the jaw (BRONJ).

Materials and Methods: Two surveys were conducted with oral and medical healthcare professionals to evaluate their knowledge and attitude about bisphosphonates and their experiences with BRONJ.

Results: A total of 385 oral and 119 medical healthcare responses were obtained. The level of knowledge of oral healthcare professionals who practiced for less than 10 years was significantly higher than that of those practicing for more than 10 years ($p<0.05$). Oral surgeons and periodontists had the highest level of knowledge ($p<0.05$). The knowledge level scores of the medical healthcare professionals exposed to the necrotic jaw during their careers were significantly higher than those who did not ($p<0.05$).

Conclusion: Within its limits, the knowledge of medical healthcare professionals was lower than expected. Knowledge of oral healthcare professionals was moderate, except for oral surgeons and periodontists. Multidisciplinary informative platforms between oral and medical healthcare professionals will benefit the management of potential risks and complications related to BPs.

Keywords: Bisphosphonate, bisphosphonate-related osteonecrosis of the jaw, awareness, survey

Öz

Amaç: Bu çalışmanın amacı, bifosfonatlarla ilişkili çene kemiği nekrozunun (BRONJ) önlenmesi, teşhisi ve tedavisi ile ilgili diş ve tıp hekimlerinin farkındalık ve bilgilerini değerlendirmektir.

Gereç ve Yöntemler: Diş ve tıp hekimlerine bifosfonatlar hakkındaki bilgi ve tutumlarını, BRONJ deneyimlerini ve farkındalıklarını değerlendirmek için iki farklı anket uygulandı.

Bulgular: Toplam 385 diş hekimi ve 119 tıp hekimi anketi yanıtladı. On yıldan az süredir çalışan diş hekimlerinin bilgi düzeylerinin, 10 yıldan fazla çalışan diş hekimlerinden anlamlı olarak daha yüksek olduğu tespit edildi ($p<0,05$). Ağız ve çene cerrahları ve periodontologların en yüksek bilgi düzeyine sahip olduğu saptandı ($p<0,05$). Kariyerleri boyunca çene kemiği nekrozu ile karşılaşan tıp hekimlerinin bilgi düzeyi skoru, karşılaşmayanlara göre anlamlı olarak daha yüksek olarak bulundu ($p<0,05$).

Sonuç: Bu araştırmanın sınırları dahilinde, tıp hekimlerinin konu ile ilgili bilgisinin beklenenden daha düşük olduğu tespit edildi. Ağız ve çene cerrahları ve periodontologlar dışındaki diş hekimlerinin bilgisi orta düzeyde bulundu. Diş ve tıp hekimleri arasındaki multidisipliner bilgilendirici platformlar, bifosfonatlar ile ilgili potansiyel risk ve komplikasyonların yönetimi üzerinde faydalı bir etkiye sahip olacaktır.

Anahtar Kelimeler: Bifosfonat, bifosfonatlarla ilişkili çene kemiği nekrozu, farkındalık, anket

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Introduction

Bisphosphonates (BPs) are used to treat malignant bone diseases, prevent metastatic bone tumors, and prevent osteoporosis-related fractures in osteoporosis and osteopenia patients (1,2). Despite their beneficial effects, they have a severe side effect of bisphosphonate-related osteonecrosis of the jaw (BRONJ), defined as exposed necrotic bone at the maxillofacial region for more than 8 weeks (3). In 2014, as the second diagnostic criteria, bone that can be probed through an intraoral or extraoral fistula was also included (4). In recent years, due to the high number of osteonecrosis cases observed after the prescription of antiangiogenic and antiresorptive medications, the nomenclature was revised to medication-related osteonecrosis of the jaw (MRONJ) (2). A growing number of cancer and osteoporosis cases make this devastating clinical condition a potential complication for medical and oral healthcare professionals. Osteonecrosis lesions may occur spontaneously or after interventional approaches (5).

Medical healthcare professionals are the first link in the chain of events, and they should warn patients about BRONJ. Awareness of oral and medical healthcare professionals about BRONJ is paramount in the prevention, early detection, diagnosis, and management of these cases. Therefore, this study evaluated oral and medical healthcare professionals' knowledge, awareness, and attitude regarding BPs and BRONJ.

Materials and Methods

This observational cross-sectional study was conducted between September 2018 and January 2019. Two different surveys were applied to oral and medical healthcare professionals. According to the sample size calculation, the estimated participation numbers were 379 and 110 for oral and medical healthcare professionals, respectively. A pilot study was performed to ensure the survey's feasibility and validity. The questionnaire applied by Alhussain et al. (6) and El Osta et al. (7) was used for oral and medical healthcare professionals surveys, respectively.

The survey for oral healthcare professionals comprised three sections. The first section included questions about professional experience and demographic data, the second focused on perceptions about BRONJ, and the third assessed knowledge acquisition. The survey for medical healthcare professionals consisted of three sections and included 20 questions. The first section gathered data on demographic and professional characteristics; the second collected information about the number of BPs prescribed monthly and the name/form/duration of the recommended BPs; and the third comprised 30 sub-questions that evaluated the experience and knowledge of professionals. The highest score that could be obtained was 29 and 30 points in oral and medical healthcare professionals surveys, respectively. A higher score indicates a higher level of knowledge. Correct response rates of less than 25%, 25-50%, 50-75%, and more than 75% were considered weak, moderate, good, and excellent, respectively.

This study was approved by the Ethics Committee of İstanbul University-Cerrahpaşa, Cerrahpaşa Faculty of Medicine (decision no: A-19, date: 05.06.2018).

Statistical Analysis

The data were collected via a web-based program (Survey Monkey, US). Normality was checked with the Shapiro-Wilks test. Intergroup differences were evaluated by Kruskal-Wallis, One-Way ANOVA, and Student's t-test. Mann-Whitney was used to determine the groups that caused the difference, and significance was assessed at $p<0.05$. Statistical analyses were conducted using SPSS Statistics 22 software (SPSS IBM, Turkey).

Results

A total of 485 dentists registered with the Turkish Dental Association responded to the survey. Due to missing answers and abandoning the test, 100 participants were excluded. A total of 200 medical health professionals working at the İstanbul University Institute of Oncology and Cerrahpaşa Medical Faculty participated in the survey and related to the abovementioned problems; 119 responded to surveys included in the study.

The average knowledge score for oral healthcare professionals was 14.25±6.39. The knowledge of oral healthcare professionals is presented in Table 1, and the knowledge score by gender, age, specialization status, type of specialty, and duration of work in Table 2. General dentists had significantly lower total knowledge scores than specialists ($p=0.000$; $p<0.05$). Among the specialties, oral and maxillofacial surgeons had significantly higher total knowledge scores than others ($p<0.05$). Additionally, the total knowledge scores of the group with a professional practice duration of 1-5 years were significantly higher than those of groups with a longer duration of work ($p<0.05$) (Table 2).

The average knowledge score for medical healthcare professionals was 19.29±6.83. Correct response rates related to BRONJ are presented in Table 3. Medical healthcare professionals' knowledge score according to demographic and professional characteristics is shown in Table 4. The knowledge level scores of the medical healthcare professionals who spent more than 51% of the day on academic work were found to have significantly higher scores than those who spent less than 25% ($p=0.025$; $p<0.05$). Medical healthcare professionals' knowledge scores according to the answers given to the questions about the use of BPs are shown in Table 5. The knowledge scores of medical healthcare professionals who prescribed BPs were found to be significantly higher than those who did not ($p=0.009$; $p<0.05$).

Table 1. Oral healthcare professionals' BRONJ knowledge

		n	%
Diseases	Osteoporosis	**364**	**94.5**
	Osteitis deformans	**119**	**30.9**
	Diabetes mellitus	3	0.8
	Bone metastasis	**317**	**82.3**
	Multiple myeloma	**254**	**66**
	Hypertension	-	-
Bisphosphonate route	Oral	**371**	**96.4**
	IV-intravenous	**334**	**86.8**
	IM-intramuscular	45	11.7
	Not sure	17	4.4
Data requested for intervention	CTX	**225**	**58.4**
	Complete blood count	24	6.2
	aPTT	8	2.1
	HbA1c	4	1
	Glycemic index	1	0.3
	No idea	123	31.9
Known bisphosphonate drugs	Zometa®	**252**	**65.5**
	Nidilat®	22	5.7
	Fosamax®	**262**	**68.1**
	Prolia®	39	10.1
	Boniva®	**194**	**50.4**
	Aredia®	**140**	**36.4**
	Osteo bi-flex®	31	8.1
	Protelos®	20	5.2
Frequency of BRONJ following oral bisphosphonate use	Common	134	34.8
	Very common	14	3.6
	Rare	50	13
	Very rare	**109**	**28.3**
	Exceptional	**51**	**13.2**
	I don't know	27	7
Frequency of BRONJ following IV bisphosphonate use	Common	**132**	**34.3**
	Rare	3	0.8
	Very rare	35	9.1
	Exceptional	95	24.7
	I don't know	120	31.2
Number of patients with BRONJ symptoms (min-max, mean ± SD)		0-100	2.17±8.3
Number of patients using oral bisphosphonates (min-max, mean ± SD)		0-100	4.72±10.76
Number of patients using IV bisphosphonate (min-max, mean ± SD)		0-70	2.6±7.19

min-max: Minimum-maximum, SD: Standard deviation, CTX: C-terminal telopeptide, BRONJ: Bisphosphonate-related osteonecrosis of the jaw, The correct answers are written in bold

Discussion

The present study aimed to evaluate the knowledge of oral and medical healthcare professionals about BP usage and BRONJ as its side effect. The oral healthcare professionals survey aimed at assessing the knowledge about BRONJ covered several key aspects. Most oral healthcare professionals know that BP is prescribed for osteoporosis and bone metastases but less for other indications. Similar results were observed in literature (8-10). Besides, in a study, 65.4% of oral healthcare professionals could not define the indications of BPs (11). Similar to the literature, the most familiar BP was alendronate, followed by zoledronic acid and ibandronate, and the least was pamidronate in this study (12). On the contrary, in another study, most responders were unaware of alendronate, zoledronic acid, and risedronate sodium (13). Obtaining a complete medical history of the patient and knowing about the information received is undeniably essential to prevent BRONJ. To date, limited data is available for suggesting C-terminal telopeptide (CTX) as a diagnostic marker for high-risk group definition of BRONJ before invasive dental treatments (14,15). Since there is no clear-cut consensus about the topic, 58.4% of participants considered obtaining CTX levels.

In this study, similar to the literature, specialists had higher knowledge scores than general dentists, and oral and maxillofacial surgeons demonstrated significantly higher

Table 2. Oral healthcare professionals' total knowledge score by gender, age, specialization status, type of specialty and duration of work

		Total knowledge score
		Mean ± SD
Gender	Female	14.76±6.59
	Male	13.68±6.12
	p[1]	0.097
Age	23-34	**15.74±6.55***
	35-44	13.03±5.39
	45-54	11.74±5.86
	55-64	11.4±5.12
	p2	**0.000***
Specialization status	No	12.96±5.65
	Yes	**17.93±6.94***
	p[1]	**0.000***
Specialty	Oral and maxillofacial surgery	**22.39±4.78***
	Oral and maxillofacial radiology	15.5±6.36
	Endodontics	15±6.96
	Oral implantology	19.5±4.04
	Orthodontics	8.67±3.32
	Pedodontics	14.14±7.4
	Periodontology	16.78±6.72
	Prosthetic dentistry	14.33±6.76
	Restorative dentistry	16.2±3.9
	p[2]	**0.000***
Profession practice (year)	1-5	**15.65±6.53***
	6-10	**16.04±6.41***
	11-20	12.47±5.55
	>20	11.8±5.57
	p[2]	**0.000***

[1]Student t-test, [2]One-way ANOVA test, *p<0.05, SD: Standard deviation

Table 3. Medical healthcare professionals' BRONJ knowledge

	Correct responses	
	n	%
Criteria for diagnosis of BRONJ-clinical conditions (n=97)		
Exposed bone that has not healed in the maxillofacial region for 8 weeks	75	77.3
No local signs of malignancy in the affected area	29	29.9
No history of radiotherapy on the affected area	16	16.5
Using oral and/or iv bisphosphonates	88	90.7
Having used oral and/or iv bisphosphonates in the past	76	78.4
Absence of necrotic bone or non-specific clinical signs and symptoms may be observed in BRONJ	62	63.9
BRONJ can be observed without infection and pain	58	59.8
Pathological fracture, extra-oral fistula and osteolysis are complications of BRONJ	70	72.2
The diagnosis of BRONJ is generally clinical	62	63.9
Predisposing risk factors in BRONJ (n=94)		
Age >65	75	79.8
Chemotherapy	67	71.3
Dentoalveolar surgery	75	79.8
Comorbidity (anemia, diabetes, kidney failure)	75	79.8
Corticosteroid therapy	84	89.4
Duration of bisphosphonate usage	84	89.4
Oral and/or dental problems can be observed during bisphosphonate usage	81	86.2
Genetic factors	53	56.4
Effect of bisphosphonate	84	89.4
Tobacco usage	65	69.1
IV bisphosphonate usage	75	79.8
Participation in the following statements about the role of diagnostic methods in BRONJ management (n=94)		
There is a little need for diagnostic imaging in patients with clear clinical findings of BRONJ	33	35.1
Panoramic and conventional radiography does not show characteristic findings in the early stages of the disease	41	43.6
Biopsy is recommended only if bone metastasis is suspected	53	56.4
Participation in the following statements in the treatment and prevention of BRONJ (n=94)		
Recovery is often difficult	72	76.6
Temporarily or permanently discontinuing bisphosphonate use is effective in BRONJ management	65	69.1
Surgical treatment turns out the clinical picture of BRONJ more difficult	32	34.0
BRONJ treatment is mainly medical	35	37.2
The effectiveness of additional treatments has not yet been proven	40	42.6
Minimally invasive dental treatments are recommended in patients using bisphosphonates	66	70.2
Prevention of BRONJ is very important	80	85.1

BRONJ: Bisphosphonate-related osteonecrosis of the jaw

knowledge than other specialty groups (16,17). The Korean group associated their result with the greater experience of oral and maxillofacial surgeons face with BRONJ and their further rate of medication history taking (16). On the contrary, in a survey any differences were not observed in the total knowledge score of responders depending on their area of expertise, which was attributed to the low turnout among specialists (18). In this study, oral healthcare professionals with more than 10 years of experience had a lower level of knowledge score when compared to less experience. Similarly, oral healthcare professionals under 30 years of age and recent graduates elucidated better knowledge scores reported about bone resorption inhibitors and BRONJ (18).

In the medical healthcare professionals survey, 76.1% mentioned osteoporosis in terms of therapeutic indication of BP, similar to other studies (19,20). The specialty of the medical healthcare professionals included in the studies can be considered as the reason for these results. As stated in the literature, although the osteoporosis indication is known

Table 4. Evaluation of the knowledge scores of medical healthcare professionals according to demographic and professional characteristics

		Knowledge score		p-value
		Mean ± SD	Median	
Academic degree	Professor	21.5±5.72	22	0.752
	Associate professor	21.4±5.4	21.5	
	Specialist	19.32±6.36	20	
	Research assistant	18.66±7.37	21	
Area of expertise	Internal medicine	21.63±5.61	22	0.108
	Physical therapy and rehabilitation	17.13±6.56	20.5	
	Hematology	18.09±7.96	19	
	Oncology	22.27±4.13	21	
	Orthopedics	19.55±7.58	22	
Age	<30	19.06±6.8	21	0.771
	30-50	18.97±7.47	19	
	>50	21.36±4.13	22	
Gender	Female	18.61±6.6	21	0.310
	Male	19.69±6.98	21	
Profession practice (year)	<5	19.02±7.34	22	0.184
	5-9	17.31±6.51	18.5	
	≥10	20.59±6.14	21	
Daily patient number	<10	20.33±2.66	20.5	0.322
	10-20	18.26±7.09	19	
	>20	19.63±7.01	22	
Clinical practice time (%)	<25%	18.73±7.02	21	0.771
	25-50%	21.17±5.11	21	
	51-75%	20.33±7.11	21	
	>75%	19.14±7.35	22	
Research activities (%)	<25%	18.51±7.06	21	0.037*
	25-50%	22.14±4.74	21.5	
	>51%	24.75±2.5	24.5	

For gender Mann-Whitney U test, for other parameters Kruskal-Wallis test was used, *p<0.05, SD: Standard deviation, BRONJ: Bisphosphonate-related osteonecrosis of the jaw

Table 5. Medical healthcare professionals' knowledge scores according to the answers given to the questions about the use of bisphosphonates

		Knowledge score		p-value
		Mean ± SD	Median	
Bisphosphonate prescription	**Yes**	20.06±6.33	22	[1]0.009*
	No	15.07±8.09	19	
Number of newly prescribed bisphosphonates per month	**<5**	20.69±5.23	21	[2]0.941
	6-10	18.69±8.32	22	
	>10	20.73±4.86	22	
Number of patients using bisphosphonates coming to the clinic (monthly)	**0**	17.90±8.63	20,5	[2]0.149
	<5	17.13±7.99	20,5	
	6-10	19.56±5.85	20	
	>10	21.55±5.55	22	
Encounter with exposed necrotic jaw during medical career	**Yes**	20.78±5.74	22	[1]0.082
	No	18.25±7.28	20	
Condition of treating exposed necrotic jaw during medical career	**Yes**	20.83±7.0	22	[1]0.267
	No	19.13±6.75	21	
Awareness of the effect of bisphosphonates on osteonecrosis of the jawbone	**Yes**	19.29±6.83	21	
	No			

[1]Mann-Whitney U test, [2]Kruskal-Wallis test, *p<0.05, SD: Standard deviation

to be high by medical healthcare professionals working in general clinical areas, specialties such as oncology and radiotherapy, responsible for the follow-up of cancer patients, had more knowledge about other indications.

In this study, a high percentage of awareness was observed about BRONJ as a side effect of BPs (82.4%) in medical healthcare professionals. Even though there seems to be a basic awareness of BRONJ, there are knowledge gaps and a need for additional education (21). Although in this study specialty of medical professionals was not significantly associated with a high knowledge score similar to Kim et al. (19), in other studies, specialty types, especially ear, nose, and throat specialists, oncologists, and physicians who counteracted with cancer patients had higher knowledge scores (7,20). Despite medical healthcare professionals' experience not contributing to the knowledge scores in this study, the highest level of awareness was observed in the group with more than 20 years of work experience in Kim et al.'s (19) study. Miranda-Silva et al. (20) reported that professionals with 5-10 years and less than 5 years of training presented significantly higher knowledge scores. In recent years, medical healthcare professionals have encountered patients using BP and have BRONJ in increasing frequencies, which may affect the relationship between experience and knowledge. Accordingly, in this study, the knowledge level scores of the medical healthcare professionals exposed to the necrotic jaw during their career were significantly higher than those who did not face this clinical condition (p<0.05). Most medical healthcare professionals who participated in this study (82.1%) could not entirely identify the BRONJ with the defined clinical criteria. Since medical healthcare professionals are one part of the equilibrium for BRONJ management, emphasis should be given to continuous professional training strategies.

A possible limitation of this study is that only Turkish oral and medical healthcare professionals were included. Therefore, the conclusions drawn from this study can only be generalized to Turkey.

BPs were included in the core basic education of Dentistry a long time ago. For this reason, dentists are expected to graduate with knowledge about the subject. This may explain differences in the responses to knowledge questions among oral and medical healthcare professionals. With the increase in the use of BP, the incidence of its side effects has also increased, and oral and medical healthcare professionals now encounter BRONJ patients more often. Understandably, healthcare professionals are more likely to encounter this situation and know more about the subject.

Conclusion

The information gathered from this study highlights the approach of oral and medical healthcare professionals to BRONJ and the need for additional training or guidelines to ensure that patients receive scientifically evidence-

based treatments. Prevention remains the most critical aspect of the management, and AAOMS re-emphasizes the importance of a multidisciplinary approach to treating patients receiving antiresorptive therapies. As stated, a continuous effort is needed to educate patients and oral and medical healthcare professionals and the coordinated work of the oral and medical healthcare professionals has the utmost importance.

Ethics

Ethics Committee Approval: This study was approved by the Ethics Committee of İstanbul University-Cerrahpaşa, Cerrahpaşa Faculty of Medicine (decision no: A-19, date: 05.06.2018).

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Authorship Contributions

Concept: Y.D., İ.Ö., Ş.D.İ., G.Ç., C.A., Design: Y.D., İ.Ö., Ş.D.İ., G.Ç., C.A., Data Collection or Processing: Y.D., Analysis or Interpretation: Y.D., G.Ç., C.A., Literature Search: İ.Ö., Ş.D.İ., Writing: İ.Ö., Ş.D.İ.

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